We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Role of Osmolytes in Amyloidosis

Sheeza Khan, Zeba Mueed, Ravi Deval, Pankaj Kumar Rai, Dinesh Kumar Prajapati and Nitesh Kumar Poddar

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.

Acknowledgements

SK is grateful to Department of Science and Technology for financial support through DST WOS-A scheme.

Author details

Sheeza Khan[1], Zeba Mueed[2], Ravi Deval[2], Pankaj Kumar Rai[2], Dinesh Kumar Prajapati[2] and Nitesh Kumar Poddar[3]*

1 School of Life Science, B.S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, India

2 Invertis University, Bareilly, Uttar Pradesh, India

3 Department of Bioscience, Manipal University, Jaipur, Rajasthan, India

*Address all correspondence to: niteshkumar.poddar@jaipur.manipal.edu

References

[1] Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN. Living with water stress: Evolution of osmolyte systems. Science. 1982;**217**:1214-1222

[2] Yancey PH. Organic osmotic effectors in cartilaginous fishes. In: Gilles R, Gilles-Baillien M, editors. Transport Processes, Iono- and Osmoregulation. Berlin, Heidelberg, NY: Springer-Verlag; 1985. pp. 424-436

[3] Yancey PH. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. The Journal of Experimental Biology. 2005;**208**:2819-2830

[4] Pollard A, Wyn Jones RG. Enzyme activities in concentrated solutions of glycinebetaine and other solutes. Planta. 1979;**144**:291-298

[5] Bowlus RD, Somero GN. Solute compatibility with enzyme function and structure: Rationales for the selection of osmotic agents and end-products of anaerobic metabolism in marine invertibrates. The Journal of Experimental Zoology. 1979;**208**:137-151

[6] Borowitzka LJ, Brown AD. The salt relations of marine and halophilic species of the unicellular green alga, Dunaliella. The role of glycerol as a compatible solute. Archives of Microbiology. 1974;**96**:37-52

[7] Wang A, Bolen DW. Effect of proline on lactate dehydrogenase activity: Testing the generality and scope of the compatibility paradigm. Biophysical Journal. 1996;**71**:2117-2122

[8] Wang A, Robertson AD, Bolen DW. Effects of a naturally occurring compatible osmolyte on the internal dynamics of ribonuclease A. Biochemistry. 1995;**34**:15096-15104

[9] Lin TY, Timasheff SN. Why do some organisms use a urea-methylamine mixture as osmolyte? Thermodynamic compensation of urea and trimethylamine N-oxide interactions with protein. Biochemistry. 1994;**33**:12695-12701

[10] Yancey PH, Somero GN. Methylamine osmoregulatory compounds in elasmobranch fishes reverse urea inhibition of enzymes. The Journal of Experimental Zoology. 1980;**212**:205-213

[11] Somero GN. Protein adaptation and biogeography: Threshold effects on molecular evolution. Trends in Ecology & Evolution. 1986;**1**:124-127

[12] Baskakov I, Bolen DW. Forcing thermodynamically unfolded proteins to fold. The Journal of Biological Chemistry. 1998;**273**:4831-4834

[13] Baskakov I, Wang A, Bolen DW. Trimethylamine-N-oxide counteracts urea effects on rabbit muscle lactate dehydrogenase function: A test of the counteraction hypothesis. Biophysical Journal. 1998;**74**:2666-2673

[14] Wang A, Bolen DW. A naturally occurring protective system in urea-rich cells: Mechanism of osmolyte protection of proteins against urea denaturation. Biochemistry. 1997;**36**:9101-9108

[15] Bagnasco S, Balaban R, Fales HM, Yang YM, Burg M. Predominant osmotically active organic solutes in rat and rabbit renal medullas. The Journal of Biological Chemistry. 1986;**261**:5872-5877

[16] Garcia-Perez A, Burg MB. Importance of organic osmolytes for osmoregulation by renal medullary cells. Hypertension. 1990;**16**:595-602

