Hydrogen Bonding of Water in Aqueous Solutions of Trimethylamine-*N*-oxide and *tert*-Butyl Alcohol: A Near-Infrared Spectroscopy Study

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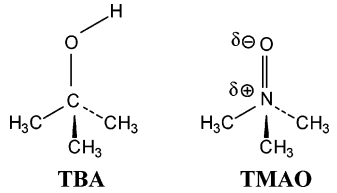
The hydration properties of two biologically relevant molecules, trimethylamine-*N*-oxide (TMAO) and *tert*-butyl alcohol (TBA), were investigated by monitoring the effects of these two solutes on the near-infrared (NIR) spectra of water. In particular, the 1450-nm $\nu_1 + \nu_3$ water combination band (ν_1 is the symmetric stretching and ν_3 is the asymmetric stretching) and the 1928-nm $\nu_2 + \nu_3$ band (ν_2 is the bending) were recorded at 25 °C in aqueous solutions of TBA and TMAO over the 0−0.1 and 0−0.05 solute mole fraction intervals for TBA and TMAO, respectively. NIR data show, in agreement with molecular dynamics simulations and other suggestions found in the literature, that on the whole water molecules are more tightly coordinated by TMAO than by TBA. Furthermore, nonadditive perturbations of the water's H-bond network are observed for TBA and are absent in the TMAO case. These results are discussed in connection to the significantly different action exerted by these two solutes on typical processes governed by hydrophobic interactions, such as protein folding and micellization of a surfactant. In these processes, the data support the assumption that the presence of TMAO or TBA modifies the extent of the free-energy contribution associated with structural reorganization of water.

Introduction

This paper is part of a series concerning the hydration properties and the association behavior of hydrophobic species in water.[1−3] This topic is of wide interest because water forms the basis of all biologically important fluids and the hydrophobic interactions are thought to be responsible for the stability of particular conformations of biopolymers in aqueous solutions and the formation of micelles and lipid membranes.[4] The series of monohydric alcohols is a commonly investigated model system used to study the effects of hydrophobic hydration. In particular, attention has been focused on *tert*-butyl alcohol (TBA) because within this series it is characterized by the largest hydrophobic group. Many of the physical properties of TBA/H_2O mixtures have already been studied in great detail, showing peculiar features in the water-rich region. Examples include the minimum in the partial volume,[5] the maximum in the excess heat capacity,[6] the large ultrasonic absorption,[7] a remarkable increase in light scattering,[8] and so forth. All of these anomalies occur in the composition range of $0.03 < X_2 < 0.05$, where X_2 is the solute's mole fraction. These results can be explained in terms of the self-aggregation of TBA molecules occurring above a threshold concentration of $X_2^* \approx 0.025$ (see ref 1 for a review), because the alcohol molecules are essentially monodisperse with their hydration structures at lower mole fractions.

In agreement with this description, molecular dynamics simulations (MD)[9] and neutron-scattering studies[10,11] on TBA/H_2O solutions indicate a small degree of association in dilute solutions ($X_2^* \approx 0.02-0.03$), whereas small aggregates, with bulky hydrophobic groups in contact, are observed at higher concentrations ($X_2^* \approx 0.06-0.08$). In recent papers, the hydration properties and the self-aggregation of TBA in water were compared with those of the isosteric molecule trimethylamine-*N*-oxide (TMAO),[2,3] which has the same alcohol hydrophobic part and a different polar head (Figure 1). The TMAO belongs to a class of small organic molecules (osmolytes) present in organisms living under conditions of water stress, where it acts principally as a regulator of the osmotic pressure in intracellular fluids.[12−14] In vitro, osmolytes typically stabilize the native state of globular proteins against thermal denaturation.[14] In particular, TMAO counteracts the denaturing effects of urea[15−18] and, like a chemical chaperone, induces the renaturation of proteins.[19,20] TBA and other monohydric alcohols cause the opposite effect because they destabilize the native conformation of proteins.[21] However, both TMAO[14] and TBA,[21] at least at low concentrations, do not interact directly with proteins. Therefore, their antithetic action on biosolutes must be correlated to differences in their respective hydration properties and/or in their association behavior. Infrared (IR) spectra[2] and compressibility and density data[3] on H_2O/TBA and H_2O/TMAO solutions have shown that although alcohol molecules progressively aggregate beyond a threshold value of the mole fraction,[1,3] for TMAO the absence of any self-aggregation behavior is also accompanied by a negligible overlapping of its hydration cages.[2,3]



TBA **TMAO**

Figure 1. Structural forms of TBA and TMAO.

