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Biochemistry and Role in Diseases

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Meet the editor

Andrei Surguchov joined Baylor College of Medicine, Houston, TX, as a faculty member in 1992, where he studied the mechanisms of the genetic control of lipid metabolism. At the University of Utah, Salt Lake City, UT, his research interests were focused on cloning of new genes encoding retinal proteins. At Washington University, St. Louis, MO, he studied molecular and cellular mechanisms of neurodegenerative diseases and retinal degeneration. Currently, his research focuses on structure-function relationship of proteins involved in neurodegeneration and ocular diseases. Using different animal and cellular models, his laboratory is studying the role of naturally unfolded proteins, their transcriptional regulation, processing, and posttranslational modifications. Recently, he became interested in the role of micro-RNA in posttranscriptional regulation of gene expression.

Contents

Preface XIII

Chapter 1 1
Introductory Chapter: Little Pigeons Can Carry Great Messages
by Andrei Surguchov

Chapter 2 5
Alpha-Synuclein Aggregation, Cholesterol Transport, and the 18-kDa Translocator Protein
by Jasmina Dimitrova-Shumkovska and Ljupcho Krstanoski

Chapter 3 21
The Relationship between Alpha-Synuclein (SNCA) Gene Polymorphisms and Development Risk of Parkinson's Disease
by Nevra Alkanli and Arzu Ay

Chapter 4 33
Therapeutic Potential of Baicalein in Parkinson's Disease: Focus on Inhibition of α-Synuclein Oligomerization and Aggregation
by Hayate Javed and Shreesh Ojha

Chapter 5 49
Role of Phenylalanine and Its Metabolites in Health and Neurological Disorders
by Muhammad Akram, Muhammad Daniyal, Aatiqa Ali, Rida Zainab, Syed Muhammad Ali Shah, Naveed Munir and Imtiaz Mahmood Tahir

Chapter 6 63
Role of Osmolytes in Amyloidosis
by Sheeza Khan, Zeba Mueed, Ravi Deval, Pankaj Kumar Rai, Dinesh Kumar Prajapati and Nitesh Kumar Poddar

Preface

Synucleins are small proteins implicated in neurodegenerative disorders and several forms of cancer. The study of synucleins brought about several amazing discoveries in addition to their role in human diseases, including their role in song bird learning, prion-like properties, vesicle trafficking during neurotransmitter release, and possible propagation of α-synuclein in the gut-brain axis. At the same time, the detailed physiological functions of synucleins remain elusive. This book provides the reader with a comprehensive overview of the current state-of-the-art in synuclein studies, featuring the most significant findings about their role in diseases and remarkable and surprising discoveries of their involvement in various processes. These include mechanism of alpha-synuclein aggregation and its connection with cholesterol transport, the role of alpha-synuclein gene polymorphisms in the development of Parkinson's disease, therapeutic potential of baicalein inhibiting alpha-synuclein oligomerization and aggregation, role of osmolytes in amyloidosis, and other topics. It is intended for specialists in neurodegenerative diseases, general practitioners, neurologists, trainees, and researchers to increase the knowledge and understanding of these complex diseases and to encourage further investigation for the benefit of the entire human community.

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Chapter 1

Introductory Chapter: Little Pigeons Can Carry Great Messages

Andrei Surguchov

1. Introduction

Synucleins are a family of proteins involved in neurodegenerative diseases and cancer. The family contains three structurally similar and evolutionary conserved proteins: α-, β-, and γ-synuclein. Synucleins are small proteins containing 140, 134, and 127 amino acids, respectively. In spite of their minor size, these proteins are implicated in numerous functions, including regulation of synaptic transmission, signal transduction, gene expression, and membrane permeability. They are localized in the cytoplasm, nucleus, and mitochondria [1]. After the discovery of the three synucleins, their investigations were primarily focused on α-synuclein for its involvement in several human neurodegenerative diseases known as synucleinopathies, including Parkinson's disease (PD), Parkinson's disease dementia (PDD), dementia with Lewy bodies, multiple system atrophy, and a number of less well-characterized neuroaxonal dystrophies [2]. Structurally α-synuclein can be subdivided into three regions: residues 1–60 belong to an amphipathic N-terminal region, containing four 11-residue repeats including the consensus motif KTKEGV. This part of the molecule has a structural alpha helix propensity similar to apolipoprotein-binding domains. The middle part of the molecule (residues 61–95) is a hydrophobic region which includes the non-amyloid-β component (NAC) region, involved in protein aggregation. Finally, C-terminus (residues 96–140) is a highly acidic, proline-rich region which is the most variable among the three members of the family.

Molecular and cellular studies of synucleins brought many exciting discoveries, such as their prion-like properties, ability to bind to DNA and change its functional state [3], and spreading and propagating between cells transducing certain signals from one cell type to another [4].

Interestingly, recent findings suggest that not only are synucleins located inside the cell but also extracellular synucleins present in the plasma, serum, cerebrospinal fluid, as well as in conditioned media and play important regulatory functions. Synucleins are secreted from cells by unconventional secretion and exocytosis or by exosomes and can spread readily between cells. Transfer can occur not only between neurons but also from neurons to glial cells, as well as between glial cells [5]. Binding of extracellular α-synuclein to CD11b integrin (the α-chain of integrin $\alpha_M\beta_2$) switches on an intracellular signaling cascade leading to the activation of microglia [6]. The results of another recent study further support the relevance of integrin CD11b to synuclein-mediated signaling. CD11b regulate intracellular signaling induced by the aggregated α-synuclein through a RhoA-dependent pathway [7]. Thus, these small proteins carry great messages inside and outside the cell.

In this book, the results of synuclein investigation by methods of biochemistry, genetics, and cell and molecular biology are presented.

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Chapter 2

Alpha-Synuclein Aggregation, Cholesterol Transport, and the 18-kDa Translocator Protein

Jasmina Dimitrova-Shumkovska and Ljupcho Krstanoski

Abstract

The molecular responses to counteract diseases, including insulting conditions such as injury and pathogen infection, involve coordinated modulation of gene expression programs. The association of alpha synuclein (α-Syn) with several progressive disorders has focused the research on its induced conformational behavior as critical for uncovering the "secrets" for progression of α-synucleinopathies. Cholesterol is one of the lipid components crucial for regular proliferation of the nervous tissue. Its interaction with α-Syn may offer other insights to α-Syn normal expression. Discovering that the molecular regulatory mechanisms responsible for prevention of α-Syn aggregation may be manifested through microRNA (miRNA) regulated gene expression is also crucial for widening the perception of neuropathology. The 18-kDa translocator protein (TSPO) localized on the outer mitochondrial membrane is able to regulate various cellular and tissue functions, with key role as cholesterol transporter for neurosteroid synthesis. TSPO up-regulation, has been connected to several diseases, including cancer, neuronal damage, and inflammation. Connection may also be established between TSPO expression and fatty acid oxidation, thus unveiling new possibilities in the research of α-Syn overexpression. However, expression of TSPO in the neuroinflammatory environment is probably the best starting point for targeting TSPO as a suitable therapeutic target.

Keywords: alpha synuclein, lipid interaction, inflammation, oxidative stress, TSPO ligands

1. Synucleins family—new insights and prospects

Synucleins are a family of small and soluble proteins expressed mostly in neural tissue and cancer cells. Existing findings have identified three members of this family: α, β, and γ—synucleins (α-Syn, β-Syn, and γ-Syn, respectively). Rather than unveiling their physiological properties and functions in normal brain tissue, the synucleins are mostly exploited as biomarkers for neurodegenerative diseases since the discovery of their involvement in proteinaceous aggregation in patients with Alzheimer's disease (AD) [1]. In the following years, synucleins have been linked with other neuronal disorders increasing the interest of elucidating their connection with these diseases.

α-Synucleinopathies are severe neurodegenerative disorders caused by abnormal accumulation and subsequent aggregation of insoluble α-Syn, a small and

intrinsically unfolded cytosolic protein localized at synaptic terminals, in structures called Lewy bodies (LBs) in neuronal or glial cells [2, 3]. Establishing its involvement in synaptic maintenance, mitochondrial homeostasis, and neurotransmitter release regulation, α-Syn impaired function is considered as a direct cause for several progressive disorders such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). Other rare diseases, mainly associated with neuroaxonal dystrophy have also shown α-Syn pathologies [4].

Despite early discovery of α-Syn as a product of SNCA gene whose dysfunction was considered as the primary cause for PD development [5], excessive research has been carried out in order to fully disclose the reasons for α-Syn aggregation. In the following years, nine other genes such as PARK, PINK, and LRRK involved in PD pathology were discovered [6–8], but so far missense mutations and multiplication of α-Syn-encoding gene are considered as the most often causation of familial form of PD [9]. Intensive research is mainly focused on discovering the effects of fibrillization, oligomerization, and misfolding of this protein as well as developing a suitable methods for its quantification in biological fluid enabling early diagnosis of PD [10–12]. Efforts are also being made to elucidate the participation of other molecules in the α-Syn altered dynamics. Namely, Abeyawardhane et al. reported the contribution of oxygen and redox active iron in conformational change and oligomerization of α-Syn, which can be useful in understanding the mechanisms of its physiological and/or pathological role [13]. The strong ability of α-Syn to form complexes with other biomolecules such as lipid moieties and cholesterol has also been reported. This capability is due to the presence of the repeats of lipoprotein-like hexamer sequence (KTKEGV) in synucleins, which may reveal other approaches for the diagnosis and therapy of neurodegenerative diseases [14, 15].

Furthermore, it has been shown that this protein was also expressed in erythroid precursors, megakaryocytes, and platelets [16, 17]. α-Syn is assumed to participate in negative regulation of calcium dependent α-granule release, thus implying that its presence is crucial for normal development and functioning of platelets [18, 19]. Relevant to this context, platelets have immense diagnostic value for neurocognitive diseases since several studies reported the significantly decreased levels of amyloid β protein precursor (AβPP) and mean platelet volume (MPV) in AD patients [20, 21]. Further supporting the concept, the presence of α-Syn in platelets impacts MPV level through the SCNA gene expression, whereas its concentration remains unaltered in patients with cognitive impairment [19]. However, α-Syn concentration in plasma supernatant is considered as a significant marker for the quality of single donor platelet samples during storage time [22].

β-synuclein (β-Syn) although somewhat smaller protein than α-Syn is also localized in presynaptic terminals, secreted and expressed in similar levels [23]. Early research concerning β-Syn properties and function suggests that this protein, even though 78% identical to α-Syn, is not present in LBs, and therefore, it is not directly involved in neurodegenerative and neurocognitive pathology. The main "advantage" of β-Syn against the induced changing of its conformation is the absence of nonamyloid β component (NAC) domain in its structure. Hence, β-Syn can significantly reduce the initiation of self-assembly and aggregation of α-Syn since it lacks this highly hydrophobic domain, which may prove beneficial against abnormal accumulation of α-Syn, thus preventing neurodegeneration [24, 25].

Several studies have shown the natural antagonism between the two molecules providing the mechanisms for inhibition of α-Syn aggregation both *in vivo* and *in vitro* [26, 27]. Janowska et al. reported that β-Syn-mediated inhibition of α-Syn aggregation occurs by direct interaction between the molecules at specific sites. This ultimately results in the formation of heterodimers further implying that balance between the specificity and affinity of α-Syn/β-Syn interactions is crucial

for maintaining favorable reduction rates of α-Syn aggregation [28]. A study by Brown et al. also suggested that β-Syn molecules can only inhibit the nucleation of lipid bound and fibril forms of α-Syn aggregates by competitive binding to the surface of vesicles prepared from the phospholipid 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (DMPS), and this inhibition is pH dependent. In addition, they confirmed that β-Syn has no effect on elongation of α-Syn aggregates [29]. It seems that further research is needed in order to highlight the exact mechanism and conditions in which β-Syn prevents the aggregation of α-Syn, therefore enabling its use as antiparkinsonian agent.

γ-Synuclein (γ-Syn) has been identified in various human tissues, and its expression is significantly upregulated in ovary, liver, and cervical cancer, with specific overexpression in breast cancer linked with tumor development and promoting of cancer metastasis through demethylation of CpG islands and activation of insulin-like growth factor pathway [30–32]. Similar to β-Syn, γ-Syn is also naturally found in peripheral neurons, and it has not been directly correlated with pathology of neurocognitive diseases, although differences have been reported in its expression [33]. Beside the existence of γ-Syn in nervous and malignant tissue, studies also reported its presence in the skin particularly in *stratum granulosum* where it could be included in modulation of keratin [34]. This synuclein member is also found in retinal ganglion cells (RGCs) where its decreased expression was first correlated with the development of glaucoma [35]. Later, research indicates that γ-Syn downregulates kinases involved in activation of pro-apoptotic signaling pathways in RGCs, therefore playing a key regulatory role in progression of this disease [36]. The protective mechanism of γ-Syn antibodies in neuroretinal cells against oxidative stress by increasing the viability and altering their apoptosis rate has also been reported [37].

2. Regulation of α-synuclein expression

Because of the genetic background of α-synucleinopathies, research must also be focused toward discovering the exact molecules and mechanisms for posttranscriptional and epigenetic regulation of SNCA gene. Up to this point, it is established that not only changes in the gene sequence (multiplications, missense mutations, and single nucleotide polymorphisms) but also activation of certain transcriptional factors and RNAs may affect α-Syn regular expression [38]. MicroRNAs (MiRNAs) are small non-coding RNA molecules encoded as independent genomic transcription units predominantly engaged as regulators of protein expression mostly through inhibition of mRNA translation or cleavage [39, 40]. Ever since their discovery, miRNA dysregulation is correlated with the pathogenesis of numerous diseases and disorders such as cancer, diabetes, nonalcoholic fatty liver disease (NAFLD), neurological and cardiovascular diseases (CVD) [41, 42]. As mentioned earlier, the main causes for PD development are mutations in genes resulting in a α-Syn overexpression and modification, so it is highly possible that PD progression and/or inhibition can be managed by alteration of certain miRNAs. So far, it has been confirmed that they can affect several signaling pathways involved in PD development, therefore enabling their use as biomarkers or alternative therapy for PD, as well as other types of dementia.

Because the oligomerization and fibrillation of α-Syn is primarily associated with increased production of reactive oxygen species (ROS) and subsequent mitochondrial dysfunction in neuronal cells, research has been conducted in order to identify the key miRNAs involved in regulation of brain mitochondrial function [43]. Namely, several studies have reported the down regulatory effects

of miR-34b and miR-34c on the expression of protein deglycase DJ-1, involved in α-Syn degradation via chaperone-mediated autophagy (CMA), thus preventing the ROS outburst from complex I or other constituents from the electron transport chain (ETC) [44–46]. Recent publication by De Miranda et al. also marked the DJ-1 as an essential for maintaining the integrity of dopaminergic neurons which is accomplished by reduction of nitrosative stress and suppression of rotenone-induced inflammatory response, thus highlighting its value as potential therapeutic target [47]. Furthermore, it was also elucidated that decrease of miR-34 b/c levels in neuronal cells leads to the loss of mitochondrial potential and reduction of ATP production. Accordingly, the depletion of these miRNAs directly contributes for decreased levels of DJ-1 in brains from PD, with a direct binding to the 3′-untranslated region (3′-UTR) of their mRNAs which proves their neuroprotective role [46]. Additionally, miR-4639 and miR-494 were identified in the list of potential inhibitors of DJ-1 expression, suggesting the measurement of their levels in human plasma as prognostic biomarkers of PD [48, 49] (**Figure 1**).