[17] Nakanishi T, Uyama O, Nakahama H, Takamitsu Y, Sugita M. Determinants

of relative amounts of medullary organic osmolytes: Effects of NaCl and urea differ. The American Journal of Physiology. 1993;**264**:F472-F479

[18] Yancey PH. Organic osmotic effectors in cartilaginous fishes, in Gilles R, Gilles-Baillien M (eds): Transport Processes, Iono- and Osmoregulation. Berlin/Heidelberg, NY: Springer-Verlag; 1985, pp 424-436

[19] Anjum F, Rishi V, Ahmad F. Compatibility of osmolytes with Gibbs energy of stabilization of proteins. Biochimica et Biophysica Acta. 2000;**1476**:75-84

[20] Foord RL, Leatherbarrow RJ. Effect of osmolytes on the exchange rates of backbone amide protons in proteins. Biochemistry. 1998;**37**:2969-2978

[21] Kim YS, Jones LS, Dong A, Kendrick BS, Chang BS, Manning MC, et al. Effects of sucrose on conformational equilibria and fluctuations within the native-state ensemble of proteins. Protein Science. 2003;**12**:1252-1261

[22] Myers JS, Jakoby WB. Glycerol as an agent eliciting small conformational changes in alcohol dehydrogenase. The Journal of Biological Chemistry. 1975;**250**:3785-3789

[23] Santoro MM, Liu Y, Khan SM, Hou LX, Bolen DW. Increased thermal stability of proteins in the presence of naturally occurring osmolytes. Biochemistry. 1992;**31**:5278-5283

[24] Taneja S, Ahmad F. Increased thermal stability of proteins in the presence of amino acids. The Biochemical Journal. 1994;**303**(Pt 1):147-153

[25] Xie G, Timasheff SN. Mechanism of the stabilization of ribonuclease A by sorbitol: Preferential hydration is greater for the denatured then for the native protein. Protein Science. 1997;**6**:211-221

[26] Xie G, Timasheff SN. Temperature dependence of the preferential interactions of ribonuclease A in aqueous co-solvent systems: Thermodynamic analysis. Protein Science. 1997;**6**:222-232

[27] Xie G, Timasheff SN. The thermodynamic mechanism of protein stabilization by trehalose. Biophysical Chemistry. 1997;**64**:25-43

[28] Leandro P, Gomes CM. Protein misfolding in conformational disorders: Rescue of folding defects and chemical chaperoning. Mini Reviews in Medicinal Chemistry. 2008;**8**:901-911

[29] Leandro P, Lechner MC, Tavares de Almeida I, Konecki D. Glycerol increases the yield and activity of human phenylalanine hydroxylase mutant enzymes produced in a prokaryotic expression system. Molecular Genetics and Metabolism. 2001;**73**:173-178

[30] Singh LR, Ali Dar T, Haque I, Anjum F, Moosavi-Movahedi AA, Ahmad F. Testing the paradigm that the denaturing effect of urea on protein stability is offset by methylamines at the physiological concentration ratio of 2:1 (urea:methylamines). Biochimica et Biophysica Acta. 2007;**1774**:1555-1562

[31] Street TO, Bolen DW, Rose GD. A molecular mechanism for osmolyte-induced protein stability. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:13997-14002

[32] Russo AT, Rosgen J, Bolen DW. Osmolyte effects on kinetics of FKBP12 C22A folding coupled with prolyl isomerization. Journal of Molecular Biology. 2003;**330**:851-866

[33] Lang F. Mechanisms and significance of cell volume regulation. Journal of the American College of Nutrition. 2007;**26**:613S-623S

[34] Steeves CL, Hammer MA, Walker GB, Rae D, Stewart NA, Baltz JM. The glycine neurotransmitter transporter GLYT1 is an organic osmolyte transporter regulating cell volume in cleavage-stage embryos. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**:13982-13987

[35] Roth J, Yam GH, Fan J, Hirano K, Gaplovska-Kysela K, Le Fourn V, et al. Protein quality control: The who's who, the where's and therapeutic escapes. Histochemistry and Cell Biology. 2008;**129**:163-177

