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Individual and combined effects of urea and trimethylamine N-oxide (TMAO) on protein structures

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ABSTRACT

In this manuscript, we perform all-atom molecular dynamics simulations of model peptides to study the molecular mechanisms accounting for individual and combined effects of two osmolytes, i.e., urea and trimethylamine N-oxide (TMAO). We find that urea, which is a denaturant osmolyte, destabilizes mainly hydrophobic and intra-backbone interactions. TMAO, which is a protecting osmolyte, stabilizes charge-charge and intra-backbone interactions whereas it destabilizes hydrophobic interactions. We show that charge-charge interactions are highly sensitive to the presence of TMAO and it may be the main interaction accounting for TMAO stabilizing effect on proteins. These charge-charge interactions are also shown to play a dominant role in how TMAO counteracts the effect of urea. These results are rationalized in terms of the preferential interaction of osmolytes.

1. Introduction

Various organisms cope with water stresses (e.g., dehydration and high salinity) by producing organic molecules known as osmolytes to maintain osmotic balances [1,2]. For example, cartilaginous fish including sharks and rays cope with the salt content of sea water by maintaining high plasma and tissue concentrations of two main osmolytes, i.e., urea (~0.3 M) and trimethylamine N-oxide, i.e., TMAO, (0.07–0.18 M) [3,4]. The elevated concentration of urea in these fish can, however, be toxic as this osmolyte is known to destabilize the functional native state of proteins [1]. It is believed that, in addition to regulating osmotic pressure, the presence of TMAO in cartilaginous fish is due to its ability to counteract the denaturant effect of urea [5], which is nullified at a concentration ratio of 2:1 urea:TMAO for some proteins [6-9]. Thus, many biochemical reactions in living systems occur in the presence of two or more osmolytes. Understanding the molecular mechanisms accounting for their combined effects on proteins is challenging and remains a question of debate, even for TMAO and urea which are the most studied osmolytes.

The molecular mechanisms accounting for the destabilizing effect of urea on native structures has been extensively studied [10]. It is now accepted that this osmolyte interacts more favorably with the protein than with solvent molecules [11-14]. This provides a preference for the unfolded state wherein the number of these favorable urea-protein interactions is larger than in the more compact native state. In particular, the strength of hydrophobic interactions between non-polar side chains has been shown to be significantly reduced by urea [11,15-19]. Conversely, TMAO's effect on proteins is often explained by its more favorable interactions with water than with the protein surface [10,20-22]. The number of these less favorable TMAO-protein interactions is smaller when proteins adopt compact conformations as opposed to extended ones. This rationalizes the preference of this osmolyte for the compact native state. Details of this mechanism remains, however, a question of debate. For example, it has been proposed that TMAO is excluded predominantly from the backbone of proteins [23,24], that it acts as a crowding agent that favors compact protein structures [25], that it weakens the strength of hydrogen bonds between the protein and water [26], and/or that it can fold hydrophobic polymers [27,28]. More recently, computational studies have shown that TMAO weakens the strength of hydrophobic interactions in proteins and this effect dominates over the exclusion of TMAO from the protein backbone [29]. Thus, unfavorable interactions of TMAO with the backbone of proteins may not be the main stabilizing mechanism of TMAO. In this study, strengthening of charge-charge interactions by TMAO was shown to contribute and could be the dominant mechanism accounting for the stabilization of native protein conformations by this osmolyte. Also, experimental studies reported that zwitterionic osmolytes (including TMAO) enhance electrostatic interactions

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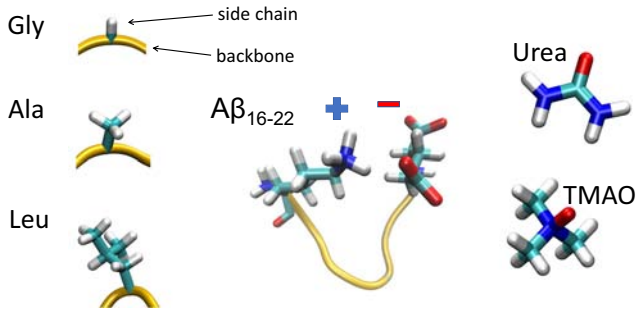

Fig. 1. Model peptides studied in this paper and atomic structure of urea (denaturant) and TMAO (protecting osmolyte). Poly-glycine is used to study effects of osmolytes on intra-backbone interactions; poly-alanine and poly-leucine to study effects of osmolytes on hydrophobic interactions; and $A\beta_{16-22}$ is used to study effects of osmolytes on charge-charge interactions. The backbone is depicted in orange and an all-atom representation is used for side chains. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

screened by salt resurrecting them to their full strength [30]. Thus, it is possible that strengthening of charged interactions by TMAO is even more pronounced in the cytosol where chemical reactions operate in the presence of different ions. These latter ideas are inline with experimental studies showing that the stabilizing effect of TMAO emerges from its more favorable interaction with the folded than with the unfolded protein surface [31].

