Urea-Induced Drying of Carbon Nanotubes Suggests Existence of a Dry Globule-like Transient State During Chemical Denaturation of Proteins

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Atomistic dynamics simulations of purely hydrophobic carbon nanotubes in 8 M urea are performed to dissect the role of dispersion interactions in the denaturing power of urea. The enhanced population of urea and a paucity of water in proximity of nanotubes suggest that the stronger dispersion interaction of urea than water with nanotube triggers drying of its interior. The preferential intrusion of urea over water within nanotube interiors irrespective of their diameters directly implies a "dry globule"-like transient intermediate formation in the initial stage of protein unfolding in urea.

Introduction

In protein folding/unfolding studies, aqueous urea solutions have been widely used as chemical denaturants.[1] Although a vast experimental literature has been devoted in dissecting the molecular origin of the effect of urea on protein's stability,[2−6] the underlying molecular mechanism of urea's action on proteins still remains a controversial issue, particularly the dominant driving force and the intermediates involved in the process. On the basis of the extensive experimental and theoretical investigations in past decades, two different mechanisms for urea-induced protein unfolding have been proposed: an indirect mechanism focusing on the disruption of water structure by urea,[7−10] thus promoting the solvation of hydrophobic groups; and a direct mechanism[11,12] implying a direct interaction between protein and urea that can be dominated by either electrostatic or van der Waals forces.[6,13−17] To further complicate the picture, a two-stage kinetic model involving a "dry-globule" transient state was proposed for protein unfolding by urea.[17] In this model, in the initial stage, urea penetrates into the largely undisrupted hydrophobic core before water, forming a "dry globule", which is then followed by global unfolding of the protein. However, the transient nature of the proposed "dry globule" ensemble along with the hard-to-define hydrophobic core region and presence of both polar and nonpolar groups in proteins make further investigations of the dry globule formation very complicated. Moreover, because of the short lifetime, the detection of this dry globule ensemble may be nontrivial in experiments. Therefore, in this study, we design some model systems with single-walled carbon nanotubes (CNTs) to investigate the important phenomena and the driving force of denaturation further. Even though the chemical structure of a carbon nanotube is not exactly the same as a protein interior, the hydrophobic environment can be similar and also the physical dimensions are of comparable size (a few nanometers). In the following studies, we carefully choose the diameters and lengths of CNTs to mimic the size of the hydrophobic cores of a wide range of natural proteins.[18] On the other hand, the flow of water molecules inside carbon nanotubes has been widely studied both in simulations[19−21] and in experiments[22,23] because of the relevance of such nanoscale water channels in chemistry, biology, and material science. We here explore the effect of urea on the water occupancy of the carbon nanotube interior that allows us to investigate the possible formation of a dry globule ensemble as an initial intermediate state and further address the unfolding mechanism. By varying the diameters of CNTs, we perform a systematic investigation of the solvent intrusion in hydrophobic cores of different sizes. Such an investigation provides us an unambiguous molecular picture of the interaction mechanism of urea with a hydrophobic core.

TABLE 1: Dimensions (diameters and lengths) of CNTs and the Number of Solvent (water/urea) Molecules in the Solvated Systems of Various CNTs in 8 M Urea

CNT	diameter (Å)	length (Å)	no. of water molecules	no. of urea molecules
(6, 4)	6.8	18.24	971	228
(6, 6)	8.12	13.50	973	225
(8, 5)	8.88	16.10	962	222
(9, 6)	10.22	18.22	939	221
(10, 8)	12.2	33.04	3292	777
(17, 8)	17.29	31.55	3253	747

Model and Methods

The initial coordinates of single-walled CNTs were generated using the nanotube builder plugin of VMD software. We used (6, 4), (6, 6), (8, 5), (9, 6), (10, 8), and (17, 8) CNTs to study nanotubes with diameters ranging from 6.8 to 17.3 Å (Table 1). The length of CNTs varies from 1.35 to 3.3 nm (see Table 1). Two different molecular dynamics (MD) simulations were run for each nanotube solvated in a cubic box of pure water or 8 M aqueous urea (see Figure 1a and Table 1). The CHARMM22 (parameter set c32b1) force field and TIP3P water model are used in this study. The MD simulations of CNTs in pure water were used as control runs. We kept the position of the nanotube restrained by applying a harmonic constraint. The MD simulations were performed at 300 K in an NPT ensemble using NAMD2 molecular modeling package with 2 fs time step. Periodic boundary conditions were applied in all directions. The particle-mesh Ewald (PME) method was used for the long-range electrostatic interactions, whereas the van der Waals interactions were treated with a cutoff distance of 12 Å. All simulations were run using IBM BlueGene/L supercomputer. The simula-

