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Effects of Cosolvents on the Hydration of Carbon Nanotubes

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Abstract: Molecular dynamics simulations of a nonpolar single-walled carbon nanotube (SWNT) solvated in aqueous solutions of urea, methanol, and trimethylamine *N*-oxide (TMAO) show clearly the effects of cosolvents on the hydration of the interior of the SWNT. The size of the SWNT was chosen to be small enough that water but not the cosolvent molecules can penetrate into its interior. Urea as a protein denaturant improves hydration of the interior of the SWNT, while the protein protectant TMAO dehydrates the SWNT. The interior of the SWNT is also dehydrated when methanol is added to the solution. The analysis of interaction energies of the water confined inside the SWNT pore shows that the stability of the confined water in the methanol and TMAO solutions mainly depends on electrostatic interactions. In contrast, both van der Waals and electrostatic interactions were shown to be important in stabilizing the confined water when the SWNT is immersed in the urea solution.

1. Introduction

How cosolvents including denaturants and structure protectants affect protein structures and solubility is one of the most fundamental questions of protein chemistry.(1,2) More than a century of research has generated a large body of biochemical and thermodynamics experimental data. Among all cosolvents in studies, urea has long been known for its denaturing effects on proteins and its effects on hydrogen carbon solubility in aqueous solutions. Trimethylamine *N*-oxide (TMAO), on the other hand, is a widely used protein structure protectant, which induces protein folding and offsets the denaturing effects of urea. Alcohols, for example, methanol, also denature proteins and dramatically affect amino acid solubility in a residue-dependent manner. Methanol was also found to induce the formation of α-helices.(3) Different mechanisms have been proposed to understand the effects of cosolvents on protein structures. In particular, both "direct" and "indirect" mechanisms have been proposed for the interaction between cosolvents and proteins. The direct mechanism states that cosolvent molecules affect the formations of protein structures via direct electrostatic/van der Waals interactions with proteins.(4−6) In contrast, the indirect mechanism postulates that the cosolvent molecules affect protein structure stabilities through disrupting or making of the water structure.(7−9) Although both direct and indirect effects have been discussed extensively in the literature, no decisive conclusion has been reached.

The molecular mechanism of the cosolvent effects on protein structure remains elusive partially because of the complex interactions among solvent, cosolvents, and the various groups of proteins. Therefore, the hydration of simple hydrophobic entities instead of proteins in the aqueous cosolvent solution was studied to clarify which mechanism, direct or indirect, is dominant in the hydration/dehydration process. For example, Zangi et al. studied the effects of urea on the solvation of hydrophobes using molecular dynamics simulations of hydrophobic plates.(10) In this study, we use a very simple and easily controlled system, a single-walled carbon nanotube (SWNT), to investigate how urea, TMAO, and methanol affect the hydration of the interior of this nonpolar and largely hydrophobic entity. From a fundamental perspective, water is expected to recede from nonpolar interfaces and cavities. However, the flux of water through carbon nanotubes has been observed earlier by both simulations(11−13) and experiments.(14,15) Hummer proposed that the confinement-induced narrowing of the interaction-energy distribution lowers the excess chemical potential of the interior of the tube.(16) Such that, water molecules can enter into the nanotube interior and effectively establish equilibrium between interior and exterior hydration. In a recent review paper,(17) Rasaiah et al. speculated that adding cosolvents that do not penetrate the tube would decrease the excess chemical potential of the bulk water and thus induces the drying of the tube. In this study, we provide a detailed quantitative study on the effects of cosolvents on the hydration of carbon nanotube

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pores. Different cosolvents, protein denaturant urea and methanol and protein structure protectant TMAO were examined for this purpose. It is found that these cosolvents have very different effects on the hydration of the interior of the nanotube. Urea enhances the water filling of the nanotube while methanol and TMAO dehydrate the interior of the nanotube. More importantly, we studied how cosolvents affect the water molecules in the bulk and those inside the carbon nanotube in order to gain deeper insight on how cosolvents change the hydration.