A similar debate surrounds the molecular explanation of how TMAO counteracts the effect of urea on proteins. In one of the earliest computational studies, this counteracting effect was proposed to emerge from opposing effects of TMAO and urea on water structure [32] wherein urea weakens water-water interactions [33-35] and TMAO enhances them [36]. However, water structuring was not sufficient to account for effects of osmolytes on proteins in other studies [37-40]. Opposing effects of urea-backbone and TMAO-backbone interactions have also been proposed as a mechanism to explain TMAO's counteracting effect on urea [7,41]. More specifically, unfavorable TMAO-backbone electrostatic interactions was proposed to compete against favorable urea-backbone van der Waals interactions [37]. In other studies, TMAO was shown to attract urea and water to its surrounding [39,42-44]. It was proposed that this would reduce the concentration of urea on the protein surface, thus, counteracting the effect of urea. This inhibition of the protein-urea preferential interaction by TMAO is supported by a more recent study in which computational models of TMAO have been refined to better reproduce experimental Kirkwood-Buff integrals [45]. In this study, the counteracting effect of TMAO had a stronger effect on the interactions between charge-charge residues in a short fragment of the Tau protein than on the interaction between non-polar residues of poly-alanine peptide. Notice, however, that for ribonuclease Tl the preferential interaction of urea was reported to be mostly unaffected by TMAO, highlighting the complexity of osmolyte-protein interactions [8].

In this manuscript, we use computer simulations to study individual as well as combined effects of urea and TMAO on the main interactions stabilizing the native structure of proteins [46-49]. We use model peptides, i.e., poly-glycine to study intra-backbone interactions, poly-leucine to account for non-polar interactions, and $A\beta_{16-22}$ for charge-charge interactions–see Fig. 1. We find that all the peptides studied here become more extended when urea is added to the solvent with conformations of poly-leucine being more sensitive to this osmolytes—see Fig. 2. Interactions between charged residues in the $A\beta_{16-22}$ peptide (see Fig. 2d) and ions (Fig. 3) are less sensitive to urea. This supports the idea that urea destabilizes the hydrophobic core of proteins as well as interactions between backbone atoms. Poly-glycine becomes more compact when TMAO is added to the solvent (Fig. 2a) which is consistent with the effect of this osmolyte on proteins. However, the opposite effect is observed when non-polar side chains are attached to poly-glycine transforming this peptide into poly-leucine. This implies that TMAO destabilizes the hydrophobic core made of leucine side chain's and this effect is not counteracted by the effect of this osmolyte on the backbone. We find that interactions between oppositely charged residues in the $A\beta_{16-22}$ peptide (Fig. 2d) and in ions (Fig. 3) are strengthen by TMAO accounting for more compact $A\beta_{16-22}$ structures. This suggests that the effect of TMAO on charged interactions contribute and may play a dominant role in stabilizing native protein structures. Supporting this view, we find that TMAO counteracts effects of urea on the $A\beta_{16-22}$ peptide in a concentration depended manner, i.e., conformations sampled by $A\beta_{16-22}$ in aqueous urea solutions become increasingly more compact as the concentration of TMAO increases–see Figs. 4c and 5. In contrast, TMAO does not counteract effects of urea on poly-leucine and it has little counteracting effect on poly-glycine–see Fig. 4 a–b. These results are rationalized by studying how these osmolytes are distributed around peptides by calculating the preferential interaction.

2. Methodology

Microsecond-long molecular dynamics simulations are performed to investigate conformations of poly-glycine, poly-alanine, poly-leucine and $A\beta_{16-22}$ monomers in pure water, binary water–urea and water–TMAO as well as ternary water–urea–TMAO solutions. Peptides are terminated with COO− and NH3+ groups. At least two independent simulations are performed starting from different extended conformations of the monomer. Systems are equilibrated for 100 ns and the remaining of the trajectory is used for analysis. A list of all simulations performed in this work is provided in Table S3 of Supplemental material.

Simulations are performed using GROMACS version 4.5 [50] with the AMBER99SB-ildn force field and TIP3P water [51]. KAST [52] and AMBER [53] force fields are used to describe TMAO and urea, respectively. The Joung and Cheatham force field is used for Na^+ and Cl^- [54]. The equations of motion are integrated using the leap-frog algorithm with a time-step of 2 fs and the neighbor list is updated every 10 steps. A cutoff of 1.3 nm is used to account for Lennard-Jones interactions. Electrostatic interactions are treated using the Smooth Particle Mesh Ewald method with a grid spacing of 0.13 nm and a 1.3 nm real-space cutoff [55]. Simulations are performed in the NPT ensemble at 1 atm and 298 K. Temperature is controlled using the v-rescale thermostat ($\tau_T = 1$ ps) and pressure using the Parrinello–Rahman barostat ($\tau_P = 1$ ps).

