Visiting Pressure and Osmolyte's Effect on Protein Solvation

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In aqueous solution, stability, structure, and functions of proteins are known to be altered by changes in environmental conditions such as temperature, pressure, and presence of co-solutes.[1] In particular, it has been known for many years that a high concentration of urea, which is one of the most commonly available osmolytes, can cause protein denaturation in solution[2] and hence can inhibit many important biological processes. In contrast to the relatively extended configuration of urea-denatured proteins, pressure-denatured proteins are found to be relatively compact and retain elements of secondary structure. It is usually believed that water penetration is the primary driving force for protein denaturation under high pressure, and there are evidences that high pressure structure has more penetrating water molecules than low pressure structure.[3]

Organisms also accumulate some other osmolytes that can bias the unfolded structure toward the folded state.[4] Trimethylamine-N-oxide (TMAO) represents the extreme among these osmolytes and has generated considerable research interest to the community of biophysicists and biochemists over the past few years. This compound is particularly known for its ability to stabilize proteins in its native conformation[5] and nucleic acids,[6] correct medicinally significant issues, such as prion aggregation[7] and cellular folding defects,[8] and counteract protein denaturation by urea,[2] heat, and pressure.[9] Although protein denaturing effects of urea and high hydrostatic pressure and TMAO's ability to counteract their deleterious effects are well-established, we are far from a generally accepted mechanism that can account their (de)stabilizing actions. To provide a molecular level picture of pressure- and osmolyte-induced protein (de) stabilization, here we focus on some of our recent molecular dynamics (MD) simulations[10-18] that investigate solvation of protein residues in solution.

Effect of pressure

Significant changes in water structure were observed at high pressure. In particular, high pressure caused crowding of water molecules, destabilized water hydrogen bonding network, and increased population of first shell water molecules that are not engaged in hydrogen bonding interactions with the central water molecule.[10,11] Extreme water crowding at high pressure restricts water movement in the bulk, and for translational relaxation, the relatively "free" water molecules in the water first shell move to the protein surface. The process gives an overall entropic profit (due to higher relaxation in water movement in the bulk) to the system and allows water to form a relatively stable hydrogen bond network. Note that the dominating contribution of translational entropy gain from relaxation of water molecules far away from the protein surface in overall entropic gain in the system upon pressure denaturation was suggested in the literature.[19] Consequent pressure-induced enhancement in number of water molecules near protein residues were also found in simulations.[10-13] The hydration shell of hydrophobic groups was significantly compressed at high pressure[10-13] and correlated with pressure-induced enhanced hydration of non-polar groups was the observation that high pressure dissolves the aggregate of neopentane.[12,13] In contrast to the highly compressed water shell in the vicinity of non-polar groups at high pressure, the water compression was found to be much weaker near the hydrogen bonding sites.[10,11] It was shown that the relative population