2. Computational Methods

The initial coordinates of the 110-carbon (6,4) single-walled carbon nanotube (13.4 Å long and 6.7 Å in diameter) were generated using the nanotube coordinate generator program (http://www.photon.t.u-tokyo.ac.jp). The carbon atoms are modeled as uncharged Lennard−Jones particles, and the bonded/nonbonded force field parameters for carbon nanotubes are taken from the simulations of Hummer et al.[11] The nanotube was solvated in a cubic box of 942 TIP3P[18] water molecules or 7 M urea, methanol, and 4 M TMAO solutions ($N_{\mathrm{urea}}/N_{\mathrm{water}} = 187/943$; $N_{\mathrm{methanol}}/N_{\mathrm{water}} = 187/941$; $N_{\mathrm{TMAO}}/N_{\mathrm{water}} = 118/942$). The atomic charges and force field parameters of the urea, methanol, and TMAO molecules were taken from refs19 and 20. All simulations were performed using the AMBER9 molecular dynamics package[21] in an NPT ensemble. The temperature of the system was maintained at 300 K using Langevin dynamics with a friction coefficient of 5 ps^{-1}, and the pressure was maintained at 1 bar using Berendsen weak-coupling algorithm[22] with a relaxation time constant of 2 ps. The particle-mesh Ewald[23] method with a real space cutoff of 10 Å was used to treat long-range electrostatic interactions. The SHAKE[24] algorithm with a relative geometric tolerance of 10^{-8} was used to constrain all bonds including hydrogen and all dynamics calculations utilized a 2 fs time step.

3. Results

3.1. Effects of Cosolvents on the Hydration of the SWNT Interior. Although the diameter of the carbon nanotube used in this study is smaller than that used in earlier studies[11] (to avoid the penetration of cosolvents into the tube and thus to obtain a clear picture on how cosolvents affect water/carbon nanotube interaction), significant occupation of the interior of the nanotube by water is again observed when it is immersed in water. The calculated number of water molecules inside the carbon nanotube is shown in Figure 1 for the last 50 ns of 125 ns simulations for each of the four systems, pure water, 7 M urea, 7 M methanol, and 4 M TMAO solutions.

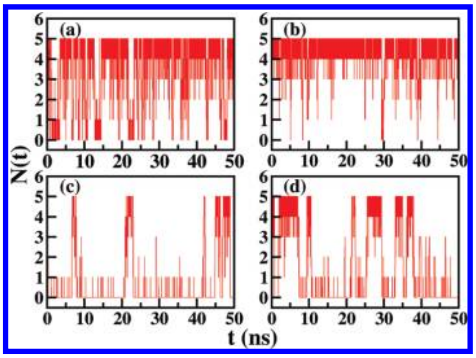

Figure 1. Number N of water molecules inside the nanotube as a function of time: (a) pure water; (b) 7 M urea solution; (c) 7 M methanol solution; (d) 4 M TMAO solution.

Figure 1 shows that multiple filling-emptying transitions occurred during the simulations, and the number of confined water molecules fluctuated between 0 and 5. The most interesting observation is that the hydration of the carbon nanotube is heavily dependent on the cosolvent. The addition of urea noticeably increases the occupation of the carbon nanotube by water molecules (Figure 1b), whereas both methanol and TMAO induce the drying of the tube interior, as can be seen from the significantly reduced water occupation number (Figure 1c, d). In pure water or urea solution, the carbon nanotube has a high probability to contain 4 or 5 confined water molecules, while in methanol or TMAO solution, it is most likely to be empty. Consistently, the averaged number of water molecules inside the carbon nanotube during the entire simulation time is 3.62, 4.03, 0.53, and 1.43 for water, urea, methanol, and TMAO solutions, respectively. Since the hydration of the carbon nanotube interior is not affected by the direct binding of cosolvent molecules inside the tunnel, as a result of their large sizes preventing them from entering, these results more clearly show the cosolvents' indirect effects on the hydration of hydrophobes.

The indirect effects of cosolvents on the hydration of the carbon nanotube can be characterized by the changes of the excess chemical potentials in the bulk and in the tube interior. As pointed by Hummer, the filling of the hydrophobic channel is mainly induced by the decreased excess chemical potential of the interior of the channel.[16] Rasaiah et al. also proposed that the addition of cosolvents that do not penetrate the nanotube decreases the excess chemical potential of the bulk water and thus induces the dehydration of the tube interior.[17] From the averaged number of water molecules inside the tube, one can estimate the excess chemical potential difference between water molecules in the nanotube pore and in the bulk:[11]

$$\Delta\mu^{\mathrm{ex}} = \mu^{\mathrm{ex}}_{\mathrm{nt}} - \mu^{\mathrm{ex}}_{\mathrm{w}} = -k_B T \ln\left(\frac{\langle N\rangle}{\rho_0 \Delta V}\right) \tag{1}$$

where k_{B} is Boltzmann's constant, T is the temperature, $\langle N\rangle$ is the average number of the water molecules inside the tube, ρ_0 is the bulk water density, and ΔV is the volume inside the nanotube. The estimated excess chemical potential difference $\Delta\mu^{\mathrm{ex}}$ is −0.82, −1.14, 0.08, and −0.51 kcal/mol for pure water, 7 M urea, 7 M methanol, and 4 M TMAO solution, respectively.

