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Biological Wonders of Osmolytes: The Need to Know More

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Abstract

Nature has selected osmolytes to protect intracellular macromolecules against denaturing stress conditions. These molecules are accumulated in the intracellular environment at considerably high concentrations. In general, osmolytes are known to stabilize proteins. However, under certain conditions, their destabilizing properties have also been pointed out. A careful qualitative and quantitative understanding of the mechanism of action of osmolytes with proteins from native to different stages of aggregation/fibrillation is extremely important in rational drug design. This review highlights the importance of naturally occurring osmolytes in protein folding, stabilization, and prevention of fibrillation/aggregation related diseases among others. Continued efforts are required to get quantitative insights into osmolyte-protein interactions along with experimental evidences for the much claimed preferential exclusion/preferential hydration phenomenon of osmolyte action. Mechanistic insights into the disease associated roles of osmolytes needs special attention.

Keywords: Osmolytes; Protein stability; Fibrillation/aggregation; Preferential hydration; Rational drug design

Introduction

Osmolytes have been fascinating to biochemists as these molecules are small solutes which manage cell volume regulation under water stress conditions. Such conditions arise due to extremes of temperature, pressure, alterations in extracellular osmotic conditions and even urea which is a protein denaturing osmolyte [1-4]. There are three major classes of organic compounds which are considered as cellular osmolytes (Figure 1) [2]. These can be further categorized into (i) osmolytes that stabilize proteins raising free energy of both the native and denatured states: trimethylamine N-oxide (TMAO), sarcosine, sorbitol, sucrose, and trehalose, (ii) osmolytes that only moderately change protein stability: glycine betaine, proline, and glycerol, (iii) denaturing osmolytes: urea, and (iv) counteracting osmolytes: mixture of urea and TMAO. Modulation of activity of molecular chaperons (heat shock proteins) due to promotion of local refolding in protein molecules has also been observed [5] which points out to linkage of chemical chaperons (osmolytes) and molecular chaperones in *in vivo* regulation of protein folding.

Significant efforts have been dedicated in understanding solution thermodynamics of protein-osmolyte mixtures [6-15]. Most of the experimental work which has led to the calculation of preferential interaction parameters is based on density measurements or thermal transition temperature of the protein by using differential scanning calorimetry or spectroscopic methods [16,17]. These experimentally determined preferential interaction parameters have been used to address the mode of action of osmolytes on proteins. Based on the free energy of transfer of protein backbone models from water to aqueous osmolyte solutions, the cause of stabilization/destabilization of the unfolded state has been suggested [6,9,10]. Molecular mechanism for osmolyte induced protein stability has been discussed extensively in literature [11,18], though their mode of action in their different roles is still not completely understood.

This mini review focuses on the mode of action of osmolytes on proteins, disease associate roles, and DNA associated effects. The unanswered questions and need for further experimental evidences on action of osmolytes in their different roles has been discussed.

Known Mode of Action on Proteins

Osmolytes are known to alter the chemical potential of proteins in the native and the unfolded states to different extents [19-22]. The difference arises due to competing protein-water and protein-osmolyte interactions [23]. One of the widely used models to explain osmolyte driven protein folding and stabilisation is based on determining free energies of interaction between osmolytes and building blocks of proteins such as amino acids or peptides [24]. This model is based on the principle that the osmolytes undergo unfavourable interactions with the peptide backbone in the unfolded state of the protein which leads to strengthening of its secondary structure (an intermediate structure in the folding pathway) before attaining the folded conformation [7,25].

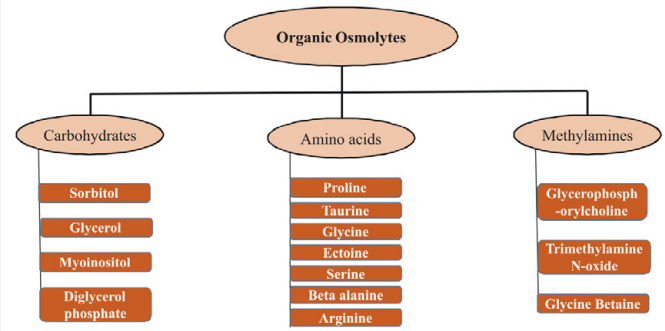

Figure 1: Major classes of organic compounds considered as cellular osmolytes.

Gibbs free energy implications of action of osmolytes have been considered in understanding mechanism of stabilization of proteins. It is believed that preferential exclusion of osmolytes increases the standard Gibbs free energy change accompanying unfolding of the protein. It is suggested that stabilization of proteins by protecting osmolytes is not due to stabilization of the native state, rather it

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arises principally from the destabilization of the unfolded state of the protein by raising its Gibbs free energy than that of the native state [26,27]. Osmolytes shift the equilibrium towards the native conformation of protein by increasing the free energy of the unfolded state. It is hypothesised that the osmolyte sequester the water molecules from the protein surroundings thus creating a hydrophobic environment which causes the protein to fold more compactly, thus driving the equilibrium to functionally active conformation [28,29].

