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Different Thermal Unfolding Pathways of Catalase in the Presence of Cationic Surfactants

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In this paper we have corroborated the usefulness of spectroscopic techniques, such as UV−visible, in the study and thermodynamic characterization of the thermal unfolding of catalase as a function of the concentration and alkyl chain length of *n*-alkyltrimethylammonium bromides (C_nTAB, $n = 8$, 10, and 12). For this reason, a thermodynamic model was used which included experimental data corresponding to the pre- and posttransition into the observable transition. It has been found that *n*-alkyltrimethylammonium bromides play two opposite roles in the folding and stability of catalase. They act as a structure stabilizer at a low molar concentration and as a destabilizer at a higher concentration. The maximum of the unfolding temperature has been found to decrease with the alkyl chain. The reason for this difference has been suggested to be the side chains involved. In the presence of C8TAB and C10TAB, Gibbs energies of unfolding ($\Delta G(T)$) decrease with concentration, whereas for C12TAB an increase has been observed. These findings can be explained by the fact that when differences in the hydrophobic nature of the surfactants exist, different pathways of unfolding may occur. Also, the presence of surfactants has been observed to affect the cold denaturation of catalase. Thermodynamic results suggest that the thermal denaturation of catalase in the presence of *n*-alkyltrimethylammonium bromides is a perfect transition between two states.

Introduction

The stability of proteins is the result of residue−residue and residue−solvent interactions. The fact is that all interactions are essentially electrostatic in origin, and traditionally they have been classified as van der Waals, electrostatic, hydrogen bonds, hydrophobic, and disulfide bridges.[1] Water-soluble proteins, which are composed of 25−30% hydrophobic amino acids, can self-assemble to form a defined structure. In general, hydrophobic amino acids are located in the interior region of the protein to avoid exposure to water.[2] Due to their amphiphilic character, the interaction between a surfactant and a protein results in changes in the natural state of the protein. The recent huge advances in biomacromolecular assembly and its applications have improved our understanding of the physical processes of these complex systems.[3]

In previous papers, we have studied the interactions between different proteins and amphiphilic ligands (lysozyme−*n*-alkyltrimethylammonium bromides,[4] lysozyme−*n*-alkyl sulfates,[5] catalase−nafcillin,[6] ovalbumin−SDS,[7] zein−SDS[8]) using a variety of physical methods including equilibrium dialysis, microcalorimetry, light scattering, and electrophoretic mobilities. The results have permitted us to obtain a thermodynamic picture of the nature of the protein−amphiphilic interaction (the binding interaction is exothermic and dominated by large increases in entropy). Recently, we have reported an investigation of the interaction of hydrogenated and fluorinated surfactants with human serum albumin (HSA).[9] Sodium octanoate, sodium perfluorooctanoate, and sodium dodecanoate were found to bind extensively to HSA in an aqueous solution. The changes in the slope of Gibbs energies were identified with the saturation of the first binding set. The surfactants were found to have different favorite adsorption sites along the protein, and the adsorption processes of perfluorooctanoate and dodecanoate were similar.

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In the present study we have examined the nature of the interaction of selected surfactants with a globular protein. This was done with several purposes in mind. First, we wanted to study the surfactant−protein interactions by optical methods which are less invasive. Second, there is a necessity for new models to quantify the thermodynamic parameters of these processes. A third consideration was to obtain information about the effect of the surfactants on the thermal stability of the proteins. The compounds used as ligands included three cationic surfactants, C_8TAB, C_{10}TAB, and C_{12}TAB *n*-alkyltrimethylammonium bromides, which allowed us to observe the effect of the alkyl chain length. The globular protein chosen was catalase. Catalase (hydrogen peroxide:hydrogen peroxide-oxidoreductase, EC 1.11:1.6), present in the peroxisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. The protein exists as a dumbbell-shaped tetramer of four identical subunits; each subunit is formed by a single polypeptide chain with hemin as a prosthetic group. Catalase was one of the first enzymes to be purified to homogeneity and has been the subject of intense study by several physical methods.