Excess chemical potentials μ^{ex} are also directly related to the distributions $P_{\mathrm{bind}}(u)$ of binding energies of individual molecules:[11,16]

$$\exp(\beta\mu^{\mathrm{ex}}) = \langle\exp(\beta u)\rangle = \int P_{bind}(u)\exp(\beta u)\mathrm{d}u \tag{2}$$

where $\beta = 1/k_{\mathrm{B}}T$, k_{B} is Boltzmann's constant, and T is the temperature. The binding energy u of a given water molecule is the potential energy difference of the system in a given configuration with and without that molecule. First, the probability distributions of binding energies of the water molecules in the bulk were calculated and shown in Figure 2a. Compared to the binding energy calculated for the system with pure water, the distributions of the binding energies are slightly shifted when

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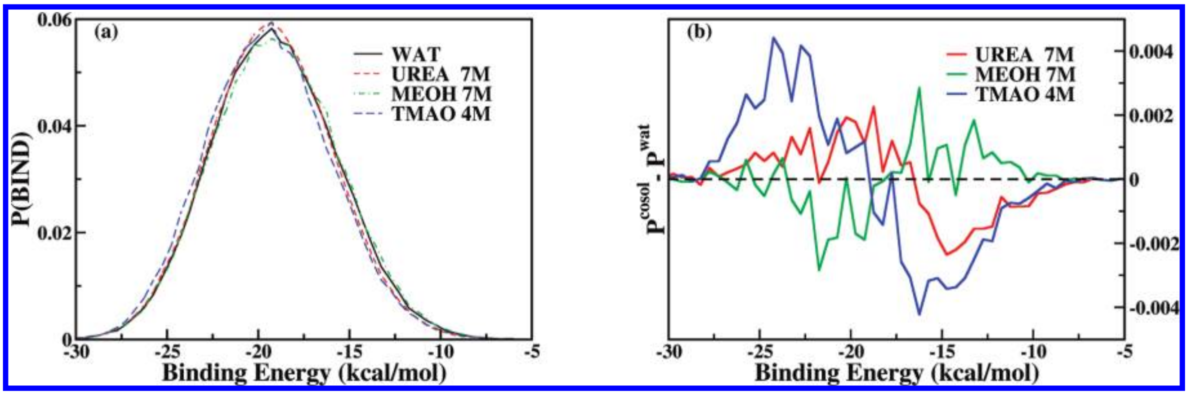

Figure 2. Probability distribution of the binding energies of the water molecules in the bulk: (a) probability distributions of the binding energies; (b) relative probability distributions of the binding energies.

the cosolvents were added to the system. To clearly check the effects of the three cosolvents, urea, methanol, and TMAO, the relative probability, that is, $P_{\text{bind}}^{\text{cosolvent}} - P_{\text{bind}}^{\text{WAT}}$, is shown in Figure 2b. From Figure 2b, we first notice that TMAO effectively stabilized the water in the bulk compared to pure water, indicated by the pronounced increased probability (positive values of $(P_{\text{bind}}^{\text{TMAO}} - P_{\text{bind}}^{\text{WAT}})$) in the low energy region and the decreased probability (negative values of $(P_{\text{bind}}^{\text{TMAO}} - P_{\text{bind}}^{\text{WAT}})$) in the high energy region. Based on eq 2, the excess chemical potential of the water molecules in the bulk is thus decreased, which has an effect of lowering the water occupancy of the nanotube. Urea has similar effects as those of TMAO in affecting the excess chemical potential of the bulk water, in that it decreases the excess chemical potential of the bulk water, however, to a lesser extent. Methanol, in contrast to urea and TMAO, increases the chemical potential of the bulk water manifested by both the decreased probabilities in the low energy region and the increased probabilities in the high energy region. Based on the chemical potential changes of the bulk water induced by the three cosolvents, urea and TMAO would dehydrate the interior of the nanotube while methanol would increase the water filling of the tube interior. Except for TMAO, the findings due to the chemical potential of the bulk water for urea and methanol obviously do not agree with what we observed in the simulations, since we know that urea actually improves the hydration of the carbon nanotube and methanol largely reduces the hydration. Certainly, the amount of water in a cavity is governed by the difference between the excess chemical potential inside and in the bulk (eq 1). The change in the chemical potential of the water inside the tube also needs to be calculated to correctly explain the filling/drying effects of the cosolvents.

