Solvent deuteration enhancement of hydrophobicity: DSC study of the inverse temperature transition of elastin-based polypeptides

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in the Stern layer are important, then it must be questioned as to why the counterion quadrupole splittings of $Co(NH_3)_6^{3+}$ do not show large changes as the amphiphile replacement occurs (Figure 4). The evidence suggests that this ion is tightly bound so it is an integral part of the Stern layer. As a point of interest in this regard, it should be noted that $Al(H_2O)_6^{3+}$ in the mixed tetradecyltrimethylammonium bromide/pyridinium bromide mesophase system showed no quadrupole splitting dependence on the replacement of one amphiphile by another.[17]

The cyanocobaltate(III) complex was also studied as a counterion in the tetradecyltrimethylammonium bromide/decylammonium bromide mixed mesophase system at pH 3 (Table V). The quadrupole splitting increased about 50% as decylammonium replaced the tetradecyltrimethylammonium while the chemical shift simultaneously underwent a change of about −55 ppm. Evidently, as similarly observed for the $Co(NH_3)_6^{3+}$/anionic mesophase, in this $Co(CN)_6^{3-}$/cationic system the ^{59}Co quadrupole splitting does not undergo a large fractional change in its magnitude.

The hydrogen-bonding properties of alkylammonium and alkyltrimethylammonium are very different. In the former, but not the latter, case, there is the possibility of direct hydrogen-bonding contact between the amphiphile and the cyanocobalt ion. The fact that there is only a small change in quadrupole splitting on going from the one system to the other means that the amount of distortion from octahedral symmetry is similar in the two cases. The large change in chemical shift indicates that the average orientation of the ion with respect to the micellar surface has undergone a large change, consistent with a change in the mode of binding. These results taken together show that in either amphiphilic system the cyanocobalt interactions with the micellar surface are mediated by molecules of water of hydration. This will allow both for the changes in the binding as reflected in the large chemical shift changes and the small changes in distortion of the ion that are reflected in the quadrupole splittings. Since the quadrupole splittings observed for the $Co(NH_3)_6^{3+}$/anionic mesophase are similar to those of the $Co(CN)_6^{3-}$/cationic mesophase, it is evident that water of hydration is also mediating the binding interactions in that system.

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Solvent Deuteration Enhancement of Hydrophobicity: DSC Study of the Inverse Temperature Transition of Elastin-Based Polypeptides

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As previously shown, the polypentapeptide of elastin, $(Val^1\text{-}Pro^2\text{-}Gly^3\text{-}Val^4\text{-}Gly^5)_n$ or simply poly(VPGVG), undergoes an inverse temperature transition which is seen macroscopically as a phase separation with a dense viscoelastic phase of about 60% water, 40% peptide by weight and which is characterized molecularly by increase in intra- and intermolecular order as evidenced by formation of specific hydrophobic contacts. Furthermore, from an extensive study of polypentapeptides of the composition poly[f_X(VPGXG), f_V(VPGVG)], where $f_X + f_V = 1$ and X is any of the amino acid residues with apolar (hydrophobic) side chains, it has been shown that the temperature of the transition decreases and the heat of the transition increases with increased hydrophobicity. In the present paper differential scanning calorimetry has been utilized to determine the effect of D_2O on the temperature and heats of the inverse temperature transitions for poly(VPGVG), poly(IPGVG), poly(LPGVG), and poly(VPAVG) and in the latter case in the presence of 0.5 and 1.0 N NaCl and of 1, 2, and 3 M urea. In all cases, the effect of D_2O as compared to H_2O is to lower the transition temperature about 2 °C and to increase the heat of the transition about 10%, and this occurs also in the presence of NaCl, which itself lowers the temperature and increases the heat, and in the presence of urea, which itself raises the temperature and decreases the heat of the transition. It is concluded that the effect of replacement of H_2O by D_2O in these polypeptides is to effect a small but consistent increase in the expression of hydrophobicity.