In addition to molecular dynamics simulations, we also use umbrella sampling to quantify effects of TMAO or urea on the interaction between Na^+—Cl^-. For the reaction coordinate, we use the distance ξ between Na^+ and Cl^- ions in the range 0.2–1.1 nm. In the different umbrella sampling window, ξ is restrained by a spring. The equilibrium distances of springs in neighboring windows differ in steps of 0.05 nm and their spring constant is 4000 kJ mol^{-1} nm^{-2}. Each window is simulated for 150 ns and the potential of mean force (PMF) is computed using the Weighted Histogram Analysis Method (WHAM) [56]. Notice that the PMF increases with $-k_bT\log(\xi^2)$ due to the three-dimensional nature of ξ, where $-k_bT$ is the thermal energy. We subtract this ξ-dependence of the PMF and the PMF at the last umbrella sampling window, i.e., $\xi = 1.1$ nm, is shifted to zero.

3. Results

In this paper, we investigate effects of TMAO or/and urea on the conformation of 10-residue poly-glycine, poly-alanine, poly-leucine as well as on the $A\beta_{16-22}$ peptide–see Fig. 1. The side chain of glycine

is made of only one hydrogen atom and, thus, poly-glycine is often used as a model of a protein's backbone. Side chains of alanine (— CH_3) and leucine (— C_4H_9) are non-polar and, thus, homopeptides made from these amino acids are often used to study hydrophobic interactions in proteins. The $A\beta_{16-22}$ peptide (amino acid sequence: K **LVFFA** E) has been used to study effects of charged interactions in proteins since its flanked residues (underline letters) have opposite charges: K is positive and E is negative. Thus, our choice of peptides allows us to study effects of osmolytes on hydrophobic and charged interactions in proteins as well as on the interaction between backbone atoms.

3.1. Individual effects of urea and TMAO on model peptides

Fig. 2 shows effects of osmolytes on the conformation of model peptides. We use the radius of gyration Rg of backbone atoms to quantify the compactness of peptides. Panel a shows that the addition of TMAO to pure water accounts for more compact conformations of poly-glycine in a concentration dependent manner, i.e., the compactness of poly-glycine increases with increasing TMAO concentration [29,57]. Conversely, poly-glycine becomes more extended at high urea concentrations [11]. These effects of TMAO and urea on poly-glycine are consistent with their respective effects on the stability of native protein structures wherein urea favors the extended unfolded state of proteins and TMAO favors the compact native state. Thus,Fig. 2a is consistent with the idea that osmolytes regulate the stability of proteins by affecting mainly interactions involving backbone atoms [24]. To test this idea further, we study effects of TMAO or urea on other peptides.

Fig. 2b shows that TMAO or urea have only a small effect on the conformation of poly-alanine. This peptide becomes only slightly more compact or extended when TMAO or urea are added to water, respectively, at concentrations of 3 M and 5 M. Thus, the addition of even the smallest non-polar side chain, i.e., CH_3 group of alanine, to the main chain reduces significantly effects of cosolvents on the backbone. In Fig. 2c, we show that poly-leucine becomes significantly more extended when either urea or TMAO are added to water. In particular, the effect of urea in extending poly-leucine is significantly more pronounced than its effect on poly-glycine implying that urea affects large non-polar side chains, i.e., C_4H_9 groups of poly-leucine, at least as significantly as it affects the backbone [11,13]. TMAO, instead of favoring more compact conformation of poly-leucine as would be expected from its effect on native protein structures, contributes in making this peptide more extended. Thus, the effect of TMAO on peptides with large non-polar side chains is opposed to and dominates over its effect on the backbone accounting for poly-leucine becoming more extended. Since proteins have a significant non-polar core, these results suggest that the stabilizing effect of TMAO on native protein structures cannot emerge solely from its effect on the backbone.

To discover effects of osmolytes on other interactions that can play a role on the stability of native protein structures, we study in Fig. 2d how osmolytes affect $A\beta_{16-22}$. On the basis of its non-polar residues alone, $A\beta_{16-22}$ is expected to become more extended when either urea or TMAO is added to the solvent. This is indeed what we observe in simulations performed in aqueous urea solutions (blue lines in Fig. 2d) [58] but not what we observe in aqueous TMAO solutions (red lines). In the latter, structures of $A\beta_{16-22}$ are significantly more compact than the ones in pure water but without favoring any specific type of secondary structures (see Fig. S1) [29]. This is consistent with the effect of TMAO on native protein structures and it suggests that effects of this osmolyte on charged residues contribute to its stabilizing effect on native protein structures. Notice that in simulations performed using the CHARMM22 force field with the CMAP modification (which is nowadays known to have a strong bias towards α-helix [59,60]), TMAO was shown to favor α-helix conformations of $A\beta_{16-22}$ driven by entropy [25].