The probability distributions of binding energies of water molecules inside the nanotube are shown in Figure 3a. Similarly, to present the effects of the cosolvents more clearly, the relative probability($P_{\text{bind}}^{\text{cosolvent}} - P_{\text{bind}}^{\text{WAT}}$) is shown in Figure 3b. Figure 3b shows that urea stabilizes the water inside the nanotube, since the pronounced positive relative probabilities in the low energy region and the negative relative probabilities in the high energy region are presented, compared to those of the system with pure water. As a result, urea decreases the excess chemical potential of the water molecules inside the nanotube. To qualitatively compare the changes in the excess chemical potentials between the water molecules in the tube channel and in the bulk, we calculated the area circumscribed by the relative probability lines (Figure 2b and Figure 3b) in the high energy region (e.g., −16 to −5 kcal/mol), since high energy states dominate the free energy as shown in eq 2. It is found that the area circumscribed by the binding energy relative probability line of the water molecules in the tube interior is 2.5 times larger than that of the bulk water. Therefore, urea decreases the chemical potential of the bulk water, but at the same time, it has a stronger effect in decreasing the chemical potential of the water inside the nanotube. Consequently, urea decreases the chemical potential of the confined water relative to the bulk and improves the hydration of the interior of the carbon nanotube. On the other hand, although methanol and TMAO affect the chemical potential of the bulk water differently, they both significantly increase the chemical potential of the water inside the tube, and the net result is that both methanol and TMAO largely reduce the hydration of the carbon nanotube interior. The analyses above show that the cosolvents affect the hydration of the nanotube interior through the interactions with the water molecules in the bulk and in the nanotube interior. There is a clear indirect effect, although this indirect effect is actually induced by the direct effect of the cosolvent partially. In Figure 7, we find that the cosolvents, especially urea and methanol, preferentially bind to the exterior of the nanotube. It is this preferential binding of the cosolvents that stabilize/destabilize the water molecules inside the nanotube.

Furthermore, we dissected the binding energy into two components, the van der Waals interaction energy (Figure 3c) and the electrostatic interaction energy (Figure 3d). It is found that, in the methanol and TMAO solutions, van der Waals and electrostatic interactions have opposite effects on the water molecules inside the nanotube. The probability distributions of the van der Waals interactions shift toward the low energy region compared to that of the pure water, representing the stabilization of the water molecules in the nanotube pores. On the contrary, the electrostatic interactions in the methanol and TMAO solutions destabilize the water molecules in the nanotube interior indicated by the larger probabilities in the high energy region as well as the smaller probabilities in the low energy region. Combining the van der Waals and electrostatic energies, we obtained the binding energy as shown in Figure 3a. Comparing Figure 3a, c, and d, it is obvious that, for the methanol and TMAO solutions, the electrostatic interactions are the dominant terms in decreasing the stabilization of the water inside the nanotube. Similarly, the van der Waals interactions in the urea solution stabilize the water inside the nanotube (the distribution of the van der Waals interactions shifts toward the low energy region). As for the electrostatic interactions, in the low energy region, the probability distribution in the urea solution is very similar to that in the pure water. Whereas, in the high energy region (approximately −8 to 0 kcal/mol), the probabilities in