Introduction

The polypentapeptide $(L\text{-}Val^1\text{-}L\text{-}Pro^2\text{-}Gly^3\text{-}L\text{-}Val^4\text{-}Gly^5)_n$, hereafter abbreviated poly(VPGVG), is the longest repeating sequence found in bovine elastin, the functional elastic protein component of connective tissue.[1-6] The synthetic polypentapeptide and its analogues (such as those obtained by substitution of one amino acid residue in the pentameric unit) have been studied extensively in this laboratory.[7] A characteristic feature of the polypentapeptide in water is that it undergoes an inverse temperature transition when the temperature is raised; i.e., the intra- and intermolecular order increase with an increase in temperature. With the polypentapeptide being hydrophobic in terms of amino acid composition, it is consistent that the inverse temperature transition be viewed as driven by mean residue hydrophobicity.[7,8] When below the transition temperature, e.g., below 20 °C, poly(VPGVG) is miscible with water in all proportions. A molecular perspective is that, with the polypeptide molecules dispersed

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in water, the side chains are exposed to water; i.e., there is formation of waters of hydrophobic hydration (clathrate-like water) surrounding the hydrophobic side chains. The clathrate-like water is viewed, when compared to bulk water, as being in a lower energy (with stronger interactions between water molecules) and lower entropy state.[9] When the temperature is raised, the more ordered, low-entropy water of hydrophobic hydration converts into less structured bulk water as the hydrophobic side chains associate with a resulting increase in order of the polypeptide part of the system.

A molecular structure for poly(VPGVG) has been put forward as a β-spiral with recurring type II Pro^2-Gly^3 β-turns as the secondary structural feature and wherein intramolecular hydrophobic side chain interactions are optimized, causing the poly(VPGVG) chain to wrap up into a loose helical structure with interturn hydrophobic contacts.[7] This β-spiral formation with experimentally demonstrated interturn hydrophobic contacts[10,11] is proposed as the explanation for the increase in order of this polypeptide with increasing temperature. Light and scanning electron microscopic observations have shown the polypentapeptide to self-assemble into fibers when the temperature is raised;[12,13] transmission electron microscopic observation using negative staining[14,15] of aggregates obtained on heating shows the presence of parallel aligned twisted filaments, and molecular mechanics calculations starting with cyclo(VPGVG)$_3$, which has a near identical conformation to poly(VPGVG),[16,17] were used to develop the β-spirals and twisted filaments.[18] Some three β-spiral chains are thought to form the twisted filament quaternary structure. There is room for considerable water within the β-spiral, and there is also space for water to occur between the associating twisted filaments. These water molecules, intraspiral and interfilament, while restricted in motion, are fewer in number and presumably less structured than the clathrate-like water surrounding the hydrophobic side chains before the transition. So the changes in the state of water can dominate the inverse temperature transition, allowing for the polypeptide ordering process.

It is apparent that waters of hydration play an important role in the functioning of the biological macromolecules. Changes in hydration play a critical role in the processes of protein folding and assembly, in which solvent-mediated solute–solute interactions (such as hydrophobic interactions) are believed to make a major contribution to the total driving force. This is graphically demonstrated, for example, by the elastin-based polypeptides in their capacity to function in thermomechanical and chemomechanical transduction.[19]

H_2O and D_2O can be viewed as forming essentially the same liquid, but small, definite differences do exist in the magnitude of physical properties.[20] D_2O is also strikingly different from H_2O in its effects on living systems. D_2O affects the growth and viability of organisms. D_2O is toxic, even lethal at higher concentrations, to higher animals and plants.[21,22] The principal basis of the metabolic disturbance is thought to be the different effects of D_2O on various enzyme-catalyzed reactions. The understanding of this, of course, must come from an understanding of D_2O interaction with polypeptides and proteins.