[36] Brown CR, Hong-Brown LQ , Biwersi J, Verkman AS, Welch WJ. Chemical chaperones correct the mutant phenotype of the delta F508 cystic fibrosis transmembrane conductance regulator protein. Cell Stress & Chaperones. 1996;**1**:117-125

[37] Brown CR, Hong-Brown LQ, Welch WJ. Correcting temperature-sensitive protein folding defects. Journal of Clinical Investigation. 1997;**99**:1432-1444

[38] Edington BV, Whelan SA, Hightower LE. Inhibition of heat shock (stress) protein induction by deuterium oxide and glycerol: Additional support for the abnormal protein hypothesis of induction. Journal of Cellular Physiology. 1989;**139**:219-228

[39] Eleutherio EC, Silva JT, Panek AD. Identification of an integral membrane 80 kDa protein of Saccharomyces cerevisiae induced in response to dehydration. Cell Stress & Chaperones. 1998;**3**:37-43

[40] Singh LR, Chen X, Kozich V, Kruger WD. Chemical chaperone rescue of mutant human cystathionine beta-synthase. Molecular Genetics and Metabolism. 2007;**91**:335-342

[41] Tatzelt J, Prusiner SB, Welch WJ. Chemical chaperones interfere with the formation of scrapie prion protein. EMBO Journal. 1996;**15**:6363-6373

[42] Welch WJ, Brown CR. Influence of molecular and chemical chaperones on protein folding. Cell Stress & Chaperones. 1996;**1**:109-115

[43] Natalello A, Liu J, Ami D, Doglia SM, de Marco A. The osmolyte betaine promotes protein misfolding and disruption of protein aggregates. Proteins. 2009;**75**:509-517

[44] Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE. Biological and chemical approaches to diseases of proteostasis deficiency. Annual Review of Biochemistry. 2009;**78**:959-991

[45] Bajorunaite E, Sereikaite J, Bumelis VA. L-arginine suppresses aggregation of recombinant growth hormones in refolding process from *E. coli* inclusion bodies. The Protein Journal. 2007;**26**:547-555

[46] Chen M, Singer L, Scharf A, von Mikecz A. Nuclear polyglutamine-containing protein aggregates as active proteolytic centers. The Journal of Cell Biology. 2008;**180**:697-704

[47] Das U, Hariprasad G, Ethayathulla AS, Manral P, Das TK, Pasha S, et al. Inhibition of protein aggregation: Supramolecular assemblies of arginine hold the key. PLoS One. 2007;**2**:e1176

[48] Singh LR, Poddar NK, Dar TA, Kumar R, Ahmad F. Protein and DNA destabilization by osmolytes: The other side of the coin. Life Sciences. 2011;**88**:117-125

[49] Blackwell E, Ceman S. Arginine methylation of RNA-binding proteins regulates cell function and differentiation. Molecular Reproduction and Development. 2012;**79**:163-175

[50] Lu SC. S-Adenosylmethionine. The International Journal of Biochemistry & Cell Biology. 2000;**32**:391-395

[51] Anfinsen CB. Principles that govern the folding of protein chains. Science. 1973;**181**:223-230

[52] Ellis RJ, Pinheiro TJ. Medicine: Danger—Misfolding proteins. Nature. 2002;**416**:483-484

[53] Berke SJ, Paulson HL. Protein aggregation and the ubiquitin proteasome pathway: Gaining the UPPer hand on neurodegeneration. Current Opinion in Genetics & Development. 2003;**13**:253-261

[54] Cohen FE, Kelly JW. Therapeutic approaches to protein-misfolding diseases. Nature. 2003;**426**:905-909

[55] Burrows JA, Willis LK, Perlmutter DH. Chemical chaperones mediate increased secretion of mutant alpha 1-antitrypsin (alpha 1-AT) Z: A potential pharmacological strategy for prevention of liver injury and emphysema in alpha 1-AT deficiency. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**:1796-1801

[56] Tamarappoo BK, Yang B, Verkman AS. Misfolding of mutant aquaporin-2 water channels in nephrogenic diabetes insipidus. The Journal of Biological Chemistry. 1999;**274**:34825-34831