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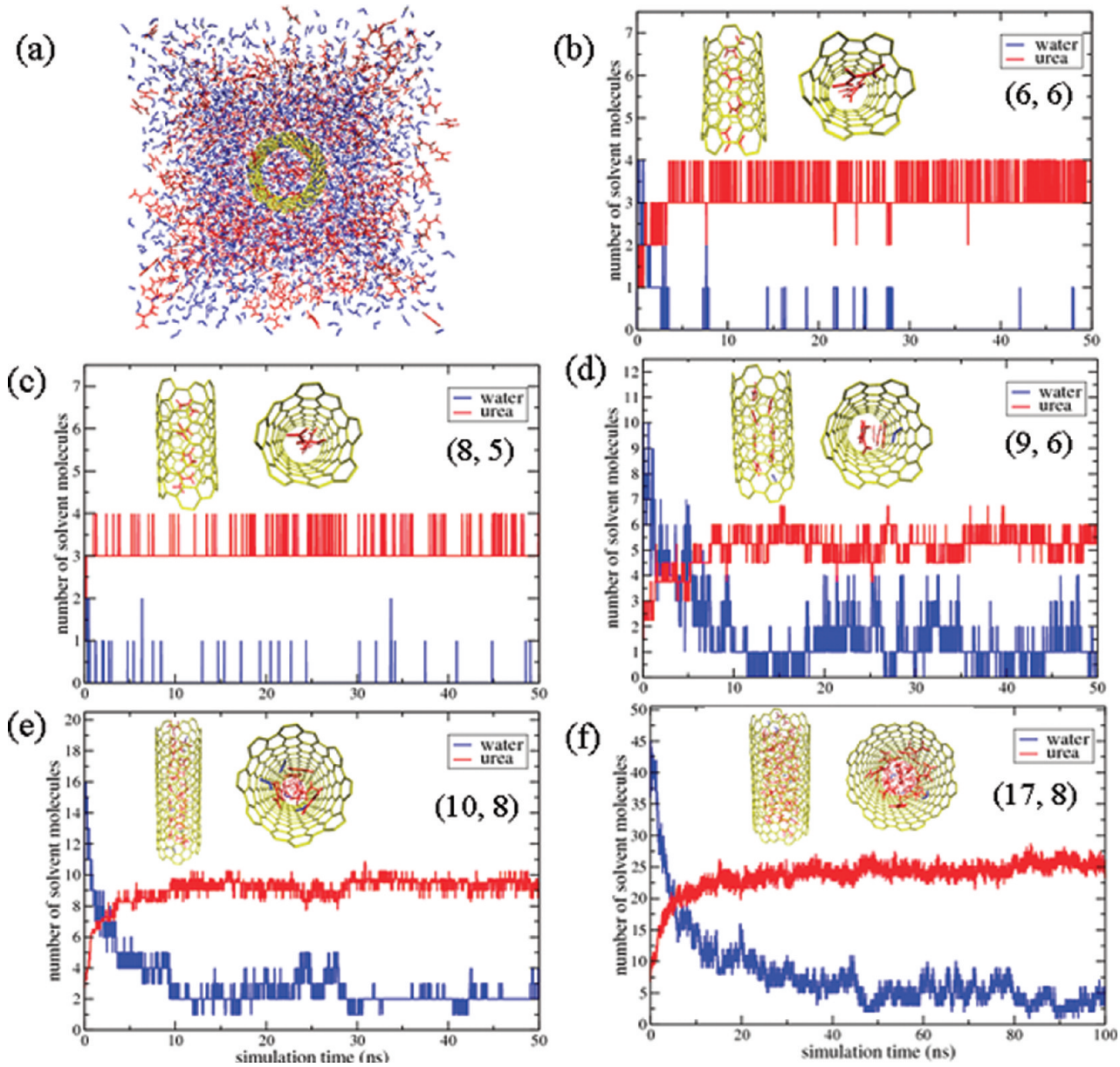