Following the suggestion from our previous work on aqueous solutions of TBA and TMAO[2], in the present paper we have principally focused our attention and the experimental data on

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the modification induced by the presence of these two solutes on the water properties by using a spectroscopic probe specific to water. The aim is to provide a quantitative estimate of the solute-induced structural perturbation as a function of the increasing mole fraction of alcohol and amine. This can be achieved by comparing experimental data with new insights coming from molecular dynamics (MD) simulation results obtained on the same systems.[22,23] To this purpose, it is worth mentioning that although MD and Monte Carlo simulations indicate that water's structuring is significantly enhanced in dilute solutions of TBA[9,22,24] the interpretation of X-ray and neutron-scattering data in terms of a structural effect on water is still a controversial question. For example, recent neutron-diffraction studies[10] on TBA−water mixtures at $X_2 = 0.03$ indicate that alcohol molecules are almost located in cavities within the hydrogen-bonded water network, with an overall small effect on the water's structure. Consistent with this result, in our recent paper on IR investigation of H_2O/TBA and H_2O/TMAO solutions, we have shown[2] that the variations in the fundamental OH stretching band profile from adding the above-mentioned solutes are substantially small. This was indicative of a distribution of hydrogen-bond energies that were only slightly perturbed, in particular, by the presence of the alcohol molecules. The extent of these effects on the fundamental OH stretching absorption band does not permit us to extract, from experimental IR spectra, a quantitative evaluation of the TBA hydration properties and their evolution with the mixture composition. Instead, in the present work, the hydration properties of TBA and TMAO are investigated and compared by recording absorption spectra in the near-infrared (NIR) spectral region. NIR spectra are particularly suitable for the detection of hydrogen bonds between protons on polar groups and free electrons pairs and are often employed to study solvent modifications in aqueous solutions.[25] In fact, NIR absorption bands are better separated in frequency, and cells of more convenient and reproducible thickness can be used. In the present investigation, the spectra of the 1450-nm band of water, assigned to the $\nu_1 + \nu_3$ (ν_1 is the symmetric stretching and ν_3 is the asymmetric stretching mode) band[26] and the $\nu_2 + \nu_3$ (ν_2 is the bending vibration) band at about 1928 nm,[27,28] were measured at 25 °C in aqueous TBA and TMAO for X_2 varying from 0 to 0.1 and 0 to 0.05, respectively. The $\nu_2 + \nu_3$ absorption band appears to be particularly suitable to the study of water perturbations that are induced by the presence of alcohol molecules because there is substantially no overlapping with alcohol absorption bands.[27] However, the $\nu_1 + \nu_3$ band is well known in the literature to be indicative of the hydrogen-bond configuration in water.[25] The spectral changes induced by TBA and TMAO have been correlated and compared with the modifications occurring in pure water spectra taken at different temperatures in the same wavelength region. The proposed analysis provides a coherent and quantitative description of the perturbation induced by the solutes on water's structure, allowing the possibility of comparing the data with the MD results.[22,23] Finally, possible consequences of these effects on the protein folding and the micellization of a surfactant are also discussed. Although the influence of TBA or TMAO on these processes is well known in the literature, new insights must be achieved to understand their microscopical mechanisms.

Experimental Section

Samples of H_2O/TBA and H_2O/TMAO were prepared by weight and by using deionized and doubly distilled water. TBA alcohol (Aldrich) and anhydrous TMAO (Aldrich) were used without any further purification.

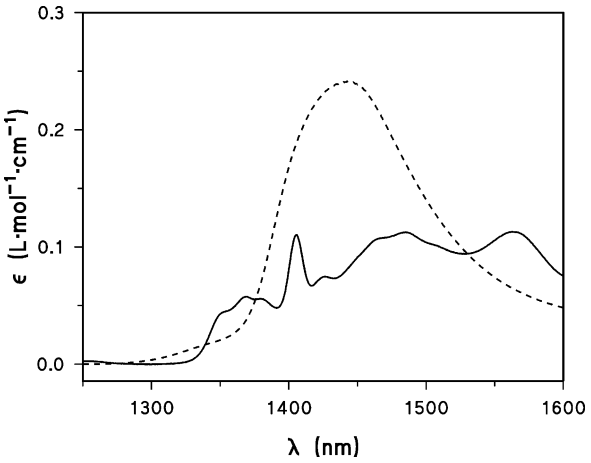

Figure 2. Molar extinction coefficient, ϵ, of (—) pure TBA and (- - -) pure H_2O in the 1250−1600 nm region.