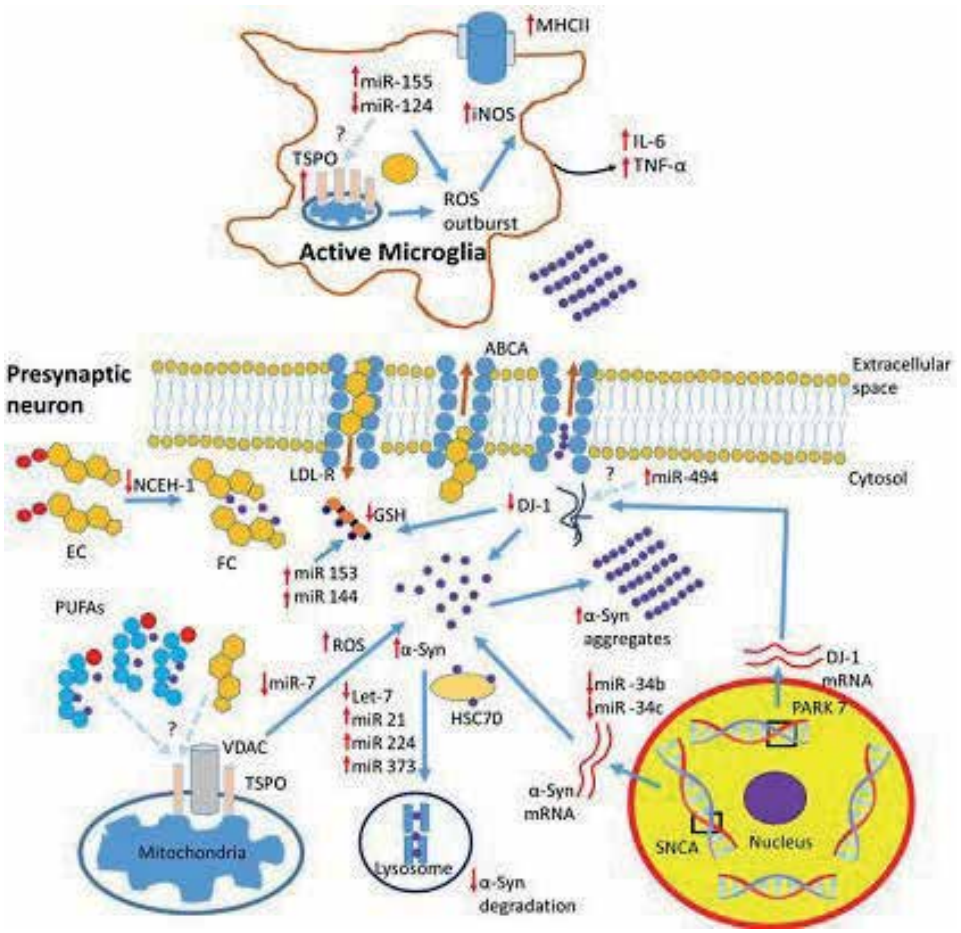

Figure 1.
Summary of signaling pathways involved in α-Syn aggregation and potential connection of 18-kDa translocator protein (TSPO) with neurodegeneration and neuroinflammatory response. Abbreviations: alpha synuclein (α-Syn), low density lipoprotein receptor (LDL-R), esterified cholesterol (EC), free cholesterol (FC), polyunsaturated fatty acids (PUFAs), heat shock cognate 71-kDa protein (hsc70), interleukin 6 (IL-6), tumor necrosis factor alpha (TNFα), neutral cholesterol ester hydrolase (NCEH-1), 18-kDa translocator protein (TSPO), major histocompatibility complex class II protein (MHC II), and ATP-binding cassette subfamily A (ABCA).

Similarly to the effects of miR-34 b/c, it was confirmed that miR-7 also plays a key role in α-Syn repression by directly binding to the 3′-UTR sequence of its mRNA in different experimental models such as SH-SY5Y cells, HEK293T cells, primary neurons, and pancreatic islets [50–52]. Moreover, Junn et al. discovered the protective role of miR-7 against hydrogen peroxide-mediated cell injury in cells expressing mutant A53T form of α-Syn [51]. The effects of MiR-7 on cell death reduction in experimentally induced PD symptomatology by various neurotoxins such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and its active metabolite MPP^+ (1-methyl-4-phenylpyridinium) was reported as well [53]. A recent study indicated that miR-7 can also achieve its protective role by regulating the expression of the voltage dependent anionic channel (VDAC) in the outer mitochondrial membrane (OMM), thereby preventing MPP^+-induced cellular damage [54]. Since VDAC is crucial part of mitochondrial permeability transition pore, its function is primarily associated with maintaining the polarization of OMM and balancing ROS production. Research has confirmed that VDAC overexpression can increase free radical generation and cause the release of pro-apoptotic proteins ultimately promoting mitochondrial swelling which inevitably triggers α-Syn aggregation [55]. The discovery that cells producing A53T had swollen mitochondria puts VDAC in the list of key molecules involved in progression of α-synucleinopathies [56]. Overall, it can be concluded that miR-7 optimal expression is directly "responsible" for regular neural development, and future research should be focused on finding suitable vectors in order to include this molecule in gene therapy for neurodegenerative diseases.

As indicated earlier, cellular mechanisms for α-Syn degradation such as CMA can also be affected by miRNAs. Studies revealed that the increase of miR-21, miR-224, and miR-373 levels leads to suppression of heat shock cognate 71-kDa protein (hsc70) which impairs α-Syn degradation via CMA [57]. Moreover, Shamsuzzama et al. reported that Let-7 miRNA knockdown might influence CMA by modulating gene expression and enhancing ROS production in *C. elegans* [58].

MiRNAs have also been connected with pathways related to synthesis and expression of enzymatic and nonenzymatic radical scavengers within brain cells effectively "delaying" any genetic aberrations and expression α-Syn mutant forms. These are included in regulation of nuclear factor erythroid 2-related factor 2-antioxidant response element (Nrf2-ARE), which is also one of the reasons for PD progression [59]. Narasimhan et al. observed that the overexpression of miR-153, miR-27a, miR-142-5p, and miR-144 weakened the antioxidant response in SH-SY5Y cells throw decreasing the activity glutathione reductase (GSR) with impact on GSH/GSSG homeostasis [60].

Progression of PD due to α-Syn aggregation results in brain inflammatory response as primary defensive mechanism against neurodegeneration achieved through microglial cells. This process is mainly associated with activation of several components in the inflammatory cascade such as interleukins, members of the complement system, and receptors or enzymes whose expression is essential for proper immune defense [61, 62]. Neuroinflammation can also be aggravated by dietary components such as artificial sweeteners who additionally enhance dopamine degeneration and gravity of the immune response [63]. Discovery of miRNAs highlighted the possibilities of regulating the intensity and severity of the immune response against α-Syn-mediated inflammation. Specifically, Thome et al. reported the pro-inflammatory effects of miR-155 in brain microglia against α-Syn fibrils manifested through elevation of inducible nitric oxide synthase (iNOS) and major histocompatibility complex class II proteins (MHCII) expression, thus keeping the integrity of dopamine neurons [64]. A recent study confirmed that miR-124 could suppress microglial activation by regulating the expression of inflammatory cytokines [65] (**Figure 1**).

Because of the complexity of brain inflammatory response, it is necessary to extend the research for other molecules that might be included in its mediation. The number of studies has suggested the 18 kDa mitochondrial translocator protein (TSPO) as potential biomarker for neurodegenerative disorders [66, 67]. This protein is included in cholesterol transport into the mitochondria where it serves as a substrate for neurosteroid biosynthesis [68]. As previously reported that brain injury increases TSPO binding affinity for its ligand PK11195 [69, 70], the connection between TSPO expression and α-synucleinopathies has not been sufficiently explained [71]. Namely, it was reported that TSPO exhibited increased striatal PK11195 binding potential in patients with PD and DLB, but its expression remained unaltered compared to healthy controls [72, 73]. Regarding neuroinflammation, TSPO overexpression is also associated with activation of microglia/macrophages, revealing yet another unexplored role of this receptor [74]. There is also overwhelming evidence that TSPO ligands and agonists possess neuroprotective properties, but so far little is known about the precise functions of TSPO itself in brain cells [75, 76]. Overall, it seems that further research is needed in order to elucidate the regulatory mechanism of miRNAs in neuroinflammation and the possible correlation with TSPO.

2.1 α-Synuclein, lipid homeostasis, and TSPO

As mentioned earlier, α-Syn possesses intriguing and still not fully characterized affinity of interacting with fatty acids, cholesterol and phospholipids, and other cell lipid molecules. This implies that high levels of polyunsaturated fatty acids (PUFAs) normally present in healthy brain tissue, which not only increase its membrane fluidity and permeability but also serve as energy sources and second messengers, could be one of the reasons for α-Syn increased expression in the nervous system [77]. Further *in vivo* investigation, revealed that α-Syn overexpression in patients with α-synucleinopathies caused an increase of PUFA levels without alteration on saturated and monounsaturated fatty acid composition [78]. The decline in n-6/n-3 ratio during aging, increased lipid peroxidation and decreased brain volume, are between main factors promoting neurodegenerative disorder. Following this assumption, many studies proved that the enhanced multimerization and interaction of this protein with PUFAs, particularly with arachidonic and docosahexaenoic acid, result in the formation and aggregation of insoluble high-molecular complexes in LBs, which might unveil new insights into PD diagnostics [79, 80]. Based on the aforementioned findings, research should be focused on discovering whether α-Syn/PUFA interactions could be a sufficient proof of the alleged scavenging activity of α-Syn in experimental models of PD. So far, results based on spectrometric analysis confirm that PUFA interacts solely with α-helical secondary structure of α-Syn in optimal protein/PUFA ratio, which strongly suggests that α-Syn may prevent the initiation of lipid peroxidation given the high autoxidation rates of PUFAs [81]. Researchers have also reported the regulatory role of α-Syn of other lipid molecules such as triacylglycerols and cholesteryl esters, considering their increased conversion to lipid droplets in α-Syn expressing cells due to the modulating activity of lipid metabolizing enzymes, such as acyl-CoA synthase [82, 83].

Other important biomarkers for neurodegenerative disorders are the phospholipase D (PLD) isoforms which are crucial enzymes mostly involved in cytoskeleton structure and cellular signaling processes in the brain. More recent studies reported that inflammation caused by oxidative stress triggers PLD signaling as part of the synaptic response in neurodegeneration indirectly insinuating a connection

between PLD and α-Syn overexpression [84, 85]. Conde et al. has confirmed this connection by proving that this protein acts as an inhibitor of PLD1 in WT α-Syn neurons [86].

Cholesterol is also one of the lipid components which homeostasis is crucial for regular proliferation of the nervous tissue, if properly regulated. It acts as an integral membrane component, improving its structure and function. As mentioned earlier, studies have already established the interaction between α-Syn and cholesterol, indirectly making a correlation between cholesterol levels and α-Syn normal expression [15]. In a study by Hsiao et al., α-Syn was described as mediator of cholesterol efflux from SK-N-SH neuronal cells enabled by an ATP-binding cassette subfamily A (ABCA1) [87]. In accordance with these discoveries, the possible neuroprotective role of enzymes included in "cellular capturing and release" of cholesterol such as *neutral cholesterol ester hydrolase* (*NCEH*) or *Acyl Co-A:cholesterol acyltransferase* (*ACAT*) was also investigated. Namely, Zhang et al. discovered that *NCEH-1* knockdown increases the aggregation of α-Syn and dopamine neural damage in *C. elegans*, while inhibition of ACAT gave the opposite effect. Moreover, they confirmed the previous hypothesis concerning normal cholesterol levels and α-Syn expression, further implying that this relation is also a highly important factor for triggering the neuroprotective role of *NCEH-1*. Finally, these authors suggested that exogenous cholesterol does not have beneficial effects against neural degeneration [88].

Taking into account that TSPO is also involved in alterations of cytosolic cholesterol levels, there is also a possibility for its involvement in modulation of α-Syn aggregation rates. Connection has also been established between TSPO binding capacity and ROS levels, which are as mentioned earlier one of the reasons for PD development [89, 90]. In accordance with these findings, Gatliff et al. reported that SH-SY5H cells exhibited enhanced ROS production after TSPO overexpression establishing connection between TSPO, VDAC, and Ca^{2+} homeostasis [91]. On the other hand, it is also suggested that TSPO expression is inversely correlated with fatty oxidation rates in steroidogenic cells [92], which may be a plausible starting point in discovering whether TSPO has the same effect in neurons and if so, could altered expression of TSPO prove beneficial against neurodegenerative disorders considering the α-Syn interactions with PUFAs and cholesterol (**Figure 1**).

3. Conclusions

A systematic research in the last two decades highlights the precise mechanisms and pathways for regulation of α-Syn expression and aggregation, involved in neuropathologies. Success has also been made in demonstrating the possible therapeutic values of miRNAs, receptors, and other bioactive molecules with specific intentions for their inclusion in modern therapy for dementias. Future research should be focused on discovering the proposed beneficial actions of the interactions between lipids and α-Syn with particular interest in the potential involvement of TSPO in cholesterol homeostasis of the neural cells (**Figure 1**).

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Abbreviations and symbols

α-Syn	alpha synuclein
β-Syn	beta synuclein
γ-Syn	gamma synuclein
NAC	non-amyloid-β component
SNCA	alpha synuclein gene
PINK	PTEN-induced kinase
PD	Parkinson's disease
AD	Alzheimer's disease
DLB	dementia with Lewy Bodies
LBs	Lewy Bodies
AβPP	amyloid β protein precursor
MPV	mean platelet volume
RGCs	retinal ganglion cells
DMPS	1,2-dimyristoyl-sn-glycero-3-phospho-L-serine
NAFLD	nonalcoholic fatty liver disease
CVD	cardiovascular disease
UTR	untranslated region
A53T	mutant form of alpha synuclein
VDAC	voltage dependent anionic channel
OMM	outer mitochondrial membrane
ROS	reactive oxygen species
CMA	chaperone-mediated autophagy
hsc70	heat shock cognate 71-kDa protein
Nrf2-ARE	erythroid 2-related factor 2-antioxidant response element
DJ-1	protein deglycase DJ-1
iNOS	inducible nitric oxide synthase
MHCII	major histocompatibility complex class II proteins
TSPO	18-kDa translocator protein
PK11195	1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline-carboxamide
PUFAs	polyunsaturated fatty acids
PLD	phospholipase D
NCEH-1	neutral cholesterol ester hydrolase
ACAT	acyl Co-A:cholesterol acyltransferase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP^+	1-methyl-4-phenylpyridinium

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Chapter 3

The Relationship between Alpha-Synuclein (SNCA) Gene Polymorphisms and Development Risk of Parkinson's Disease

Nevra Alkanli and Arzu Ay

Abstract

Parkinson's disease (PD) is a neurodegenerative disorder affecting the motor system and occurring in the central nervous system. One of the symptoms of PD is accumulation of Lewy bodies and Lewy neurites. The alpha-synuclein (SNCA) gene is part of the protein complex called Lewy body. The SNCA gene encoding a presynaptic protein product is thought to play a role in PD-related important pathways. It is suggested that there is a relationship between the risk of PD development and SNCA levels, and it is suggested that SNCA level is an important marker in PD diagnosis. Various polymorphisms have been identified in the 5′ and/or 3′ UTR regions of the SNCA gene, and as a result of these polymorphisms, changes occur in the binding of transcription factors. The identification of the roles of SNCA gene polymorphisms in PD development may enable the development of new methods for the treatment of PD.

Keywords: Parkinson's disease, neurodegenerative disorders, SNCA gene polymorphisms, Lewy bodies, SNCA levels

1. Introduction

The most important feature of age-related neurodegenerative diseases is the slow and irreversible deterioration of brain function. PD affects the motor system and is a neurodegenerative disorder of the central nervous system [1]. The prevalence of PD, which is known to be the second progressive neurodegenerative disease, increases with age. PD, which causes severe morbidity, has motor symptoms such as tremors, bradykinesia, muscle stiffness, and postural instability and non-motor symptoms, i.e., autonomic dysfunction, sensory symptoms, sleep disturbances, and fatigue. These symptoms occur as a result of the progressive neurodegeneration of dopaminergic neurons in the substantia nigra pars compacta, clustering of proteins within the brain, Lewy bodies, and Lewy neurites [2, 3]. As a result of degeneration of dopaminergic neurons, movement disorder occurs, and the cause of this movement disorder is the neurotransmitter dopamine deficiency. Although the basic mechanism of neuronal death in PD is unknown, genetic and environmental factors have been found to be effective in the pathogenesis of the disease [4].

It has been reported that many genes play a role in the pathogenesis of PD, and polymorphisms in these genes may be genetic risk factors for PD development [5]. A large number of different DNA variants have been identified in disease genes associated with familial PD in molecular genetic analyses. These genetic variants include SNCA, Parkin (PARK2), PTEN-induced putative kinase 1 (PINK1), DJ-1 (PARK7), and leucine-rich repeat kinase 2 (LRRK2) variants [6].

The SNCA gene plays a role in important pathways associated with PD. It is suggested that there is a significant relationship between SNCA levels and the risk of PD development, and SNCA levels are thought to be an important marker in the diagnosis of PD. Several studies have been conducted to explain the relationship between genetic polymorphisms in different regions of the SNCA gene and risk of PD [7].

Polymorphisms that occur in different regions of the SNCA gene, an important gene for PD etiology, have been identified in relation to PD [7, 8]. Changes in SNCA expression levels are one of the main mechanisms of SNCA to cause PD [7, 9, 10]. As a result of polymorphisms in the 5′ and 3′ UTR regions of the SNCA gene, the binding of transcription factors and miRNAs can be altered, and promoter activity is affected. Thus, gene expression can be regulated. According to the general results from the studies aimed to investigate the relationship between SNCA gene polymorphisms and the risk of PD development, the SNCA gene and some polymorphisms of this gene have been identified as genetic risk factors in PD development [7].

The purpose of this chapter is, in addition to giving general information about PD, to summarize the studies that investigated on the relationship between SNCA gene polymorphisms and the risk of developing PD.