3.2. Individual effects of urea and TMAO on charged interactions

To provide a better understanding of the effects of TMAO or urea on the interaction between opposite charged residues, we depict in Fig. 3 the PMF of Na^+ and Cl^- ions in different aqueous

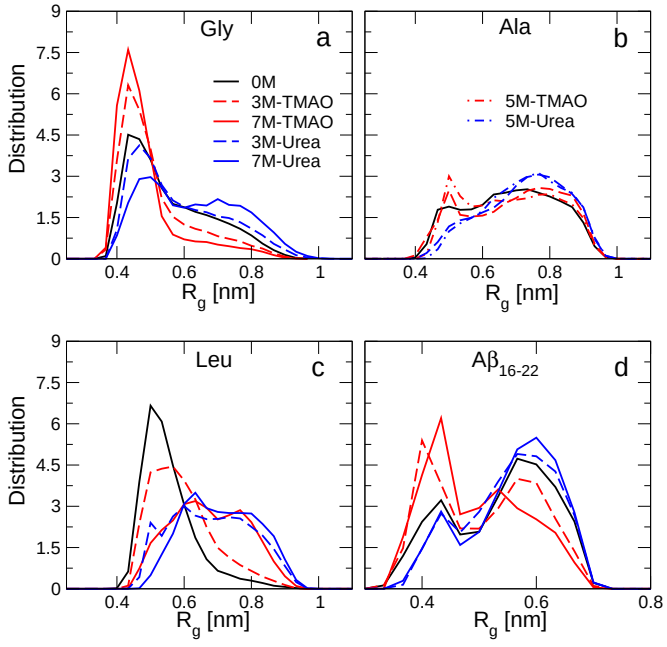

Fig. 2. Distribution of the radius of gyration Rg of backbone atoms for (a) poly-glycine, (b) poly-alanine, (c) poly-leucine, and (d) $A\beta_{16-22}$ in pure water (black), TMAO (red) and urea (blue) solutions at 298 K and 1 atm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

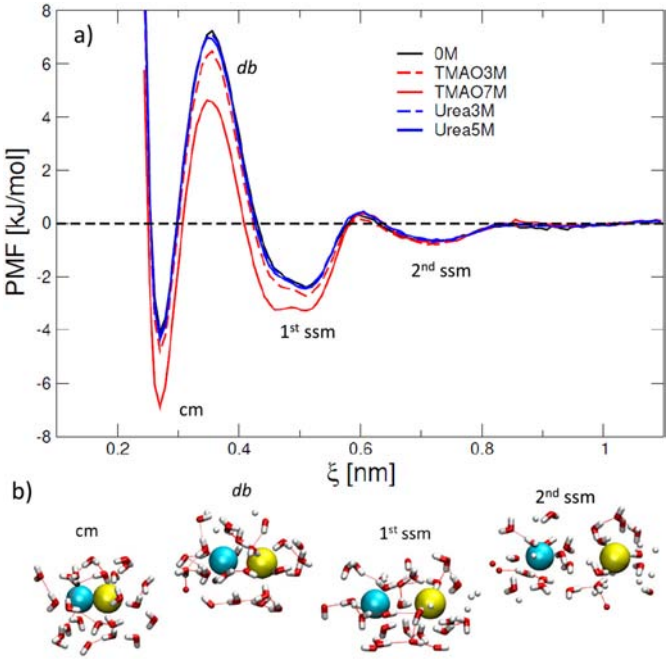

Fig. 3. Potential of mean force (PMF) for the interaction between Na^+ and Cl^- ions in pure water (black), aqueous TMAO (red), and aqueous urea (blue) solutions. PMF values computed at $\xi = 1.1$ nm are used as our reference, i.e., zero value. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

solutions as a function of the distance ξ between them. These PMF are characterized by the presence of a contact minimum (cm), a desolvation barrier (*db*), a first-solvent-separated minimum (1st ssm), and a second-solvent-separated minimum (2nd ssm) [61-65]. At cm, Na^+ and Cl^- ions are in direct contact with each other at a distance $\xi = 0.27$ nm. At the 1st and 2nd ssm, which occur at $\xi = 0.51$ nm and $\xi = 0.72$ nm, respectively, one and two layers of water molecules are found in the space between ions. At *db*, i.e., $\xi = 0.35$ nm, water molecules cannot fit within the space between ions which is characterized by the presence of cavities. Fig. 3b, shows characteristic conformations of these states obtained from simulations performed in pure water.

Values of the PMF computed in aqueous TMAO solutions (red lines in Fig. 3) at cc and 1st ssm are more negative than in pure water. Similarly, the PMF computed at *db* is smaller than in pure water. In other words, TMAO strengthens charge-charge interactions, i.e., it favors conformations in which opposite-charged ions are at close proximity from each other. The implication of this result for Aβ_{16-22} is that TMAO will favor small end-to-end distances of this peptide and, thus, more compact conformations which is consistent with simulation results in Fig. 2d. Recently, effects of TMAO on charged interactions have been rationalized by showing that this osmolyte weakens ion-solvent interactions [65]. Notice that the number of these ion-solvent interactions decreases when ions are brought close to each other accounting for an increase in the internal energy (or enthalpy) of the system. Thus, a weaker ion-solvent interaction due to the presence of TMAO accounts for a smaller increase in enthalpy and, accordingly, a more negative (or more favorable) PMF. Fig. 3 shows that urea (blue lines), when added to water, does not affect significantly the interaction between Na^+ and Cl^-. In particular, the PMF computed in aqueous urea solutions does not change significantly when compared to the PMF in pure water—see also reference [66]. The implication of this result for Aβ_{16-22} is that urea will interfere mainly with backbone and non-polar residues favoring the extended state of this peptide without affecting significantly the strength of the interaction between its terminal charged residues.