All absorption spectra were recorded versus the nonabsorbing CCl_4 with a Jasco V-570 spectrophotometer equipped with a temperature control system for the sample cell and with digital output for computer analysis. The spectra of the H_2O/TBA and H_2O/TMAO samples were recorded at $T = (25.0 \pm 0.1)$ °C. Pure-water spectra were taken at different temperatures in the range of 3−60 °C. All of the difference spectra were calculated with respect to the pure-water spectrum at 25 °C. Quartz cells with thicknesses of 10^{-1} and 10^{-2} cm we used for the absorption band at 1450 and at 1928 nm, respectively. The molar extinction coefficient of the pure components was calculated using the usual expression $\epsilon = A/dc$, where A is the absorbance, c is the concentration in moles/L, and d is the cell thickness in cm. This requires data for the density of TBA and TMAO solutions, which were taken from refs 3 and 29 and appropriately interpolated.

Results and Discussions

NIR Absorption Spectra. The molar absorption coefficients of pure water and pure TBA in the 1250−1600-nm region are shown in Figure 2. No extinction coefficient of TMAO is reported because the molecule does not absorb in this wavelength interval. The intense water band centered at 1450 nm is attributed to the combination of symmetric (ν_1) and antisymmetric (ν_3) O−H stretching modes (first overtone).[26] The absorption band of TBA indicated in Figure 2 is the intense alcohol OH stretching first overtone,[30] which shows a marked overlap with the $\nu_1 + \nu_3$ water band. This interference was then taken explicitly into account, as will be discussed below, to subtract the alcohol contribution appropriately.

Figure 3 shows the molar extinction coefficients of water and of pure TBA in the 1800−2150-nm wavelength region. The broad water band is the $\nu_2 + \nu_3$ combination of the asymmetric stretching and the bending vibration. No absorption of TMAO is present in this spectral region, whereas bands belonging to the TBA molecule lie above 2000 nm and do not cause interference with the absorption of water.[27] The spectra analysis was carried out by writing the absorbance A of a sample containing c_1 moles/L of water and c_2 moles/L of solute through the following relation:

$$A = d(\epsilon_1 + \Delta\epsilon_1)c_1 + d(\epsilon_2 + \Delta\epsilon_2)c_2 \qquad (1)$$

where $\Delta\epsilon_1$ and $\Delta\epsilon_2$ are the variations in the molar extinction coefficients of water and of the solute with respect to the corresponding values ϵ_1 and ϵ_2 of the pure components.

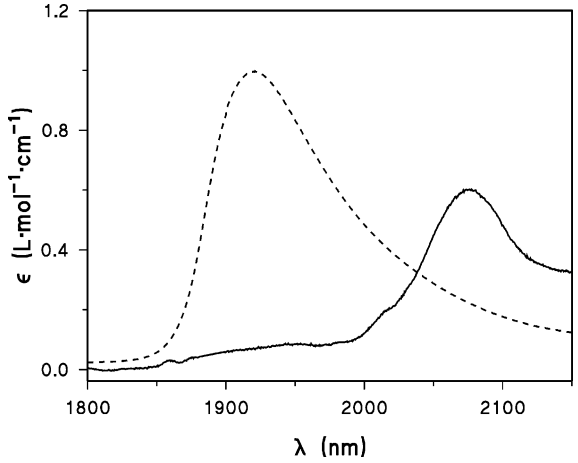

Figure 3. Molar extinction coefficient, ϵ, of (—) pure TBA and (- - -) pure H_2O in the 1800−2150-nm region.

Equation 1 can be rewritten in this way:

$$\epsilon = \frac{A - d\epsilon_2 c_2}{dc_1} = \epsilon_1 + \Delta\epsilon_1 + \frac{\Delta\epsilon_2 c_2}{c_1} \qquad (2)$$

where ϵ is defined as the apparent molar extinction coefficient of the water in the mixture.