2. Parkinson's disease

Parkinson's disease (PD), the second most common neurodegenerative disease after Alzheimer's disease, affects approximately 1–2% of individuals over 65 years of age [11, 12]. The incidence of PD usually begins after 50 years of age and increases more after 60 years of age [13]. The prevalence of PD increases approximately 4% in people over 85 years of age [6]. PD is a neurological disorder associated with increased morbidity and reduced survival [14, 15].

PD is characterized by bradykinesia, resting tremor, rigidity, and postural instability. PD is associated with the formation of Lewy bodies [16]. Lewy bodies of postmortem that are determined in brain autopsy specimens are the distinguishing features of PD. These bodies are observed in intense eosinophilic nuclei and cytoplasmic inclusions. The presence of intraneuronal protein inclusions, called Lewy bodies or Lewy neurites, is one of the neuropathological features of PD [17].

In PD, in addition to motor symptoms, non-motor symptoms occur. Motor symptoms in PD are due to the selective loss of dopaminergic neurons in the midbrain and the axon terminals reflected in the dorsal striatum [18]. Motor symptoms occur when neuronal cell loss reaches 80% or more as a result of progressive loss in the dopaminergic neurons of substantia nigra pars compacta [19]. Non-motor symptoms of PD are autonomic dysfunction and cognitive impairment [20]. The motor and non-motor symptoms of PD are caused by the loss of the dopaminergic neurons of the substantia nigra [21–23]. PD is defined as a syndrome because it is a complex disease characterized by motor and non-motor symptoms [20].

The majority of PD cases are sporadic cases; however, it is known that 10–15% are familial cases, and the majority of these cases are hereditary. Environmental and genetic factors play a role together in the pathogenesis of PD, which has a complex etiology [24].

3. Protein encoded by SNCA gene

SNCA protein is the major protein of Lewy bodies. This protein is a presynaptic phosphoprotein that has a specific tendency to aggregation that plays an important role in both hereditary and idiopathic PD [25]. SNCA protein and fibrils constitute Lewy bodies [16]. In Lewy bodies, SNCA protein is predominant and is therefore known to be associated with the etiology of PD. SNCA also has an important function in the pathological process of PD [26, 27]. SNCA, an important component of Lewy bodies, is one of the distinctive features of PD [28–30].

The SNCA protein, which contains 144 amino acids, found as a soluble protein, not naturally folded in the cytoplasm, is encoded by three different SNCA transcripts. The function of the SNCA protein in the brain is still not fully elucidated, but it has been found to play an important role in the neurotransmitter release and vesicle cycle at presynaptic terminals [6]. SNCA plays an important role in the regulation of neurotransmitter release, synaptic function, and plasticity of dopaminergic neurons [31–33].

Dopamine from presynaptic vesicles plays an important role in the normal functioning of a presynaptic complex [34–36]. Function of the SNCA protein, which is highly expressed in the brain; is the vesicle formation required for storage and transport of dopamine. Dopamine transported from the presynaptic neuron to the postsynaptic neuron is important for smooth and coordinated movements of the body. As a result of mutations in the SNCA protein, the vesicle that required for the dopamine transport cannot form, and the aggregates are formed. These aggregates are identified as important distinguishing features in PD pathogenesis. Genetic changes known to be effective in PD pathogenesis disrupt normal function of SNCA protein [37, 38].

4. Gene of SNCA

The SNCA gene is localized on the fourth chromosome in the human genome. It is the first gene associated with PD that contains a pathogenic missense mutation (Ala53Thr) responsible for the disease in a large Italian family [6]. The SNCA gene plays a role in important pathways associated with PD and encodes the presynaptic protein product. Therefore, this gene is one of the genes that are extensively studied among PD susceptibility genes [39].

It is the first causal gene in familial PD, and it encodes SNCA with main component of Lewy bodies. Lewy bodies accumulate in neural cells in familial PD cases. The reason of this accumulation is overproduction of SNCA. Overproduction of normal SNCA also plays an important role in the pathogenesis of sporadic PD [40].

The chromosomal location is presented in **Figure 1**.

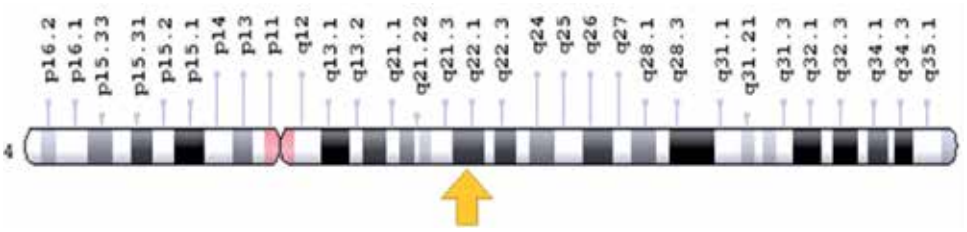

Figure 1.
Chromosomal location of SNCA gene [41].

5. Gene polymorphisms of SNCA

Genetic predisposition plays an important role in the etiology of PD. Multiple linkage and genome-wide association studies (GWAS) were performed to determine genetic polymorphisms in the SNCA gene. Single-nucleotide gene polymorphisms in the SNCA gene were found to be associated with increased PD risk. Single-nucleotide polymorphisms in the SNCA gene can lead to a change in PD susceptibility, resulting in increased or decreased PD risk. Several genes and several polymorphisms of the SNCA gene have been identified in GWAS. It has been determined that some of these gene polymorphisms may be important risk factors especially for sporadic PD [26]. Polymorphisms in different regions of the SNCA gene have been studied in various studies with different populations [7]. Genetic risk factors are very important in the development of PD, and up to now, 16 loci have been identified, which are known to be associated with the development of PD. Very few of the PD, which is mostly sporadic, are familial. The cause of familial PD development is autosomal recessive or dominant mutations. In particular, mutagenic mutations (SNCA, LRRK2, PRKN, DJ-1, and PINK1) occurring in five genes were found to be related to familial PD. Six loci such as MAPT, SNCA, HLA-DRBS, BST1, GAK, and LRRK2, which are associated with sporadic PD, are also defined. In a meta-analysis of various GWAS studies, five new loci identified to be associated with idiopathic PD were identified. ACMSD, STK39, MCCC1/LAMP3, SYT11, and CCDC62/HIP1R loci are found among these loci [20]. SNCA mutations contribute to PD development as a result of amino acid displacements and configuration changes in the encoded protein [41, 42].

Mata et al. found that SNCA gene polymorphisms were important risk factors for PD development risk. In a study conducted by Mata et al., a significant relationship was found between SNCA plasma levels and rs356219 single-nucleotide gene polymorphism [43]. In a study conducted with the Korean population, it was determined that the G allele of rs356219 gene polymorphism was associated with the risk of PD development [44]. In studies conducted with populations of North America, Spain, Russia, and China, rs356219 gene polymorphism was identified as a genetic risk factor for PD development risk [26].

In several families of Greek origin and in families of Asian, Swedish, and Polish origins, p.Ala53Thr polymorphism, one of the rare mutations in the SNCA gene, was detected [6].

In a study that performed to investigate the relationship between rs2301134, rs2301135, rs356221, and rs11931074 gene polymorphisms and PD development risk, the significant relationship was determined between two promoter polymorphisms of the SNCA gene (rs2301134 and rs2301135 gene polymorphisms) and PD development risk. In this study, genotype frequencies in rs11931074 gene polymorphism in 3′ UTR region of SNCA gene were found to be significantly different in patient and control groups. In this study conducted with the Iranian population, SNCA gene polymorphisms were identified as a genetic risk factor in PD development. In another study, the relationships between the rs2301134, rs2301135, rs11931074, and rs356221 gene polymorphisms and the risk of PD development were determined in the SNCA gene. In this study, CC genotype and C allele of rs2301134 gene polymorphism were found to be related to increased PD risk. In addition, CC genotype and C allele of rs2301135 gene polymorphism and GG genotype of rs11931074 gene polymorphism were determined to be associated with decreased PD [6]. In another study conducted with the Han-Chinese population, rs2301135 gene polymorphism was found to be an important risk factor for sporadic PD development. However, rs356221 gene polymorphism was not effective in PD development [45]. Mata et al. also found that SNCA gene polymorphisms

were important risk factors for PD development risk [43]. A meta-analysis study to determine 10 candidate single-nucleotide polymorphisms of the SNCA gene showed a significant relationship between rs11931074 gene polymorphism and PD development risk [28]. In a study conducted in the United States, it was found that some single-nucleotide gene polymorphisms in the SNCA gene were associated with the risk of PD development, but no significant relationship was determined between rs11931074 gene polymorphism and PD development risk [46]. In a study conducted with a South American Brazilian population, the relationship between rs2583988, rs356219, rs2736990, and rs11931074 gene polymorphisms and the risk of PD development was investigated, and the significant relationship was determined between rs356219 gene polymorphism and increased cognitive disorder in PD patients [13]. In studies conducted with European and North American populations, rs2736990 gene polymorphism has been identified as a genetic risk factor in PD development [47]. In another study conducted with a Chinese population, a significant relationship was found between the T allele of the rs11931074 polymorphism of the SNCA gene and the G allele and PD risk of the rs894278 polymorphism. G allele of gene polymorphism of rs11931074 was found associated with decreased progression PD risk [29]. Primer sequences for rs2301134, rs2301135, rs356221, and rs11931074 gene polymorphisms are presented in **Table 1** [7].

In a study conducted by Yu et al. with Han-Chinese population, a significant relationship was found between the risk of PD development and the polymorphism of rs7684318, which is the intronic polymorphism of the SNCA gene [48].

Rep 1, a complex microsatellite repeat of about 10 kilobases in length, is located in the translation start region of the SNCA gene. In some studies, a significant relationship was found between specific SNCA-Rep 1 alleles and the risk of late onset idiopathic PD development. However, there are also studies indicating that Rep 1 risk alleles are not a genetic risk factor for the development of idiopathic PD or that these risk alleles and PD development are inversely related [49]. Rep 1 polymorphic microsatellite repeat is localized in the promoter region (above 10 kb the transcription start site) of the SNCA gene [49]. Rep 1–261 is a microsatellite polymorphic variant associated with an increase in SNCA mRNA levels [47]. There are two common Rep 1 alleles as 251 and 261 bp in length, and functional assays about these alleles were performed. According to these functional analyses, while a significant relationship was found between the risky allele with a length of 261 bp and the upregulation of SNCA gene expression, a significant relationship was found

SNPs	Forward primer (5′-3′)	Reverse primer (5′-3′)
rs2301134	F_1: AAAGGGTCCTGAGGGTGCAA F_0: CTGAAATTTAATCACGGTC ACAGGTTA	R_1: CCTGTGACTCTTCCTTAGTAG TCTCACC R_0: GAAAAGCCTTAGGACCGCTTGT
rs2301135	F: TCCACAAGAGTGCTCGTGAC	R_1:CTGATTTGTCAGCGCTTCTG R_2: CTGATTTGTCAGCGCTTCTC
rs356221	F_1: GTTCATAAGAGAAG CCATCCTACTA F_0: CATGGGTTAGGTTTCATTTTGT	R_1: GTTGATCTGCAACTATAGGT TAAGAA R_0: ATTGGAAGCAGTTAAACCACAT
rs11931074	F_1: AATTGTGAATATGTCTTTGACCGG F_0: ATTCTGTCACTGGGTAGGCAGA	R_1: CAGCCTTCCAAATCATAAT TCCTTA R_0: TCTGTAGAAAGAACCCATTTGGC

PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

Table 1.
Primer sequences used in PCR for single-nucleotide polymorphisms (rs2301134, rs2301135, rs356221, and rs11931074).

between protective variant 259 bp length and decreased SNCA gene expression [49]. In addition, variability in the length of the Rep 1 repeat sequence is associated with PD susceptibility. Genotypes containing 263 bp allele of this sequence were associated with increased risk of PD development, and genotypes containing 259 bp allele were associated with decreased risk of PD development [42]. Changes in the promoter region and genetic variations in 30 untranslated SNCA gene regions have been associated with susceptibility to PD [49]. In the SNCA promoter (SNCA-Rep 1), it was determined that the localized Rep 1 and 3′ untranslated region polymorphisms interfered with the transcription binding regions and played an important role in increasing PD susceptibility. Thus, target regions of miRNAs that alter SNCA gene expression are formed or disappear [26]. The significant difference was determined between Rep 1–259 allele and low levels of SNCA mRNA in post-mortem brain tissues of patients with PD. Rep 1 microsatellite repeat and rs2583988, rs2619363, rs2619364 gene polymorphisms were found to be a relationship between. In a study conducted with a population selected from Belgium, Germany, and Serbia, the relationship was found between Rep 1 microsatellite repeat; rs2583988, rs2619363, and rs2619364 gene polymorphisms; and PD. In a study conducted with German and Japanese populations, it was observed that there was a link between intron 4 and 5′ and 3′ untranslated regions (UTRs) in the SNCA gene [6]. In a Russian population study, TT genotype of rs2583988 and rs2619363 gene polymorphisms and the GG genotype of rs2619364 gene polymorphism were found to be associated with higher levels of SNCA mRNA [47]. There are many small-scale studies aimed at investigating this relationship, and also, in a meta-analysis study conducted by Maraganore et al., in a large population selected from 11 different regions, the relationship between the rep 1 gene polymorphism and the risk of PD development has been proven [42].

In an analysis with the Caucasus population to investigate the relationship between 12 single-nucleotide polymorphisms and PD, including the whole SNCA gene region, it was determined that signals were collected in a 24 kb-long region in the middle of intron 4 of the SNCA gene, and it was determined that these signals were confirmed by haplotype analysis showing the presence of a strong protective allele [42].

In addition to these gene polymorphisms, SNCA gene polymorphisms which are known to be associated with PD include rs104893875 (G > A), rs104893877 (G > A), and rs104893878 (G > C) gene polymorphisms. It has been reported that the polymorphism rs104893875 (G > A) has been identified primarily in a multinational Spanish family. The significant relationship was determined between rs104893875 (G > A) gene polymorphism and increased SNCA protein aggregation. In another study conducted with the Swedish population, as a result of rs104893877 (G > A) mutation, PD and encephalopathy with cortical involvement develop. Immunoreactive Lewy neurites were detected in brain stem pigmented nuclei, the hippocampus, and temporal neocortexes of PD patients in whom this gene polymorphism was detected. In a study that is significant relationship between rs104893878 (G > C) gene polymorphism and autosomal dominant PD, hypometabolism was determined in frontal, parietal, and left temporal cortexes of PD patients in whom this polymorphism was detected. As a result of postmortem examination of brain slices of these patients, Lewy bodies and neurodegeneration developed and correspondingly identified. Primer sequences for rs104893875 (G/A), rs104893877 (G > A), and rs104893878 (G > C) gene polymorphisms are presented in **Table 2** [38].

There are three missense mutations, A53T, A30P, and E46K, as the most common pathogenic changes of the SNCA gene. In a study conducted with the Mexican Mestizos population, a significant relationship was determined between the rs1801133 and rs3857059 allelic variations of the SNCA gene and the risk of PD development. In addition, GG genotype of rs3857059 gene polymorphism was found to be

SNPs	Forward primer (5′-3′)	Reverse primer (5′-3′)
rs104893875 (G/A)	GGCCCCGGTGTTATCTCAT (SN-75-CF) TTGTAGGCTCCAAAACCATGG (SN-75-GF)	AATTCAAAGCCCTCATTA TTCTTGG (SN-75-CR) CACCATG CACCACTCCCTT (SN-75-AR)
rs104893877 (G > A)	GGCCCCGGTGTTATCTCAT (SN-75-CF) GGAGTGGTGCATGGTGAGA (SN-77-AF)	AATTCAAAGCCCTCATTATT CTTGG (SN-75-CR) GCACAATGGAGCTTACCTGTAGC (SN-77-GR)
rs104893878 (G > C)	TCCGTGGTTAGGTGGCTAGA (SN-78-CF) ACCAAACAGGGTGTGGCAGAAGCAG (SN-78-GF)	CACACGTTCACATTCACCTACCT (SN-78-CR1) ACCCTCTTTTGT CTTTCCAGC (SN-78-CR2)

PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

Table 2.
Primer sequences used in PCR for single-nucleotide polymorphisms [rs104893875 (G/A), rs104893877 (G > A), and rs104893878 (G > C)].

a genetic risk factor for PD development [50]. While A18T and A29S missense mutations in patients with sporadic PD; A53T, A30P, E46K, and H50Q missense mutations in familial PD patients have been described. Dual and triple copies of the SNCA locus known to be associated with PD severity cause familial Parkinsonism [26].

There are studies showing that there are significant relationships between some polymorphisms in the SNCA gene and the risk of sporadic PD development. It is also known that these polymorphisms are associated with increased levels of plasmatic SNCA. Tyrosine hydroxylase activity and dopamine release decrease as a result of SNCA overexpression. In order to investigate the relationship between SNCA gene polymorphisms and the risk of PD development, different results have been obtained in studies performed with different populations [13].