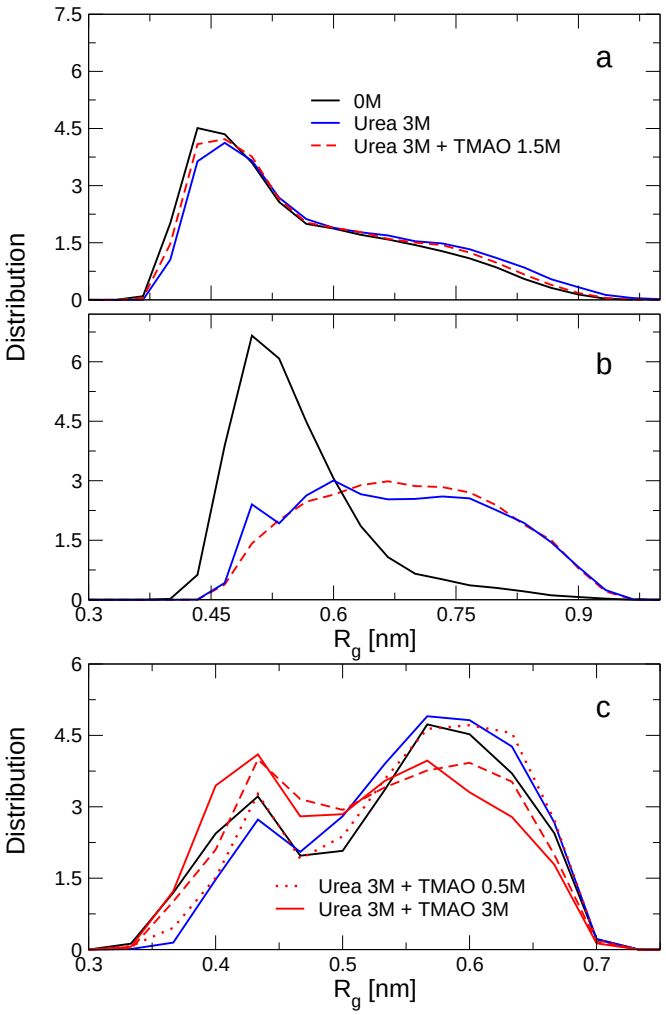

Fig. 4. Probability distributions of the radius of gyration Rg of backbone atoms for (a) poly-glycine, (b) poly-leucine, and (c) Aβ_{16-22} in pure water (black), 3 M urea solutions (blue), and ternary water-urea-TMAO solutions (red). Dotted, dashed, and full red lines correspond to concentration ratios of 3:1, 2:1, and 1:1 urea-TMAO, respectively. Simulations were performed at 298 K and 1 atm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Combined effects of urea and TMAO on model peptides

In Fig. 4, we study the combined effects of urea and TMAO on the conformation of poly-glycine, poly-leucine, and Aβ_{16-22}. We compare conformations of these peptides in three aqueous solutions: pure water (black line), 3 M urea (blue line), and a solution containing both 3 M urea and 1.5 M TMAO (red line). The latter solution corresponds to a 2:1 mixture of urea:TMAO which is the experimental condition wherein TMAO is expected to best counteract the destabilizing effect of urea on native protein structures [8]. Distributions of R_g in pure water and 3 M urea solution have already been reported in Fig. 2 and they are used here as a reference.

Fig. 4a shows that the addition of urea to water, accounting for a 3 M urea solution, does not affect significantly conformations of poly-glycine. Similarly, the addition of 1.5 M TMAO to the 3 M urea solution has no significant effect on the conformation of poly-glycine. Thus, although effects of urea or TMAO on poly-glycine are qualitatively consistent with their respective effects on the stability of native protein structures (see discussion of Fig. 2a), this peptide is not very sensitive to these osmolytes. Fig. 4b shows that the addition of 3 M urea to a pure water solution has a significant destabilizing effect on compact poly-leucine structures and this effect is not counteracted by TMAO at a concentration of 1.5 M. In other words, R_g distributions of poly-leucine computed in a 3 M urea solution and in solutions containing both 3 M urea and 1.5 M TMAO are not significantly different from each other. This behavior of poly-leucine is expected since individually urea or TMAO favor extended conformation of poly-leucine–see Fig. 2c. These results reinforces the idea that effects of osmolytes on backbone interactions are not strong enough to dominate over their effects on side chains. Moreover, the effect of TMAO on hydrophobic interactions, which is an important interaction driving protein folding, cannot explain their effects on the stability of native structures.