From eq 2, it follows that the difference spectrum, $\Delta\epsilon = \epsilon - \epsilon_1$, coincides with $\Delta\epsilon_1$ if $\Delta\epsilon_2 = 0$. This is the case for TMAO in the 1250−1600- and 1800−2150-nm wavelength regions, whereas for TBA, it holds almost exactly only in the 1800−2000-nm region (Figures 2 and 3). However, in all of the investigated concentration intervals $c_2/c_1 \ll 1$ for TBA samples, so it should be expected that $\Delta\epsilon \approx \Delta\epsilon_1$. The quantity $\Delta\epsilon$, calculated from eq 2 by using the experimental spectra in the 1250−1600-nm wavelength region. is shown in Figure 4a and b for H_2O/TMAO and H_2O/TBA mixtures, respectively. The changes with respect to the pure-water spectrum are very similar for the two molecules and closely resemble the variations previously observed in the fundamental OH stretching region of water[2] and in the decoupled OH stretching band of the HDO solvent, the latter by adding TMAO.[31] In fact, for both solutes, negative peaks can be identified in the difference spectra near 1410 nm, where the absorption of weakly bound molecules with more distorted H bonds is reported. Furthermore, positive peaks appear at higher wavelengths, where the absorption of strongly bonded water molecules belonging to fairly regular tetrahedral structures is observed.[25,32−34] Thus, the effect of adding TBA or TMAO to pure H_2O can be described as a transfer of OH oscillators from lower to higher wavelengths of the spectrum, this phenomenon being quantitatively more pronounced for TMAO solutions. It has been noted that some spectroscopic and dynamic properties of water in the presence of hydrophobic solutes, in particular, TBA molecules, are similar to those of bulk water at low temperature.[35,36] Figure 4c shows the influence of temperature on the extinction coefficient of water in the 1250−1600-nm range. As the temperature decreases, the absorbance at 1410 nm diminishes, whereas the absorbance at longer wavelengths increases. The shape and positions of the main peaks in the difference spectra shown in Figure 4a,−c are very similar, particularly in the short-wavelength region. This striking resemblance indicates that the effect of TMAO or TBA on the hydrogen-bonding equilibrium of water is analogous to that observed by decreasing the temperature; that is, it can be thought to consist of an enhancement of water−water interactions.

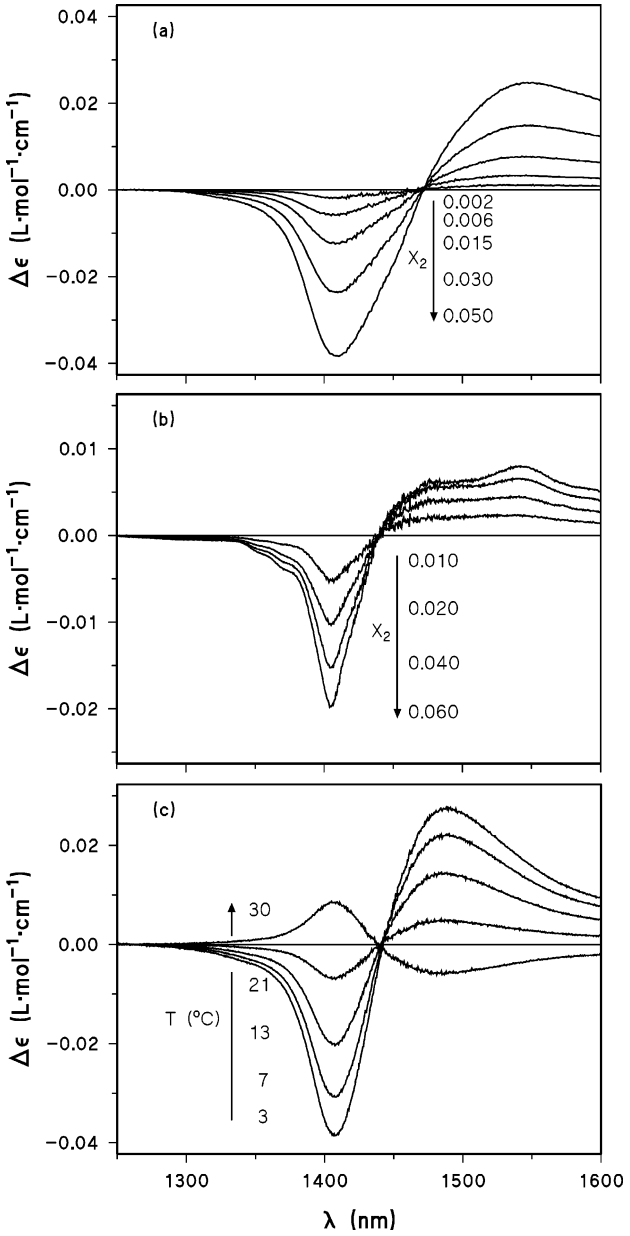

Figure 4. Difference spectra, $\Delta\epsilon$, in the 1250−1600-nm region for (a) H_2O/TMAO, (b) H_2O/TBA samples at selected values of solute mole fraction X_2, and (c) pure H_2O at selected temperatures T.

Figure 5a and b show the quantity $\Delta\epsilon$ calculated from eq 2 using the measured absorbance in the wavelength region of 1800−2050 nm for H_2O/TMAO and H_2O/TBA mixtures, respectively; the influence of temperature on the extinction coefficient of water in the same spectral range is reported in Figure 5c. Again, the changes observed in the water band profiles are very similar for the two solutes, and strong analogies appear with the pure water spectrum by decreasing the temperature. The principal features of the difference spectra consist of decreased absorption at ca. 1898 nm (negative peak) with a concomitant increased absorption at higher wavelengths. This effect is more evident in the case of H_2O/TMAO samples.