Extensive experimental observations on osmolytes-protein interactions have been explained in terms of preferential hydration/preferential interaction phenomenon by Timasheff, Shellman and other researchers [8,16,30-33]. The ability of organic osmolytes to stabilize and protect intracellular proteins under denaturing environmental stresses has been demonstrated by different experiments [2,34,35]. Osmolytes exert a dramatic influence on protein folding process without making new or breaking existing covalent bonds. Protein stabilization is ability of the osmolytes to push the native (N) = denatured (D) equilibrium towards left. An increase in the thermal unfolding transition temperature up to 22°C has been reported for ribonuclease A in the presence of 8.2 M sarcosine which translates to 45000 fold increase in stability of the native form of the protein over that in the absence of the osmolyte. Similarly, the osmolytes led to an increase in the thermal unfolding temperature of lysozyme up to 23°C [36]. Exceptional increase in the thermal stability of lysozyme up to 26.4°C and myoglobin up to 31.8°C was obtained in the presence of hydroxyproline [17].

The increase in the thermal stability of proteins or refolding of unfolded polypeptides by osmolytes also permits the latter to be classified as "chemical chaperones". Regulation of molecular chaperones (Heat Shock Proteins or HSP's) *in vitro* and in cells by chemical chaperones probably by promoting local refolding within chaperone protein molecules under combined salt and heat stresses is well documented in literature [5]. This suggests a link between the chemical and molecular chaperones in supporting protein folding in cells. [28]

Disease Associated Roles of Osmolytes

Role in immunological processes

Several key immunological processes are regulated by osmolytes. Specific examples include immunoglobulin assembly and folding, immune cell proliferation, immune cell function regulation, inflammatory response and also protection against photo-immunosupression [37]. Therefore osmolytes based therapeutic strategies in the treatment of several immunological disorders needs a special attention.

Role in cancer

Specific inhibitory effect of a 240s plasma exposure in the presence of osmolytes against T98G brain cancer cells has been observed without affecting HEK normal cells [38]. The use of non-thermal plasmas has increased recently in the treatment of living tissues [39-43]. It is observed that sucrose, glycerol and TMAO exhibit inhibitory effect on T98G brain cells only. The importance of work can further be realised if we understand the mechanism of action of osmolytes on these cell lines. For example, it will be important to understand whether the properties of cell lines are changed due to interaction with osmolytes or plasma leads to alteration in the properties of osmolytes.

Role in kidney related diseases

The role of organic osmolytes (such as glycine betaine, myoinositol, sorbitol, and glycerophosphoryl) in human and other mammalian kidneys has also been reported [44]. The concentration of osmolytes was observed to be different in inner medulla and cortex tissue samples [44]. Myoinositol, sorbitol, and glycine betaine have been found to be components of human urine [45,46]. These results strongly point out physiological importance of organic osmolytes in humans and need further investigation in understanding a general mammalian osmoprotectant strategy.

Role in cardiovascular risk factors and urinary excretion

Deficiency of the osmolyte betaine is a possible cardiovascular risk factor [47], and its urinary excretion is increased in diabetes. It is reported that almost 30% of patients affected with diabetes have unusually higher levels of urinary betaine excretion [48]. The correlation of the osmolyte deficiency with fibrate treatment can help in designing proper dietary intake supplements for patients. The contribution of betaine to transmethylation of homocysteine to methionine is reported to be important since BHMT1 pathway is a major route for the elimination of monocysteine which has a role in the development of cardiovascular disease [49]. Thus betaine is an important nutrient in the prevention of chronic disease [50]. Natural osmolyte trimethylamine *N*-Oxide (TMAO) has been shown to correct assembly defects of mutant branched-chain α-ketoacid decarboxylase in maple syrup urine disease [51]. TMAO has also been recently described as risk factor for cardiovascular disease and the mechanism of its accumulation in hemodialysis patients has been discussed [52]. Glycine betaine and proline betaine are present as osmoprotectants in urine. They act as potents for inhibiting growth of bacteria and thus in the treatment of urinary tract infections [46].

Role in lungs and skin related issues

Several antimicrobial substances which kill constantly deposited bacteria in the lungs are present in the thin layer of airway surface liquid. The role of osmolyte xylitol in enhanced killing of such bacteria and hence in prevention of onset of bacterial infection in cystic fibrosis has been hypothesized [53]. It is suggested that delivery of xylitol to airway surface may lead to enhancement of innate bacterial defence system. The effect of glycerol and urea have been explored on permeability of excised skin membranes [54]. It was observed that these two osmolytes (glycerol and urea) penetrate the skin membrane and retain skin permeability characteristics even at low water activity. Being chemical chaperone, the increased uptake of osmolytes by uv-irradiated keratinocytes was correlated with their defence strategy against detrimental effects of such irradiations [55]. The role of taurine in prevention of surfactant induced dry and scaly skin by modulation of proinflammatory response and stimulation of epidermal lipid synthesis has also been reported [56]. *Staphylococcus aureus (S. aureus)* is a major cause of skin and soft tissue infections. Osmolyte transport in *Staphylococcus aureus* and its role in pathogenesis have recently been described [57].