Figure 1. Urea-induced drying of carbon nanotubes. (a) The solvated system of (17, 8) CNT in 8 M aqueous urea. (b−f) Number of urea (shown in red) and water (shown in blue) molecules within nanotube core as a function of simulation time for CNTs with different diameter. The number of solvent molecules is normalized for a 1.35 nm long CNT to make easy comparison among different CNTs. Inset: Structure of urea/water molecules inside nanotubes from a radial and axial view. Solvent molecules are shown using licorice representation with urea in red and water in blue. CNT is shown in yellow.

tions of (6, 4), (6, 6), (8, 5), (9, 6), and (10, 8) CNTs solvated in either pure water or 8 M urea were run for 50 ns. Since the systems containing (17, 8) CNT took longer to reach equilibrium the simulation length for those systems was 100 ns. For comparison among results obtained for different CNTs, the number of solvent molecules was normalized with that for a 1.35 nm long CNT.

Results and Discussion

Our simulations indicate that the smaller CNTs [(6, 4) and (6, 6)] are hydrated with single-file water chains, whereas the larger CNTs are occupied with one or more two-dimensional layer(s) of water columns, consistent with previous investigation.[24] Figure 1b−f shows the number of water/urea molecules inside the nanotube core in 8 M aqueous urea during the course of MD simulation for CNTs with diameters larger than 6.9 Å. Surprisingly, all of these CNT cores become exclusively occupied by urea molecules. Even the core of the (6, 6) CNT is filled with 3−4 urea molecules within first 4 ns of MD by expelling all of the single-file water molecules. For larger nanotube cores, longer time is needed to replace most of the water molecules by urea. This remarkable observation clearly demonstrates the preferential intrusion of urea into the purely hydrophobic core of a nanotube. We find hydrogen-bonded urea molecules inside CNTs with a wide range of arrangements from single-stranded to double-stranded to disordered bulk-like depending on the diameter of CNT (Figure 1b−f, inset). This result evidently shows that urea penetrates a hydrophobic core before water, thus providing strong evidence to a dry-globule-like intermediate formation in the early stages of protein denaturation by urea.

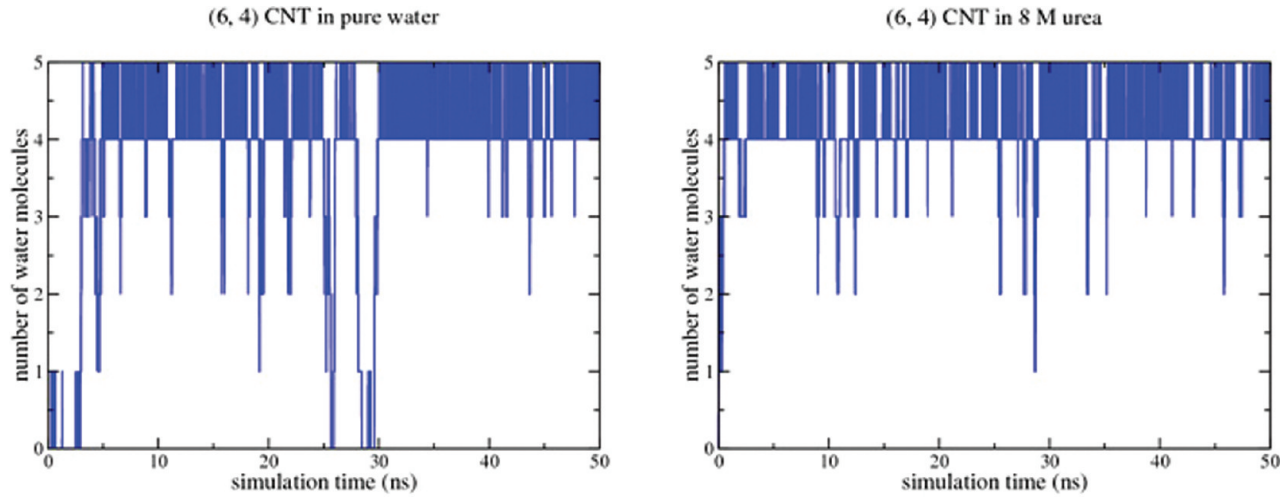

Figure 2. Number of water molecules within (6, 4) nanotube core in pure water (left) and in 8 M urea (right) as a function of simulation time. The (6, 4) nanotube interior becomes slightly more "wet" as a result of the electrostatic interaction between single-file water molecules inside the nanotube and accumulated urea molecules at the nanotube surface.