6. Conclusions

It is known that environmental and genetic factors play a role together in PD pathogenesis. Several studies have been carried out to investigate the relationship between gene polymorphisms and PD development risk, which are important among genetic factors, and different results have been obtained in these studies. It is thought that the differences in the results of these studies are due to of PD patient and healthy control groups' different selection criteria and different race and populations. The identification of genetic polymorphisms that play an important role in the development of PD will enable us to have knowledge about the mechanism of PD. In addition, new treatment methods can be improved in order to prevent PD. By increasing the number of PD patients and healthy controls, different results can be obtained in studies with larger populations. As a result, in studies aimed at the relationship between SNCA gene polymorphisms and the risk of PD development, some of the SNCA gene polymorphisms were found to be genetic risk factors for PD development and play an important role in the pathogenesis of the disease.

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Conflict of interest

We declare that there is no conflict of interest with any financial organization regarding the material discussed in the chapter.

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Chapter 4

Therapeutic Potential of Baicalein in Parkinson's Disease: Focus on Inhibition of α-Synuclein Oligomerization and Aggregation

Hayate Javed and Shreesh Ojha

Abstract

Parkinson's disease (PD) is the most common neurodegenerative diseases, which affects the people in old age. The neuropathological symptoms of PD include the degeneration of dopaminergic neurons in the substantia nigra pars compacta, and presence of intracellular inclusions of α-synuclein (α-syn) aggregates. α-Syn, a natively unfolded protein, has been found to play a key role in PD pathology. Several mechanistic studies revealed the numerous aspects of α-syn fibrillation and aggregation process that lead to dopaminergic neurodegeneration in PD. Till to date, there is no complete cure of PD, but some therapeutic agents are able to halt the disease progression. Scutellaria baicalensis Georgi is a traditional Chinese medicine commonly used to treat the central nervous system diseases. Recently, it has been confirmed that root of S. baicalensis Georgi contains baicalein (5,6,7-trihydroxyflavone) as a major bioactive flavone constituent. Baicalein possess numerous pharmacological properties such as antiaggregation of amyloid proteins including α-syn, antioxidant, anti-inflammatory, and antiapoptotic. In the light of these properties, baicalein has potential therapeutic efficacy for PD. In this chapter, we explored the pharmacological protective actions of baicalein against α-syn fibrillation and aggregation that make it suitable for PD treatment and also discussed the possible mechanisms underlying the effects.

Keywords: Parkinson's disease, α-Synuclein, baicalein, flavonoids, dopaminergic neurons

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease involving movement disorders [1, 2]. Worldwide, about 10 million people are living with PD and it affects 3–4% of individuals over the age of 65 years [3, 4]. Symptomatically, PD is characterized by the movement and behavioral disorders and pathologically characterized by the deterioration of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies [5, 6]. The diagnosis of PD, in general, includes motor disorders such as bradykinesia, cogwheel rigidity, resting tremors, postural instability, and slowness or absence of voluntary movements along with neuropsychiatric manifestations [7, 8]. The Lewy bodies in PD result from accumulation of intracellular α-synuclein (α-syn) inclusions in neurons,

and aggregates of α-syn spread through the brain following a specific pattern [9]. The neuron-to-neuron transfer of α-syn is considered critical for the propagation of Lewy body pathology. The definitive postmortem diagnosis and pathological character of PD constitute the presence of aggregated (i.e., fibrillar, spherical, and oligomeric) forms of α-syn protein in the soma and processes including axons and dendrites of affected neurons. The familial form of PD is caused by mutations in the *SNCA* gene, which is the first determinant of PD [10]. Moreover, inherited forms of PD result from the genetic overexpression *via* gene duplication or triplication [11, 12]. Importantly, more α-syn gene copies result in highly aggressive form of the disease, which suggests a direct relationship between disease severity and the expression level of α-syn [13]. The fibrillated form of α-syn protein is the abundant component of Lewy bodies, which are the pathological hallmarks of sporadic form of PD and present as cytoplasmic inclusions [14]. Consequently, α-syn is believed to play a crucial and significant causative role in most, if not all, forms of PD.

In the last 30 years, the approach in the treatment of PD is based on enhancing the deteriorating levels of dopamine neurotransmitter through administering dopamine precursor L-dopa. Until now, the replacement of dopamine by L-dopa administration is the standard treatment for PD management [15]. However, L-dopa appears to provide only symptomatic relief in correcting the motor abnormalities and did not inhibit or reverse the PD progression [16]. However, the long-term use of dopamine precursor drugs found to produce numerous adverse effects and induce complications that ultimately flop to treat PD patients if disease progresses [17]. Thus, there is a great requirement to develop new drugs for the management of PD, which are not only therapeutic but can also prevent the initiation or delay or stop the progression of the disease.

In the past few years, there is an enormous emphasis on the medicinal use of plant extracts, which are also reputed for their therapeutic claims in a variety of traditional medicines. The traditional preparations usually contain many medicinal plant extracts that have been shown better therapeutic efficacy on neurological diseases, such as cerebral stroke, dementia, and paralysis, in clinical setup for decades [18, 19]. Though all these herbs-based medicinal preparations are used in the traditional practice of medicine with perceived safety and efficacy, the precise pharmacological and molecular mechanisms involved in the therapeutic efficacy of these traditional Chinese medicines remain unclear. Majority of the mechanisms purported behind the clinical efficacy of these herbal medicines are based on generalized antioxidant and anti-inflammatory property, rather a well-established pharmacological target. They are therefore not well accepted in modern medicine, which is based on rigorously documented evidence-based randomized clinical data. It is challenging to systematically identify these mechanisms with modern biochemical and pharmacological techniques [20]. Simultaneously, medicinal plants are well acknowledged as a source of modern drugs, which provides a drug discovery approach and further leads to drug development. It is well documented that enhanced dietary consumption of herbal medicines reduces the risk of developing neurodegenerative diseases [21].

In an approach to identify a novel molecule form of traditional medicine, the root of *Scutellaria baicalensis* Georgi garnered enormous attention in drug discovery for neurodegenerative diseases. *Scutellaria baicalensis* Georgi, widely grown in Mongolia, Korea, Siberia, China, Japan, and the Russian Far East, represents one of the important ingredients in the decoction of traditional herbal preparation employed for the treatment of CNS diseases [21]. Pharmacokinetic study confirmed that baicalein, a flavone chemically known as 5,6,7-trihydroxyflavone, is one of the prominent bioactive components of the roots of *Scutellaria baicalensis* Georgi [22]. Baicalein is one of the widely studied compounds and

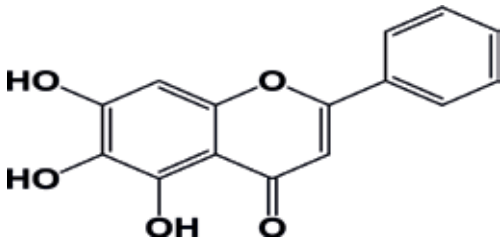

Figure 1.
The chemical structure of baicalein.

reputed for its multiple pharmacological actions including antibacterial, antiviral, anticarcinogenic, and anti-inflammatory activities in traditional Chinese medicine [23–26]. Baicalein has a wide safety of margin and appears to be able to cross the blood-brain barrier [27]. Several studies based on animal model of AD and PD have shown that baicalein exhibits neuroprotective properties [26–29]. Therefore, baicalein has been the central molecule to investigate its versatility as therapeutic agent for neurological diseases [21]. In this chapter, we will describe the therapeutic importance of baicalein against the PD with a focus in the amelioration of α-syn, which is central protein in the pathogenesis of PD. In addition, we will also highlight the potential underlying biochemical mechanisms of action of baicalein (**Figure 1**).

2. α-Syn a major mediator in PD pathogenesis

α-Syn is a 140 amino acid protein, which is encoded by *SNCA* gene located on human chromosome 4 and most abundantly expressed in presynaptic terminals and helps in the maintenance of neurotransmitter systems in CNS [28]. Regardless of its abundant distribution in numerous areas intricated in multifaceted activities, α-syn pathology exists in selectively vulnerable areas rather than its site of expression in the brain [28, 29]. Furthermore, red blood cells are also abundant in α-syn [30] and many other CNS tissues [31, 32], which indicates broader range of α-syn functions throughout the body. Almost two decades have been spent to find out the exact role of α-syn physiology; its mechanism of action is still unknown, and complex dynamics of this protein is characterized by its ability to achieve the toxic effect as well as flexibility to adapt. α-Syn has gained much consideration as important factor in PD pathophysiology. α-Syn exists in a dynamic equilibrium from monomeric to oligomeric states, and this equilibrium prevents formation of fibrils in physiological conditions. Importantly, multifunctional properties of α-syn are predicted by its structure that has been attributed to this protein [33]. The structure flexibility property of α-syn allows it to accept a wide range of conformations depending on binding partners and environment [34, 35]. Because of its abundant presence at presynaptic terminals, chaperone function of α-syn is the key cellular function, which controls the exocytosis through trafficking and organizing the synaptic vesicle pool. Mutations in α-syn coding gene *SNCA* lead to functional changes of SNAP REceptor (SNARE) protein, which is a receptors family that binds soluble N-ethylmaleimide sensitive fusion attachment proteins (SNAP) receptor (SNARE) proteins and controls its assembly [36]. Moreover, α-syn also targets presynaptically dopamine active transporter (DAT) [37].

Several approaches occur to prevent the oligomerization of α-syn [39], which includes hydrophobic interactions between C- and N-terminals of α-syn [40]. Interestingly, α-syn exhibits a polar C-terminal tail, which can bind to

hydrophobic region of other denatured protein, having functional and structural homology with different molecular chaperones. Therefore, the flexibility of α-syn depends on the capacity of this protein to autoassemble and function as intramolecular chaperone [41]. Autochaperone property was found absent in C-terminus of truncated α-syn and that will increase the formation of α-syn aggregates compared with the full-length α-syn [39]. At present, the exact biological functions of α-syn are unknown; but there is a substantial amount of ongoing research that helps us to understand this gap in our knowledge. The functional repertoire of α-syn is largely studied through determining the irregularities following overexpression, expression of mutant forms of α-syn, or loss of expression. The number of studies indicates α-syn involved in the formation of synaptic vesicles, mitochondrial function, and/or dopamine synthesis and metabolism. Furthermost characteristics strongly argue for important role in synaptic plasticity and neurotransmitter release. Chaperone plays an important role in the folding of polypeptide during proteins translation, and assembly misfolding is common with aging yet they are generally partial by numerous quality control machineries that degrade the misfolded and denatured proteins [42]. With the complex organization of α-syn expression and its function of great adaptability, homeostatic functions failures do not bring to uneven function gain, instead trigger a sequence of neurodegenerative process in the intracellular system.

Several studies conducted in PD patients and animal models support the idea that the proteostasis of α-syn has a critical role in the PD pathogenesis. This concept backs to two decades when two discoveries provided the evidence that PD is linked to α-syn mutations. The first report published by Polymeropoulos and colleague for the identification of a missense mutation in α-syn gene causing early onset of familial form of PD [9]. In the same year, an experimental report showed that Lewy bodies contain higher amount of α-syn that appears as intracytoplasmic inclusion of α-syn aggregates and considered as classical pathological hallmarks of PD [14]. Soon after these discoveries, the presence of Lewy body in the brain parenchyma of sporadic idiopathic forms of PD was also found [43]. To examine the probable causal links, pathological and physiological functions of α-syn and different misfolding proteins have been investigated in relative with documented features of PD. Several risk factors for PD have been explored that are genetic and environmental. Point mutations, mitochondrial dysfunctions, oxidative stress, neuroinflammation, multiplications, and specific polymorphisms are the genetic causes that may lead to develop suitable environment for progressing PD. Remarkably, these factors are equally responsible to exert α-syn toxicity. Despite having good quality control structures to confirm a precise α-syn assembly and the capability to control α-syn oligomerization, this protein also expresses its neurotoxic effects upon formation of oligomers from soluble monomers, and then gradually formed protofibrils to large α-syn aggregates which eventually leads to Lewy body formation [44]. A greater chance of α-syn aggregation is accessible by various posttranslational covalent modifications including conformational changes, which direct α-syn more vulnerable to aggregation [35]. α-Syn truncation at the C-terminus and tyrosine nitration (Tyr125) are usually observed in α-syn aggregates and often found to enhance its in vitro fibrillation [45]. Additionally, aging diminishes the proteolytic efficiency that plays an important additive role in the accumulation of α-syn. These finding supporting the data showing enhanced α-syn levels in nigral neurons in aged brain. In the normal brain, intracellular homeostasis of α-syn is confirmed by the accumulative functions of lysosomal autophagy, and ubiquitin-proteasome systems with the previous one play a key role in the clearance of oligomeric α-syn. Irregularities in both systems lead to excessive production and accumulation of α-syn that eventually end up to formation of α-syn aggregates.

3. Abnormal accumulation of α-syn causes dopaminergic neurodegeneration

In PD, decreased striatal dopamine occurred following selective neurodegeneration of dopaminergic neurons of the substantia nigra par compacta (SNc) and impairment of several basal ganglia functions. It is still unknown through which mechanism α-syn causes dopaminergic neurons vulnerable and it needs to be fully elucidated. α-Syn is associated to dopamine neurons for its capability to control homeostasis of dopamine in the synapses and effects DAT activity, but the exact mechanism is still debatable [46]. α-Syn plays a key role as a modulator for dopamine synthesis and metabolism by decreasing the tyrosine hydroxylase phosphorylation and stabilizes in inactive state [47]. Therefore, α-syn absence employs considerable effect on dopaminergic systems because it reduces dopamine levels in the striatum and decreased DAT functions. Absence of α-syn is also linked with reduced striatal dopamine uptake, decreased number of TH-positive nigral dopaminergic neurons, and nerve terminals in the striatum [48]. However, the sensitivity of dopaminergic neurons also conferred to intrinsic and excitotoxic insult and does not rely on absence of dopamine metabolism. There are two characteristic features of dopaminergic neurons which makes them especially more susceptible to excitotoxic challenge. First dopamine neurons show a prolonged axonal branch, which offer extensive neurotransmitter release sites. Enhanced mitochondrial impairment is observed in the axons of dopaminergic neurons and this is one of the reasons to show elevated susceptibility. Second, dopamine neurons act as autonomous pacemakers and display spontaneous activity. The activity of dopaminergic neurons is considered through oscillations in intracellular calcium (Ca^{2+}) levels, which is operated by voltage-dependent L-type Ca^{2+} channels opening to maintain a rhythmic (2–10 Hz) spiking [49]. This capability is connected to decreased intrinsic Ca^{2+} levels and required a strict quality control for Ca-mediated processes from intracellular reservoir, endorsing Ca^{2+} entry into the mitochondria and ATP production by oxidative phosphorylation [50]. All these processes are required to complete the requirements of bioenergetics and to prevent the unnecessary activation of ATP-sensitive potassium channels that may inhibit the spontaneous activity of neurons. Nigral dopaminergic neurons and different brain nuclei neurons involved in sensorimotor integration are capable with mechanisms that maintain to rapidly implement a correct strategy upon environmental stress. As a result, for this adaptive ability is the vulnerability of the system to environmental toxins, age, and genetic mutations or that can increase the reactive oxygen species production which ca warrant the proteostasis, DNA damages, especially in mitochondria. Impairment in mitochondria ultimately leads to cause impaired mitophagy and also compromised. Recently, Burbulla and colleagues [51] showed inactivation of DJ-1 causes additive toxic effect of elevated α-syn levels, mitochondrial dysfunction, and stimulation of dopaminergic receptors in mice. Remarkably, overexpression of human α-syn A53T [52] in dopaminergic neurons and constitutive DJ-1 deficiency in mice showed enhanced levels of oxidized dopamine in dopaminergic neurons and decreased lysosomal activity.

4. Baicalein as a potential molecule of natural origin to target α-syn

In the recent years, a growing effort has been taken in the development of innovative neuroprotective molecules of natural origin with high efficacy and low side effects to prevent neuronal deaths [10, 19]. Several studies have been attempted to

examine the effects of extracted components from different plants on neurotoxicity. Among the numerous compounds from plants, flavonoids are the utmost effective components with a broader range of pharmacological and health-promoting properties [20, 21]. To date, almost 8000 different flavonoid compounds have been discovered and are classified into various subgroups, including flavonols, flavones, flavanols, flavanones, isoflavones, etc. [22]. Numerous vegetables and fruits, flavonoid compounds are the major classes of natural polyphenols. The consumption of flavonoid-rich fruits and vegetables significantly reduces the risk of many diseases in humans [23, 24].

