Enhanced Pair Hydrophobicity in the Water−Dimethylsulfoxide (DMSO) Binary Mixture at Low DMSO Concentrations

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We observe a surprisingly sharp increase in the pair hydrophobicity in the water−dimethylsulfoxide (DMSO) binary mixture at small DMSO concentrations, with the mole fraction of DMSO (x_D) in the range 0.12−0.16. The increase in pair hydrophobicity is measured by an increase in the depth of the first minimum in the potential of mean force (PMF) between two methane molecules. However, this enhanced hydrophobicity again weakens at higher DMSO concentrations. We find markedly unusual behavior of the pure binary mixture (in the same composition range) in the diffusion coefficient of DMSO and in the *local* composition fluctuation of water. We find that, in the said composition range, the average coordination number of the methyl groups (of distinct DMSO) varies between 2.4 and 2.6, indicating the onset of the formation of a chain-like extended connectivity in an otherwise stable tetrahedral network comprising of water and DMSO molecules. We propose that the enhanced pair hydrophobicity of the binary mixture at low DMSO concentrations is due to the participation of the two methane molecules in the local structural order and the emerging molecular associations in the water−DMSO mixture.

I. Introduction

The effects of cosolvents in biological systems have received considerable attention in recent years. Cosolvents (with water) like methanol and trifluoroethanol (TFE) lead to protein denaturation,[1] whereas cosolvents like trehalose and glycerol prevent denaturation.[2,3] Cosolvents can also markedly enhance the activity of an enzyme. Such behavior of cosolvents has been engineered in a variety of ways to reflect several important properties of proteins, enzymes, and other biopolymers. Interesting properties of the cosolvents have been exploited in several other situations,[4] such as the cryopreservation of sperm and stem cells, the development of highly reliable organ-preserving solution, and so forth. Understanding the basic nature of such behavior can be used to exploit more specific "co-solvent engineering" and create biological and nonbiological polymers with desired functionalities.

Dimethyl sulfoxide (DMSO) behaves as an anomalous cosolvent.[4] It increases enzymatic activity at low concentrations but decreases the same at higher concentrations. It is a protein stabilizer at low concentrations,[5,6] whereas it is a protein destabilizer at higher concentrations.[7] In experiments it has been found that this crossover takes place at very low concentrations of DMSO, in the range of DMSO mole fraction, $x_D \leq 0.15$. Understanding the anomalous behavior of DMSO might help us in revealing the underlying mechanism of stabilizing and destabilizing behaviors, especially of the crossover. In the recent past, a considerable amount of research has been devoted to study the interactions in water−DMSO binary mixtures.

The binary mixture of water and DMSO exhibits strongly nonideal behaviors. The nonideality has been reflected in a number of physical properties such as viscosity, density, dielectric constant, excess mixing volume, translational and rotational diffusion constants, and heat of formation, to mention a few. However, it has been found that most of these deviations occur at 30−40% of DMSO by composition, much higher than the composition range where the crossover from "protein-stabilizer" to "protein-destabilizer" takes place.

Several simulation studies have been directed toward the understanding of such anomalous behavior. Vaisman and Berkowitz[8] simulated diluted mixtures ($x_D = 0.005, 0.04, 0.2$). They observed that the lone pairs of the electron on the oxygen atom of DMSO form two hydrogen bonds with two water molecules. This association leads to certain local ordering.[8] These hydrogen bonds are found to be more stable than the hydrogen bonds among two water molecules in the bulk.[10] Soper and Luzar[9] investigated the structure of water in concentrated aqueous DMSO solution ($x_D = 0.21$ and 0.35) by neutron diffraction. They determined the partial structure factors and pair correlation functions and found that the water structure is not strongly affected by the presence of DMSO. However, the percentage of water molecules which hydrogen bond to themselves is substantially reduced compared to pure water, with a large proportion of the hydrogens available for bonding associated with the lone pairs on the DMSO. Following the neutron diffraction study, Luzar and Chandler[10] performed molecular dynamics (MD) simulations at those two compositions ($x_D = 0.21$ and 0.35). They studied the structural properties, H-bond distribution, and H-bond dynamics. They found that the local tetrahedral ordering of the water molecules is preserved at both the concentrations. They also found the presence of 1DMSO−2H$_2$O complexes of nearly tetrahedral geometry. Borin and Skaf[11] presented simulation results of water−DMSO mixtures over the whole range of composition, focusing on the study of local structures, H-bond distribution, and dynamical properties. However, the anomalous behavior of DMSO in the low concentration regime could not be explained by the above simulation studies. We anticipated that hydrophobicity can be one of the many reasons for such behavior of DMSO. Thirumalai et al.[12] have studied hydrophobic effects in water−urea mixture, but there has been no systematic study of the hydrophobicity in water−DMSO mixture.