In Fig. 4c, we show that the addition of 3 M urea to a pure water solution has a small destabilizing effect on Aβ_{16-22}. This effect is overcompensated by effects of TMAO when 1.5 M of this cosolvent is added to the 3 M urea solution. In particular, the Aβ_{16-22} peptide samples more compact conformations in the combined 3 M urea and 1.5 M TMAO solution than in the 3 M urea solution. Fig. 4c also shows that this counteracting effect of TMAO is concentration dependent as increasing the concentration of TMAO from 0.5 M to 3 M in an aqueous 3 M urea solution favors increasingly more compact Aβ_{16-22} conformations. To further highlight the role of charge interactions in the counteracting effect of TMAO, we show in Fig. 5 the distribution of charge-charge distances at the different urea-TMAO aqueous solutions. These distributions are characterized by three peaks which have an amplitude that is dependent on the concentration of TMAO. In particular, increasing the concentration of TMAO

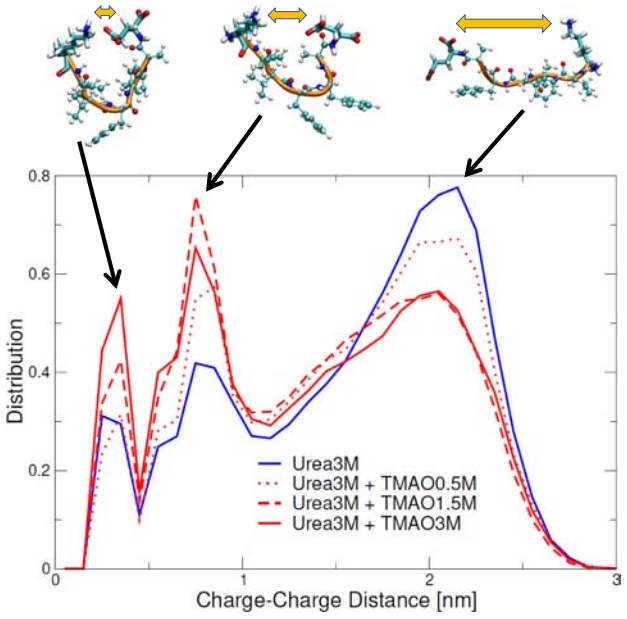

Fig. 5. Probability distributions of the distance between charged hydrogen atoms of Lysine residue (K) and charged oxygen atoms of glutamic acid (E) for $A\beta_{16-22}$ in 3 M urea (black), and ternary water-urea-TMAO solutions. Characteristic conformations of flanked peptides at the peak with shortest, medium and longest charge to charge distance are also showing.

reduces the magnitude of the peak corresponding to extended peptide conformations, i.e., large charge-charge distances. Concurrently, the magnitude of the peak corresponding to compact conformation, i.e., small charge-charge distances, increases. Thus, effects of TMAO on the interaction between charge residues in pure water as well as in aqueous urea solutions are consistent with their effects on native protein structures. Moreover, the magnitude of charge-charge interactions are significantly more sensitive to cosolvents than the other interactions probed in this work.

3.4. Spatial distributions of urea and TMAO around model peptides

Effects of osmolytes on the stability of native protein structures are often related to their spatial distribution in the solution [8,67]. Loosely speaking, osmolytes that are found with a higher concentration close to the surface of proteins are expected to interact more favorably with the protein surface than with water molecules. Accordingly, these osmolytes tend to favor protein conformations that have a larger solvent accessible surface area (SASA), i.e., the unfolded state, to increase the number of these favorable interactions. This characterizes denaturing osmolytes. In contrast, osmolytes that are found with a higher concentration in the bulk solution, i.e., away from proteins, interact more favorably with the solvent than with the protein. They behave as protecting osmolytes as they will favor compact protein conformations, i.e., the native state, to avoid interacting with proteins.

This behavior of osmolytes around proteins can be quantified using the preferential interaction $\Gamma(r)$ [67-69]. This quantity is computed by counting the number of osmolytes $N_{\mathrm{osm}}(r)$ and water molecules $N_{\mathrm{water}}(r)$ that are within a minimal distance r to the protein. In the bulk solution, the same number of water molecules are surrounded by an estimated number $\left(N^b_{\mathrm{osm}} / N^b_{\mathrm{water}}\right) N_{\mathrm{water}}(r)$ of osmolytes—where N^b_{osm} and N^b_{water} are the number of osmolytes and water molecules in the bulk, respectively. The preferential interaction compares the actual number of osmolytes within a distance r to the protein, i.e., $N_{\mathrm{osm}}(r)$, with this estimated number:

$$\Gamma(r) = N_{\mathrm{osm}}(r) - \left(\frac{N^b_{\mathrm{osm}}}{N^b_{\mathrm{water}}}\right) N_{\mathrm{water}}(r) \,. \tag{1}$$

The average $\cdots$ is performed over different frames of the trajectory. Negative and positive $\Gamma(r)$ values are expected for osmolytes that have lower and higher concentrations close to the protein, respectively, compared to the bulk solution. In solutions containing both urea and TMAO, $\Gamma(r)$ can be computed by counting separately the number of urea or TMAO molecules within a minimal distance r to the protein which gives rise to $\Gamma_{\mathrm{urea}}(r)$ and $\Gamma_{\mathrm{TMAO}}(r)$, respectively. According to Eq. (1), $\Gamma(r)$ is additive, i.e., $\Gamma(r) = \Gamma_{\mathrm{urea}}(r) + \Gamma_{\mathrm{TMAO}}(r)$.