D_2O is often used as a probe to study water structure and macromolecular hydration and specifically hydrophobic interactions. Various experimental and theoretical studies have been reported. In regard to the relative strength of hydrophobic interactions in H_2O and D_2O, different conclusions have resulted when different methods were used on different molecular systems. Critical micellar concentrations (cmc) of detergents,[23] for example, are lower in D_2O than in H_2O, suggesting that hydrophobic interactions are stronger in D_2O than in H_2O. Conductometric studies of association equilibria in solutions of pairs of molecules with long hydrocarbon chains have led to the same conclusion.[24] In the macromolecular assembly processes of synthetic polyriboadenylic and polyribouridylic acids, of agarose, and within sickel cells, which are all characterized by the removal of hydrophobic groups from exposure to the solvent, the solvent deuteration effects have also been interpreted as suggesting that hydrophobic interactions are stronger in D_2O than in H_2O.[25] On the other hand, arguments based on the relative solubility of hydrocarbons in the two kinds of water have led to the inverse conclusion.[26,27] For proteins, results are somewhat more controversial or less conclusive.

An advantage of studying elastin-based polypeptides such as poly(VPGVG) and related analogues resides in their simple amino acid composition with a dominantly hydrophobic character and without the complication of the more complex amino acid composition involved in the proteins usually studied. The inverse temperature transition exhibited by these elastin-based polypeptides provides a window through which to characterize expression of hydrophobicity and the effects of various means whereby hydrophobicity may be modulated.

Differential scanning calorimetry (DSC) provides direct, model-independent determinations of the transition properties, such as enthalpy, entropy, and critical temperature, in thermally induced structural and phase transitions of biomolecules.[28] DSC studies of the inverse temperature transition of the elastin-based polypeptides add another experimental dimension with which to resolve the issue of expression of hydrophobicity in D_2O and in H_2O. For this purpose and as a part of a systematic investigation of the elastin-based polypeptides, this paper reports differential scanning calorimetric results on the aqueous solvent deuteration effect.

Materials and Methods

The studies were carried out on the Micro 1710 differential scanning calorimeter (Hart Scientific, Provo, UT). The instrument includes four removable cells; one is used as reference, and the other three can be used for samples. The thermopile response as a function of energy input was calibrated by the built-in calibration heater of the instrument. For each cell, a temperature-dependent calibration constant was determined every 10 °C from −5 to 105 °C and fit by the least-squares method to a second-order polynomial in temperature. The polynomials are used in the experiment to convert the measured electrical signal to the power required on heat absorption. The detailed calibration and checking of the performance of the instrument have been presented elsewhere.[8]

Preparation of the Polypentapeptides. The synthesis and characterization of poly(VPGVG), (L-Val^1-L-Pro^2-Gly^3-L-Val^4-

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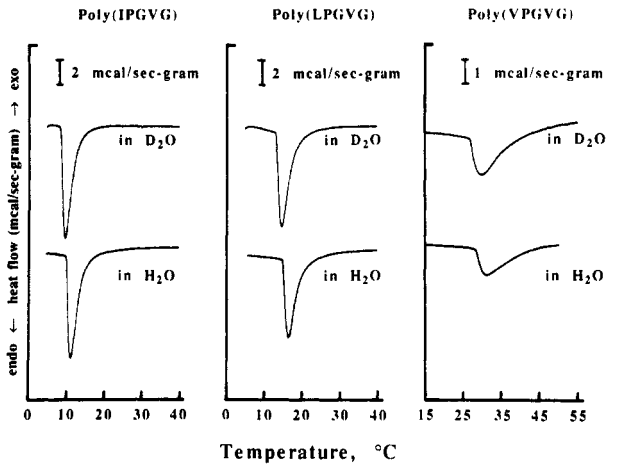

Figure 1. DSC scans for the synthetic poly(IPGVG), poly(LPGVG), and poly(VPGVG) in H_2O/D_2O.

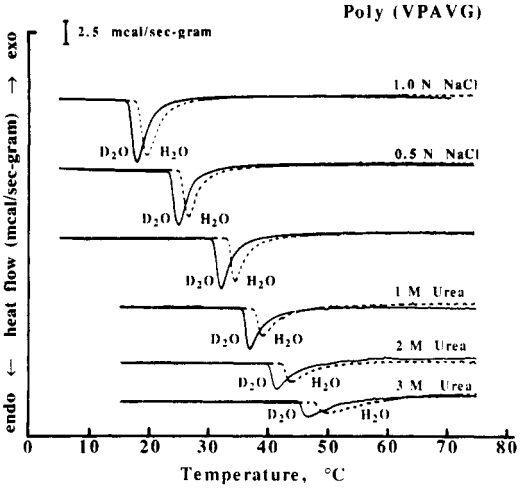

Figure 2. DSC scans for the synthetic poly(VPAVG) in H_2O plus urea and NaCl and in D_2O plus urea or NaCl.