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The hydrophobic effect needs to be understood at several levels. First is the so-called bulk hydrophobicity or hydrophobic hydration, which is conventionally expressed in terms of solubility of the solute in water. One can construct a hydropathy scale by considering the enthalpy of transfer of a mole of solute from ethane to water.[13,14] Such a hydropathy scale has been proven to be useful in the study of protein folding.[15,16] However, in understanding the aggregation of polymers and also the collapse transition in polymers and proteins, a more detailed view is obtained by studying the pair hydrophobicity. The latter can be expressed in terms of a potential of mean force (PMF) between two solute atoms or molecules.[17] The pair hydrophobicity, conventionally known as hydrophobic interaction, can sometimes offer a different view than what one would expect from the knowledge of hydrophobicity of the individual groups, due to the involvement of many-body interactions. For example, the PMF calculated from protein data bank shows the presence of a weak attractive interaction between two polar amino acid residues, like arginine, which is not anticipated from bulk hydrophobicity. This attraction is due to bridged hydrogen bonding by water molecules.[17] Such three-body interactions can be particularly important when the solvent is a binary mixture because of several interactions that are available to the system.

In this paper, we report the *first ever* molecular dynamics (MD) study of methane molecules in an explicit model of the water−DMSO mixture. To understand the effect of DMSO as a cosolvent, we study the PMF between two methane molecules in the water−DMSO mixture with a varying composition range. The PMF for the methane−methane association is a good measure of the hydrophobic interaction of the solvent. The first minimum in the PMF (also known as contact minimum, CM) is a measure of the pair hydrophobicity, whereas the second minimum or solvent separated minimum (SSM) is directly related to the solvent effects. The CM and SSM are separated by a desolvation barrier.[18−23] Our study reveals that the hydrophobic effect of water−DMSO mixture initially increases with increasing concentration, undergoes a transition at $x_D \sim 0.15$, and then decreases again. Such behavior can be helpful to explain the anomalous behavior of DMSO at the low concentration regime. The anomalous behavior of DMSO in the low concentration region has been attributed to a phase-transition-like behavior of the binary mixture. The simulation studies explicitly reveal that the motion of the DMSO molecules does not change in the transition region, which provides an insight to the origin of nonideality in the water−DMSO mixture. We have found a local structural ordering among the DMSO molecules at $x_D \sim 0.15$. The transition starts at $x_D \sim 0.125$, until which the DMSO molecules added to the mixture are used up to form the structural orientation, and hence the collapse of the biopolymers is reinforced. Beyond $x_D \sim 0.15$, the DMSO molecules are no longer used in the structure formation and are free to break the structure of other foreign molecules like enzymes, proteins, and so forth. The invariance in the motion of the DMSO molecules with the change in concentration is also reflected in the anomalous behavior of the diffusion coefficient of the DMSO molecules near $x_D \sim 0.125$. Our results are also supported by the anomalous behavior of composition fluctuation of water at the same concentration.

The organization of the rest of the paper is as follows. In the next section we discuss the systems and the simulation details. In Section III we discuss the results, both on pair hydrophobicity (or hydrophobic interaction) of two methane molecules and also on pure binary mixture. In Section IV we discuss an explanation of the reason for the enhanced pair hydrophobicity observed

here for the first time. Section V concludes with a summary and a discussion of the future problems.