[57] Martinez J, Georgoff I, Levine AJ. Cellular localization and cell cycle regulation by a temperature-sensitive p53 protein. Genes & Development. 1991;**5**:151-159

[58] Hawthorne DC, Friis J. Osmotic-remedial mutants. A new classification for nutritional mutants in yeast. Genetics. 1964;**50**:829-839

[59] Higgins CF. ABC transporters: From microorganisms to man. Annual Review of Cell Biology. 1992;**8**:67-113

[60] Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annual Review of Biochemistry. 1993;**62**:385-427

[61] Kuchler K, Thorner J. Functional expression of human mdr1 in the yeast Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**:2302-2306

[62] Figler RA, Omote H, Nakamoto RK, Al-Shawi MK. Use of chemical chaperones in the yeast Saccharomyces cerevisiae to enhance heterologous membrane protein expression: High-yield expression and purification of human P-glycoprotein. Archives of Biochemistry and Biophysics. 2000;**376**:34-46

[63] Fink AL. The aggregation and fibrillation of alpha-synuclein. Accounts of Chemical Research. 2006;**39**:628-634

[64] Wetzel R. Mutations and off-pathway aggregation of proteins. Trends in Biotechnology. 1994;**12**:193-198

[65] Troulinaki K, Tavernarakis N. Neurodegenerative conditions associated with ageing: A molecular interplay? Mechanisms of Ageing and Development. 2003;**126**:23-33

[66] Munishkina LA, Ahmad A, Fink AL, Uversky VN. Guiding protein aggregation with macromolecular crowding. Biochemistry. 2008;**47**:8993-9006

[67] Ben-Zvi AP, Goloubinoff P. Review: Mechanisms of disaggregation and refolding of stable protein aggregates by molecular chaperones. Journal of Structural Biology. 2001;**135**:84-93

[68] Uversky VN, Li J, Fink AL. Evidence for a partially folded intermediate in alpha-synuclein fibril formation.

The Journal of Biological Chemistry. 2001;**276**:10737-10744

[69] Gustafsson M, Thyberg J, Naslund J, Eliasson E, Johansson J. Amyloid fibril formation by pulmonary surfactant protein C. FEBS Letters. 1999;**464**:138-142

[70] Hayden MR, Tyagi SC, Kerklo MM, Nicolls MR. Type 2 diabetes mellitus as a conformational disease. Journal of the Pancreas. 2005;**6**:287-302

[71] Kholova I, Niessen HW. Amyloid in the cardiovascular system: A review. Journal of Clinical Pathology. 2005;**58**:125-133

[72] Kosinski-Collins MS, King J. In vitro unfolding, refolding, and polymerization of human gammaD crystallin, a protein involved in cataract formation. Protein Science. 2003;**12**:480-490

[73] Levy CB, Stumbo AC, Ano Bom AP, Portari EA, Cordeiro Y, Silva JL, et al. Co-localization of mutant p53 and amyloid-like protein aggregates in breast tumors. The International Journal of Biochemistry & Cell Biology. 2011;**43**:60-64

[74] Ross CA, Poirier MA. Protein aggregation and neurodegenerative disease. Nature Medicine. 2004;**10**(Suppl):S10-S17

[75] Taylor JP, Hardy J, Fischbeck KH. Toxic proteins in neurodegenerative disease. Science. 2002;**296**:1991-1995

[76] Williams RA, Mamotte CD, Burnett JR. Phenylketonuria: An inborn error of phenylalanine metabolism. Clinical Biochemist Reviews. 2008;**29**:31-41

[77] Xu J, Reumers J, Couceiro JR, De Smet F, Gallardo R, Rudyak S, et al. Gain of function of mutant p53 by coaggregation with multiple tumor suppressors. Nature Chemical Biology. 2011;**7**:285-295

[78] Dobson CM. Protein misfolding, evolution and disease. Trends in Biochemical Sciences. 1999;**24**:329-332

[79] Selkoe DJ. Folding proteins in fatal ways. Nature. 2003;**426**:900-904

[80] Uversky VN, Fink AL. Conformational constraints for amyloid fibrillation: The importance of being unfolded. Biochimica et Biophysica Acta. 2004;**1698**:131-153