Fig. 6 shows the preferential interaction around poly-glycine, poly-leucine, and $A\beta_{16-22}$ in two different 3 M urea solutions: one containing no TMAO molecules (dotted lines) and the other with 1.5 M TMAO (full lines). $\Gamma_{\mathrm{urea}}(r)$ and $\Gamma_{\mathrm{TMAO}}(r)$ are shown in blue and red, respectively, and $\Gamma(r)$ computed without distinguishing between these osmolytes is shown in black. Notice that Γ oscillates as r increases due to layering of osmolytes and water molecules around the protein. Moreover, due to the larger sizes of urea and TMAO, only water molecules can be found at the smallest r distances from the proteins. This accounts for negative values of the preferential interaction at small r distances between ~0.2–0.35 nm for all the systems studied.

Fig. 6a shows that for most r distances to poly-glycine, $\Gamma_{\mathrm{urea}}(r)$ and $\Gamma_{\mathrm{TMAO}}(r)$ are positive and negative, respectively. This is consistent with the effect of these osmolytes on the conformation of

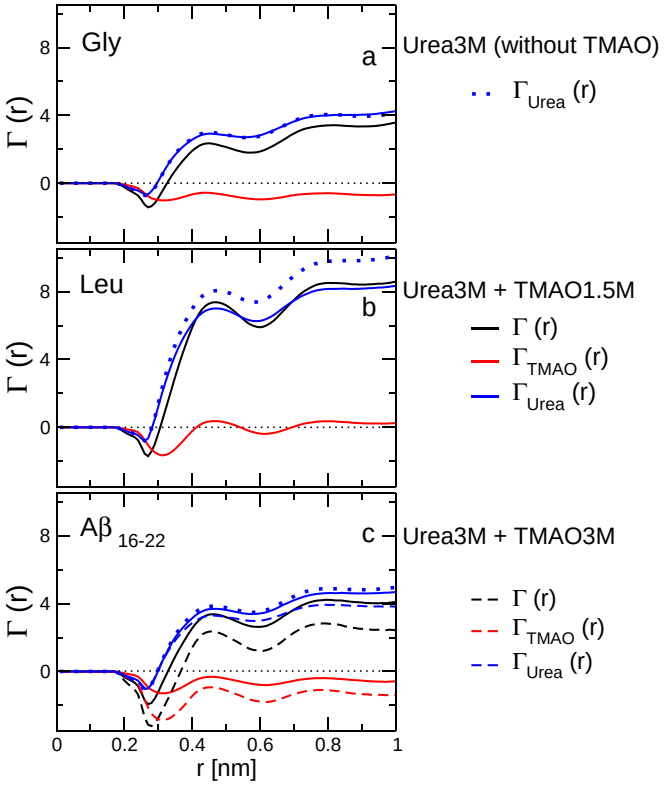

Fig. 6. Preferential interactions $\Gamma(r)$ of (a) poly-glycine, (b) poly-leucine, and (c) $A\beta_{16-22}$ in solutions containing urea and TMAO molecules. $\Gamma(r)$ is decomposed into contributions from TMAO (red) and urea (blue). Dotted lines correspond to 3 M urea solutions. Solid and dashed lines represent urea-TMAO ratios of 2:1 and 1:1, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

poly-glycine. In particular, a positive $\Gamma_{urea}(r)$ implies that urea interacts more favorably with the surface of poly-glycine than with water molecules and, thus, it will favor extended structures of this peptide (see Fig. 4a) to increase the number of these favorable interactions. Conversely, a negative $\Gamma_{TMAO}(r)$ implies that TMAO prefers to interact with the solvent than with the surface of poly-glycine and, thus, it will favor more compact structures of this peptide (see Fig. 4a) to reduce the number of less favorable TMAO-polyglycine interactions. Panel *a* also shows that the addition of TMAO does not affect significantly the distribution of urea around poly-glycine, i.e., $\Gamma_{urea}(r)$ computed in 3 M urea and in 3 M urea + 1.5 M TMAO solutions are not significantly different from each other. This suggests that effects of TMAO on the backbone are not enough to counteract the effect of urea on proteins.

Fig. 6b shows that urea is highly attracted to poly-leucine and TMAO exhibits only a small attraction to this peptide, i.e., $\Gamma_{urea}(r) \gg 0$ and $\Gamma_{TMAO}(r)$ oscillates around a small positive value. Moreover, this figure shows that the addition of 1.5 M TMAO to the 3 M urea solution significantly decreases $\Gamma_{urea}(r)$. These results suggest that when TMAO is added to the solution, it competes with urea for positions at the peptide's surface leading to a reduced urea concentration at the surface of poly-leucine. At first sight, it might appear counterintuitive that this reduced urea concentration does not account for more compact conformations of poly-leucine–see Fig. 4b. However, this is what is expected if the reduced urea concentration is compensated by an increased TMAO concentration at the surface of poly-leucine.