II. Details of Simulation

All of the simulations have been done at 300 K and 1 bar pressure. The extended simple point charge model (SPC/E) model[24] was employed to study the water−water interaction. Full atomistic details have been retained for every molecule in the simulation except for the hydrogen atoms bound to carbon atoms. The hydrogen atoms attached to the carbon atoms were treated as united atoms within the GROMOS96 53a6 force field.[25] To perform the MD simulation, we have chosen GROMACS (version 4.0.5), which is a highly efficient and scalable molecular simulation engine.[26−29]

The Jarzynski relation[30] can be employed for obtaining the equilibrium free energy difference ΔG between two distances from many nonequilibrium simulations.

$$\Delta G_{AB} = -k_B T \log \langle e^{-\beta W_{AB}} \rangle_A \qquad (1)$$

where W_{AB} is the work performed to force the system along one path from state A to B, the angular bracket denotes averaging over a canonical ensemble of the initial state A, and $\beta = 1/k_B T$. In our simulation, the Jarzynski relation was used to calculate the PMF using MD, coupled with constraint pulling— a method described below.

For calculating the PMF, the simulation box consisted of two methane molecules and corresponding solvent molecules. MD simulation was performed for steepest descent energy minimization and equilibration of the NPT system. After equilibration, the distance between the center of mass of the two methane molecules was determined. Next, position restraint MD simulation was performed to equilibrate the NPT system with the positions of the methane molecules restrained. In the subsequent production run by constraint pulling method, the SHAKE algorithm[32] was employed for calculating the constraint force. We obtained the mean constraint force and also the error estimate from that simulation. We performed the constraint simulations at constantly spaced distances between 0.28 and 1 nm. Each simulation, put in a separate trajectory, started from the last conformation of the previous distance and was allowed to run for 20 ns. The mean constraint force, thus obtained, was corrected for entropic contribution and integrated to obtain the PMF.

For calculating the radial distribution functions,[31] simulation boxes consisting of 60 methane molecules were generated along with corresponding solvent molecules. The system was equilibrated by NVT simulation, followed by NPT simulation, both at 300 K and for 20 ns. The production run was carried out using the final NPT system.

For the calculation of the solvent properties like composition fluctuation and diffusion coefficient we have used cubic solvent boxes, with sides 3.0 nm filled with the same explicit model water and DMSO. NVT simulation followed by NPT simulation was performed for the equilibration using the same force field (GROMOS96 53a6). Subsequently the production run was carried out for 20 ns at 300 K with the final NPT system.

III. Results and Discussion

A. CH₄−CH₄ Potential of Mean Force (PMF). Since the theoretical work of Pratt and Chandler[19] in 1977, there has been growing interest to study the hydrophobic effect using the PMF between methane molecules. In an earlier work, Dang[33] had
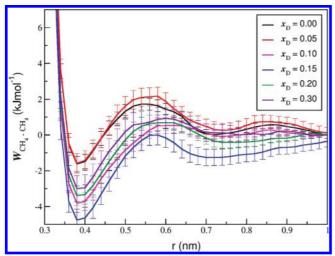
Hydrophobicity in Water−DMSO Binary Mixture

J. Phys. Chem. B, Vol. 114, No. 40, 2010 **12877**

Figure 1. CH$_4$−CH$_4$ PMF in the water−DMSO binary mixture. Results are shown for varying concentrations of DMSO in the water−DMSO binary mixture. The plots at various concentrations are shown in different colors, as described in the legend (x_D is the mole fraction of DMSO in the binary mixture). The X-axis is the distance of separation between the methane groups, and the Y-axis is the CH$_4$−CH$_4$ PMF ($W_{CH_4−CH_4}$). The error bars represent the variance from the mean value of W, calculated over the entire trajectory. All PMFs are asymptotic to zero at large r. It is interesting to note that the minima of the PMF appears at the same distance for different concentrations of DMSO, but the value of the PMF changes with concentration.

reported the PMF between two methane molecules in pure water at 300 K and 330 K, using the SPC/E model of water. The first minimum (contact minimum) of the PMF was found to be at r = 0.39 nm. In another recent work,[12] Thirumalai et al. have studied the CH$_4$−CH$_4$ PMF in the water−urea mixture. In this paper we report the study of PMF between two methane molecules in the water−DMSO mixture.