The $\Delta\epsilon$ values at 1410 and 1898 nm, corresponding to minima in H_2O/TBA and H_2O/TMAO difference spectra, are plotted in Figures 6 and 7, respectively. A comparison of the two Figures shows the parallel behavior of $\Delta\epsilon$ in the two spectral regions for both solutes with almost linear trends in $\Delta\epsilon$ versus X_2 for TMAO but not for TBA solutions. To this end, it is worth mentioning that quantitative agreement is obtained for the

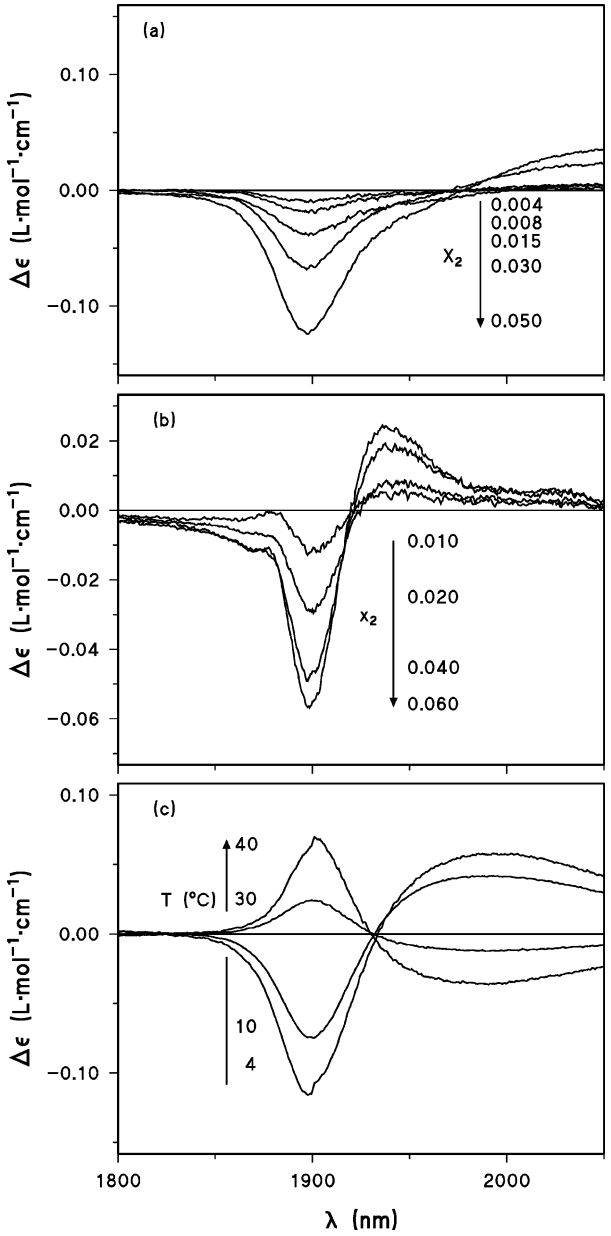

Figure 5. Difference spectra, $\Delta\epsilon$, in the 1800−2050 nm region for (a) H_2O/TMAO, (b) H_2O/TBA samples at selected values of solute mole fraction X_2, and (c) pure H_2O at selected temperatures T.

corresponding $\Delta\epsilon$ series versus X_2; in fact, the data belonging to the same solute, normalized to the $\Delta\epsilon$ values at the highest concentrations, coincide within the experimental errors (not shown). The consistency of the normalized $\Delta\epsilon$ values indicates that the correction factor $\Delta\epsilon_2 c_2/c_1$ is negligible for H_2O/TBA spectra near 1410 nm in the concentration interval of $0 < X_2 < 0.1$.

Figures 6 and 7 show significant differences in the hydration properties of TBA and TMAO. The decrease of $\Delta\epsilon$ versus X_2 is less pronounced in the TBA case than in the TMAO case. This is in agreement with previous suggestions[2,3,22,23,37] in which the interaction with water is stronger for TMAO than for TBA. Furthermore, for alcohol samples, $\Delta\epsilon$ decreases significantly at low X_2 whereas at higher mole fractions, it tends to level off to a saturation value. In contrast, for H_2O/TMAO samples, a roughly linear trend is observed for $\Delta\epsilon$ versus X_2. This means that nonadditive perturbations of water's H bonds are observed in the case of TBA solutions but not in the TMAO case. The continuous lines in Figures 6 and 7 are an interpolation of the