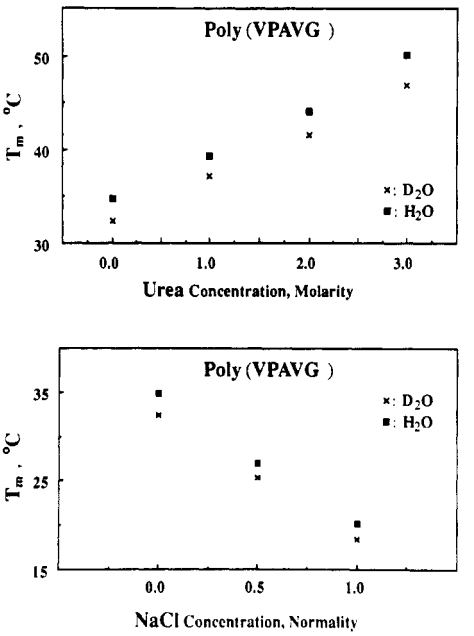

Figure 3. Plot of T_m vs concentration of urea and NaCl in D_2O and in H_2O.

$Gly^5)_n$, have been extensively reported elsewhere.[29,30] The syntheses and characterizations of poly(IPGVG), i.e., (L-Ile^1-L-Pro^2-Gly^3-L-Val^4-$Gly^5)_n$, of poly(LPGVG), i.e., (L-Leu^1-L-Pro^2-Gly^3-L-Val^4-$Gly^5)_n$, and of poly(VPAVG), i.e., (L-Val^1-L-Pro^2-L-Ala^3-L-Val^4-$Gly^5)_n$, have also been described previously,[31-33] Molecular weights of the four synthetic polypeptides are greater than 50000 as all have been retained within 50000 MW cutoff dialysis membranes after many days of dialysis at 4 °C with regular changes of the dialysate.

Low-conductivity H_2O (with a resistance greater than 15 MΩ) and D_2O (99.83%, Cambridge Isotope Laboratories, lot no. E-2227) were used as solvent for poly(VPGVG), poly(IPGVG), poly(LPGVG), and poly(VPAVG). The NaCl (Baker, Phillipsburg, NJ) and urea (Rascher & Betzold) used were of reagent grade. Stocks of concentrated NaCl and urea in H_2O and D_2O were prepared, and more dilute solutions were obtained by dilution. In all of the experiments, each weighed amount of sample was dissolved in the amount of H_2O (or D_2O) required to obtain a particular concentration, that is, 40 mg of polypeptide/mL of solvent for poly(VPGVG), poly(IPGVG), and poly(LPGVG), and 10 mg of polypeptide/mL of solvent for poly(VPAVG). The solutions were then allowed to stand at low temperature (2 °C) for about 12 h to ensure that the sample was completely dissolved in the solvent. The samples were preheated up to 70 °C with a scan rate of 14 °C/h (0.23 °C/min) such that the polypeptide systems will have passed once through the inverse temperature transition before the data were recorded on the second run. The inverse temperature transition was found to be essentially reversible, but the reverse process is slow. The experiments began after the samples had re-equilibrated at 2 °C for approximately 90 h. The scan rate used was again 14 °C/h (0.23 °C/min). A baseline scan with solvent was obtained following each DSC scan involving sample. The sample scan and baseline scan were run at the same scan rate and against the same reference cell, the content of which remained the same for the two runs. The reported curve is the result of subtracting the baseline run from the sample run.

The heat of transition ΔQ (ΔH) is obtained by direct integration of the DSC scan. Since the DSC scans are recorded as a function of time, the integrations were carried out with time as the integrand variable. The baseline used in calculating ΔQ is a straight line joining the initial and final temperatures of the transition as schematically shown in Figure 3 of ref 8. The baselines are drawn from 2 °C below the transition onset temperature, T_b, at which the thermal profile departs abruptly from the pretransition baseline to a point tangent to the curve on the high-temperature side. The uncertainty introduced by the means of choosing baselines is negligible when compared to the observed differences in magnitudes of the calculated thermal parameters for the peptides in the salts of different concentrations.