If $g_{AA}(r)$ denotes the pair correlation function of two spherical nonpolar species (A) dissolved in water, then the PMF

$$W_{AA}(r) = -k_B T \ln g_{AA}(r) \qquad (2)$$

is a good measure of the solvent-induced interactions between the particles. $W_{AA}(r)$ is the reversible work required to bring the two particles from infinite separation ($r = \infty$) to r. In our study, we have calculated the PMF for the CH$_4$−CH$_4$ association to account for the hydrophobic effect of water−DMSO mixture. This method is rigorous and reliable and provides more detail about the association process of the hydrophobic solutes.[34]

Figure 1 shows the plot of the CH$_4$−CH$_4$ PMF with varying concentrations of DMSO. It shows a strong nonideality in the behavior with a considerable increase in the depth of the first minimum (contact minimum) when the mole fraction of DMSO is $x_D \sim 0.15$ in the mixture. The contact minimum appears at r = 0.38 nm. We note that the position of the contact minimum shows no change with the increasing concentration of DMSO. The depth of the contact minimum, however, shows significant changes. The magnitude of the depth initially increases with increasing concentration of DMSO in water up to $x_D \sim 0.15$ and then starts decreasing again. To understand this transition, the depth of the contact minimum has been plotted against the concentration of DMSO in Figure 2. The anomalous behavior in the depth of the contact minimum becomes clearer from this figure. Thus, there is a significant increase in the hydrophobic interaction of the methane molecules until $x_D \sim 0.15$, and then
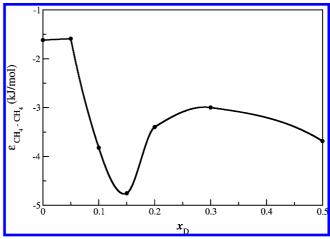

Figure 2. Minima ($\varepsilon_{CH_4−CH_4}$) of the CH$_4$−CH$_4$ PMF in the water−DMSO binary mixture. The corresponding minima ($\varepsilon_{CH_4−CH_4}$) for the various concentrations of the water−DMSO binary mixture were obtained from the PMF plots in Figure 1. x_D is the mole fraction of DMSO in the binary mixture.
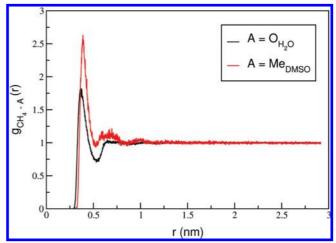

Figure 3. Site−site radial distribution functions (rdf) between different groups in the solution of methane in water−DMSO binary mixture. The black curve shows the rdf ($g_{CH_4−O_{H_2O}}$) of methane and oxygen of water (O_{H_2O}). The red curve ($g_{CH_4−Me_{DMSO}}$) shows the rdf of methane and the methyl (Me$_{DMSO}$) group of DMSO. The concentration of the binary mixture is given by DMSO mole fraction, $x_D = 0.15$.

the interaction starts decreasing with increasing DMSO concentration. The second minimum, which is better known as the solvent-separated minimum, however follows no trend but has a significant dip for $x_D = 0.15$. The hydrophobic interaction is directly related to the hydrophobic effect of the solvent. Hence, Figure 2 reflects an unusual change in the hydrophobic effect of the water−DMSO mixture with varying concentration.

To analyze the situation in more detail, we studied the radial distribution functions between various groups in the solvent. In Figure 3, we present a comparison of the radial distribution function of the methane molecules with respect to the methyl groups of DMSO and the O atoms of water, at $x_D = 0.15$ at 300 K. It clearly shows the prevalence of DMSO molecules in the first hydration shell of the methane molecules.

B. Probing Water−DMSO Mixture. In an effort to find the origin of the anomaly in the hydrophobic effect of water−DMSO mixture, we did a systematic study of the solvent. We have found anomalous behavior in the diffusion coefficient of DMSO and average composition fluctuation of water. We have tried to explain the origin of the anomaly by proposing a
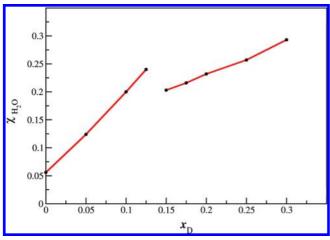
Figure 4. Average relative composition fluctuation (χ_{H_2O}) of water at varying composition of the water−DMSO binary mixture. x_D is the mole fraction of DMSO in the binary mixture.

