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 in 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/distabilization 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 not still 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].

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...
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
hypothesized 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 poisons 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
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 fibrosis, 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
intrinsic disorders are disordered in a more extended conformation [76]. 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 IDPs 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 IDPs 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 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 fibriilar/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.

References