The temperature dependence of the transitions can be characterized by two specific temperatures, the temperature of maximum heat absorption (T_m) and the temperature for the onset of the transition (T_b). T_m can be obtained directly from a DSC scan; T_b can be obtained from the initial extremum of the derivative of the DSC scan with respect to temperature, as shown in Figure 1 for poly(VPAVG) in ref 34. Either one of these two temperatures can be used to characterize the effect of solvent deuteration on the inverse temperature transition of the elastin-based polypeptides, with T_b providing the most accurate value especially for relatively broad transitions.

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TABLE I: DSC-Derived Thermodynamic Data of the Inverse Temperature Transition Exhibited in H_2O and D_2O by Poly(VPGVG), Poly(IPGVG), and Poly(LPGVG)[a]

sample	solvent	T_b, °C	T_m, °C	ΔH, kcal/mol	ΔQ,[b] cal/g
$(VPGVG)_n$	D_2O	27.2	29.8	1.33	3.25
	H_2O	28.8	31.2	1.07	2.62
		ΔT_b = 1.6 °C	ΔT_m = 1.4 °C		
$(IPGVG)_n$	D_2O	9.0	10.0	2.86	6.76
	H_2O	10.6	11.6	2.61	6.17
		ΔT_b = 1.6 °C	ΔT_m = 1.6 °C		
$(LPGVG)_n$	D_2O	13.7	14.7	2.83	6.70
	H_2O	15.5	16.4	2.55	6.02
		ΔT_b = 1.8 °C	ΔT_m = 1.7 °C		

[a] T_b, temperature of the beginning of the transition; T_m, temperature of maximum heat absorption. The reproducibility of the temperature is within 0.1 °C in D_2O/H_2O and 0.5 °C in D_2O/H_2O plus urea or plus NaCl. [b] Per mole of repeat unit, i.e., pentamer.

TABLE II: DSC-Derived Thermodynamic Data of the Inverse Temperature Transition Exhibited in H_2O and D_2O by Poly(VPAVG)[a]

sample	solvent	T_b, °C	T_m, °C	ΔH, kcal/mol	ΔQ, cal/g
$(VPAVG)_n$	D_2O	31.7	32.4	3.04	7.17
	H_2O	34.2	34.8	2.73	6.46
		ΔT_b = 2.5 °C	ΔT_m = 2.4 °C		
	1 M urea				
	D_2O	36.5	37.1	2.57	6.07
	H_2O	38.5	39.3	2.23	5.27
		ΔT_b = 2.0 °C	ΔT_m = 2.2 °C		
	2 M urea				
	D_2O	40.8	41.5	2.16	5.09
	H_2O	43.0	44.0	1.85	4.37
		ΔT_b = 2.2 °C	ΔT_m = 2.5 °C		
	3 M urea				
	D_2O	45.8	46.8	1.67	3.94
	H_2O	48.5	50.1	1.51	3.55
		ΔT_b = 2.7 °C	ΔT_m = 3.3 °C		
	0.5 N NaCl				
	D_2O	24.5	25.3	3.17	7.50
	H_2O	26.1	27.0	2.93	6.93
		ΔT_b = 1.6 °C	ΔT_m = 1.7 °C		
	1.0 N NaCl				
	D_2O	17.6	18.4	3.91	9.24
	H_2O	19.1	20.1	3.72	8.79
		ΔT_b = 1.5 °C	ΔT_m = 1.7 °C		

[a] For definitions, see Table I.