Figure 5. Diffusion coefficient of DMSO (D_{DMSO}) at varying compositions of water−DMSO binary mixture. x_D is the mole fraction of DMSO in the binary mixture.

weak phase-transition-like behavior due to the ordering of the DMSO molecules.

i. Anomaly in Transport Properties of Binary Mixture. To understand the local structural orientation in the water−DMSO binary mixture, we have calculated the composition fluctuation of water molecules within a sphere of 0.7 nm defined in the simulation box. In Figure 4 we have plotted the average relative composition fluctuation (χ) of water against the varying concentration of DMSO in the solvent mixture.

The average composition fluctuation, $\langle\chi\rangle$, is calculated by averaging over the instantaneous fluctuation χ_i obtained from every i^{th} step:

$$\chi_i = \frac{|N_{w_i} - \langle N_w \rangle|}{N_{w_i}} \tag{3}$$

$$\langle\chi\rangle = \frac{1}{N_{step}} \sum_{i=1}^{N_{step}} \chi_i \tag{4}$$

where N_{w_i} is the number of water molecules found in the specified sphere at the ith step and $\langle N_w \rangle$ is the average number of water molecules within the sphere over the whole range of simulation.

Figure 4 shows an abrupt fall in the average of instantaneous composition fluctuation of water in going from $x_D \sim 0.125$ to $x_D \sim 0.15$. The average of instantaneous composition fluctuation of the water molecules increases smoothly up to $x_D \sim 0.125$, abruptly falls at $x_D \sim 0.15$, and then increases again but at a slower rate. This indicates that there might be some local structural ordering in the system, which starts forming at $x_D \sim 0.125$ and prevents the free motion (or local fluctuation) of the water molecules. The onset of such local ordering also becomes evident from the plot of the diffusion coefficient of DMSO over the wide spectrum of water−DMSO mixture, as shown in Figure 5.

The diffusion coefficients were calculated from the slopes of the plot of mean square displacement against time and were verified with results from the velocity autocorrelation plots. Figure 5 also shows a deviation at around $x_D \sim 0.15$. It may also be noted here that the slope of the plot up to $x_D \sim 0.125$ is different from the slope of the line starting from $x_D \sim 0.15$. Thus the rate of decrease of the diffusion coefficient also
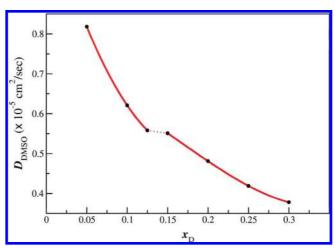
undergoes a change in this region of interest, that is, $x_D \sim 0.15$. It is evident from these anomalous behaviors of the water−DMSO binary mixture that the motions of the DMSO molecules become invariant with the change in concentration ($x_D \sim 0.125-0.15$). This can be due to some sort of structural ordering which we will discuss in the next subsection.

ii. Origin of the Anomaly in the Water−DMSO Binary Mixture. Until now we have discussed the anomalous hydrophobic effect of the water−DMSO mixture, as measured by the CH_4-CH_4 PMF. The hydrophobic interaction becomes most prominent at $x_D \sim 0.15$. We have also found anomalous behaviors in the diffusion coefficient of DMSO and the average of instantaneous composition fluctuation of water. Surprisingly, all of these anomalies were observed in the range $x_D \sim 0.125-0.15$. This is a clear indication of structural ordering in the system in that concentration range.

Examples of anomalous behavior in binary mixtures are plenty and have been extensively studied. However, the anomalous behavior at $x_D \sim 0.15$ seems to be out of the ordinary and demands proper explanation. So we have tried to explore the reason for such anomaly. One possible origin is some sort of association, or aggregation, of the DMSO molecules because the diffusion coefficient of DMSO becomes invariant when the concentration of the binary mixture is changed from $x_D \sim 0.125$ to $x_D \sim 0.15$, and subsequently there is a slower rate of decrease of the diffusion coefficient of DMSO. The formation of $1DMSO-2H_2O$ clusters in this concentration range is a well-known fact. However, the formation of a network with these clusters requires 33% DMSO in the mixture (but, see the discussion below). Hernandez−Perni and Leuenberger have *speculated* that the percolation phenomena is responsible for observed anomaly in the dielectric spectra of the binary water−DMSO mixtures. We predict here the formation of association of DMSO molecules, via the methyl (Me) groups of DMSO. In a tetrahedral system, the percolation transition occurs at a critical concentration (P_c) given by the Flory formula,