Baicalein (5,6,7-trihydroxyflavone; $C_{15}H_{10}O_5$) is an important flavonoid compound mainly isolated from the roots of *S. baicalensis* Georgi (Labiatae). Earlier studies clearly showed that baicalein has various pharmacological properties such as antioxidant, anti-inflammatory, antidiabetic, anticancer, antiulcerative colitis, antithrombotic, antiviral, eye protective, cardioprotective, neuroprotective, and hepatoprotective properties [25]. It also possesses anticonvulsive, anxiolytic, and mild sedative actions [26]. Many studies have clearly demonstrated that baicalein protected 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenylpyridinium (MPP+), glutamate, amyloid-β (Aβ), hydrogen peroxide (H_2O_2), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and methamphetamine-induced neurotoxicity in animal models and cell lines [27–33]. Earlier, some authors clearly reviewed the anticancer, anti-inflammatory, cardioprotective properties, ocular disorders, and mitochondrial function [34–37]. Li et al. [38] briefed the therapeutic properties of baicalein against PD. Baicalein halts PD progression by reducing oxidative stress, inhibiting excitotoxicity, inhibiting aggregation of disease-specific amyloid proteins, and stimulating neurogenesis and antiapoptosis as well as anti-inflammatory properties. In addition, Gasiorowski et al. [21] reviewed the neuroprotective actions of flavones (baicalein, oroxylin A, and wogonin) from the root of *S. baicalensis*. However, scientific literature review on the neuroprotective effects of baicalein is still missing.

5. Baicalein inhibits aggregation of α-syn protein

PD develops with the aggregation of disease-specific proteins in amyloid nature. Amyloid proteins are predominantly rich in β-sheet fibrillar aggregates composed of self-assembly of different proteins including α-syn. Abnormally folded aggregates of α-syn are the key component of Lewy bodies and present as intracellular inclusions in nigral dopaminergic neurons in the PD brain [14].

Familial early-onset PD is potentially linked with α-syn mutations [53]. Therefore, α-syn is the potential therapeutic target for PD therapy to prevent the progression and development of this devastating disease. It is well evidenced that preformed α-syn fibrils, oligomeric α-syn, are considered more toxic than mature fibrils in arbitrating α-syn-induced neurotoxicity [54, 55]. The underlying mechanism for protein aggregation remains to be elucidated; phytochemicals or molecules of natural origin that can halt or slow down the fibrillation process of α-syn could be potentially important as new therapeutic strategy for the prevention of PD. Remarkably, reports have shown that aggregation of disease-specific α-syn protein can be inhibited by baicalein. Baicalein, as well as its oxidized forms in lower micromolar concentrations, inhibits formation of α-syn fibrils. Moreover, baicalein also showed the ability to disaggregate the existing α-syn fibrils [56]. Biophysical and experimental (in vitro/in vivo) studies showed that baicalein is capable of modifying α-syn aggregation and decreasing the cytotoxicity [57, 58]. In cellular as well as cell-free systems, baicalein was found to inhibit the α-syn oligomerization

and effectively inhibits α-syn fibrillation. It has been reported that inhibition of α-syn oligomer formation was achieved by baicalein treatment in Hela cells and SH-SY5Y cells and later was protected from the toxicity induced by α-syn oligomer [57]. Further, inhibition of aggregation and cytotoxicity of wild-type α-syn and baicalein showed decreased aggregation of different mutant form (E46K A30P and A53T) of α-syn in vitro and represented a neuroprotective effect in N2A cellular model of familial forms of parkinsonism [58, 59]. Moreover, α-syn aggregation was augmented by baicalein in the nigrostriatal dopaminergic system of MPP+-treated rat brain [60]. In the intragastrically rotenone-injected chronic mouse model of PD, baicalein did not decrease the expression of α-syn mRNA, but significant decrease

Experimental model system	Effects/mechanisms observed	References
α-Syn aggregation assay	• Inhibits formation of α-syn fibrils • Disaggregates α-syn fibrils after binding to Tyr residues in α-syn	[56]
Dopaminergic cell lines (SN4741) overexpressing wild-type α-syn or A53T mutant-type α-Syn	• Inhibits α-syn fibrillation by covalent binding • Promotes degradation of α-syn fibrils and polymerization to reduce its propagation and transmission • Enhances cell viability and increases macroautophagy	[76]
Intranigral infusion of MPP+ in rat brain	• Attenuates α-syn aggregation • Inhibits inflammasome activation and cathepsin B production • Inhibits apoptosis (caspases 9 and 12), and autophagy (LC3-II)	[60]
α-Syn aggregation in infused substantia nigra of rats	• S/B formulation containing *Scutellaria baicalensis* Georgi attenuates inflammation, apoptosis, oxidative, mitochondrial and ER stress and preserves glutathione • Attenuates astrocytosis/microgliosis, improves dopamine levels • Inhibits α-syn aggregation in SNc	[77]
PC12 and primary dopaminergic neurons	• Dichloromethane and n-butanol extract of *Scutellaria pinnatifida* reduces α-SN aggregation and scavenges free radicals	[78]
Biophysical and biochemical assays on 39 polyphenols including baicalein	• Inhibits α-syn aggregation	[79]
Biophysical and biochemical assays on 14 polyphenols including baicalein	• Inhibits α-syn aggregation	[80]
Biophysical and biochemical assays on 12 polyphenols including baicalein	• Inhibits α-syn aggregation	[59]
Biophysical and biochemical assays on four polyphenols including baicalein	• Inhibits α-syn aggregation	[81]

Table 1.
The experimental studies showing the effect of baicalein in PD.

of α-syn oligomers was observed in the thoracic spinal cord, midbrain, and ileum. In the light of this, baicalein could be able to prevent the PD progression through inhibiting the aggregation and formation of α-syn oligomers [61]. Although, the exact molecular mechanism of baicalein through which inhibition of aggregation of α-syn proteins takes place is yet to be elucidated, but characteristic molecular structure-based mechanism of baicalein has been proposed. It is well known that polyphenols, including baicalein or other flavonoids, are readily oxidized to quinones by oxygen because of their reductive nature, although quinones are readily reactive with the side-chain amino groups of proteins. Oxidized form of baicalein, that is, baicalein quinones, plays a key role in carrying α-syn inhibitory reactions, and the resulting product is mostly soluble α-syn oligomers. In this process, baicalein quinone covalently modified the protein molecules to form a Schiff base with a lysine side chain in α-syn, and Tyr is involved in the interaction of α-syn with baicalein [56]. Moreover, analysis of structure-activity demonstrated that quinone formation required vicinal dihydroxyphenyl moieties of baicalein to bind α-syn, and for maximum inhibitory effects of baicalein on α-syn fibrillation, three vicinal hydroxyl groups are more beneficial compared with two vicinal hydroxyl groups [59]. The in vitro inhibitory effects of baicalein on α-syn fibrillation are correlated with its antioxidant activities [62]. Recent study showed that non-baicalein-treated α-syn oligomers fibrillation can be inhibited by baicalein-stabilized α-syn oligomers. This suggests that some forms of soluble oligomer formation can be beneficial because baicalein-stabilized α-syn oligomers do not disrupt the integrity of the biological membrane [63]. Hence, these results indicated that baicalein would be a therapeutic agent for PD treatment through inhibition of α-syn accumulation and aggregation (**Table 1**).

6. Blood-brain barrier (BBB) penetrating ability

The BBB is an important network that plays a key role in maintaining homeostasis of the central nervous system, and its disturbance is prominently recognized for many neuronal disorders [27]. The BBB is made up of microvessel endothelial cells, in the arrangement of basement membrane, pericytes, neurons, and astrocytes. The BBB brings a hard barrier between the blood and brain parenchyma cells. However, several transport systems help to ease the channel of selective constituents across the BBB. Tsai et al. [64] reported that baicalein crosses the BBB 20–30 min following injection. Wang et al. [65] showed that baicalein remarkably decreased the BBB permeability after 24 hours in animal model of subarachnoid hemorrhage.

7. Bioavailability of baicalein

The deprived oral bioavailability and aqueous solubility are the major drawback for the pharmaceutical formulations and clinical practice of baicalein. Previous reports showed that baicalin and baicalein 6-O-sulfate are the metabolic product of baicalein in the blood [66, 67]. Several studies have demonstrated that oral administration of baicalein endures widespread glucuronidation within the wall of intestine and liver in humans and rats [68]. Additionally, baicalein was found less absorbed by colon when compared to stomach and small intestine [69]. Lai et al. [70] reported that 75.7% of the baicalein dose injected intravenously was found to be circulating in the form of conjugated metabolites in rats. Based on the earlier studies, it was documented that intravenous and oral administration of baicalein is instantly metabolized into baicalin in the animal's blood [71, 72].

In another study, conducted on monkeys, it has been reported that the different doses of baicalein bioavailability range 23.0 and 13.1% through intravenous and oral routes, respectively [73].

8. Clinical trials with baicalein

Baicalein has been shown a novel potential therapeutic agent for the treatment of neurological diseases. Therefore, clinical trials are essential for baicalein in order to confirm the potential therapeutic efficacy against neurodegenerative diseases. Two phase I clinical trials have been done based on chewable tablets of baicalein in Chinese healthy adult volunteers.

A phase I (2014), randomized, double-blind test for pharmacokinetic properties of baicalein with single-dose trail (100–2800 mg) was investigated on 72 healthy Chinese adults [74]. Without any additional treatment, mild adverse effects were fixed. Blood pressure and electrocardiogram were found normal during the entire period of study. No sign of toxicity observed in liver and kidney or any other serious adverse effects were not observed [74].

In 2016, another study in Chinese subjects based on placebo-controlled, single-center, and double-blind parallel group investigated the pharmacokinetics, safety, and tolerability of baicalein following multiple-ascending-dose protocol [75]. Volunteers were randomly divided to get placebo treatment (n = 2 per dose regimen) or baicalein (n = 8 per dose regimen). The selected dose regimens (200, 400, and 800 mg) were given once daily on days 1 and 10, and twice on days 3–9. High-performance liquid chromatography–tandem mass spectrometry methods were employed to assay the baicalein and its metabolite in urine, feces, and plasma samples. On day 8, plasma samples are given steady-state concentration of analytes after repeated dosing. The analytes concentration increased with increasing dose. The dose proportionality constant (b) for the area under the plasma concentration-time curves of baicalein and baicalin was 0.922 (90% confidence interval 0.650–1.195) and 0.942 (90% confidence interval 0.539–1.345), respectively.

Phase I clinical trials of baicalein showed that oral administration is safe to humans. But additional studies of baicalein are needed in the patients of neurodegenerative diseases to strengthen its therapeutic importance.

9. Conclusion

In the recent years, the pathophysiological understanding of α-syn has advanced rapidly from small unfolded protein located into nerve terminals and possesses the quality to self-aggregate into fibrils, resembling to Lewy bodies, a characteristic pathological hallmark of PD. Moreover, the endpoints of PD are not this long structure but an intermediate and transferable agent of the disease and is the most potent therapeutic target for PD prevention/treatment. Baicalein has showed the neuroprotective effects against PD in both in vitro/in vivo studies. Baicalein possesses the antioxidant activity and inhibition of α-syn aggregation. This suggests baicalein to be considered as solid treatment tool for neurodegenerative diseases including PD. Importantly, baicalein is safe and sufficiently well tolerated by healthy volunteers as evidenced by two phase I clinical trials based on Chinese healthy adult volunteers [74, 75]. Therefore, the abovementioned properties of baicalein indicate its potential therapeutic implications in slowing down/halting the progression of PD.

Conflict of interests

The authors declare that there is no conflict of interest.

Author's contribution

HJ wrote and revised the chapter; SO critically proofread the final version and provided the important inputs to prepare the final version of the chapter.

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Chapter 5

Role of Phenylalanine and Its Metabolites in Health and Neurological Disorders

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Abstract

Phenylalanine, an amino acid, is a "building block" of protein. Phenylalanine is a component of food sources and also derived through supplementation. In current treatment, phenylalanine is prescribed as anti-depressant agent. The present study reviewed the possible antidepressant potential of phenylalanine. We reviewed data using the major databases, namely, Web of Science, SciFinder, Google Scholar, and PubMed. This manuscript provides a brief overview of the role of phenylalanine in depressive disorders. Phenylalanine possesses anti-depressant potential. Significant anti-depressant activities have been studied both in-vitro and in-vivo models. Based on current data, phenylalanine could be recommended as a potential candidate for clinical anti-depressant trials. Phenylalanine hydroxylase (PAH) deficiency results in intolerance to the dietetic consumption of the phenylalanine and a variety of syndromes such as deep and permanent logical disability, impaired cognitive development.

Keywords: depression, phenylalanine, tyrosine, metabolic product of phenylalanine, diseases related to phenylalanine

1. Background

Phenylalanine is consumed either through food sources or through supplementation including wheat germ, oats, milk products, and meats. Phenylalanine is a fundamental amino corrosive and is convertible into tyrosine, however not the other way around. Transformation of phenylalanine to tyrosine is catalyzed by phenylalanine hydroxylase which is a blended capacity oxygenase [1]. Adrenaline, noradrenaline, and dopamine are subordinates of tyrosine. The transformation of tyrosine in dihydroxyphenylalanine (DOPA) is catalyzed by tyrosine hydroxylase. Decarboxylation of DOPA to dopamine is catalyzed by DOPA decarboxylase conversion of neither dopamine to nor epinephrine catalyzed by dopamine—beta-hydroxylase. Transformation of neither dopamine to nor epinephrine by transmethylation is catalyzed by phenylethanolamine-N-methyl transferase. The protein phenylethanolamine N-methyltransferase is prompted by glucocorticoids in the adrenal medulla, which acquires glucocorticoids from the adrenal cortex through entryway course in gigantic amounts. With the goal, that the adrenal medulla has

100 times a greater number of glucocorticoids than the plasma. α-Methy I—DOPA is a drug, which aggressively hinders the chemical DOPA decarboxylase, in this manner restrains the arrangement of catecholamine and is utilized as a part of the administration of hypertension. Adrenaline and noradrenaline are the hormones of the adrenal medulla and have a place with the gathering of mixes called catecholamine [2]. They are likewise discharged at postganglionic thoughtful nerve endings and go about as neurotransmitters [3]. Dopamine has imperative physiological properties, e.g., incitement of the myocardial contractility (inotropic activity). It is additionally a neurotransmitter in the focal sensory system and its inadequacy in the basal ganglia delivers additional pyramidal illness known as Parkinsonism [4]. Dopamine of the hypothalamic starting point additionally goes about as prolactin discharge restraining factor in the front pituitary organ.

1.1 Food sources

Food sources are lentils, chickpeas, pecans, soybeans, whole grains, sesame seeds, pumpkin seeds, peanuts, nuts, lima beans, cheese, cottage, corn, brewer's yeast, bananas, almonds, dairy products, and eggs [5].

1.2 Role of phenylalanine in the body

This supplement is necessary to the usual working of the central nervous system; particularly regarding manifestations like chronic pain and depression along with numerous other disorders that have been associated with the nervous system malfunction. It is involved in formation of neurotransmitters such as norepinephrine, epinephrine, and dopamine. Nervous system requires all these chemicals for proper functioning. As a nootropic, phenylalanine has numerous valuable properties improved motivation, increased concentration and focus, anxiety relief and mood enhancement [6].

1.3 Role in vitiligo

L-phenylalanine in combination with UVA exposure or application of L-phenylalanine in combination with UVA exposure to the skin appears to be useful in the treatment of vitiligo in children and adults [7].

1.4 Weight loss benefits

Phenylalanine regulates the discharge of the hormone cholecystokinin (CCK). Phenylalanine conveys signals to the brain that person is satisfied after eating. If someone is attempting to reduce some weight, incorporate supplementary diets that possess this essential amino acid into food. A person may feel more pleased after eating less.

1.5 Parkinson's disease

Restricted study recommends that administration of D-phenylalanine might reduce manifestations of Parkinson's disease [8].

1.6 Metabolic role of tyrosine

Metabolic products of tyrosine are tyrosine-O-sulfate, cresol, phenol, tyramine, melanin pigment, catecholamine, tri-iodotyrosine and thyroxine [9, 10].

1.7 Tyrosine becomes essential

Because the tyrosine can no longer be formed in phenylketonuria, it becomes an essential amino acid.

1.8 Development of melanin

It is the main color of the skin and is additionally present in the eye, even in the cerebrum (e.g., substantia nigra). In the skin, it is delivered by particular cells called melanocytes, which are located in the limit between the epidermis and dermis and in hair globules. Melanin is a co-polymer of dopa-quinine, indole 5, 6 quinone, and indole quinine 2—carboxylic corrosive in the proportion 3:2:1. It is delivered on the surface of intracellular granules called melanosomes, which are rich in the catalyst tyrosinase. It is exceedingly insoluble substance. Zinc particles are important for the melanin development. Arrangement of melanin is animated by light (e.g., tanning), ACTH and MSH. Nonappearance of Cu-containing protein tyrosinase (tyrosine hydroxylase) produces tyrosinase-negative oculocutaneous albinism. Human skin is presented to bright light that can harm the skin. Melanin keeps harm of skin from bright light [11].

1.9 Homogentisic acid

Homogentisic acid is a metabolite in the breakdown process of amino acids such as tyrosine and phenylalanine. In normal condition, it is not detected in urine and blood.