Distributions of urea and TMAO around $A\beta_{16-22}$ (see Fig. 6c) are qualitatively similar to the respective distributions around poly-glycine, i.e., urea is attracted to the surface of this peptide whereas TMAO is depleted from it. However, for this peptide $\Gamma_{urea}(r)$ is more sensitive to the presence of TMAO decreasing when TMAO is added to the solution. This effect on $\Gamma_{urea}(r)$ is small when 1.5 M TMAO is added to a 3 M urea solution and it increases with increasing TMAO concentration. Concurrently, $\Gamma_{TMAO}(r)$ becomes more negative with increasing TMAO concentration. Thus, concentrations of both TMAO and urea at the surface of $A\beta_{16-22}$ decrease when TMAO molecules are added to the solvent.

4. Conclusion

The prevalent theory of osmolytes is that these molecules favor either less or more compact protein conformations depending on whether they prefer to interact with the protein backbone or the solvent, respectively. Denaturant osmolytes prefer to interact with the protein backbone and, thus, favor less compact protein conformations to increase the number of these backbone-osmolyte interactions. In contrast, protecting osmolytes prefer to interact with the solvent and, thus, they favor more compact protein conformations to reduce the number of backbone-osmolyte interactions. This backbone based theory of osmolytes may explain the universal effect of these molecules on native protein structures as the backbone is the common ingredient of all proteins. Computer simulations using peptide models of the protein backbone, i.e., poly-glycine, is consistent with the backbone theory of osmolytes. However, these simulations alone cannot determine if effects of osmolytes on the backbone are more important/dominate over effects of these molecules on side chains of proteins.

There is now a growing body of evidence, both computational and experimental, supporting the view that TMAO (and possibly other protecting osmolytes) plays a more complex role on protein stability which requires understanding its effects on the different chemical groups of proteins. Here, we show that TMAO favors both the exposure of non-polar side chains to the solvent and it increases the magnitude of charge-charge interactions. These effects dominate over the effect of TMAO on the backbone, and they suggest that TMAO can favor unfolded protein surfaces, which have non-polar residues exposed to the solvent, as well as native structures that have charged residues on their surfaces. Our simulations using the $A\beta_{16-22}$ peptide shows that effects of TMAO on charge-charge interactions dominate over effects of this osmolyte on non-polar residues. In the same vein, in a previous study we showed that charged residues in the Trp-cage miniprotein are at closer proximity in aqueous solutions containing TMAO than in pure water whereas the non-polar core of this protein is more swollen [29]. This highlights a new possible mechanism of action of TMAO wherein this osmolyte stabilizes charge-charge interactions on the surface of native structures. Notice that observed effects of TMAO on hydrophobic and charge-charge interactions can be rationalized by an increased relative permittivity of the solvent due to the addition of TMAO which has been measured experimentally [70].

Here, we also study how TMAO counteracts effects of urea on proteins. We show that TMAO counteracts effects of urea on the $A\beta_{16-22}$ peptide, which is our model peptide for charge-charge interactions, but not on our model peptide for non-polar interactions, i.e., poly-leucine. Also, we find that poly-glycine is not very sensitive to TMAO in order to allow this osmolyte to counteract effects of urea. Thus, the only interaction in our simulations of model peptides that can significantly counteract effects of urea in proteins are charge-charge interactions. This provides further support for a mechanism in which TMAO affects proteins by stabilizing charge-charge interactions.

Despite the insights brought up by this work, it is also important to highlight its limitations. In particular, it is desirable to test the validity of the ideas brought forward by this study by performing additional simulations on other peptide sequences. The extensive computational cost of such efforts (see Table S3) has limited us from doing so. Notice, however, that other studies have reported on the strong effect of TMAO on charge-charge interactions for the Trp-cage miniprotein [29] and a fragment of the tau protein [71]. TMAO was also reported to fold a general polymer populated with positive and negative beads although this effect was depended on the distribution of charge on the polymer [72]. Currently, there are various models to simulate TMAO at the atomic level. We used the more traditional Kast model which was develop based on quantum mechanics calculations of partial charges [52]. In a previous study, we have also studied effects of TMAO using osmotic and Netz model [29]. Notice that the latter model was parametrized to reproduce transfer-free energies of poly-glycine from pure water to an aqueous TMAO solution [20]. We showed that TMAO stabilizes charge-charge interactions in $A\beta_{16-22}$ as well as other short non-polar peptides flanked by charged residues. Combined effects of urea and TMAO was not studied in the previous study. It would be desirable to test the ideas in this paper using the modified-Netz model that was develop while we were writing this manuscript and which takes into account ternary water-urea-TMAO mixtures in a more realistic manner [45]. The latter study also reported stronger charge-charge interactions in aqueous TMAO solutions compared to pure water.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2019.111443.

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