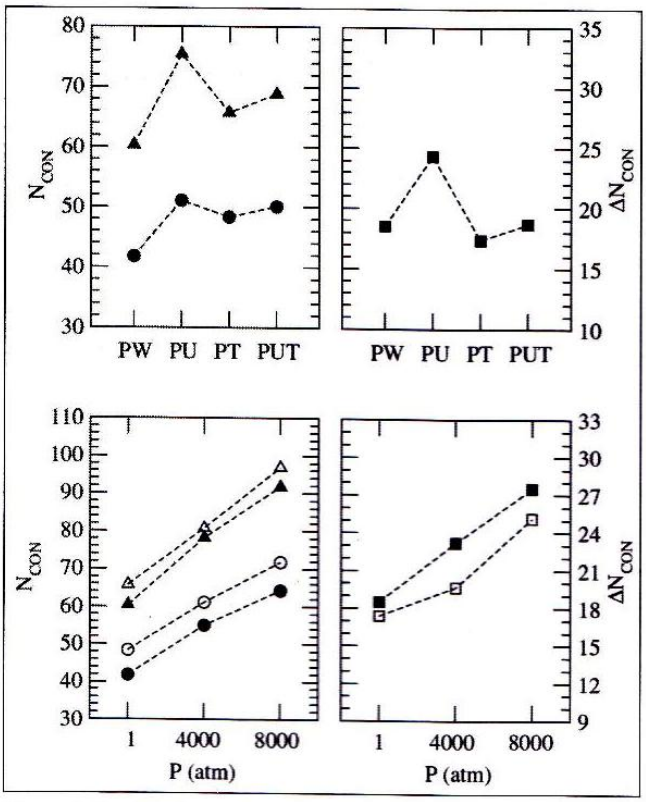

Figure 1. Top: Total number of heavy atom contacts (NCON) between polypeptide and solution species (left) for the helical (circle) and extended (triangle) states and their difference (right) at 1 atm in pure water (PW), binary urea (PU), binary TMAO, and urea/TMAO mixture (PUT). Data from ref. 17. Bottom: NCON (left) for the helical (circle) and extended (triangle) states and their difference (right) in PW (closed) and PT (open) as a function of pressure . Data are obtained from ref. 11.

of water molecules in protein solvation shell that are involved in hydrogen bonding interactions with protein residues reduces at high pressure.[11] Moreover, while pressure-induced enhancement of hydration number was found to be higher for the unfolded state as compared to the folded state (Figure 1), we did not see much difference in protein-water hydrogen bond number enhancement between the folded and unfolded states (Figure 2), making the pressure-induced protein denaturation through enhancement of hydrogen bonds between protein and water an unlikely possibility.[11] Protein denaturation by high pressure is most likely to be associated with enhanced hydration of non-hydrogen bonding sites, which results in a relatively compact structure as compared to thermally-denatured structure.

Effect of urea

Although a slight collapse of water second shell in the presence of urea was observed from computations of water oxygen-oxygen site-site radial distribution function,[14,15] indicating urea's ability to act as a water structure breaker; our analysis of hydrogen bond properties did not provide any evidence for the water structure breaking capacity of urea.[16] Rather, urea was found to slightly increase water-water hydrogen bond energy and lifetime.[16] Water did lose some hydrogen bonds to identical species upon addition of urea, but those hydrogen bonds were replaced by hydrogen bonds between urea and water, and urea appeared to fit well in the water cavities.[16] Therefore, by no means can the water structure breaking property of urea which, in turn, increases protein hydration, be considered to be a primary factor in urea-conferred protein denaturation.

Preferential accumulation of urea near large hydrophobic solute and urea-induced dispersion of neopentane molecules was clear from our simulations.[14] While urea was found to remove large number of water molecules from protein solvation shell with consequent reduction of protein-water hydrogen bonds, total number of heavy atom contacts as well as the hydrogen bond number between protein and solution species increased in the presence of urea (Figures 1 and 2) due to its direct interaction with protein residues.[17] The two solvation sites of backbone oxygen (which were occupied by water alone in pure water) were shared by water and urea in binary urea solution.[16] Of significant importance in the context of urea-induced protein

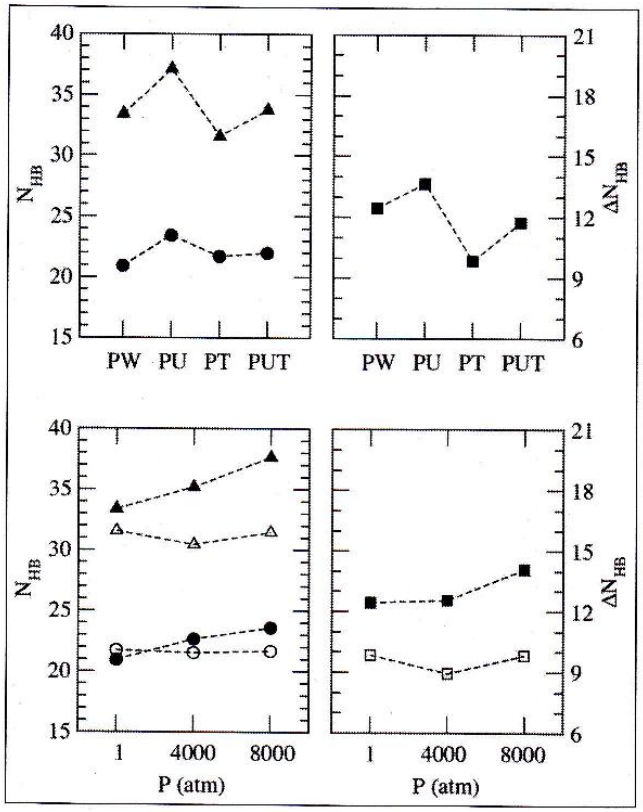

Figure 2. As in Figure 1 except total number of hydrogen bonds (N_{HB}) between polypeptide and solution species. For top panel, data from ref. 17. For bottom panel, data from ref. 11.