Role in prevention of aggregation/fibrillation of protein

Osmolytes have also found important role in the prevention of fibrillation/aggregation of proteins. They can be utilized as therapeutic targets for diseases mainly related to protein misfolding. Protein fibrillation is responsible for several amyloidogenic disorders including diseases such as Alzheimer's, Parkinson's, mad cow, diabetes type II, cystic fibroisis, and dialysis related amyloidosis [58,59]. Though there have been several studies describing the effects of osmolytes on fibrillation/aggregation of proteins [60-66], quantitative understanding in terms of energetics of interaction has only recently been addressed

[67,68]. Trehalose has been currently used for the treatment of Huntington's disease in transgenic animal mice [69]. Such studies allow identification of functional groups on potential inhibitors of fibrillation/aggregation and hence in deriving guidelines for novel drug synthesis.

Role in intrinsically disordered proteins (IDPs)

Many domains or regions in proteins are intrinsically disordered (ID) under native conditions. Such ID proteins or IDP's are found disproportionately in cell signalling proteins and transcription factors. These regions in signalling proteins tend to promote molecular recognition by binding specific protein partners [70,71]. The effect of osmolytes on IDP's is known to be opposite to that of globular proteins. For instance, several types of osmolytes induce aggregation/fibrillation in intrinsically disordered protein α-synuclein which is associated with Parkinson's disease [72-74]. Other intrinsically disordered proteins which undergo aggregation/fibrillation by some osmolytes include tau protein [75], the prion protein [76], Alzheimer's amyloid β-peptides [77,78] and glucagon hormone peptide [64].

Osmolytes and DNA

The destabilization of DNA by osmolytes has also been observed [79] and role in modulating protein-DNA interactions has been discussed [80]. For example, mitigation of the binding of ERG1 to DNA in a differential manner has been reported. The mechanism proposed involved interaction of osmolytes with the DB domain of ERG1 instead of its conjugate DNA. The negative modulation of ERG1-DNA interaction can have important therapeutic implications. The role of osmolytes in the regulation of biological activity of transcription factors needs to be seriously examined. The effect was observed to be concentration and/or solvent condition dependent. Potential of osmolytes in trapping DNA-protein binding reactions with natural osmolytes has been reported [81]. The reason for this trapping has been assigned to slowing down of the rate of dissociation of the complex of the nucleic acid with the protein.

Even though we understand the effect of osmolytes on the overall conformation of protein, the mechanism of the osmolytic effect has mostly been attributed to preferential hydration or preferential exclusion phenomenon [82]. In general, contrasting theories of direct interaction mechanism [83-86] and indirect mechanism [82,87-90] have been proposed. Synergy in osmolyte mixtures has potential applications in medicinal and agricultural fields. It is important to understand whether the counteraction of chemical denaturing stress by osmolytes is due to synergy between additive molecules or due to direct interaction with the protein. Thus the counteraction mechanism needs extensive experimental and theoretical proof [91]. Experimental proofs for these direct or indirect mechanism are lacking and there is a need to focus more in this direction.

Conclusion and Future Perspectives

The important role of osmolytes not only in the counteraction of stress conditions for proteins, but the disease associated roles require a thorough understanding of the related mode of action. It will be important to know if the known preferential exclusion phenomenon is able to explain all the observed effects or the mode of action changes depending upon the role of the osmolyte. There is a still lot more to be done to understand the effect of osmolytes on protein conformation, fibrillation and many other processes specifically quantitatively. This requires extensive experimental approaches which can help in establishing whether the mechanism of action of osmolytes on proteins in the native, denatured, and fibrillar/aggregated state is direct, indirect, or a combination of the two processes. Establishing nature of interactions with the protein at different stages from nucleation to fibrillation can possibly provide more information on the mechanism of action of osmolytes under such conditions.

The mode of action of osmolytes on DNA has been not been addressed to the extent as it has been done for the proteins. The questions which still need to be completely understood are as to whether the protein models with respect to osmolytes can also be extended to nucleic acids or not. Further experimental investigations on osmolytes-nucleic acids interactions, especially addressing the energetics of interactions, can perhaps throw more light on the commonality shared by proteins and nucleic acids with respect to the mode of action of osmolytes on these biological macromolecules.

A thorough understanding of these mechanisms can lead to development of osmolytes as effective therapeutic molecules and hence rational drug design for the prevention and cure of diseases which result due to protein misfolding/fibrillation/aggregation among other factors. Thus extensive efforts are needed in complete understanding of these biological wonders of osmolytes.

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