$$P_c = \frac{1}{z - 1} \tag{5}$$

where $z = 4$ for a tetrahedral lattice. Hence, if the Me groups undergo a percolation-like transition, then it should occur at $P_c \sim 0.33$ of the Me groups. Since each DMSO molecule has two Me groups, it is expected that such transition should occur at

Hydrophobicity in Water−DMSO Binary Mixture

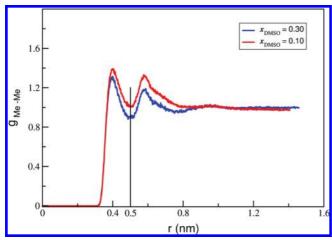*J. Phys. Chem. B, Vol. 114, No. 40, 2010* **12879**

Figure 6. Radial distribution function (rdf) of two methyl (Me) groups of distinct DMSO molecules. The qualitative nature of the plot remains same at two different concentrations of the water−DMSO binary mixture, $x_D = 0.30$ (blue line) and $x_D = 0.10$ (red line). The first peak appears at 0.39 nm, and the first minimum appears at 0.5 nm. The vertical black line denotes the position of the first minimum.

Figure 8. Total fraction of contacts between Me groups of DMSO molecules in the water−DMSO binary mixture. Π_{Me-Me} is the total fraction of contacts within a cutoff of 0.5 nm per unit volume per DMSO. x_D is the mole fraction of DMSO in the binary mixture. The total fraction at each concentration of the binary mixture was obtained by integrating over the corresponding histograms (see Figure 7).

Figure 9. Average number of Me groups ($\langle N_{Me} \rangle$) surrounding each Me group within a cutoff radius of 0.5 nm. The second methyl group of the central DMSO molecule has been excluded from the counting. This plot shows the variation of $\langle N_{Me} \rangle$ with the change in concentration of the water−DMSO binary mixture. x_D is the mole fraction of DMSO in the binary mixture.

$x_D \sim 0.165$. The predicted transition region is thus very close to the concentration range of the observed anomalies.

As mentioned above, a percolation with 1DMSO−2H$_2$O structural units is difficult to imagine unless the water molecules themselves participate by H-bonding. In such a situation, an alternative picture of percolation can be developed by considering 1DMSO−2H$_2$O as a distinct structural entity. As this entity has a functionality of 8 (i.e. $z = 8$), Flory theory would predict a percolation threshold to occur at $P_c = 1/(8-1) \approx 0.143$ of the structural motifs. Since each DMSO consumes 2H$_2$O molecules, the mole fraction of 0.143 of the structural units corresponds to a mole fraction of 0.111 of the DMSO molecules in the binary mixture. Therefore, this model also produces a value of DMSO concentration in the observed range of anomalies, albeit a little low.

It is always difficult to capture weak phase transitions from a finite size simulation. We have carried out the following analyses to support the predicted percolation-like transition. We have searched for DMSO aggregations via methyl groups (Me) in the system. We calculated the radial distribution function (rdf), $g(r)$, of the Me groups. The plot is shown in Figure 6. This confirms that there is no shift in the peak of $g(r)$ at various concentrations. It also provides us with a cutoff of 0.5 nm for our subsequent calculations with the Me groups. We then looked for pattern formation by plotting the histogram of the average number of contacts between Me groups of distinct DMSO
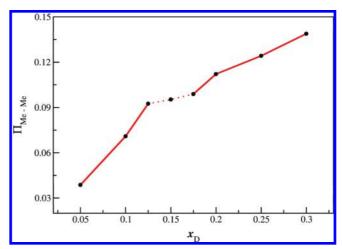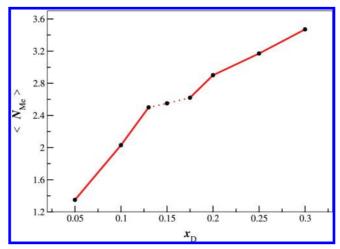
molecules within a cutoff of 0.5 nm. Results are shown in Figure 7. It shows the histogram of fraction of contacts per unit volume per DMSO molecule. Apparently there is no significant difference or anomaly in the histogram plots. However, when we
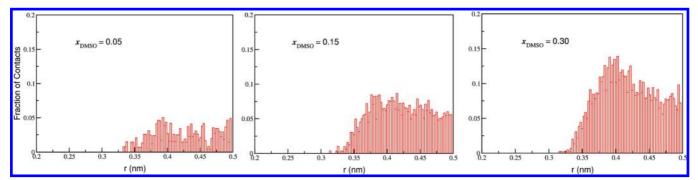