Results and Discussion

Figure 1 presents the DSC thermograms for poly(VPGVG), poly(IPGVG), and poly(LPGVG) in H_2O and in D_2O. Figure 2 contains the DSC experimental results of poly(VPAVG) in D_2O plus urea, in H_2O plus urea, in D_2O plus NaCl, and in H_2O plus NaCl. The corresponding thermodynamic data are reported in Tables I and II. The larger enthalpies for the transition and the larger isotope-induced shifts in the transition temperature exhibited by poly(VPAVG) are considered to be due to a different conformation exhibited by this polypentapeptide; in particular, for poly(VPAVG) in H_2O the Val^4NH appears to be the most exposed, whereas for the other polypentapeptides the Val^4NH is the most shielded and participates in a hydrogen bond with residue 1 C–O (unpublished studies).

From the data presented here, small but substantial differences in the thermodynamic parameters in D_2O and in H_2O are observed. Independent of the hydrophobicity of the four different polypeptides, D_2O consistently shifts the value of T_m lower than in H_2O by about 2 °C. For poly(VPAVG) (whether in D_2O or H_2O), the effect of increasing urea concentration was to increase T_m and addition of NaCl (whether in D_2O or H_2O) caused the values of T_m to decrease progressively. For the NaCl effect in D_2O, T_m is again about 2 °C lower than that in NaCl of the same concentration in H_2O. This is also the case for the poly(VPAVG) peptide in urea. So the effects of D_2O and NaCl or D_2O and urea are essentially additive, and whether the perturbation lowers (NaCl) or raises (urea) the value of T_m, the sign of the D_2O shift is the same and the magnitude is nearly the same.

It is well-established that poly(VPGVG), and related linear analogues, poly(VAPGVG), and cyclic analogues, cyclo(VAPGVG)$_2$ and cyclo(VPGVG)$_3$, increase intra- and intermolecular order when the temperature of solutions of these peptides in water is raised[7] as reviewed in part for poly(VPGVG) under Introduction. This is qualitatively explained by the existence of waters of hydrophobic hydration of lower entropy surrounding hydrophobic moieties at lower temperatures that become higher entropy bulk water as the polypeptide becomes more ordered when the temperature is raised through that of the inverse temperature transition. A particularly striking demonstration is the reversible crystallization of cyclo(VAPGVG)$_2$ as the temperature is raised and the dissolution of crystals as the temperature is lowered.[35] The disordering of the waters of hydrophobic hydration (seen in special ordered cases as clathrate water)[36] allows that the total entropy of the system, polypeptide plus water, increases with increasing temperature in accordance with the second law of thermodynamics. Accordingly, the basis of an inverse temperature transition is often referred to as the hydrophobic effect.

In such systems, therefore, other things being equal, changes in the hydrophobicity can be expected to result in predictable shifts in the temperature and heats of the inverse temperature transition. This is indeed observed. Ile and Leu are generally considered to be more hydrophobic than Val and replacement of Val^1 by Ile^1 results in a lowering of the temperature of the transition and an increase in the heat of the transition.[8] Going the other direction, replacement of Val^4 by Ala^4 causes an increase in the temperature of the transition and a decrease in the heat of the transition; e.g., for poly[2(VPGVG)(VPGAG)] T_b is raised to 35 °C and ΔH is lowered to 0.74 kcal/mol (unpublished results). More striking is the substitution of Val by an aromatic residue; for example, just 1 Trp in 50 residues as in poly[9(VPGVG)(VPGWG)] lowers the temperature of the transition to 11 °C and increases the heat of the transition to 1.74 kcal/mol[37] (unpublished results).

The changes in heat capacity, ΔC_p, of the polypeptides studied are negative in both H_2O and D_2O; that is, the C_p of the polypeptide solution decreases after the transition. The differences in ΔC_p between H_2O and D_2O, however, are sufficiently small in the present study that the results do not warrant a discussion of the comparisons.

From the above analogues, a chemical variation which lowers the temperature of the transition and increases the heat of the transition may be viewed as increasing the hydrophobicity of the polypeptide.

On the basis of this perspective, the effect of D_2O can be considered. The characteristic transition temperatures, T_b (or T_m) for poly(VPGVG) and its three analogues in D_2O are consistently lower than those in H_2O by about 2 °C, and the heats of transition are increased by about 10% in D_2O. For a given temperature the polypeptides have stronger tendency to associate in D_2O than in H_2O. The apparent hydrophobicity of the polypeptide is greater in D_2O than in H_2O. It is therefore not unreasonable to state that the hydrophobic effect is stronger in D_2O than in H_2O.