1.10 Homogentisic acid accumulation

In deficiency of homogentisic acid dioxygenase, homogentisic acid builds up in the blood and excretes in urine. When come in contact with air, homogentisic acid reacts with oxygen and cause the urine to become black. This is because of black pigment knows as alkapton and termed as alkaptonuria. This same black pigment in a procedure known as ochronosis causes bone and tissue to darken and degenerate. This causes disabling and painful joint disease called as osteoarthritis [12].

1.11 Alkaptonuria

It is an inborn error of metabolism, a genetic disorder caused by a deficiency of enzyme homogenetic acid dioxygenase. Without this enzyme, persons cannot break down the amino acids such as tyrosine and phenylalanine, which cause accumulation of homogentisic acid in urine, cartilage, and bone. The characteristics of Alkaptonuria are black urine, ochronosis (black cartilage and bone), and degenerative arthritis of the joint [13].

1.12 Homogentisic acid dioxygenase deficiency

The deficiency of homogentisic acid dioxygenase occurs due to the mutation in the homogentisic acid dioxygenase gene. It occurs in children when both father and mother are the carriers of mutated gene. This is known as autosomal recessive disease [14].

1.13 Hypopigmentation

Pigmentation loss is usually seen in patients with phenylketonuria due to reduction in amino acid tyrosine which is utilized by melanocytes to form melanin [15].

1.14 Catabolism of phenylalanine and tyrosine

Phenylalanine is changed over to numerous subsidiaries, which are discharged in pee. These incorporate phenyls lactic corrosive, phenyl acidic corrosive, ortho-hydroxyphenylpyruvic corrosive and ortho-hydroxyphenylacetic corrosive. In any case, this is ordinarily a minor pathway of phenylalanine and it turns out to be quantitatively more critical just when phenylalanine is not changed over to tyrosine, which is the real pathway of phenylalanine catabolism. The catabolic results of tyrosine incorporate homogentisic corrosive which is additionally separated to fumaric corrosive and acetoacetic corrosive [16].

1.15 Diseases associated with an abnormal metabolism of phenylalanine and tyrosine

1.15.1 Phenylketonuria

It is likewise called phenylpyruvic oligophrenia. It is because of absence of the chemical phenylalanine hydroxylase, which changes over phenylalanine to tyrosine. Phenylalanine is redirected to its ordinarily minor metabolic pathway framing para-hydroxy phenylpyruvic corrosive, para-hydroxy phenyl lactic corrosive, para-hydroxy phenyl acidic corrosive, and phenyl acetylglutamine all of which gather in the body alongside phenylalanine. These are discharged in pee in vast sums, which causes mental hindrance. The infection ought to be determined early because to have appropriate treatment (low phenylalanine abstains from food) the impediment of mental improvement can be halted. The best test is finding a raised blood level of phenylalanine. Nonetheless, it can likewise be analyzed prenatally (before birth) by DNA ponders as the quality for phenylalanine hydroxylase has been cloned. The name of the illness phenylketonuria is because of the discharge of parahydroxyphenyl pyruvic corrosive, which is a keto corrosive. This infection is currently gathered under the term hyperphenylalanemia of which there are numerous assortments [17].

1.15.2 Manifestation

Manifestations include psychiatric disorders, behavioral problems, delayed development, seizures, and intellectual disability, lighter hair, skin, musty, or mouse-like odor, microcephaly. Studies propose that untreated phenylketonuria in pregnancy is linked to attention-deficit hyperactivity disorder, intellectual disability, and microcephaly.

1.15.3 Musty or mousy body odor

Aromatic amino group is present in phenylketones, which is responsible for musty or mousy odor in patients which is feature for phenylketonuria [18].

1.15.4 Maternal PKU

Maternal PKU is developed when there is increased concentration of phenylalanine in a female's blood during gestation. This goes to the developing fetus. These high levels significantly enhance the danger for a baby to be born with behavioral problems, characteristic facial features, heart defects, growth retardation, and a small head size (microcephaly). For female with phenylketonuria, it is significant that they follow a low phenylalanine diet if they plan to develop expectant or are expectant. The bad effects of high levels of phenylalanine can be stopped if this diet is followed before conception and during the pregnancy [19].

1.15.5 Adults with PKU

Adults with PKU carry on taking care throughout whole life. Older adults with phenylketonuria who may have stopped the PKU food in their teens may advantage from an appointment with their physicians. Returning to the food may increase intellectual working and performance and gentle impairment to the central nervous system in adults with increased levels of phenylalanine [20].

1.15.6 Calcium homeostasis

Calcium homeostasis is vital for brain activity and its dysregulation in phenylketonuria was recommended by numerous studies. In this background, dehydrocholecalciferol, osteocalcin and parathyroid hormone were found enhanced in blood of infants with phenylketonuria, but level of calcitonin was low. These changes were not returned by Phenylalanine restricted food. In additional work, Yu and colleagues proved that Phenylalanine modifies intracellular free calcium levels by altering plasma membrane Ca^{2+}-ATPase in cortical neurons [21].

1.15.7 Diagnosis

Phenylketonuria is analyzed by examining the amino acids in the plasma. Screening programs have been introduced in numerous countries that permit identifying the illness in neonates within the first few days of birth. The objective of these recognition programs is to manage the babies prior to the initiation of exhibiting manifestations of the illness. Once identified, the children will be referred to a reference hospital for differential diagnosis with other less frequent forms of diseases, which can cause a rise of blood phenylalanine levels and initiate the management. The analysis of the PAH gene mutations approves the diagnosis [22].

1.15.8 Tracking pH levels

Children and young kids with phenylketonuria require having consistent blood tests for measurement of phenylalanine levels. If there is too much or too little phenylalanine in the blood, the formula and diet may require to be attuned [23].

1.15.9 Molecular testing

It is usually unnecessary for a diagnosis of phenylketonuria. However, restricted genotype-phenotype association has been designated. In addition, molecular testing is necessary for prenatal diagnosis [24].

1.15.10 Screening

Blood is taken from a 2-week-old infant to test for phenylketonuria [25]. Phenylketonuria is generally incorporated into the infant screening board of various nations, with various identification systems. Most infants in created nations are screened for phenylketonuria not long after birth. Screening for phenylketonuria is finished with bacterial hindrance test (Guthrie test), immunoassays utilizing fluorometric or photometric location, or amino corrosive estimation utilizing pair mass spectrometry (MS/MS) [26]. Estimations done utilizing MS/MS decide the grouping of phenylalanine and the proportion of phenylalanine to tyrosine, the proportion will be hoisted in phenylketonuria.

1.15.11 PKU carriers

Sisters and brothers who do not have phenylketonuria still have a chance to be carriers like their parents. Except in special cases, the carrier testing should be done merely in persons older than 18. Each of the parents' sisters and brothers has a 50% chance to be a carrier. It is significant for other family members to be said that they could be carriers. There is a minor chance they are also at danger to have offspring with phenylketonuria. When both parents are carriers, newborn screening outcomes are not adequate to rule out the disorder in a neonatal baby. In this situation, special investigative testing should be done in addition to newborn screening [27].

1.15.12 Foods to be avoided in phenylketonuria

Nuts, beans, eggs, dairy, meats, chocolate, ice cream, cheese, yogurt, regular bread, birthday cake, and pizza.

1.15.13 Management of phenylketonuria

Treatment ought to be begun as right on time as conceivable after birth. Phenylalanine is a basic amino corrosive and, in this manner, cannot be completely barred from the eating routine. Eating routine containing low phenylalanine with included tyrosine is suggested. Phenylalanine levels ought to be kept up between 6 and 9 mg%. Extremely serious limitation of phenylalanine prompts tissue breakdown. Strict limitation is suggested until eighth year of life. After this, confinement may not be so unbending [28].

1.15.14 Medical formula

Even though they require less phenylalanine, children with phenylalanine still require a definite quantity of protein. The medical formula gives children and the babies with phenylketonuria the protein and nutrients they require while keeping their phenylalanine amount within a nontoxic array. The dietician and metabolic physicians will tell you what kind of formula is greatest and how much to use. Administering a phenylketonuria formula for life is to make sure patient gets sufficient essential protein (without phenylalanine) and nutrients that are vital for general health and growth.

1.15.15 Foods that are allowed (excessive use is not allowed)

Fruits allowed include strawberries, apples, grapefruit, oranges, grapes, melons, bananas, and peaches. Vegetables allowed include tomatoes, radishes, lettuce, cucumber, celery, cauliflowers, carrots, and French beans.

1.15.16 Food not allowed

All meats including fish, fish products, chicken, bacon, pork, lamb, organ meats (liver, heart, kidney) and all dairy products including pudding, ice cream, yogurt, milk, cheese, cottage cheese, seeds, nuts, legumes, biscuits and flour cakes.

1.16 Prevention

1.16.1 Follow a low-phenylalanine diet

Female with phenylketonuria can inhibit birth defects by sticking to (or returning to) a low-phenylalanine diet prior to the becoming pregnant. Female with PKU should consult to physician prior to conception.

1.16.2 Prognosis

The consequence is predictable to be very good if the food is carefully monitored, beginning soon after the birth of child. If management is late or the disorder remains untreated, damage of brain will occur. School working may be slightly reduced. If proteins comprising phenylalanine are not evaded, phenylketonuria can lead to intellectual incapacity by the completion of the first year of life.

1.16.3 Hypertyrosinemia

It is because of the shortage of tyrosine aminotransferase [29].

1.16.4 Hereditary tyrosinemia

It occurs due to deficiency of fumarylacetoacetate hydrolase [30].

1.16.5 Alkaptonuria

This results from the lack of the enzyme homogentisate 1,2-dioxygenase. This leads to buildup in the body of homogenetisic acid, which is expelled in urine. Oxidation of homogentisic acid occurs that causes the urine to become dark. Patients with alkaptonuria are also affected with arthritis. This disease was discovered by Archibald Garrod in early 1900s. This was the major disorder in which an association between an enzyme and inheritable trait was established [31].

1.16.6 Attention deficit disorder

In the solitary double-blind, crossover study available in this part, quantities of up to DL-phenylalanine (1200 mg) were prescribed in 19 patients with attention deficit disorder. After 2 weeks, a substantial alteration in mood lability and mood was detected in the treatment cluster. After 2–4 months, however, patients who had improved with the use of DL-phenylalanine developed tolerant and did not respond to higher quantities [32].

1.16.7 Dopamine history

Dopamine was first made in 1910 by James Ewens and George Barger and at Wellcome Laboratories in London, England. In 1958, Nils-Åke Hillarp and Arvid Carlsson at the Laboratory for Chemical Pharmacology of the National Heart Institute of Sweden, found the dopamine work as a neurotransmitter. Arvid Carlsson was introduced the 2000 Nobel Prize in Medicine or Physiology for demonstrating that dopamine is not only an antecedent of epinephrine and norepinephrine however a neurotransmitter, too [33].

1.16.8 Role of dopamine in pain

Dopamine has an impact in handling of agony in various levels of the focal sensory system, for example, cingulate cortex, basal ganglia, thalamus, periaqueductal dark, and the spinal line. Diminished centralization of dopamine has been connected to excruciating signs that regularly saw in Parkinson's infection. Distortions in dopaminergic neurotransmission likewise occurs in various difficult clinical issue, for example, fretful legs disorder, fibromyalgia and consuming mouth disorder [34].

1.16.9 Drug-nutrient interactions

Phenylalanine has been revealed to contest with levodopa for passage through the blood-brain barrier [35]. Tyramine, dopamine, norepinephrine, epinephrine is derived from phenylalanine. Supposedly, attention is necessary with the concomitant use of phenylalanine and monoamine-oxidase inhibitors.

1.16.10 Side effects and toxicity

LD-50 of D-phenylalanine in rats is higher than 10 g/kg. No tissue toxicity was observed in murine studies at dose of 1 mg/kg daily for 6 months. Short time adverse effects are insomnia, aggressiveness, irritability, headache, and increase of blood pressure [32].

1.16.11 Dosage

Dosages differs with the disorder; 1–4 g daily for pain treatment and 1–14 g daily for depression.

1.16.12 Warnings and contraindications

Phenylalanine supplementation should be avoided in phenylketonuria [36]. Phenylalanine can affect efficacy and absorption of levodopa [37]. Phenylalanine use is contraindicated in patients with schizophrenia [38].

2. Research study

2.1 Impact of co-trimoxazole on phenylalanine metabolism in man

An investigation was completed to assess impact of co-trimoxazole on phenylalanine digestion. It was discovered that phenylalanine level stays high in the wake of taking co-trimoxazole. Proportion between serum-phenylalanine and tyrosine was likewise high. In a few patients, serum phenylalanine levels were marginally brought up in fasting conditions. As a conclusion, it was proposed that the trimethoprim/sulfamethoxazole blend has a synergistic activity in offending phenylalanine resistance [39].

2.2 An open study on phenylalanine in depressed patients

In a clinical trial, phenylalanine was regulated to 20 patients with gloom. Length of treatment was 20 days. Measurement of phenylalanine was 75–200 mg/day. Toward the finish of treatment, 12 patients were dealt with and there was no further need of treatment for these patients. Mellow to direct reaction was seen in 4 patients. Four patients did not react at all to phenylalanine. This examination shows that phenylalanine is significant in depressive patients [40].

2.3 Schizophrenia and blockage of dopaminergic neurotransmission

Phenylalanine is hydroxylated to tyrosine and tyrosine to dopa and dopa to dopamine. Dopamine has been embroiled for a long time in the pathophysiology of schizophrenia, and the run of the mill antipsychotics, by means of bar of dopaminergic neurotransmission, have furnished help for patients with positive

manifestations [41]. In any case, just dopamine blockage is not sufficient to ease manifestations of schizophrenia in the way it is viewed as those different neurotransmitters are additionally associated with pathophysiology of schizophrenia. Dopamine partiality for dopamine receptor is diminished by expanding adenosinergic transmission. Adenosine level might be expanded by presentation of allopurinol that is xanthine oxidase inhibitor, at last prompting antipsychotic and anxiolytic impacts. Confirmation for this treatment has been accounted for in both case reports and little clinical trials. Different investigations demonstrate that allopurinol is valuable in those patients who are ineffectively receptive to existing treatment for schizophrenia. Nevertheless, additionally study ought to be completed to discover its viability and wellbeing as a standard treatment for schizophrenia. In any case, exhibits think about demonstrate that allopurinol at 300 mg day by day is sufficient to assuage side effects of schizophrenia [42].

2.4 Impact of loading measurements of phenylalanine in unipolar discouraged patients with and without tardive dyskinesia (TD)

In a clinical trial, phenylalanine was managed to three distinct gatherings. Dosage of medication was 100 mg/kg phenylalanine. Eleven patients were in first gathering (discouraged patients with tardive dyskinesia). Ten patients were in second gathering (discouraged patient presented to neuroleptics yet without TD), 10 patients were in third gathering (patients never presented to NLs). There was no critical factual contrast among three gatherings. A relationship was found between automatic development and fasting, and phenylalanine stacking following 2 hours. Three TD patients indicated surprisingly expansive increments in phenylalanine level in plasma. This examination demonstrated that variations from the norm in digestion of phenylalanine add to the improvement and seriousness of TD in some NL-treated unipolar discouraged patients [43].

3. Conclusion

Phenylalanine hydroxylase (PAH) deficit consequences in intolerance to the dietetic consumption of the important amino acid phenylalanine and produces a variety of syndromes. The hazard of antagonistic consequence fluctuates based on the grade of PAH deficit. Deprived of effective management, maximum individuals with severe phenylalanine hydroxylase deficit, recognized as classic phenylketonuria, develop deep and permanent logical disability. Affected patients on an unrestricted food who have phenylalanine concentration above normal but below 1200 μmol/L (20 mg/dL) are at much lower hazard for impaired cognitive development in the lack of management. Phenylalanine is prescribed for alcohol withdrawal symptoms, vitiligo, weight loss, depression, rheumatoid arthritis, osteoarthritis, pain, multiple sclerosis, depression, Parkinson's disease and attention deficit-hyperactivity disorder.

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Chapter 6

Role of Osmolytes in Amyloidosis

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Abstract

Osmolytes are naturally occurring small organic molecules present in all kingdoms of life. These organic molecules are accumulated by living systems to circumvent stress conditions. A number of human diseases have been grouped under the protein-misfolding diseases. These entire diseases share the same hallmarks of the presence of cellular inclusions and plaques that are deposited in the cells and tissues affected by the disease. These misfolded forms of protein are responsible for initiating toxic cascades in the cell, causing vesicle dystrafficking, synaptic and cell organelle dysfunction, and ultimately cell death. Published results suggest that cells regulate many biological processes such as protein folding, protein disaggregation, and protein-protein interactions via accumulation of specific osmolytes. Since, as of now, complete cure for these protein-misfolding disorders does not exist; therefore, it becomes increasingly important to review the recent works on this aspect to develop strategies for therapeutics. It has been shown that certain osmolytes can prevent the proteins from misfolding. Thus, osmolytes can be utilized as therapeutics for such diseases. In this review article, we discuss the role of naturally occurring osmolytes in various forms of amyloidosis associated with human diseases.