[81] Mittal S, Singh LR. Macromolecular crowding decelerates aggregation of a beta-rich protein, bovine carbonic anhydrase: A case study. Journal of Biochemistry. 2014;**156**:273-282

[82] Ignatova Z, Gierasch LM. Inhibition of protein aggregation in vitro and in vivo by a natural osmoprotectant. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:13357-13361

[83] Walker FO. Huntington's disease. Lancet. 2007;**369**:218-228

[84] Tanaka M, Machida Y, Niu S, Ikeda T, Jana NR, Doi H, et al. Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. Nature Medicine. 2004;**10**:148-154

[85] Samuel D, Kumar TK, Ganesh G, Jayaraman G, Yang PW, Chang MM, et al. Proline inhibits aggregation during protein refolding. Protein Science. 2000;**9**:344-352

[86] Park YD, Wu BN, Tian WX, Zhou HM. Effects of osmolytes on unfolding of chicken liver fatty acid synthase. Biochemistry (Mosc). 2002;**67**:914-917

[87] Meng F, Park Y, Zhou H. Role of proline, glycerol, and heparin as

protein folding aids during refolding of rabbit muscle creatine kinase. The International Journal of Biochemistry & Cell Biology. 2001;**33**:701-709

[88] Ratnaparkhi GS, Varadarajan R. Osmolytes stabilize ribonuclease S by stabilizing its fragments S protein and S peptide to compact folding-competent states. The Journal of Biological Chemistry. 2001;**276**:28789-28798

[89] Macchi F, Eisenkolb M, Kiefer H, Otzen DE. The effect of osmolytes on protein fibrillation. International Journal of Molecular Sciences. 2012;**13**:3801-3819

[90] Liu R, Barkhordarian H, Emadi S, Park CB, Sierks MR. Trehalose differentially inhibits aggregation and neurotoxicity of beta-amyloid 40 and 42. Neurobiology of Disease. 2005;**20**:74-81

[91] Singer MA, Lindquist S. Multiple effects of trehalose on protein folding in vitro and in vivo. Molecular Cell. 1998;**1**:639-648

[92] Davies JE, Sarkar S, Rubinsztein DC. Trehalose reduces aggregate formation and delays pathology in a transgenic mouse model of oculopharyngeal muscular dystrophy. Human Molecular Genetics. 2006;**15**:23-31

[93] Dobson CM. Principles of protein folding, misfolding and aggregation. Seminars in Cell & Developmental Biology. 2004;**15**:3-16

[94] Rousseau F, Schymkowitz J, Serrano L. Protein aggregation and amyloidosis: Confusion of the kinds? Current Opinion in Structural Biology. 2006;**16**:118-126

[95] Dobson CM. Protein folding and disease: A view from the first horizon symposium. Nature Reviews. Drug Discovery. 2003;**2**:154-160

[96] Kelly JW. The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. Current Opinion in Structural Biology. 1998;**8**:101-106

[97] Librizzi F, Rischel C. The kinetic behavior of insulin fibrillation is determined by heterogeneous nucleation pathways. Protein Science. 2005;**14**:3129-3134

[98] Ahmad FB, Williams PA. Effect of sugars on the thermal and rheological properties of sago starch. Biopolymers. 1999;**50**:401-412

[99] Arakawa T, Timasheff SN. Stabilization of protein structure by sugars. Biochemistry. 1982;**21**:6536-6544

[100] Arakawa T, Timasheff SN. Preferential interactions of proteins with salts in concentrated solutions. Biochemistry. 1982;**21**:6545-6552

[101] Dong XY, Huang Y, Sun Y. Refolding kinetics of denatured-reduced lysozyme in the presence of folding aids. Journal of Biotechnology. 2004;**114**:135-142

[102] Kar K, Kishore N. Enhancement of thermal stability and inhibition of protein aggregation by osmolytic effect of hydroxyproline. Biopolymers. 2007;**87**:339-351