Qualitatively similar effects, of course, have been observed in proteins[38,39] and in polymers such as hydroxypropylcellulose[40] and in other polymers.[41] In proteins in particular, it has been difficult,

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however, to make conclusions on the effect of H_2O replacement by D_2O because of the many interactions and the complexities of the side chains. In fact, qualitatively similar results on a per residue basis as obtained here have been interpreted as arising from hydrogen bonding between polar side chains.[38]

D_2O is usually considered to be inherently more structured than H_2O;[20] i.e., the correlation among D_2O molecules is greater than the correlation among H_2O molecules. It should be made clear that the statement that D_2O is more structured than H_2O does not necessarily lead to the conclusion that hydrophobic effect is stronger in D_2O than in H_2O. Ben-Naim, for example, concurs that D_2O is more structured than H_2O but believes the hydrophobic interaction to be stronger in H_2O than in D_2O.[42] It is not simply the comparison of the relative degree of correlation of the molecules of the two solvents but rather the relative change in the enhancement of the correlation on going from bulk D_2O to D_2O of hydrophobic hydration with that on going from bulk H_2O to H_2O of hydrophobic hydration, i.e.

$$^{\delta}D_2O\ (\text{structure}) = \text{structure (hydration } D_2O) - \text{structure(bulk } D_2O)$$

$$^{\delta}H_2O\ (\text{structure}) = \text{structure (hydration } H_2O) - \text{structure(bulk } H_2O)$$

To say that the hydrophobic effect in D_2O is stronger than in H_2O means that

$$^{\delta}D_2O\ (\text{structure}) > {}^{\delta}H_2O\ (\text{structure})$$

In other words, the solute-induced correlation enhancement for molecules in the hydration shell can be greater in D_2O than in H_2O. This enhancement has been found in the computational efforts of Rossky and co-workers.[43]

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Nanosecond Reorganization of Water within the Interior of Reversed Micelles Revealed by Frequency-Domain Fluorescence Spectroscopy

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The fluorescence properties of 1,8-ANS (1-anilino-8-naphthalenesulfonic acid) in AOT (Aerosol-OT; 1,4-bis[2-ethylhexyl] sulfosuccinate) reverse micelles have been studied. Using steady-state and time-resolved methods we show that water content and temperature play key roles in the photophysics of the emission process. For example, a continuous red shift of the emission spectrum is observed as the water content within the AOT reverse micelle increases. New multifrequency phase and modulation experiments show clearly that the 1,8-ANS emission spectrum is continuously evolving on a nanosecond time scale. From these experiments, we find that the solvation correlation functions ($S(t)$) are characterized by a pair of solvent relaxation rates when the water content is at or below $R \approx 2.5$ (R, molar ratio of water to AOT). Above $R \approx 2.5$ ($r_w \approx 2R \approx 5$ Å, r_w is the core radius of the water droplet) we observe a single relaxation process. We propose that, at lower water levels, the two solvation rates are a consequence (in part) of interfacial (type 1) and core (type 2) associated water, each characterized by its own relaxation rate. Upon increasing the water content within the reverse micelle, the relative fraction of type 2 water increases and the solvent relaxation process collapses into a single relaxation rate. From temperature-dependent studies we determine the activation barriers for each of these solvent reorganization events.

Introduction

Over the past few decades, surfactant aggregates in nonpolar solvents, termed reversed micelles, have attracted increased attention.[1-7] Of all the possible systems, reverse micelles formed by the surfactant sodium bis(2-ethylhexyl) sulfosuccinate (Aerosol-OT; AOT) in alkanes (e.g., heptane or isooctane) have been the most widely investigated.[8-11] As a direct result of these types of detailed studies, reverse micelle systems are now used routinely to solve practical problems in biotechnology,[12] separation sciences,[13] microparticle synthesis,[14] and oil recovery technology.[15]

The AOT-alkane-water system, in particular, is interesting for several reasons. AOT solutions are homogeneous and optically

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