Keywords: osmolytes, misfolding, aggregation, therapeutics

1. Introduction

Organisms in all the phylum of life experience stress conditions (heat, salinity, drought, etc.) of various kinds at one or more point in their life cycle. To survive such environmental assaults, the living forms during the course of evolution have developed a number of strategies. One such strategy that has been employed nearly by all kingdoms of life is the accumulation of osmolytes to evade various forms of stress. The osmolytes are low molecular weight compounds of organic nature [1–3]. These osmolytes are also termed as chemical chaperones due to their inherent ability to cause proper protein folding. These stabilizing molecules, osmolytes, have been grouped into various categories by different classification schemes. The most commonly followed classification of these molecules is based on their chemical nature: polyols (sorbitol, myo-inositol, glycerol, etc.), amino acids and their derivatives (taurine, glycine, alanine, etc.), and methyl ammonium compounds (glycine betaine, TMAO, sarcosine, etc.). Another most common way of classifying these molecules is based on their ability to modulate protein function and stability: compatible osmolytes and counteracting osmolytes. It has been observed that the compatible class of osmolytes at 25°C causes changes in the stability of protein but does not have significant effect on the function [4–8]. In contrast to the compatible

class of osmolytes, the counteracting osmolytes modulate both the stability and functional activity of proteins. It has also been observed that counteracting osmolytes protect proteins (in terms of both stability and function) by neutralizing the inactivating and destabilizing effects of urea on cellular proteins [9–14]. Lower vertebrates such as cartilaginous or elasmobranch fishes and coelacanth maintain their ionic homeostasis by accumulating large concentration of urea. Similarly, in the mammalian kidney, the cells of the inner medulla are normally exposed to high urea and salt concentration. In order to counteract the deleterious effect of urea on the structure and function of macromolecules, it has been proposed that marine fishes and cells of renal medullary cells adopt their unusual environment by accumulating osmolytes such as TMAO, glycine betaine and glycerophosphorylcholine, etc. [15–18]. The term "protective" has also been used to define osmolytes since these molecules poses the capability to stabilize proteins under conditions, which are deleterious to their structure [19–27]. Osmolytes have also been implicated in stabilizing proteins that are less stable and also cause refolding of proteins that have been misfolded [28–30]. By using different experimental approaches such a differential scanning calorimetry or spectroscopic methods, researchers have calculated the preferential interaction parameters, which are based on free energy transfer methods of protein backbone in the presence and absence of osmolytes and, on this account, they proposed different protein-solvent mechanisms such as preferential hydration, preferential exclusion, preferential binding mechanism of stabilization, and destabilization of the unfolded state of the proteins [31].

Many reports describe the application of osmolytes in a number of mammalian disease models. The fact is that osmolytes play an important role in the protein quality control system (PQC) for maintaining homeostasis of the cell. Additionally, osmolytes have also been implicated in modulating the proteostasis, in controlling the degradative pathways of normal proteins and also the aggregation pathways of misfolded proteins. These osmolytes have also been reported to be able to act as therapeutic agents for the treatment of various pathophysiologic conditions associated with formation of protein aggregates. Importance of osmolytes in various types of diseases related to amyloidosis has also come to light. This review is designed to address all the clinical implications of osmolytes toward amyloidosis-related diseases with special emphasis on the amyloidosis related to α-synuclein.

2. How osmolytes modulate structure and stability of proteins?

The cells remain viable only when the protein residing in them retain their native structure, under normal conditions of pH and temperature [1]. The proteins adopt numerous three-dimensional arrangement of their polypeptide chains as is required by the cell to carry out its functions and, hence, maintain its viability [32]. The polypeptides, which are to be degraded, are always found to adopt a less stable conformation as compared to more stable and functional proteins [32]. For the cell to maintain homeostasis, it is very essential that a constant balance should be maintained between rate of degradation and production of proteins. A small change in this balance will lead to the development of disease [1, 33, 34]. Osmolytes have an inherent property to either stabilize or sometimes destabilize the three-dimensional polypeptide conformations; hence, they are able to modify protein homeostasis. A very fine relationship that exists between degradation and proper folding of proteins that are either unstable or has misfolded is what makes cell viable. The quality of the polypeptides is dictated by the events that occur in the early pathways of secretion, nucleus, and cytoplasm where the polypeptide assumes its proper functional native conformation and assembly. The proteins, which

attain conformationally less stable structures, undergo proteasomal degradation [35]. In addition, the misfolded or pathological protein aggregates downregulate proteasomal activity, which leads to neurodegenerative diseases. Now the role of osmolytes comes into action. They act as molecules of surveillance, trying to find such misfolded molecules and enabling them to attain proper functional conformation, leading to increment in homeostasis of less stable proteins. But many of the osmolytes come under the category of destabilizers, which promote protein degradation of highly stable or fibrillar conformations. It, therefore, seems very logical for cells to accumulate osmolytes, which have both the property of stabilizing and destabilizing, to regulate proteins homeostasis. Numerous research groups have demonstrated significant role of osmolytes as regulators of protein homeostasis [28, 32, 36–42]. It has been clearly demonstrated that in the presence of the stabilizing osmolytes, the misfolded proteins can be saved from proteolytic pathways by making them refold back to native structures. If we can modulate the cell's ability to accumulate osmolytes through any process, then it will have a profound impact on the stability and functions of proteins and enzymes in maintaining the homeostasis of the cell. The methylamine group of osmolyte such as betaine has dual role at two different molar concentrations; it acts as a denaturant at high molarity and as a stabilizer at low molarity [43]. The pathological conditions associated with age or that have genetic relations are all due to the errors involved in the breakdown of misfolded polypeptides. These misfolded polypeptides are associated together to form nucleation-dependent polymerization to form fibrillar structures, which leads to amyloid-associated diseases [44]. These pathophysiological conditions can be combined under one title, i.e., the protein conformational diseases. Many diseases fall under this category and include serpin-deficient disorders, Alzheimer's disease, cystic fibrosis, transmissible spongiform encephalitis, hemolytic anemia, Huntington disease, diabetes type II, amylotropic lateral sclerosis, dialysis-related amyloidosis, and Parkinson's disease [9, 23]. Therefore, if we could devise certain methods to use these osmolytes so that it will shift the changes from non-native conformations to native conformations of protein, then many misfolded disorders can be treated. Even the destabilizing osmolytes can be utilized to remove the fibrillar structures of polypeptides formed inside the cell. Two amino acids, lysine and arginine, are often used in the solubilization of inclusion bodies and fibrillar structures [45–47]. Thus, osmolytes that stabilize the polypeptides are also known as good refolders [48]. Additionally, many proteins are known to bind to particular proteins, subsequently modifying the native conformation, as in posttranslational modification reactions [49]. A large number of polypeptides are shown to bind with arginine and the methyl group of betaine to regulate their biological function [50].

3. Osmolytes induce misfolded protein to attain functional folded structure

It is a very well-known fact that the primary sequence of amino acid residues in the polypeptide chain code for the three-dimensional structure of the protein, which in turn determines the functional structure and activity of protein through different complex pathways of protein folding [51]. It becomes very essential that no error occurs during the protein folding through this pathway, but if it will move to off-pathway, then it can lead to the formation of a misfolded protein. Since these misfolded proteins lose their original function, they are deleterious to function and survival of cell [52]. The formation of such misfolded proteins may be due to mutation in its gene or error in the pathway of protein folding, and these are recognized by the cells as abnormal and are subjected to undergo degradation in the PQC

system. The loss of proteins by subjecting them to degradation may result in loss of function [53]. The other reason cited for the loss of function was that misfolded proteins tend to accumulate in the endoplasmic reticulum (ER), which is considered to be a type of defect associated with trafficking pathway and resulting in functional deficiency. Functional deficiency can be explained by taking the example of α1-antitrypsin (α1-AT), which is an extracellular protein synthesized in the ER. The mutant form of this protein is due to altered structure, which aborts the transportation mechanism of the protein in the ER and hence gets accumulated in ER leading to α1-antitripsin deficiency. Many reports have been shown that misfolded protein accumulated in the ER may lead to the development of environmental stress and subsequently, developing into the ER-associated disease [28]. Another hypothesis put forward to explain the functional deficiency is caused due to the presence of mutant protein. The mutant protein fails to fold to its properly folded native structure and forms a misfolded structure that may oligomerize and may further form aggregates. These oligomers and aggregates induce oxidation stress in the cells, thereby, deleteriously affecting the physiological processes of cells; i.e., they may cause enhancement in function of certain proteins, which is the case in pathophysiological state that causes neurodegeneration [54].

Many reports demonstrated that when osmolytes were added to the solution containing misfolded mutant proteins, their native function was restored. Many of these studies have also shown that specific osmolytes are able to assist correct folding of misfolded proteins. This in turn prevents their degradation, thus increasing their intracellular function [32, 36, 38, 39, 41, 42]. A study had shown that treating the mammalian fibroblast cell line with media containing 4-PBA and glycerol reduced the concentration of mutant form of α1-ATZ in the cells [55], but this effect was not observed when TMAO, D_2O, and betaine were added to the culture media and resulted in the rise in the levels of native α1-ATZ in the cells when osmolytes were present [28, 55]. Similarly, different classes of osmolytes were found to correct the defects in folding of protein and restore protein function of phenylalanine hydroxylase [29]. For instance, in the protein p53, which is a protein involved in suppression of tumor formation, mutation of p53, A125V causes temperature sensitivity and when such cells are cultured at 32°C, the permissive temperature, the p53 protein is active and is found localized in the nucleus [37]. However, cells, which are grown at higher temperatures (nonpermissive condition), the p53 protein becomes inactive and gets localized in the cytosol. It was also observed that when 75 mM TMAO, 0.6 M glycerol, or D_2O, were added to the culture media, the p53 protein is relocated in the nucleus even at higher temperature. This could be explained as the osmolytes cause the correct folding of the mutant protein; therefore, at higher temperature also, the p53 protein remains correctly folded and hence functional in the presence of TMAO, glycerol, and D_2O. Interestingly, when the osmolytes where removed from these cells, the p53 protein became temperature sensitive. Aquaporin-2 (AQP-2) is a vasopressin protein found in the kidney collecting duct. It functions as a water channel that carries water molecules across the cell membrane. The mutation in AQP2 gene results in the misfolding of Aquaporin-2 protein which leads to the development of diabetes insipidus in mammals. But when glycerol (1 M) was added in the cell culture, glycerol restores the folded structure and hence the proper translocation of this protein in the cell [56]. The cells, which have the mutant protein Src kinase (pp60src), do not attain the correct functional conformation at higher temperatures (nonpermissive), and therefore, it is unable to modify the cells. Interestingly, when the cells grow at nonpermissive temperatures in the presence of glycerol, this mutant protein is still capable of transforming the cells. Thus proving that glycerol is able to restore the correct conformation of protein and hence its function. Another study showed that the mutant form of ubiquitin ligase, ts-E1, which remains nonfunctional at higher temperature, causes loss of cell growth because of unspecific

degradation of proteins. But, when glycerol or TMAO was added, these osmolytes restored the correct conformation of the protein and hence normal growth of cells [57]. In all the above experiments, which were performed in the mammalian cell culture, bacterial, or yeast systems, the addition of glycerol to media assisted correct folding of mutant proteins and restored their normal biological activity [36, 58]. These osmolytes have also been implicated in increasing the expression of certain proteins (P-glycoprotein) in cancer cells [59], which acted as pumps for exporting the drugs out of the cells [60]. The human P-glycoprotein expression levels were observed to be very low [61], but when osmolytes were added in the culture media, the expression level of the protein in cells was enhanced [62].

4. Osmolytes play important role in preventing protein aggregation

When the proteins get misfolded, the hydrophobic groups present in the polypeptide chain, which earlier had remained buried in the three-dimensional structure of native protein, are exposed to the surrounding solvent. The exposure of the hydrophobic groups to the exterior of the protein causes intermolecular binding between these exposed groups. When these misfolded proteins reach a critical concentration inside the cell, the process of oligomerization and subsequent aggregate formation starts. The sequence of amino acid residues in the polypeptide chain changes when the proteins are exposed to harsh conditions, for example, high temperature or exposure to oxidation stress. These changes in the primary structure of proteins may be caused due to modification of RNA or defects during translation process of proteins, and all of these factors increase the chances for the protein to aggregate [63, 64]. These aggregation products of protein have very low solubility in the surrounding physiological solvent. These protein aggregates remain stable metabolically, and they may exist in the cells in the form of amyloids or in conformations, which are amorphous [65]. Many research groups have focused their work on the key factors involved in the process of aggregation of proteins and subsequent development of the disease. It is well known that the kinetics of aggregate formation follows a sigmoid curve and comprises of three stages: (i) lag phase that corresponds to structural transformation within monomer leading to the appearance of aggregation-prone partially folded species, (ii) exponential phase that corresponds to self-association leading to the formation of oligomers, and (iii) stationary phase that leads to the formation of aggregates [66]. The amount of aggregation products that are formed and deposited inside the cell is determined by the biosynthesis rate, stability, compactness of the protein, and also the number of hydrophobic groups present in the protein that are exposed to the exterior environment [67]. One of the characteristic features of all the diseases, which involve formation of protein aggregates, is the increase in the β-helical content of the protein with subsequent decrement in the α-helical content of the protein secondary structure [68]. In the field of medicine, this phenomenon of aggregate formation of protein has now acquired a lot of attention due to its implication in many of the human diseases including, neurodegenerative, metabolic, cardiovascular disorders, etc. [69–77]. It has also been reported that a particular amino acid sequence or three-dimensional structure of protein is involved in a specific disease that is associated with aggregate formation [78–80]. Surprisingly, many of the peptides and proteins have not been found associated with any case of disease until and unless they are subjected to specific conditions or stress conditions to form aggregates. Hence, it is safe to say that it is the inherent property of all proteins to get aggregated [78–80].

Researchers have shown that proline acts as a wonder osmolyte against aggregation of protein. In this instance, it was found that the aggregation of bovine

carbonic anhydrase inhibited at three molar concentrations of proline [81]. The inhibition of aggregation in the presence of proline can be explained that proline, a charged amino acid, might introduce an electrostatic repulsion in the interactions of the polypeptide chains at the very earlier stages of the aggregation process [82]. The protein huntingtin, which is polyglutamine-rich (polyQ), is reported to get accumulated in the nucleus of cell. This type of amyloid deposition is a hallmark feature for the patients affected by Huntington disease (HD) [83]. An experiment with 21 days old mice, which were kept on trehalose solution diet till the day of killing, was observed with a significant reduction in the accumulation of misfolded protein Huntingtin [84]. Because of this, motor dysfunction associated with the disease was improved, which resulted in the extension of the life of HD transgenic mice used as models for the disease [84]. Many other laboratories reported the inhibition of aggregation in many different types of protein in the presence of proline for instance, chicken egg lysozyme [85], chicken liver fatty acid synthase [86], and rabbit skeletal muscle creatine phosphokinase [87]. But the existing reports also demonstrated that these osmolytes may in some instances promote the misfolding of protein leading to the aggregates formation or sometimes they have no effect at all on the process of protein aggregation. TMAO, an osmolyte, is one the best examples of such case. When RNase was subjected to refolding in the presence of TMAO, it increased the formation of aggregates [88]. In another study, it was found that glycine (at very high molarity) had no significant effect on the aggregate forming ability of chicken liver fatty acid synthase [86]. In addition, it could not also refold the chicken egg lysozyme [85]. Taurine, though was able to delay the fibrillation [89], had no effect on the aggregate morphology of glucagon [89]. Trehalose, on the other hand, has shown good potential to inhibit the fibrillation of many Aβ40 [90], yeast prion protein Sup35 [91]. In the models of oculopharyngeal muscular dystrophy in mouse, trehalose lowers down the levels of aggregation of protein [92]. In addition to the general role played by osmolytes in PQC system and hence in the disease etiology of multiple PQC-related diseases, there are some specific diseases that have been paid more attention and osmolyte strategy has been successful in their case, which are described below.