[103] Mishra R, Seckler R, Bhat R. Efficient refolding of aggregation-prone citrate synthase by polyol osmolytes: How well are protein folding and stability aspects coupled? The Journal of Biological Chemistry. 2005;**280**:15553-15560

[104] Yang DS, Yip CM, Huang TH, Chakrabartty A, Fraser PE. Manipulating the amyloid-beta aggregation pathway with chemical chaperones. The Journal of Biological Chemistry. 1999;**274**:32970-32974

[105] Yoshimoto N, Hashimoto T, Felix MM, Umakoshi H, Kuboi R. Artificial chaperone-assisted refolding of bovine carbonic anhydrase using molecular assemblies of stimuli-responsive polymers. Biomacromolecules. 2003;**4**:1530-1538

[106] Tanaka M, Machida Y, Nukina N. A novel therapeutic strategy for polyglutamine diseases by stabilizing aggregation-prone proteins with small molecules. Journal of Molecular Medicine (Berlin). 2005;**83**:343-352

[107] Dobson CM, Evans PA, Radford SE. Understanding how proteins fold: The lysozyme story so far. Trends in Biochemical Sciences. 1994;**19**:31-37

[108] Choudhary S, Kishore N. Addressing mechanism of fibrillization/aggregation and its prevention in presence of osmolytes: Spectroscopic and calorimetric approach. PLoS One. 2014;**9**:e104600

[109] Needham LM, Weber J, Fyfe JWB, Kabia OM, Do DT, Klimont E, et al. Bifunctional fluorescent probes for detection of amyloid aggregates and reactive oxygen species. Royal Society Open Science. 2018;**5**:171399

[110] Muttthukattil AN, Reddy G. Osmolyte effects on the growth of amyloid fibrils. The Journal of Physical Chemistry. B. 2016;**120**:10979-10989

[111] Ueda T, Nagata M, Monji A, Yoshida I, Tashiro N, Imoto T. Effect of sucrose on formation of the beta-amyloid fibrils and D-aspartic acids in Abeta 1-42. Biological & Pharmaceutical Bulletin. 2002;**25**:375-378

[112] Tomiyama T, Asano S, Furiya Y, Shirasawa T, Endo N, Mori H. Racemization of Asp23 residue affects the aggregation properties of Alzheimer amyloid beta protein analogues. The Journal of Biological Chemistry. 1994;**269**:10205-10208

[113] McLaurin J, Franklin T, Chakrabartty A, Fraser PE. Phosphatidylinositol and inositol involvement in Alzheimer amyloid-beta fibril growth and arrest. Journal of Molecular Biology. 1998;**278**:183-194

[114] McLaurin J, Golomb R, Jurewicz A, Antel JP, Fraser PE. Inositol stereoisomers stabilize an oligomeric aggregate of Alzheimer amyloid beta peptide and inhibit abeta-induced toxicity. The Journal of Biological Chemistry. 2000;**275**:18495-18502

[115] McLaurin J, Kierstead ME, Brown ME, Hawkes CA, Lambermon MH, Phinney AL, et al. Cyclohexanehexol inhibitors of Abeta aggregation prevent and reverse Alzheimer phenotype in a mouse model. Nature Medicine. 2006;**12**:801-808

[116] Fenili D, Brown M, Rappaport R, McLaurin J. Properties of scyllo-inositol as a therapeutic treatment of AD-like pathology. Journal of Molecular Medicine (Berlin). 2007;**85**:603-611

[117] McLaurin J, Chakrabartty A. Membrane disruption by Alzheimer beta-amyloid peptides mediated through specific binding to either phospholipids or gangliosides. Implications for neurotoxicity. Journal of Biological Chemistry. 1996;**271**:26482-26489

[118] Auton M, Bolen DW. Predicting the energetics of osmolyte-induced protein folding/unfolding. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:15065-15068

[119] Koppaka V, Axelsen PH. Accelerated accumulation of amyloid beta proteins on oxidatively damaged lipid membranes. Biochemistry. 2000;**39**:10011-10016

[120] McLaurin J, Chakrabartty A. Characterization of the interactions

of Alzheimer beta-amyloid peptides with phospholipid membranes. European Journal of Biochemistry. 1997;**245**:355-363