Figure 7. Histogram of the fraction of contacts between methyl (Me) groups of DMSO molecules in the water−DMSO binary mixture. The average numbers of contacts between Me groups of DMSO were calculated within a cutoff radius of 0.5 nm. The second methyl group of the central DMSO has been excluded from the counting. The histogram shows the fraction of such contacts per unit volume per DMSO, with increasing distance.

Figure 10. Energy-minimized (EM) structures of the water−DMSO binary mixture. The EM structures are shown at various compositions, (a) $x_D =$ 0.05, (b) $x_D = 0.10$, (c) $x_D = 0.15$, and (d) $x_D = 0.20$. The red beads represent water molecules, and the yellow beads represent DMSO molecules.

integrate the plots to find the total fraction of contacts per unit volume per unit DMSO, we get the first direct evidence of clustering of Me groups of DMSO. This is shown in Figure 8. It shows that the fraction of contacts below 0.5 nm steadily increases up to $x_D \sim 0.125$, and then it remains almost steady in the range $x_D \sim 0.125-0.175$. It again increases after $x_D \sim 0.175$ but at a much slower rate.

Thus, it is evident that the number of contacts reaches a saturation, or a critical value at $x_D \sim 0.125$. We have also calculated the average number of Me groups surrounding each Me group (excluding the Me group in the same DMSO), within a cutoff of 0.5 nm, as shown in Figure 9. The average number of Me groups, from distinct DMSO molecules, surrounding each Me group within a cutoff of 0.5 nm, shows a critical point at $x_D \sim 0.125$. Near the "critical-point" (at $x_D \sim 0.125-0.15$) the average number of "other" Me groups surrounding each Me group is $\sim 2.4-2.6$.

iii. Energy-Minimized Structures. The energy-minimized structures of the water−DMSO binary mixture also show the signature of the percolation, as shown in Figure 10. The energy-minimized structures were obtained by conjugate gradient energy minimization of the parent system at 300 K. Here we have viewed the water and DMSO molecules as beads to obtain a clearer view of the percolation. In Figure 11, we have shown only the methyl groups of DMSO at various concentrations of the binary mixture. All methyl groups which are within 0.39 nm have been connected. Hence the methyl groups of isolated DMSO molecules appear as rods, whereas the methyl groups of the network appear as branched chains. We note here that there is an emergence of a cluster-like structure at $x_D = 0.15$, which supports our speculation of a percolation transition.

IV. Reason for the Anomaly in CH_4-CH_4 Potential of Mean Force

Figures 4−5 and 8−9 clearly indicate that the water−DMSO binary mixture itself exhibits a weak phase-transition-like

behavior in the same composition range where we find the strong enhancement in the pair hydrophobicity. All of these properties are highly unusual and still elude a quantitative explanation. In the following we attempt to correlate the observed anomaly in the water−DMSO mixture with the enhanced pair hydrophobicity.

We note here that the contact minimum between the two methane molecules appears at $r = 0.38$ nm (Figure 1) and the first peak of the $g(r)$ of two Me groups of two distinct DMSO molecules appears at $r = 0.39$ nm (Figure 6). This extreme closeness clearly suggests that the hydrophobic CH_4 molecules are congruent with the structural orientation of the DMSO molecules and appear as substitutes for the Me groups. The CH_4 molecules help the aggregation of the DMSO molecules until $x_D \sim 0.15$, beyond which the inclusion of CH_4 molecules is no longer favored, as the DMSO molecules are self-sufficient to form the network. Free energy gained by accommodating more CH_4 molecules, due to their inherent relatively weak interaction with water molecules, might not be as rewarding as when it was below the critical aggregation composition.

