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Roles of osmolytes in protein folding and aggregation in cells and their biotechnological applications

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ABSTRACT

Nature has selected osmolytes to protect intracellular macromolecules exposed to denaturing conditions and stabilize proteins. Osmolytes are small naturally occurring compounds that act as chemical chaperones under changing environmental conditions and in disease states, and are present in microorganisms, animals, and plants. In the intracellular environment osmolytes naturally accumulate at high concentrations when cells/tissues are exposed to stressful conditions, which is important because protein aggregation, misfolding, and destabilization underlie the pathogenesis of several life-threatening neurodegenerative disorders. The chaperone abilities of osmolytes suggests they may be therapeutically used for the treatment of several diseases associated with protein misfolding, and their abilities to protect proteins against denaturing stresses impinges on the fundamental problem of protein stabilization, which plagues the pharmaceutical industry, biotechnologists, and researchers. We hope that this review will encourage further research in this area and catalyze increased collaboration at the interface of chemistry and biology to decipher the mechanisms and roles of protein folding, misfolding and aggregation in the fields of health and disease.

Contents

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1. Introduction

Nature has developed many strategies to ensure that the complex and challenging protein folding reaction occurs *in vivo* with adequate efficiency and fidelity for the success of the organism. Among the strategies widely employed in a huge range of species and cell types is the elaboration of small organic molecules called osmolytes that offset the potentially damaging effects of osmotic stress. Many organisms have evolved to respond to such conditions by regulating levels of small organic compounds, called osmolytes [1]. Mannitol, glycine betaine, proline, etc. are examples of the many compatible solutes that can accumulate intracellularly without inhibiting enzyme activities [2]. These osmolytes are also known as compatible solutes or non-perturbing solutes owing to their compatibilities with cellular functions at high cytoplasmic concentrations. Osmoadaptation is a defense mechanism against hyperosmotic and hypoosmotic shocks encountered by animals, and involves the accumulations of a wide variety of organic solutes, such as, sugars, polyols, amino acids and their derivatives that protect biomolecules from osmotic vicissitudes [3]. Osmolytes protect and stabilize proteins thermodynamically by regenerating native forms from unfolded states by interacting with protein surfaces. Additionally, the accumulation of heat shock proteins helps organisms with heat resistance by refolding denatured polypeptides and preventing protein aggregations [4,5]. Changes in the cytoplasmic concentrations of osmolytes play a crucial roles in the regulation of translocation processes, such as, trans epithelial transport, cell passage, proliferation, and death [6]. During osmotic up shock, the concentrations of compatible solutes increase within cytoplasm by *de novo* intracellular synthesis or by uptake from surroundings, and these concentrations can increase to >400 mM without interfering with cellular metabolism [7]. Molecular structures of betaine, citrulline, proline and sorbitol are shown in Fig. 1 based on polarity, charge distribution, and their H-bonding abilities for inhibition of protein aggregation.

2. Natural selection of osmolytes

Intracellular accumulations of organic solutes induce osmotic pressures that can affect cell volumes, and these solutes are often referred to as organic osmolytes [8]. Proteins are dynamic entities that constantly interact with their surrounding environments, and several components present in their environments, such as, solvents [9], crowding agents [10], small- and macro-molecular ligands [11–13] and osmolytes [14], influence protein folding [15] and function. By acting as chemical chaperones, osmolytes constitute a defense mechanism against intense physiological stresses, such as, high or low temperatures [16], high osmotic and hydrostatic pressures [1,17], and dehydration [18]. Osmolytes can be categorized into three types based on their protein stabilizing functions (Fig. 2).

Urea is a major metabolic byproduct and is used as an osmolyte by various marine vertebrates [1], some amphibians [16,19], and air-breathing teleosts [20]. In mammalian kidneys urea facilitates the generation of a medullary osmolality gradient, which is sustained even in diuresis, although its magnitude is less than that during antidiuresis [21]. Nevertheless, urea accumulation even at physiologically significant concentrations has a strong denaturing effect on proteins [1,22]. In urea rich biological systems the detrimental activity of urea is impeded by various defensive osmolytes, such as, methylamines and polyhydric alcohols [1,23,24].

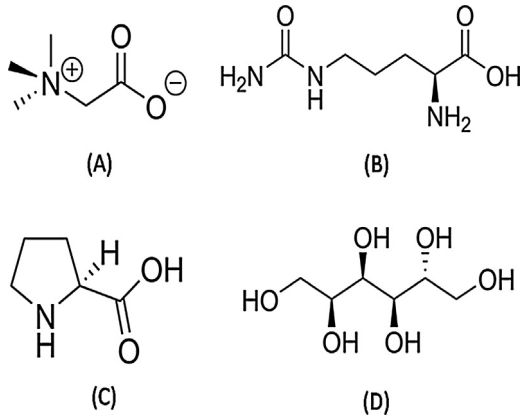

Fig. 1. Chemical structures of osmolytes (A) betaine, (B) citrulline, (c) proline and (d) sorbitol.

Trimethylamine N-oxide (TMAO) is a major defensive and the most effective methylamine found in many marine vertebrates [25]. A counter action hypothesis, proposed by Yancey et al., indicates these protecting osmolytes counteract the denaturing effects of urea [1,25]. The contra-activities of urea and TMAO have been studied in various systems, both *in vivo* and *in vitro* [26]. *In vitro,* TMAO has been used to reverse the effects of denaturants on proteins [27–29] and to fold mutation-destabilized [30] and partially folded or unfolded proteins [31]. It has also been reported recently TMAO can fold mutant proteins *in vivo* and counter the effects of mutations in *Escherichia coli* [32].

3. Osmolytes in natural environment

Organisms use organic osmolytes to protect cellular structures from harsh environmental circumstances like dehydration during hypertonic conditions [1]. Upon exposure to hyper-osmotic conditions cells begin to synthesize defensive organic osmolytes, although it worth noting, not all osmolytes protect cells and cellular proteins. Intracellular accumulations of these osmolytes generate osmotic pressures that affect ideal cell volumes. Parameters that affect the natural selection of osmolytes include osmotic pressure, the accessibilities of substrates and osmolytes in the environment, and differential buffering potential [26]. In marine organisms organic osmolytes accumulate in tissues and extracellular fluids (predominantly in elasmobranchs) to counterbalance the high osmolality of sea water and elevated urea concentrations [1,27]. The dissimilarity between protein denaturants (e.g., urea) and osmolytes (e.g., methylamines) is attributable to their contrary effects on protein backbone configurations [33]. One popularly accepted hypothesis is that when osmolyte concentration reaches twice that of urea, they suppress the denaturing effect of urea [34,35]. Yeast and fungi produce and/or accumulate different polyols from aqueous surroundings [36,37], and in these organisms intracellular glycerol production is increased under hypertonic conditions to reduce cellular stress and water efflux from cell [36,37]. Normally proteins aggregate and form sediments when