[121] Barth S, Huhn M, Matthey B, Klimka A, Galinski EA, Engert A. Compatible-solute-supported periplasmic expression of functional recombinant proteins under stress conditions. Applied and Environmental Microbiology. 2000;**66**:1572-1579

[122] Kregel KC. Heat shock proteins: Modifying factors in physiological stress responses and acquired thermotolerance. Journal of Applied Physiology (Bethesda, MD: 1985). 2002;**92**:2177-2186

[123] Sun Y, MacRae TH. The small heat shock proteins and their role in human disease. The FEBS Journal. 2005;**272**:2613-2627

[124] Yamin G, Ono K, Inayathullah M, Teplow DB. Amyloid beta-protein assembly as a therapeutic target of Alzheimer's disease. Current Pharmaceutical Design. 2008;**14**:3231-3246

[125] Murray B, Rosenthal J, Zheng Z, Isaacson D, Zhu Y, Belfort G. Cosolute effects on amyloid aggregation in a nondiffusion limited regime: Intrinsic osmolyte properties and the volume exclusion principle. Langmuir. 2015;**31**:4246-4254

[126] Surguchov A. Molecular and cellular biology of synucleins. International Review of Cell and Molecular Biology. 2008;**270**:225-317

[127] Gallea JI, Celej MS. Structural insights into amyloid oligomers of the Parkinson disease-related protein alpha-synuclein. The Journal of Biological Chemistry. 2014;**289**:26733-26742

[128] Jamal S, Kumari A, Singh A, Goyal S, Grover A. Conformational ensembles of α-synuclein derived peptide with different osmolytes from temperature replica exchange sampling. Frontiers in Neuroscience. 2017;**11**:1

[129] Gregersen N, Bolund L, Bross P. Protein misfolding, aggregation, and degradation in disease. Molecular Biotechnology. 2005;**31**:141-150

[130] Emamzadeh FN, Surguchov A. Parkinson's disease: Biomarkers, treatment, and risk factors. Frontiers in Neuroscience. 2018;**12**:612

[131] Burre J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Sudhof TC. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science. 2010;**329**:1663-1667

[132] Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. Annual Review of Neuroscience. 2005;**28**:57-87

[133] Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997;**388**:839-840

[134] Yancey PH, Somero GN. Counteraction of urea destabilization of protein structure by methylamine osmoregulatory compounds of elasmobranch fishes. The Biochemical Journal. 1979;**183**:317-323

[135] Uversky VN, Li J, Fink AL. Trimethylamine-N-oxide-induced folding of alpha-synuclein. FEBS Letters. 2001;**509**:31-35

[136] Mane JY, Stepanova M. Understanding the dynamics of monomeric, dimeric, and tetrameric alpha-synuclein structures in water. FEBS Open Bio. 2016;**6**:666-686

[137] Park SB, Yoon J, Jang S, Lee K, Shin S. Computational study on oligomer formation of fibril-forming peptide of a-synuclein. Bulletin of the Korean Chemical Society. 2012;**33**:848-858

[138] Ferreon AC, Moosa MM, Gambin Y, Deniz AA. Counteracting chemical chaperone effects on the single-molecule alpha-synuclein structural landscape. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:17826-17831

[139] Levine ZA, Larini L, LaPointe NE, Feinstein SC, Shea JE. Regulation and aggregation of intrinsically disordered peptides. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**:2758-2763

[140] Poddar NK, Ansari ZA, Singh RK, Moosavi-Movahedi AA, Ahmad F. Effect of monomeric and oligomeric sugar osmolytes on DeltaGD, the Gibbs energy of stabilization of the protein at different pH values: is the sum effect of monosaccharide individually additive in a mixture? Biophysical Chemistry. 2008;**138**:120-129

[141] Pradhan N, Jana NR, Jana NR. Inhibition of protein aggregation by iron oxide nanoparticles conjugated with glutamine- and proline-based Osmolytes. ACS Applied Nano Materials. 2018;**1**:1094-1103