Experimental Section

Materials. Crystalline bovine liver catalase (Product No. C-9322) was obtained from Sigma. The surfactants octyltrimethylammonium bromide (C8TAB), decyltrimethylammonium bromide (C10TAB), and dodecyltrimethylammonium bromide (C12TAB) were obtained from Lancaster MTM Research Chemicals Ltd. All materials were of analytical grade, and solutions were made using doubly distilled and degassed water.

Difference Spectroscopy. Difference spectra were measured using a Beckman spectrophotometer (Model DU 640), with six microcuvettes, which operates in the UV−visible region of the electromagnetic spectrum wavelength. All measurements were

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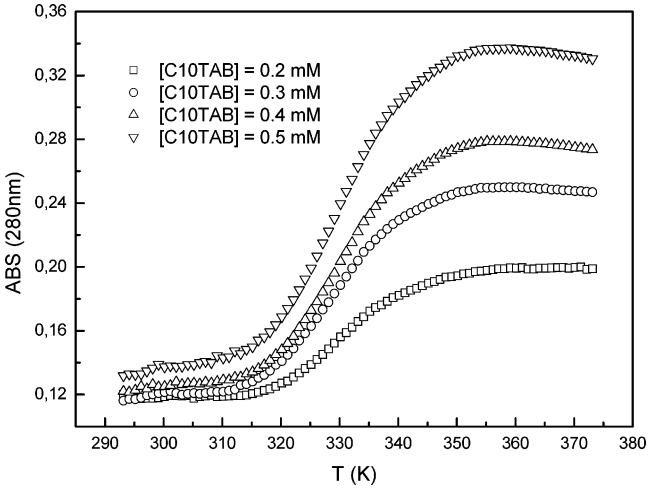

Figure 1. Thermal unfolding curves for catalase using the differences in absorbance at 280 nm in the presence of decyltrimethylammonium bromide.

made using catalase solutions with a fixed concentration of 0.125 g dm^{-3} in carefully matched quartz cuvettes (50 μL capacity). For absorbance difference spectra, just two cells were used. The first microcuvette contained only protein in the corresponding medium and was used as a blank reference; meanwhile, the other was filled with the corresponding protein plus surfactant solution. The microcuvettes were filled and placed in the same orientation for all the tests. Measurements were made after protein and surfactant had been incubated for over 30 min, after which the difference spectra did not change. For absorbance measurements with changing temperature, a Beckman (DU Series) temperature controller was used, following the Peltier methods of controlling temperature, in the range 20−100 °C.

Results and Discussion

Plots of the absorbance of catalase at 280 nm, as a function of temperature, in the presence of different decyltrimethylammonium concentrations are shown in Figure 1. Similar plots have been obtained for the rest of the concentrations and for octyl- and dodecyltrimethylammonium bromides. The data show that there is a transition region over which the absorbance changes with temperature. Thus, for all our systems under study, a two-state mechanism was assumed in order to evaluate the thermodynamic parameters obtained from spectroscopic techniques.

From the thermodynamic point of view, the denaturation process can be described as a transition between two macroscopic states,[10,11] that is, from the native state (N) to a denaturated state (D).

$$\mathrm{N} \Leftrightarrow \mathrm{D} \tag{1}$$

The stability of a globular protein is usually quantified with Gibbs energy values, since ΔG is the work required for disruption of the native protein structure. For that reason, the difference in Gibbs energy at a given temperature can be expressed by the Gibbs−Helmholtz equation:

$$\Delta G(T) = \Delta H_{\mathrm{m}}(1 - T/T_{\mathrm{m}}) - \Delta C_p(T_{\mathrm{m}} - T + T\ln(T/T_{\mathrm{m}})) \tag{2}$$

where T_{m} refers to the temperature at the midpoint of the transition ($T_{\mathrm{m}} = T$ at $\Delta G = 0$), ΔH_{m} is the enthalpy of denaturation at the T_{m}, and ΔC_p is the heat capacity change between the folded and unfolded conformations.