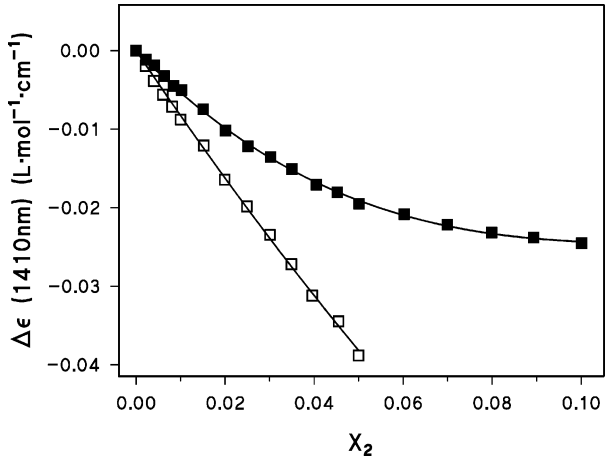

Figure 6. Values of difference spectra, $\Delta\epsilon$, at 1410 nm for (■) H_2O/TBA and (□) H_2O/TMAO samples vs solute mole fraction X_2. The continuous line (—) is a fit of the experimental data with a third-degree polynomial (TBA) and a second degree polynomial (TMAO). The data point dimensions take into account the experimental errors.

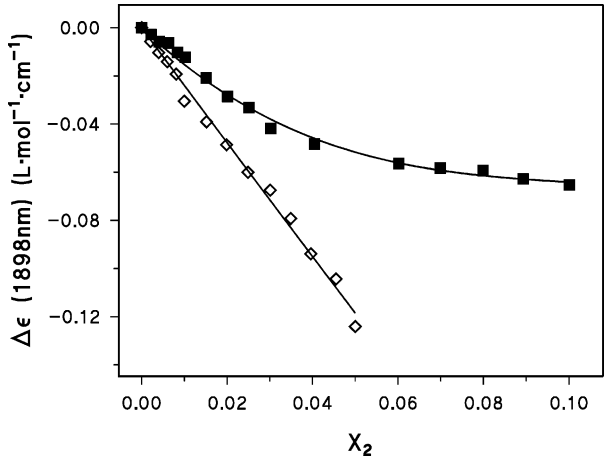

Figure 7. Values of difference spectra, $\Delta\epsilon$, at 1898 nm for (■) H_2O/TBA and (□) H_2O/TMAO samples vs solute mole fraction X_2. The continuous line (—) is an interpolation of the experimental data with a third-order polynomial (TBA) and a second-order polynomial (TMAO). The data point dimensions take into account the experimental errors.

experimental data with a third-order polynomial in the case of TBA and a second-order polynomial for TMAO.

The overall behavior of $\Delta\epsilon$ versus X_2 is consistent with that of other properties of TBA and TMAO aqueous solutions[3] and, in particular, with the shifts in the fundamental frequencies of the asymmetric and symmetric alkyl stretching bands of TBA that accompany the association of its hydrophobic groups in water.[1] For TMAO samples, this band maintains the characteristic typical of a monomeric solute dispersion (no variation in frequency or in other parameters).[3] This result substantially agrees with the roughly linear trends in $\Delta\epsilon$ versus X_2, as reported in Figures 6 and 7. In contrast, the CH stretching frequency in the TBA case is constant for $X_2 < 0.025$, decreases rapidly in the range of $0.025 < X_2 < 0.13$, and for higher X_2 progressively saturates toward the pure-alcohol value.[1] This behavior has been interpreted in terms of the self-aggregation of the alcohol molecules occurring beyond a threshold value of the alcohol mole fraction ($X_2^* = 0.025$). The slight variation in the CH stretching frequency for $X_2 > 0.13$ indicates that the TBA hydrophobic parts are essentially surrounded by other alcohol molecules, leaving the water−alcohol interfaces nearly unchanged. Such a description is consistent with the $\Delta\epsilon$ data reported in this work and, in particular, with the saturation value

attained by $\Delta\epsilon$ at higher TBA mole fractions. However, it should be stressed that here the $\Delta\epsilon$ data provide evidence for the nonlinear behavior of the H_2O/TBA mixture by looking at the modifications induced in water spectra, rather than in a solute band.