Urea molecules cannot penetrate the (6, 4) nanotube core because of its small size. Interestingly, in 8 M urea solution the (6, 4) CNT core becomes slightly more wet (Figure 2), as has been found by Gao and co-workers as well;[25] the average number of water molecules inside the nanotube increases from 3.94 in pure water to 4.36 in 8 M urea. This enhanced hydration would seem to support the indirect mechanism at first glance (i.e., urea acts as a water breaker, therefore, more water molecules "escape" into the carbon nanotube). However, a closer examination reveals that the enhanced hydration of the (6, 4) nanotube interior is due to the stronger attractive interactions between the inner water with the rest of the system, i.e., the environment. Compared to the pure water, each inner water gained an average energy of −0.28 kcal/mol in 8 M urea, mainly electrostatic (in pure water: van der Waals energy $E_{vdW} = -0.35$ kcal/mol, electrostatic energy $E_{elec} = -12.26$ kcal/mol; in 8 M urea: $E_{vdW} = -0.36$ kcal/mol, $E_{elec} = -12.53$ kcal/mol). This is due to the accumulation of urea molecules at the surface of the nanotube, which attracts favorably the water molecules inside the nanotube. Furthermore, if indirect mechanism dominates in the solvation of CNTs in 8 M urea, all CNTs regardless of their diameters should show similar enhanced wetting of the nanotube interiors, which is contrary to what we have observed in larger CNTs.

We further characterize the dynamics of the solvent molecules inside nanotube core by computing their residence time. Figure 3 shows the cumulative distributions (and the normalized histograms in Inset) of the residence times of water and urea within the (17, 8) CNT following a similar approach used by Hummer et al.,[19,26] as its interior remains wet for a considerably long time even in 8 M urea due to the larger size. Clearly, the residence time of urea is significantly longer than that of water inside nanotube, suggesting urea prefers to stay longer inside nanotube than water. We observed similar dynamic behavior of solvent molecules in all CNTs studied here.

Next, to explain the observed urea-induced dehydration of CNTs from an energetic perspective, we first computed the interaction energy of each water/urea solvent molecule with the environment, during the first 1 ns (long before the inner water molecules are replaced by urea in equilibrium) of the MD simulation in 8 M urea (Figure 4a). Below, we present the results of such analysis for (6, 6) CNT, the smallest nanotube that can fit urea molecules inside its core (similar results have been found

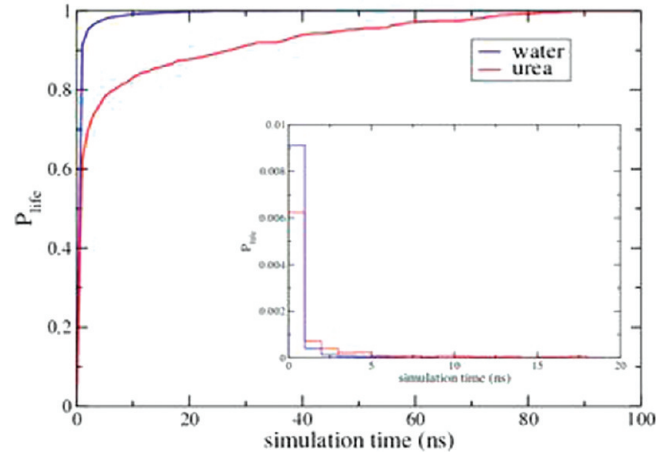

Figure 3. Cumulative distributions of the residence times (P_{life}) of the urea (red) and water (blue) inside a (17, 8) CNT. The normalized histograms of P_{life} are shown in the inset.

for other larger CNTs as well). We found that the loss in electrostatic interaction energy with rest of the system for a urea molecule (∼7.5 kcal/mol) is significantly larger than for a water molecule (∼5.0 kcal/mol) as the solvent molecule approaches the nanotube (Figure 3a, dashed lines). Thus, the electrostatic interaction clearly cannot be the driving force for preferential occupancy of urea within nanotube. On the other hand, there is a significant stabilization in the van der Waals (vdW) interaction energy for urea (approx. −9.5 kcal/mol) compared to water (approx. −4.5 kcal/mol) as it moves closer to the CNT (Figure 4a, solid lines). Thus, each urea gains about −2.0 kcal/mol (−9.5 + 7.5 kcal/mol) on average as it penetrates the CNT, whereas each water loses about 0.5 kcal/mol (−4.5 + 5.0 kcal/mol) for the same process. This driving force with stronger vdW interactions can also be seen from the interaction energy of each water/urea molecule with the nanotube (CNT only in this case) in Figure 4b. The average CNT-urea vdW interaction energy (approx. −17.5 kcal/mol) within the core is significantly higher than the CNT-water interaction energy (approx. −5.0 kcal/mol) (on the other hand, no electrostatic interactions between urea/water and the CNT, because there is no charge on CNT)). The much stronger dispersion interaction energy of urea than water with CNT results in an enhanced urea accumulation not only in the CNT interior but also at the CNT surface, as seen in our