The evaluation of thermodynamic parameters, obtained from spectroscopic techniques, is based on the equilibrium constant K for a transition between the native state and the denaturated state. The equilibrium constant was deduced from the equation

$$K = \frac{[\text{unfolded}]}{[\text{native}]} \tag{3}$$

or as a function of spectroscopic parameters:

$$K = \frac{A_{\mathrm{N}} - A_0}{A_0 - A_{\mathrm{D}}} \tag{4}$$

where A_{N} is the absorbance of the pure native state, A_{D} is the corresponding absorbance of the pure denatured state, and A_0 is the observed absorbance at any temperature in the transition zone.

After denaturation, the protein solution was cooled to room temperature to measure the absorbance and to compare with that of the unheated sample. The concordance of absorbance values is taken as a measure of the reversibility of the heat-induced denaturation. In all the cases, the thermal transitions were reversible and hence amenable to thermodynamic analysis.

To avoid large errors in the estimation of the thermodynamic parameters, we have followed the procedure created by Kaushik et al.[12] Thus, we have used all the experimental data points obtained and fitted them into the equations in the following manner:

$$A_0 = \frac{A_{\mathrm{N}} + KA_{\mathrm{D}}}{1 + K} \tag{5}$$

On the other hand, the equilibrium constant can be expressed by a Gibbs energy function:

$$K = \exp(-\Delta G/RT) \tag{6}$$

where R is the gas constant and T is the temperature in kelvin. Substituting the value of K in eq 5, the following equation is generated:

$$A_0 = \frac{A_{\mathrm{N}} + A_{\mathrm{D}}\mathrm{e}^{-\Delta G/RT}}{1 + \mathrm{e}^{-\Delta G/RT}} \tag{7}$$

Finally, substituting the $\Delta G(T)$ expression for eq 2, the result is

$$A_0 = \frac{A_{\mathrm{N}} + A_{\mathrm{D}}\exp\left[-\frac{1}{R}\left(\Delta H_{\mathrm{m}}\left(\frac{1}{T} - \frac{1}{T_{\mathrm{m}}}\right) - \Delta C_p\left(\frac{T_{\mathrm{m}}}{T} - 1 + \ln\left(\frac{T}{T_{\mathrm{m}}}\right)\right)\right)\right]}{1 + \exp\left[-\frac{1}{R}\left(\Delta H_{\mathrm{m}}\left(\frac{1}{T} - \frac{1}{T_{\mathrm{m}}}\right) - \Delta C_p\left(\frac{T_{\mathrm{m}}}{T} - 1 + \ln\left(\frac{T}{T_{\mathrm{m}}}\right)\right)\right)\right]} \tag{8}$$

The concern about baselines follows from the way in which progress through the unfolding transition is determined: pre- and posttransition baselines are extrapolated into the observable transition zone and the relative concentrations of native protein and denatured protein are determined from the distances between the observed and extrapolated spectral values. For proper evaluation of fitting errors, terms for baselines should be included in any equation used to fit the spectroscopic data.[11] Since A_{N} and A_{D} have been found to be linear functions of temperature, they can be written as

$$A_N = a_N + m_N T \quad (9)$$

$$A_D = a_D + m_D T \quad (10)$$

Finally, the difference in free energy between folded and unfolded conformations, ΔG, depends mainly on the first term at temperatures near T_m. The second term becomes important at temperatures below T_m.[13] Plotting ΔG against temperature, the temperature of maximum stability can be obtained by means of

$$T_s = T_m \exp(-\Delta H_m / T_m \Delta C_p) \quad (11)$$

Figure 2 shows the unfolding temperature, T_m, of catalase as a function of the concentration of the surfactants under study. T_m was calculated by fitting the experimental absorbance difference to eq 8. The numerical analysis of the data was made by means of the Levenberg−Marquardt least squares fitting algorithm which consists of the combination of two classical methods to minimize χ^2, the approximation to the minimum by a Taylor expansion and by a gradient approximation.