Comparison with Simulation Results. The above description agrees with recent studies of TBA and TMAO hydration properties through MD simulation.[23] Statistical analyses of the trajectories evidence quantitative agreement with experimental findings, indicating that TBA starts to self-aggregate in the same solute concentration range that is suggested by the experiment, whereas TMAO does not appreciably self-aggregate up to the highest concentration considered. In particular, MD data show that as X_2 increases the number of water molecules in the first hydration shell decreases uniformly for TMAO, whereas for TBA, it decreases more rapidly in a concentration range where the alcohol starts to self-aggregate. To compare MD and experimental data quantitatively, we try to describe NIR spectra in terms of a simple two-state model, which is found to account satisfactorily for the temperature-induced variations in pure-water band profiles.[33,34] Note that this description is supported by the presence of almost well-defined isosbestic points (Figures 4 and 5), which indicate a possible interconversion essentially between two spectral components corresponding to two different oscillator states. Let us decompose the volume of a solution containing n_1 moles of water and n_2 moles of TBA or TMAO into two regions: the free-water region with n_1^{free} moles of water, and the hydration region encompassing n_1^{hydr} water molecules statistically belonging to the solute's hydration structures. The hydration number $S(X_2)$ can be defined as follows:

$$S(X_2) = \frac{X_1^{\text{hydr}}}{X_2} \tag{3}$$

where X_1^{hydr} is the mole fraction of hydration water.

On the basis of the assumed two-state model,

$$\epsilon_1 + \Delta\epsilon_1 = \frac{\epsilon_1^{\text{hydr}} X_1^{\text{hydr}}}{X_1} + \epsilon_1\left(1 - \frac{X_1^{\text{hydr}}}{X_1}\right) \tag{4}$$

From eqs 3 and 4 one obtains

$$S(X_2) = k\frac{X_1}{X_2}\Delta\epsilon_1 \tag{5}$$

where $k = (\epsilon_1^{\text{hydr}} - \epsilon_1)^{-1}$ is a constant. The quantity $S(X_2)$ calculated by means of eq 5 using for $\Delta\epsilon_1$ the experimental $\Delta\epsilon$ values of Figures 6 and 7 is shown in Figure 8. In the same Figure, the results obtained from the simulation[23] for the number of water molecules in the first hydration shell of TBA and TMAO molecules are also reported. The two sets of experimental data have been normalized to the MD values corresponding to $X_2 = 0.0349$ for TMAO and 0.0699 for TBA samples, respectively. It is evident from the Figure that there is excellent agreement between the experimental $S(X_2)$ and the MD calculated number of water molecules in the first hydration shell. In the TMAO case, the data show a slight and roughly linear decrease in S on increasing X_2. This is in line with a picture of compact and well-organized hydration structures without the presence of self-aggregation phenomena.[3,23,37] On the contrary, the hydration number of TBA appears to be strongly dependent on X_2, as expected from the presence of mutual interference of the hydration shells and alcohol−alcohol aggregation phenomena.[1,2,23] In particular, the agreement with the simulation data indicates that NIR spectra detect essentially a water perturbation induced by TBA and TMAO that extends over almost all of the first hydration shell.

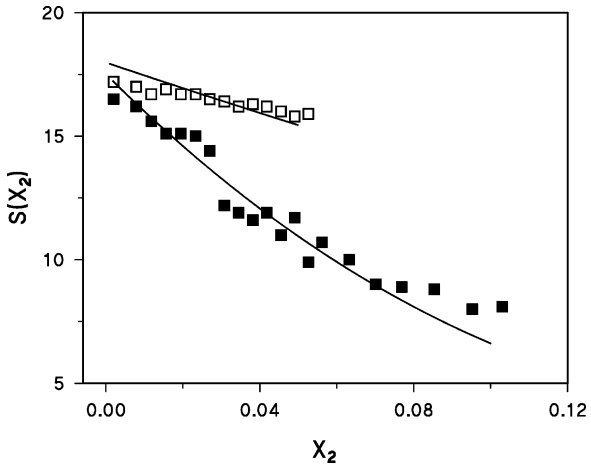

Figure 8. Hydration number calculated by MD simulation[23] for (■) TBA and (□) TMAO. (—) Experimental hydration number from NIR data (see eq 5).

Protein Folding and Micellization of Surfactants. The results described in the previous sections are of interest not only in themselves but also for their relevance in understanding the effects of solvent perturbation on biologically and chemically interesting processes, such as protein folding or micellization of a surfactant.[1] Actually, the effects of alcohol/water mixtures on the conformation of proteins are very complex. Although the general effect is the destabilization of the protein native state, low concentrations of alcohol in water seem to promote a more tightly folded conformation.[38,39] Similar behavior appears also by analyzing the effect of alcohols on the micellization process.[40] For example, the critical micelle concentration (cmc) of sodium dodecyl sulfate (SDS) in the presence of TBA decreases at low alcohol mole fractions and increases at high alcohol concentrations, passing through a minimum at $X_2 \approx 0.025$, where the hydrophobic clustering of TBA molecules starts.[3] Furthermore, in the same concentration range where the cmc and thus the free energy of micellization show a minimum, a maximum is observed for the transition enthalpy and entropy in the case of lysozyme thermal denaturation in H_2O/TBA mixtures.[21] Thus, the stabilization of both the protein and the micellar structures in the H_2O/TBA mixtures appears to be closely linked to the properties and the anomalous behavior of the mixed solvent. On the contrary, TMAO does not affect the micellization process[3] and does not significantly perturb the Gibbs energy of stabilization of proteins near room temperature.[41]

These results suggested that the addition of some small hydrophobic molecules such as TBA or TMAO may affect the unfolding of proteins or the micellization of surfactants by modifying the entity of free-energy contributions associated with the structural reorganization of water in these processes.[3,21] In other words, it seems that at low concentrations the alcohol essentially modulates the hydrophobic effect by inducing a concentration dependence of the hydration number S.