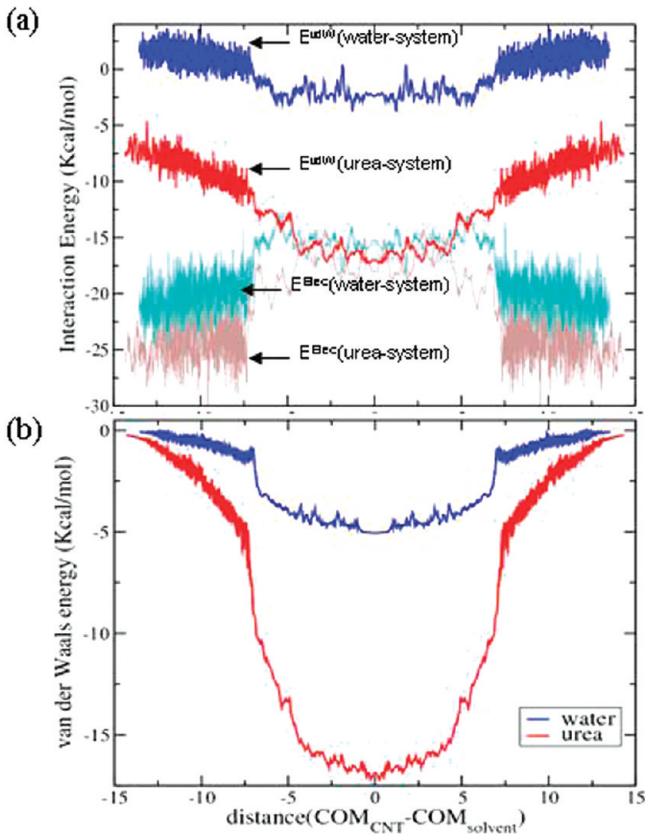

Figure 4. (a) Interaction energies (kcal/mol) with the rest of the system in 8 M urea for a water or a urea molecule as a function of its distance (Å) from the center of mass of the (6, 6) nanotube. The van der Waals interaction energy of water with the rest of the system is shown in blue, whereas the same for urea is shown in red. The electrostatic interaction energies with rest of the system are shown in cyan for water and in brown for urea. (b) van der Waals energy (in kcal/mol) of a water (in blue) or a urea (in red) molecule with the (6, 6) nanotube (CNT-only) during the first nanosecond of MD simulation in 8 M urea as a function of its distance from the center of the mass of the nanotube.

MD simulations. It should be noted that the replacement of initially constrained water molecules by larger urea inside the nanotube core is also favorable because of a solvent entropy gain. Thus, this interaction energy decomposition analysis shows the higher affinity of urea than water to the nanotubes as a result of stronger dispersion interaction of urea than water with hydrophobic moieties. Such enhanced population of urea with scarcity of water has been also observed in the first solvation shell of the protein during initial stages of lysozyme unfolding in 8 M urea.[27] Thus, our results provide strong support to the direct interaction mechanism of protein unfolding in presence of urea, in which the stronger dispersion interaction between protein and urea is the primary driving force.

Conclusions

In conclusion, a striking phenomenon of urea-induced drying of the hydrophobic nanotube cores is observed in MD simulations. The results of this study demonstrate the role of stronger dispersion interaction of urea than water with hydrophobic carbon nanotubes as the primary driving force of this phenomenon. The dehydration of the nanotube interiors in aqueous urea provides direct evidence to a "dry-globule"-like transient intermediate formation in the initial stages of urea-induced protein unfolding. Thus, this study offers an unambiguous molecular picture of the hydration of a hydrophobic core in aqueous urea that is driven by the preferential direct dispersion interaction of urea than water with the hydrophobic core.

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