The phase-transition-like behavior, coupled with the anomalous hydrophobic effect, can be used to explain the protein stabilizing and destabilizing behavior of DMSO. It can as well account for the anomalous behavior of DMSO as a cosolvent in studies of enzymatic activity. Until $x_D \sim 0.125$ DMSO does not aggregate completely, and hence the added DMSO might be completely used up in forming structures among itself. The hydrophobic segments of the biopolymer may also assist in the structure formation. This point needs further exploration. Hence the biopolymers are stabilized at lower concentration of DMSO. Subsequently, after $x_D \sim 0.15$, free DMSO molecules, which are not involved in the association, start increasing in number and interact with the exposed hydrophobic regions of the biopolymers, stabilizing them and making the further opening of the hydrophobic core energetically favorable. This facilitates the denaturation of the biopolymers.
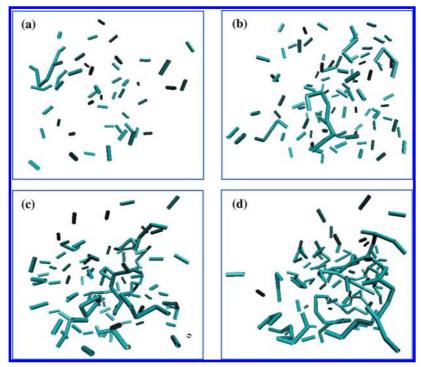
Figure 11. Network formation among methyl groups of DMSO molecules in the EM structures. The figures show only the methyl groups of DMSO in the EM structures of the water−DMSO mixture at various concentrations, (a) $x_D = 0.05$, (b) $x_D = 0.10$, (c) $x_D = 0.15$, and (d) $x_D = 0.20$. All methyl groups within 0.39 nm have been connected. Hence, the single rods are methyl groups of the same DMSO molecule. The branched chains are the network of the methyl groups.

V. Conclusion

This work reports a series of studies motivated by the need to understand the anomaly of the water−DMSO binary mixture. DMSO is a unique cosolvent which helps in stabilizing proteins at low concentrations but denatures proteins at higher concentrations. We studied the PMF between two methane molecules in the water−DMSO mixture over a wide concentration range and found that the depth of the contact minimum increases up to ∼15% concentration of DMSO ($x_D \sim 0.15$) and then decreases. In a systematic attempt to find the origin of this anomaly, we studied the average of instantaneous composition fluctuation of water over a range of concentrations ($x_D \sim 0.05−0.30$) and found interesting deviations at the same concentration range ($x_D \sim 0.15$). The diffusion coefficient of DMSO also shows a weakening dependence on composition, in the same concentration range. To explain all of these anomalous behavior of the water−DMSO binary mixture we have proposed a percolation-like phase transition. We have indeed observed the formation of a chain-like association of DMSO molecules via the methyl groups in DMSO. We have presented plots of the fraction of contacts between Me groups below 0.5 nm, as well as a plot of average coordination number of the Me groups. These plots reveal a weak diffuse phase transition starting at $x_D \sim 0.125$ and ending at around 0.15.

This work provides a new explanation and mechanism to understand the origin of anomalies in the water−DMSO mixture. Whether this provides a general explanation (applicable to other binary mixtures) toward understanding the coexistence of both stabilizing and destabilizing behavior of cosolvents remains to be verified. Our study also provides an explanation for the observed increased stability of hydrophobic macromolecules in water−DMSO mixtures.

The present study suggests several future problems. We have not been able to conclusively prove the nature of the onset of association in the water−DMSO binary mixture—this would require a more elaborate study which should involve the calculation of the inherent structures of the mixture. Second, it would be interesting to study the altered nature of polymer collapse in this mixture. Third, it would be highly worthwhile to develop microscopic approach, similar to Pratt−Chandler theory,[19] for hydrophobicity in the binary mixture. Investigations on these problems are under progress.

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