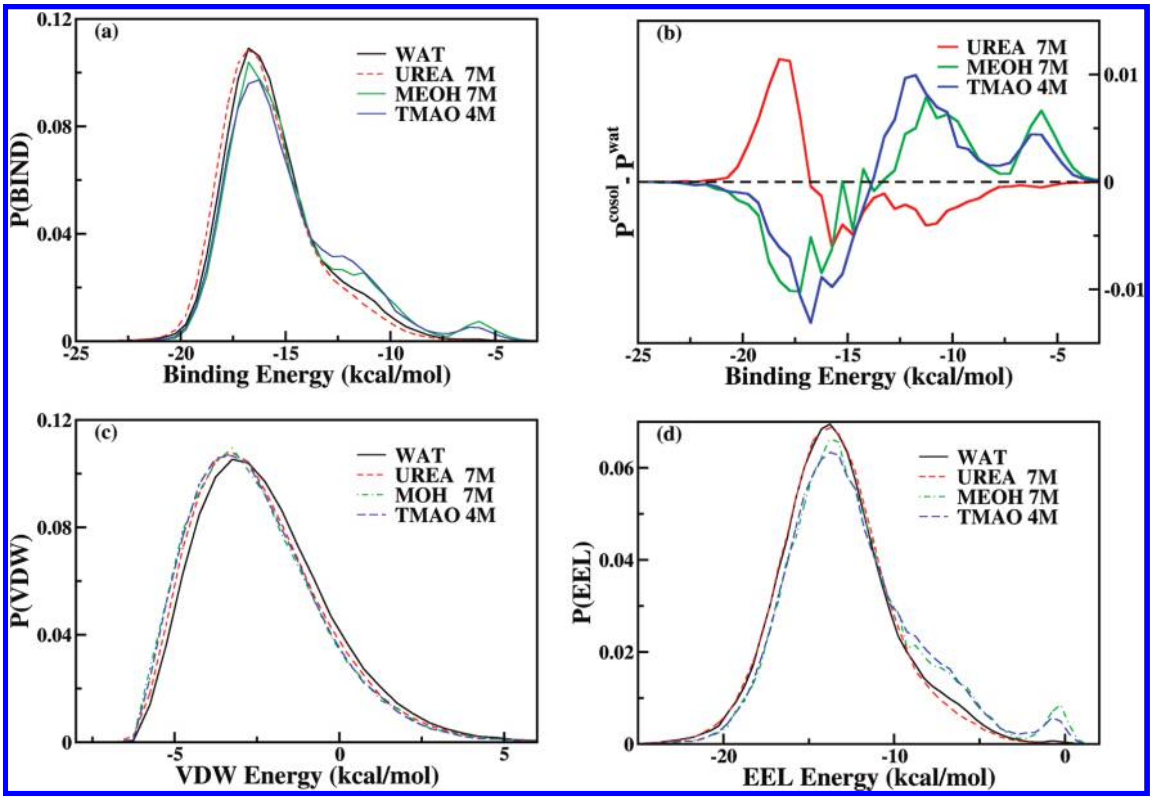

Figure 3. Probability distribution of the binding energies of the water molecules inside the nanotube:. (a) probability distributions of the binding energies; (b) relative probability distributions of the binding energies; (c) probability distributions of the van der Waals energies; (d) probability distributions of the electrostatic energies.

the urea solution are apparently smaller than those in the system of pure water. In summary, both van der Waals and electrostatic interactions are improved in urea solution and stabilize the water molecules in the interior of the nanotube.

3.2. Effects of Cosolvents on the Water Structural Properties. The indirect effects of cosolvents on the hydration of the nanotube interior can also be shown by the structural properties and hydrogen-bond network analyses of water. Although there is still debate on the usefulness of the water structure perturbation ideas in predicting the protein stability, for the specific system studied in this paper, when considered together with the binding energy analyses of water, the analyses on the water structure does provide more physical details on the cosolvents' effects on the hydration of the nanotube interior. In a previous paper studying urea denaturing the short peptide, Wei et al. already showed that 5 M urea, 5 M TMAO, and 4 M TMU solutions affect the water structure and hydrogen-bond network differently due to their water structure perturbation abilities and there is significant indirect cosolvent effect on the protein backbone hydration.[25] Here, for the simple nonpolar hydrophobic channel, the carbon nanotube, solvated by pure water, 7 M urea, 7 M methanol, and 4 M TMAO solutions, we performed the similar analyses and attempt to correlate the effects of the cosolvents on the hydration of the hydrophobic channel with the water structure perturbation abilities of the cosolvents. (Water structure perturbation refers to the structure distortion of the water outside the first hydration shell induced by cosolvents, which can be seen from the two-body radial distribution function $g(r)$ of water–water pairs. That is, the prominent and outward peak of $g(r)$ compared to pure water represents the more ordered water structure,; while the lowered and inward peak of $g(r)$ represents the more disrupted water structure.)