5. Osmolytes and process of amyloidosis

The process of fibril/aggregate formation in the process of amyloid formation is determined by the thermodynamic solubility of peptides. The solubility of the peptides depends on the structure and concentration relative to the critical concentration of the peptides. Above the solubility limit, the peptides formed a series of crystals and fibril structures to form amorphous aggregates. Two types of aggregates are formed based on their morphology: ordered (amyloids with β-rich proteins) and disordered (amorphous) [93, 94]. It was evident that the partial unfolding of the native state results in the formation of ordered aggregate structures [93, 95]. The intermediate formed during this partial unfolding has hydrophobic patches exposed to the exterior. These hydrophobic patches bind each other and lead to the formation of amyloids [80, 96, 97]. Several groups have reported that osmolytes have a great potential to suppress/inhibit this misfolding and subsequent fibril formation by proteins [98–102]. It has been very well demonstrated that these osmolytes increase the stability of proteins under conditions of heat stress by the mechanism of preferential hydration [99, 100, 103–105]. A number of reports exist, which demonstrate the use of these small molecule osmolytes as good therapeutic agents for the treatment of several protein-misfolding diseases [84, 106]. It is very interesting to note that in the presence of some osmolytes, proteins can also be

induced to form fibrils or aggregates under controlled conditions. One such enzyme is lysozyme, a model protein. This enzyme has been widely used for such kind of studies due to its small size and also since a lot of information related to structure and stability exists in the literatures. Since long, lysozyme has been considered a model protein to study the complexities of structure of protein as well as its function [107]. It also has been extensively studied to get information on the kinetics of enzyme action as well as on markers for protein-misfolding diseases [108].

Choudhary and coworkers prepared several amyloidogenic forms of lysozyme and subjected them to ThT-binding fluorescence spectroscopy [108]. Then they monitored the intensity of ThT fluorescence emission of lysozyme at the various stages of fibril and aggregate formation: nucleation, elongation, and saturation. This enabled them to assess the different stages of the fibril formation. To get a better understanding of the energetics of interaction of a particular osmolyte with the fibril at different stages of its formation, they performed isothermal titration calorimetry (ITC) studies. They also used transmission electron microscopy to see the morphology of these fibrils at different stages [108]. They found that osmolytes such as sarcosine, proline, TMAO, and 4-hydroxy-L-proline were able to inhibit the fibril formation of lysozyme [108]. Sarcosine and 4-hydroxy-L-proline were found to significantly prolong the start of the elongation stage of fibril formation. Interestingly, lysozyme showed amorphous aggregates in the presence of TMAO for more than 5 hours of incubation at a stretch. Similar results were obtained when observed under TEM. In another example, they used proline as a polar osmolyte and observed that an exothermal interaction was occurred in between lysozyme and proline at the stage of nucleation. However, at the elongation stage, the association of protein with proline was found to be less exothermic as compared to the presence of sarcosine [108]. Therefore, this study clearly demonstrated that osmolytes basically interact with polar protein at the nucleation stage. This is accounted as the major step for the inhibition of fibril formation [108].

Researchers found many difficulties in the detection of protein aggregates with respect to conformational patterns, nature of aggregates, and various structural fibrils forms. There are different spectroscopic methods such as UV visible, circular dichroism (CD), NMR, X-ray diffraction, etc., used in characterizing the conformational changes in the protein. Fluorescence dyes such as Thioflavin T and Congo red binding assays as well as antibodies are used as external probes for evaluating the physical properties of the aggregates.

Recently, Needham and coworkers developed bifunctional sensors for simultaneous detection of amyloid aggregates of α-synuclein and pathophysiological H_2O_2 concentrations for oxidative stress. They also developed a new imaging method called single-aggregate visualization by enhancement (SAVE) to detect single-β-sheet containing amyloid aggregate of α-synuclein using ThT [109].

Another group studied the fibrillation process in the presence of TMAO of the peptide NNQQNY (of Sup35 prion) by molecular dynamics simulation (MDS) method [110]. When the free surface energy of the formation of protofibril was observed, it showed three main basins which corresponded to the time when peptide was in solution, known as free state, the time when the peptide in the solution was interacting with the surface of the protofibril, known as the docked state and the last stage when the peptide gets tightly bound to the protofibril and thus becoming a part of fibril known as locked state [110]. When these studies were performed in the presence of TMAO, it was found that this osmolyte stabilized the locked state and thus acting as an aid in the process of aggregation [110]. When the associative reactions of TMAO with each amino acid residue in the protein were studied in detail, it was found that TMAO interacted with each amino acid residue either directly or indirectly, and this was determined by nature of their side chain. It was also found

that the TMAO's methyl groups interacted very strongly with the aromatic ring of tyrosine residues. In this study, they proposed that in the locked state, the tyrosine's surface area of the peptide was not available to interact with TMAO. As a result of this, the tyrosine residue flips in such a way that it fluctuates the locking state of the protofibril of peptide, and this leads to destabilization of the folded and also the fibril state of proteins. The increase in the stability of peptide, which is locked in protofibril state in the presence of TMAO, is because of interactions, which are entropic in nature and indirectly with the peptide backbone of residues Gln and Asn. Both of these residues are important part of NNQQNY peptide [110].

Many research groups studied the aggregation properties of amyloid plaques, which are a central feature of Alzheimer's disease. In this context, Aβ residues represent a common seed element for the nucleation of fibrillar structure, which leads to amyloid fibrils.

Researchers have shown various effects of osmolytes on the aggregation pathway of Aβ. For example, Trehalose was found to be a potential osmolyte, which reduces the Aβ-cytotoxicity by inhibiting its aggregate formation [90]. Another report demonstrated that sugar (sucrose) was able to slow down the growth of Aβ fibril. This osmolyte was found to block the racemization reaction of D-aspartic acid [111], which is the main contributor to the formation of deposits of Aβ [112]. Cyclohexanehexol (inositol), a naturally-occurring cyclic polyol compound belonging to myo-inositol family of osmolyte, is found in foods such as nuts, beans, and fruits. McLaurin and coworkers [113, 114] had very clearly demonstrated that stereoisomers of cyclohexanehexol, for instance, myocyclohexanehexol, scyllo-cyclohexanehexol, and epi-cyclohexanehexol, are not only responsible for preventing the assembly of fibrils of Aβ, but also they cause the disassembly of fibrils that had been formed in earlier stage. They have also reduced the toxicity, which is caused by the formation of oligomeric structure in primary cultures of neuronal cells. It was also observed that out of three cyclohexanehexol stereoisomers, myo-cyclohexanehexol was the most abundant stereoisomers found in the brain, but the other two stereoisomeric forms, i.e., epi-cyclohexanehexol and scyllo-cyclohexanehexol, were found to be more effective in reducing the fibrillation of Aβ and its associated toxic effects [114]. When the epi-and scyllo-cyclohexanehexol stereoisomers were fed to the mouse models for TgCRND8, it was observed that it prevented the accumulation of Aβ oligomers (soluble and insoluble aggregates) in a dose-dependent way and subsequently, it reduced the toxicity associated with its deposition [115]. This caused a decrease in the symptoms and mortality associated with AD in these mice [115]. Administration of scyllo-cyclohexanehexol also caused reduction in the number of amyloid plaques in mice having an advanced stage of AD [116]. Another compound that is slightly different from the inositol is the inosose stereoisomer, which has a keto-group instead of hydroxyl group. These inososes are able to prevent Aβ fibrillation [117]. These osmolytes are found to modify the folding pathway of Aβ [118], and secondly, they may also compete for binding sites on Aβ. The stereoisomers of inosose and inositol act as competitors for the phosphatidylinositol binding sites on Aβ [115]. These lipids (phosphatidylinositol) are known to induce the process of oligomerization and fibril formation of Aβ [113, 117, 119, 120]. Additionally, osmolytes may cause upregulation of HSPs, chaperone expression [121], and that will cause increments in the efficiency of folding of proteins under conditions of stress [122, 123]. Therefore, we can say that these compounds (cyclohexanehexols) are potential candidates for the treatment of AD-like pathological events in mice [124].

A study tried to investigate the role of osmolytes in the amyloid-associated aggregation model based on insulin (human) hormone protein. They found that

sorbitol, TMAO, and glycerol cause reduction in the rate of fibril formation by slowing down the process of unfolding of monomers. These experimental results showed a good correlation with volume exclusion principle applicable to polymer crowding. This voted for the need of rearrangement (conformational) of monomers prior to nucleation. This group used fluorescence correlation spectroscopy and showed that the aggregation of amyloid is not limited by diffusion, except under conditions where elevated levels of long-chain polymers of fibril are present. They demonstrated that the osmolytes, which had neutral charge at physiological conditions, affected the *tlag* (i.e., the time required for the initiation of fibril formation) in a surface area (of osmolyte)-dependent fashion. This phenomenon is a result of the preferential interaction phenomenon of osmolytes [125].

6. Effects of osmolytes on α-synuclein

Human α-synuclein consists of 140 amino acid residues [126]. High concentrations of this protein are found in brain, particularly, in the terminals of presynapse. This protein is expressed in thalamus, hippocampus, cerebellum, and substania nigra regions of brain [127]. This protein has three well-differentiated portions: the N-terminal domain, which is amphipathic in nature (residues 1–60), the nonamyloid-β component (NAC), which is hydrophobic in nature that constitutes the central region (residues 61–95), and C-terminal domain, which is acidic in nature (residues 96–140). The proteins belonging to the synuclein class do not have an ordered three-dimensional conformation; therefore, they are known as intrinsically disordered proteins (IDP) [128]. IDPs have a very random and unfolded structure, which induces the formation of protein aggregates, subsequently causing various human diseases [129]. This protein comprises a very significant part of Lewy's bodies and lead to the development of PD and other pathologies that result from fibrillation of proteins [130]. Numerous reports have shown that when α-synuclein (monomer) undergoes a conformational change, which causes the beginning of aggregation in the brains affected with PD. Since, IDPs are very dynamic with regard to conformation as they attained, the design of drugs to inhibit their aggregation becomes difficult. Now, researcher groups are using REMD computational technique to investigate the folding and misfolding of different IDPs. The protein α- synuclein in its monomeric form has the features of typical IDPs. The main role of α-synuclein inside the cell is to act as a chaperone to assist in forming larger proteins [131], trafficking of vesicle, and neurotransmitters secretion [128]. In the adverse condition, α-synuclein undergoes changes in its conformation, which results in the beginning of the fibrillation process and this cause for the advent of PD [132, 133]. Therefore, it becomes very important to get a basic knowledge of mechanism of unfolding monomer of α-synuclein in order to prevent its fibrillation process.

Since it is very clearly reported in literature that urea denatures protein even at low concentrations, this effect of urea can be counteracted by many of the counteracting osmolytes [128, 134]. Based on this fact, Jamal and coworkers [128] performed REMD simulations with the peptide of α-synuclein in the presence of urea and TMAO. Interestingly, they found that urea present with the peptide existed in an expanded conformation. But in the presence of TMAO, the peptide assumed a more compact conformation. They performed REMED on the TGVTAVA segment, of α-synuclein, in the presence of water ($Synuclein_{water}$), urea ($Synuclein_{urea+water}$), and TMAO ($Synuclein_{TMAO+water}$). They studied the different conformations attained by the segment, TGVTAVA, in the presence of water, urea, and TMAO. They obtained 18 replicas of the

protein segment (TGVTAVA) from the REMED analysis performed in temperature range of 300–350 K. When they calculated the root-mean-square deviation (RMSD) for all the three systems, they obtained variation in the values with each system, i.e., $Synuclein_{water}$, $Synuclein_{urea+water}$, and $Synuclein_{TMAO+water}$. These variations indicated that peptide assumed multiple conformations, under different conditions [128]. In this study, they demonstrated that 2 M TMAO and 5 M urea had significant different impact on the conformation adopted by protein in their presence. Urea promoted the formation of extended structures for $Synuclein_{urea+water}$ peptide, whereas, TMAO favored the formation of compact and folded forms of the $Synuclein_{TMAO+water,}$ peptide. The population density plot created as a function of average end-to-end distance (R_{ee}) and radius of gyration (R_g) for all the three systems ($Synuclein_{water}$, $Synuclein_{urea+water}$, and $Synuclein_{TMAO+water}$) also demonstrated that extended structures were more populated in the presence of urea. On the contrary, only few extended conformations of peptide were observed in the presence of TMAO [128].

Uversky and coworkers [135] studied the effect of TMAO on the α-synuclein that was unfolded. They found that TMAO induced the α-synuclein to fold back to compact conformation and suggested a biphasic mechanism of α-synuclein [135]. Another group used an all-atom molecular dynamic simulations and essential dynamics approach to study the dynamics of folding of unfolded α-synuclein present in water [136]. They used a monomer, dimer, and a tetramer of α-synuclein forms in their study. They found that the α-synuclein adopted globular conformation, which consisted of random coils in addition to β-bridges for the monomers used in the study. When dimers were used, it comprised mainly of stable β-sheets to attain stable conformation. But in case of the tetramers, it had less number of β-sheets. Another group used REMD approach to investigate the oligomer formation by α-synuclein peptide (residues 71–82), trimers, and tetramers. They found that with increase in size of the peptide, i.e., from dimer to tetramer, the conformation was stabilized [137]. It was found that in the system consisting of synuclein with urea and water, the number of hydrogen bonds that were formed with the external surface of peptide was decreased.

Ferron and coworkers [138] studied the counteraction of urea-induced denaturation of α-synuclein by TMAO with the help of single-molecule Förster resonance energy transfer (FRET) technique. They found that TMAO indeed had the ability to induce the shifting of the expanded α-synuclein toward a more compact form in the presence of urea [138]. Interestingly, their study clearly demonstrated that 2:1 molar ratio of urea and TMAO had negligible effect on the dimensions of the protein. This study explains that a simple interplay of interactions exists between urea and TMAO with the protein [138]. Through this report, the group demonstrated that α-synuclein tends to adopt structures (expanded or compact) in the presence of osmolytes (denaturing or protecting). But in doing so, a very clear cooperative transition in structure was not observed [138]. This study has also suggested that osmolytes protect the protein against the deleterious effects of urea in specific ratios. These osmolytes in this case are able to protect the function and structure of proteins by accommodating the changes in the ratios of osmolytes to urea, and this does not require any kind of regulation on the part of the molar concentrations of these solutes present in the cell [138]. Again, the results in the study demonstrated that the counteraction phenomenon holds true even for the protein, which have not evolved in the urea-osmolyte systems [138]. The hypothesis of "superposition of ensembles" [139], which states that when osmolytes are added to a system, they do not induce the formation of a new state,

but they induce the changes in the population of protein conformations present in a particular state. For example, TMAO has been found to promote the formation of tau protein's ordered structures by merely shifting the populations of existing monomer conformations [139].

7. Conclusion and perspective

It is now clear that osmolytes would be of immense application in large number of human diseases. It is now important to look for the strategies for tissue-specific delivery of osmolytes. Since osmolytes are accumulated under different disease conditions, identifying specific osmolytes with respect to upregulation or downregulation in the cell under particular diseases will be useful for the selective use of osmolytes against a disease. This will benefit for the use of osmolytes as a strategy for the diagnostic purposes. Large volume of the work carried out to investigate the effect of individual osmolyte on the aggregation behavior or misfolding of proteins is still confined to case-by-case analysis. Understanding the effect of one osmolyte against the different properties of protein aggregation, misfolding, or folding pathway will yield several additional insights for the therapeutic intervention of conformational diseases. Since accumulating a particular osmolyte may become toxic to cells [48], using mixtures of different osmolytes in specific molar ratios may help to increase the efficacy and/or reduce toxicity of accumulated osmolytes. In this respect, Poddar et al. [140] showed that the sum effect of individual sugars is always more effective or stabilizing on the protein than its higher respective sugars. Thus, using different specific mixtures of osmolytes against a particular amyloid aggregates such as α-synuclein may provide a good strategy for therapeutic treatment of neurological disease.

Large number of literatures showed the mechanism of the effect of specific osmolytes on different fibrillar/aggregated states, native, and denatured state of proteins through different experimental approaches directly or indirectly or through a combination of the two methods. Thus, the detailed information on the mechanism of action of each osmolytes with the protein at different stages from nucleation to fibrillation under such condition and this information would help in making a rational drug as pharmaceutical chaperones for the prevention and cure of diseases. In this respect, recently, Pradhan and coworkers designed nanoparticle forms of sugar/amino-based osmolytes that not only inhibit the lysozyme aggregation in vitro but also inhibit the mutant huntingtin protein aggregation in vivo, and thus, the chaperoning activity of these nanoparticle-conjugated osmolytes against protein aggregation increased a 1000 order of magnitude [141].

Thus, extensive efforts are still needed in the direction of mechanism of osmolytes, their unusual properties against each protein, new methods for investigating a correlation between aggregation of protein with other metabolic function in the cells, and designing new conjugated or drugs based on osmolytes which show better chaperoning activity for prevention of aggregation-associated diseases.

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Neurodegenerative diseases are debilitating conditions that result in degeneration and death of nerve cells. A significant group of these diseases is the synucleinopathies, which are characterized by the accumulation of aggregates of alpha-synuclein in neurons, nerve fibers, or glial cells. There are three main types of synucleinopathies: Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. Synucleins are small, naturally unfolded proteins prone to aggregate and form intracellular inclusions, which impair normal cellular functions. This book presents new data on synuclein aggregation and its effect on cholesterol transport. It also discusses the role of genetic contribution to these diseases and approaches to inhibition of synuclein aggregation.

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