denaturation was the observation that the relative enhancement of both the heavy atom contact and hydrogen bond number were higher for the unfolded state than the compact state of protein, stabilizing the extended state better, and hence causing protein unfolding.[17]

Effect of TMAO

Investigation of solvation characteristics of TMAO by solution species revealed TMAO solvation by both water and urea.[14-16] TMAO molecules, which participated in 2 to 3 hydrogen bonds with water in its binary solution, showed its inability to maintain these hydrogen bonds as urea was added and these hydrogen bonds were replaced by TMAO-urea hydrogen bonds. We observed that hydrogen bonds formed by TMAO with water and urea were energetically very attractive and they relaxed slowly with time.[16] TMAO formed strong hydrogen bonds with water at high pressure as well and although the TMAO-water hydrogen bond number was almost unaffected by pressure, hydration shell of TMAO methyl groups was significantly compressed at high pressure and number of water molecules near these hydrophobic groups increased.[11,13]

TMAO was found to influence water properties in a manner that opposes the effects of pressure.[10] It not only reduced number of nearest identical neighbors of water but also increased population of more stable hydrogen bonds and the relative number of first shell water molecules that participate in water hydrogen bonding network.[10] Solvation of TMAO by water in the bulk thus prevents pressure-induced crowding of water molecules (in terms of identical neighbors), and this indirect effect of TMAO on water structure greatly reduces the need of water molecules to move to the protein surface. TMAO also increases the penalty of transferring water molecules from its hydrogen bonding network to the protein surface by making the network stronger. As compared to pure water, relaxation of water-water hydrogen bonds reduced noticeably in binary solution of TMAO both at low and high pressures and also in urea/TMAO mixture.[10,16] Additionally, TMAO was seen to make water second shell more pronounced and hence counteract urea-induced slight collapse of water second shell.[14,15]

Just like the protein denaturing osmolyte urea, TMAO removed water molecules from the solvation shell of hydrophobic groups and interacted directly with these solutes both at low and high pressures.[13-15,18] TMAO's effect on propensity of hydrophobic association was, however, insignificant, and it did not show any

tendency to prevent the pressure- and urea-induced dissolution of neopentane aggregate.[13,14] Our simulations also indicated existence of hydrogen bonding interaction between TMAO oxygen and protein hydrogen bond donating sites.[11,16,17] On the other hand, despite the unlikely hydrogen bonding interactions, TMAO methyl groups occupied the space around backbone oxygen and other hydrogen bond acceptor sites in protein, reducing efficiency of these atomic sites to form hydrogen bonds with solution species dramatically.[16,17] Consequent TMAO-induced reduction in hydrogen bond number between protein and solution species was observed in simulations.[10,11,16,17] The observed TMAO-induced higher reduction in hydrogen bond number for the unfolded state than the folded state of protein (Figure 2) can be taken as evidence of relatively higher destabilization of the extended state in the presence of TMAO.[11,17] Protein adopts the compact state to reduce the number of inefficient interaction with solution species and to increase the number of efficient intra-protein interactions. Note that protein folding also allows TMAO to maximize the number of favorable contacts with water in the bulk and to stabilize water hydrogen bonding network, giving further stability to the system that contains the folded protein. Hence, without excluding the importance of direct interaction of TMAO with water (and urea in urea/TMAO mixture) and its indirect effect on water structure enhancement, we conclude that the ability of TMAO to protect proteins both at low and high pressures in the presence or absence of urea arises due to the relatively higher inefficient interactions between hydrogen bond acceptor sites and solution species for the extended proteins.

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