In Figure 4a, the two-body radial distribution functions (RDF) of water oxygen atoms g_{oo} are shown. It is easy to notice that the g_{oo} for water has a more pronounced first peak at 2.77 Å upon the addition of cosolvents. The increasing of the peaks in the methanol and TMAO solutions is more prominent than that in the urea solution. However, the comparisons of g_{oo} at the second peak, the third peak, the first valley, and the second valley show more meaningful results of the effects of urea, methanol, and TMAO to the water structure. In order to present more clearly the comparisons of g_{oo} at the regions mentioned above, the same radial distribution functions were redrawn as Figure 4b with the second/third peak and the first/second valley focused on. First, a noticeable rise of the first valley in the g_{oo} can be observed when urea is added. Thus, it leads to a much less pronounced second peak, corresponding to the collapse of the water structure in the second water shell.[26] This finding reveals that urea disrupts the water structure, which is consistent with its effect of reducing the penalty in moving water molecules into the nanotube. In contrast, TMAO shows the deeper first and second valleys at 3.5 and 5.8 Å. Compared to that for pure water, g_{oo} for the TMAO system also has the more pronounced second and third peaks. In earlier simulations, the increasing of the height of the peaks for TMAO was also observed.[27] This

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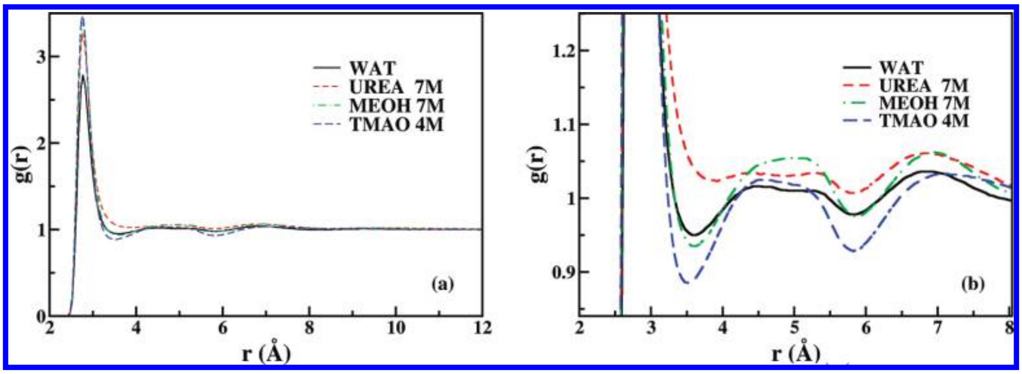

Figure 4. Comparisons of water structure in 7 M urea (red), 7 M methanol (green), and 4 M TMAO (blue) with that in pure water (black). (a) Radial distribution function (RDF) of water oxygen to water oxygen; (b) the same RDF with the second and third peaks zoomed in.

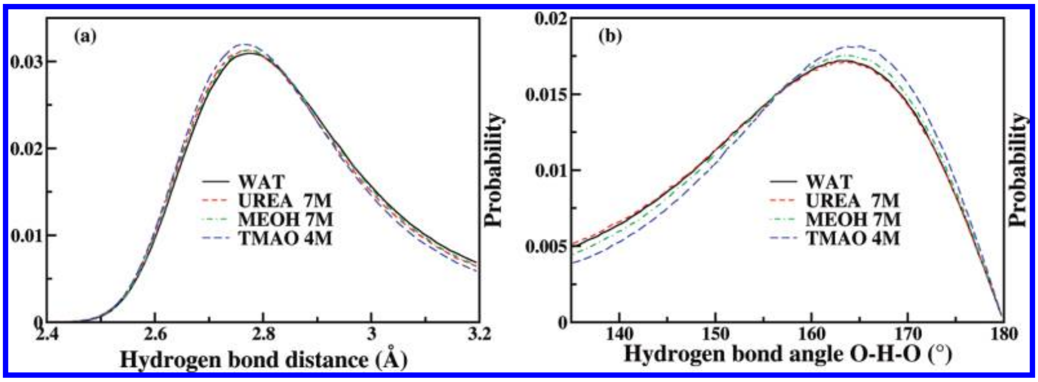

Figure 5. Comparisons of water hydrogen-bond properties in 7 M urea (red), 7 M methanol (green), and 4 M TMAO (blue) with those in pure water (black). (a) Hydrogen-bond length distribution; (b) hydrogen-bond angle distribution. The definition of hydrogen bonds is that the distance between donor and acceptor is no greater than 3.2 Å and the angle of donor−H−acceptor is no smaller than 135°.

behavior of TMAO is an indicator of its effect on changing the water activity. In addition, the positions of the second and third peaks in the TMAO solution are ~0.1 and ~0.3 Å further outward than those of the pure water. These observations again suggest a more ordered water structure. As a consequence, it is more difficult to move a water molecule into the channel of the nanotube in TMAO solutions. As for the third colsovent methanol, although it is usually used as the denaturant, the g_{oo} for water in the methanol solution shows that methanol changes water structure to that being consistent with a more ordered one. Methanol not only makes the first valley deeper but also makes the second and third peak of g_{oo} more pronounced and outer-positioned compared to those of pure water. These results indicate that methanol may not denature protein through enhancing hydration. Actually, it should induce dehydration like TMAO does. This observation is on the other hand consistent with the capability of methanol in inducing secondary structure formation.[28]