More precisely, the demicellization process as well as the unfolding of a protein can be described to a first approximation as the introduction of an equivalent number Δn_2 of hydrophobic groups into the solvent. The concomitant transfer of water molecules from the free to the hydration region is given by

$$\Delta n^{\mathrm{hydr}} = \Delta n_2\left(S + n_2\frac{\mathrm{d}S}{\mathrm{d}n_2}\right) \quad (6)$$

The exposure of the hydrophobic groups during the unfolding or demicellization process can therefore be associated with the following free-energy change:

$$\Delta G^{\mathrm{hydr}} = \Delta G_1^{\mathrm{hydr}}\left(S + n_2\frac{\mathrm{d}S}{\mathrm{d}n_2}\right)\Delta n_2 \quad (7)$$

where $\Delta G_1^{\mathrm{hydr}}$ is the free energy needed to transfer one mole of water from the free to the hydration region. In the case of TMAO aqueous solutions, the data show that $\mathrm{d}S/\mathrm{d}n_2 \approx 0$ so that ΔG^{hydr} is not appreciably affected by the solute concentration. Adding TMAO to an aqueous solution of surfactants or proteins illustrates this situation. In contrast, for the TBA molecules $\mathrm{d}S/\mathrm{d}n_2 \neq 0$, and a modulation of the hydrophobic effect and of ΔG^{hydr} is expected. In ref 21, it has been suggested, consistent with Shinoda's point of view,[42] that the hydrophobic interaction at a low concentration of hydrophobic groups is repulsive and disfavors the aggregation of nonpolar species. Therefore, the attenuation of these interactions, due to alcohol addition, should favor the clustering of hydrophobic groups and more compact structures. This is consistent with the effect of TBA on the micellization process and protein folding at low alcohol concentration. At high alcohol concentration, the contents' direct interactions between the hydrophobic groups of the protein and the alcohol molecules become increasingly favorable and progressively replace the interactions of these groups with water molecules, leading finally to the unfolding of the protein.

Conclusions

NIR spectra of H_2O/TBA and H_2O/TMAO samples in the 1250−1600- and 1800−2150-nm wavelength regions provide a coherent description of the water structure perturbations induced by these two solutes. By using NIR spectroscopy, the perturbation of the water structure due to the presence of TBA or TMAO is evident and reproducible. This allows us to make a quantitative evaluation of the solute hydration structures as a function of X_2 that can be easily correlated with the MD results. According to MD simulations, the experimental spectra show that the TMAO molecules tend to stabilize highly ordered, noninteracting hydration structures and do not show any self-aggregation behavior. This is reflected, in turn, in a negligible dependence of the hydration number S from X_2. In contrast, the TBA molecules perturb the water's structure less, but the alcohol hydration shells interact with each other and the solute molecules show marked self-association. This allows the corresponding alcohol hydration number to be strongly dependent on X_2. Furthermore, the experimental values obtained for the hydration number versus X_2 of both TMAO and TBA numerically agree with MD simulation data. This result is of notable interest because, despite the intensive simulation work, the experimental data reported in the literature concerning the structural properties of alcohol or amine hydration shells are somewhat contradictory. Finally, the concentration dependence of the hydration number could suggest a possible microscopic mechanism underlying the effects of TBA and TMAO on macroscopical processes governed by the hydrophobic effect, such as protein folding or micellization of surfactants. The data support the assumption that the presence of TBA or TMAO affects these processes by modifying the extent of the free-energy contribution associated with the structural reorganization of water, that is, by modulating the hydrophobic effect. This phenomenon has been related, at low alcohol concentration, to the X_2 dependence of the TBA hydration number. At high alcohol concentrations, the hydrophobic clustering of TBA molecules is likely accompanied by surfactant−TBA or protein−TBA hydrophobic associations, which destabilize the micelle aggregate or the native structure of the protein. However, for TMAO molecules, the hydration number is almost independent of X_2, and no modulation of the hydrophobic effect is expected in the entire investigated mole fraction range.

References and Notes

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