The distribution functions for the water−water hydrogen-bond length and angle are shown in Figure 5. For the 7 M urea solution, both hydrogen-bond length and angle of water are only slightly perturbed comparing to those of pure water. These observations illustrate that urea fits well into the water hydrogen-bond network, which is consistent with the finding in the earlier simulations.[29,30] Again, this feature of urea reduces the penalty of transferring water molecules from the bulk to the interior of the nanotube. First, the preferred interaction (largely van der Waals[6]) between urea and the nanotube brings urea close to the nanotube. Second, urea molecules are also good hydrogen-bond donors as well as acceptors for water. As a result, these interactions lead to the stabilization of water in the nanotube pores, by reducing the penalty of removing water from its hydrogen-bonded environment of the bulk. Among the three cosolvents used in this study, TMAO has the strongest effects on the distributions of the water hydrogen-bond length and angle. The probability distributions at the hydrogen-bond length of 2.75 Å and at the hydrogen-bond angle of 160−175° are significantly increased in TMAO solution compared to those of the pure water system. These results suggest that, on average, the individual water−water hydrogen bonds become stronger (shorter hydrogen-bond lengths and larger hydrogen-bond angles) upon the addition of TMAO. They also imply that TMAO stabilizes the bulk water, which has been shown earlier in Figure 2. Methanol also slightly increases the proportion of the strong water hydrogen bonds. Although it only slightly changes the hydrogen-bond length distribution, methanol does increase the probability of the hydrogen bonds with the larger O−H−O angle. This result again shows that methanol strengthens water/water interaction so that it denatures protein through a very different way from urea, in that it is expected to strengthen the secondary structures. On the other hand, methanol tends to reduce the hydrophobic effect and thus could increase the solvation of the hydrophobic domains of a protein.

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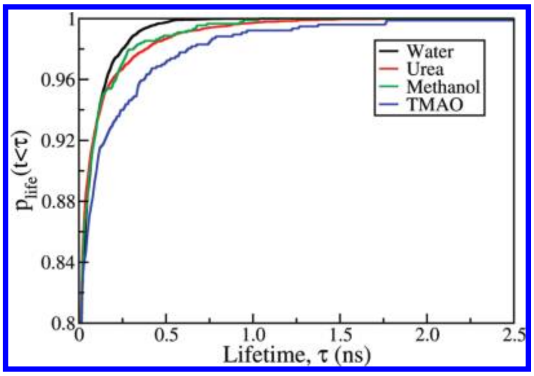

Figure 6. Cumulative distributions $P_{\mathrm{life}}(t < \tau)$ of the lifetime τ of the water molecules inside the nanotube.

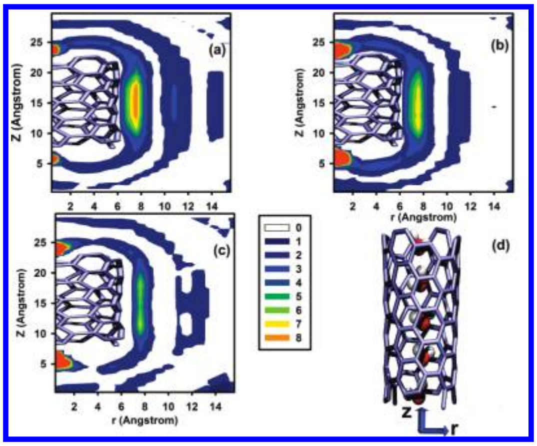

Figure 7. Relative distribution function of cosolvent/water as a function of radius r and length z: (a) 7 M urea solution; (b) 7 M methanol solution; (c) 4 M TMAO solution. (d) Structure of water chain inside the nanotube.

3.3. Dynamics of the Interior Water Molecules. Furthermore, we characterize the effect of cosolvents on the dynamics of interior water molecules by calculating the residence time of water inside the nanotube. As shown in Figure 6, all cosolvents increase the residence time of water molecules.

These results are easily understood from the relative distribution function of cosolvent/water around the carbon nanotube (Figure 7). The relative distribution function represents the preferential binding of cosolvent molecules over water molecules near the carbon nanotube pore opening. Based on the coordinates of their center of mass, the molecules are sorted into a histogram (HIST) where each bin has a radius dr (extends from r to $r +$ dr) and a height dz (extends from z to $z +$ dz). The histogram is normalized by dividing the total number of frames and the volume of the bin ($\pi[r^2 - (r + \mathrm{d}r)^2]\mathrm{d}z$). Then, the relative distribution function is obtained from the ratio of histogram ($\mathrm{HIST}_{\mathrm{cosolvent}}/\mathrm{HIST}_{\mathrm{water}}$) multiplied by the ratio of the number of molecules ($N_{\mathrm{water}}/N_{\mathrm{cosolvent}}$). The relative distribution function shows pronounced peaks at the opening of the tube, indicating a high probability for the cosolvent to reside at these sites. These cosolvent molecules residing at the opening of the carbon nanotube, especially methanol and TMAO, function as seals for the nanotube and significantly slow down the flow of water across its tunnel. Both urea and methanol also show significant preferential binding to the carbon nanotube, consistent with them being denaturants and their possible roles in reducing the hydrophobic effects in protein solvation. In particular, it needs to stress again that the preferential binding of urea to the nanotube together with the feature of urea fitting well into the water hydrogen-bond network reduce the penalty of removing water from its hydrogen-bonded environment of the bulk. TMAO, the renaturant, binds to the carbon nanotube exterior to a much lesser extent (Figure 7c).

4. Conclusion

In this paper, we examined the effects of three different cosolvents, two denaturants, urea and methanol, and one renaturant, TMAO, on the hydration of the interior of a nonpolar hydrophobe, the carbon nanotube. Since the diameter of the carbon nanotube used in the simulations is small enough to avoid the direct binding of cosolvent molecules to the nanotube interior, the results revealed indirect cosolvent effects on the hydration of the interior pore of the hydrophobe.

Supplementary to the earlier study,[17] our simulation results showed that different cosolvents have very different effects in changing the excess chemical potentials of the water molecules in the bulk and in the nanotube interior. As a result, urea improves the hydration of the interior of the nanotube while methanol and TMAO dehydrate the interior of the tube. Analyses of the structural properties of water, which is closely related to the cosolvents' abilities of changing water activity, were also conducted and revealed the indirect effects of cosolvents on the hydration of the nanotube interior. In particular, the observations on the water structure perturbation abilities of the cosolvents may be correlated to the indirect effects of the cosolvents on the hydration of the more complex hydrophobic cavities existed in proteins, although these effects are associated strongly with their direct effects. The fact that urea increases the occupation of the carbon nanotube by water is remarkable, given that the water density in the 7 M urea solution is only ~70% of that in pure water. This improved hydration of the carbon nanotube by urea is consistent with urea's role as a protein denaturant. It implies that, besides direct effect, urea is capable of denaturing proteins through an indirect mechanism, of which urea enhances the hydration of the interior hydrophobic cores of proteins. The drying of the carbon nanotube induced by TMAO is consistent with its effect on increasing the water activity inside the hydrophobic pore with respect to bulk water. Extended to proteins, TMAO actually protects protein structures by preventing the protein interior from being hydrated. The fact that methanol, although it is sometimes also categorized as a protein denaturant, dehydrates the carbon nanotube interior, indicates that it denatures proteins through a mechanism different from urea. In fact, it was found that methanol induces the formation of secondary structures, although it does denature proteins at high concentrations.[28] These results indicate that methanol does not change protein structure by enhancing hydration. Instead, it is likely to induce dehydration (such as that observed in this study). It likely denatures protein at high concentrations through direct protein/methanol interactions and reduction of the hydrophobic effects. It is also needed to mention that the widely used Jorgenson model of urea is applied in this study. A recent paper[31] shows that the Jorgenson model might have an incorrect activity coefficient with respect to concentration. However, compared to pure water and the other two cosolvents solutions, the distinct trend of the effect of urea on the hydration of the nanotube interior is well captured. We hope that more realistic urea models will allow

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(31) Kokubo, H.; Rosgen, J.; Bolen, D. W.; Pettitt, B. M. *Biophys. J.* **2007**, *93*, 3392.

us to make quantitatively more correct chemical potential calculations that can be tested by experiments.

The effects of the cosolvents on the dynamics of the interior water molecules were also studied. It was found that the residence time of water inside the nanotube is increased upon the addition of the cosolvents. Further analyses of the dynamics to clarify in details the effects of the cosolvents might prove to be interesting, especially for the usage of nanotubes as transportation devices.

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