For the three surfactants under study, unfolding temperature exhibits the same pattern and the experimental points have been fitted to a second degree polynomial. On the basis of these facts, these results can be interpreted as that the *n*-alkyltrimethylammonium bromides play two opposite roles in the folding and stability of catalase. They act as a structure stabilizer at low molar concentrations (enhancing T_m) and as a destabilizer at higher concentrations (diminishing T_m). A similar behavior has been corroborated with BSA and SDS.[14,15]

Although both cationic and anionic surfactants coincide with that, protein unfolding typically occurs above the critical micelle concentration. In general, cationic amphiphiles bind cooperatively to most proteins to form complexes similar to those formed by SDS, but with diminished affinity. The reason for this difference has been suggested to be the side chains involved. Favorable electrostatic interactions for cationic amphiphiles are Glu and Asp, which have only two and one methylene side chain groups, whereas the corresponding side chains for anionic amphiphiles are Lys and Arg, amino acids with four and three methylene groups. This makes a significant hydrophobic contribution to binding as well as a favorable electrostatic interaction.[16] Thus, changes in unfolding temperature have been interpreted on the basis that small amounts of surfactants induced

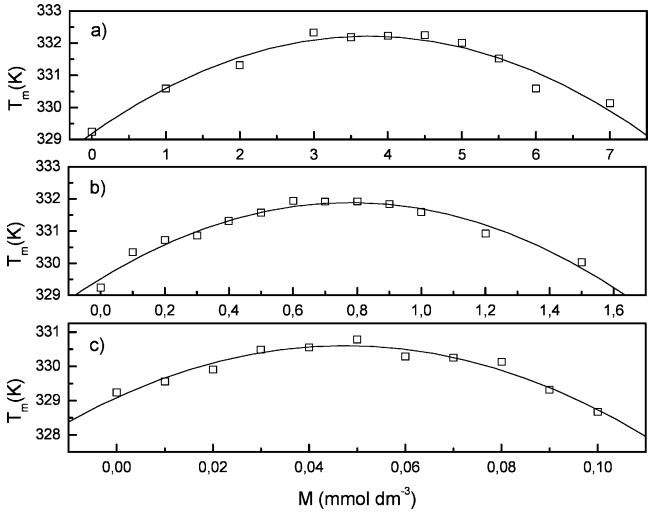

Figure 2. Catalase unfolding temperature (T_m) as a function of concentration of (a) octyltrimethylammonium bromide, (b) decyltrimethylammonium bromide, and (c) dodecyltrimethylammonium bromide.

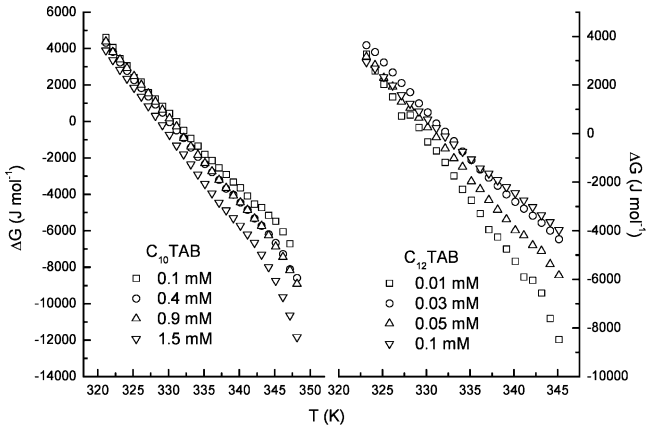

Figure 3. Gibbs energy of unfolding (ΔG) of catalase (0.125 g dm^{-3}) as a function of temperature, in the presence of different surfactants: decyltrimethylammonium bromide (left) and dodecyltrimethylammonium bromide (right).

a protective effect due to some cross-linking function of the surfactant ions. In other words, the native conformation is stabilized by a cross-linking function of the surfactant ion between a group of nonpolar residues and a positively charged residue located on different loops of the protein.[17,18]

From the plots in Figure 2 the effect of the alkyl chain length on the variation of the maximum of the unfolding temperature can be noticed. Values of 332.26, 331.88, and 330.61 K have been obtained in the presence of octyl-, decyl-, and dodecyltrimethylammonium bromides, respectively. These values are higher than the value obtained for the catalase without surfactants, 329.24 K, indicating a decrease in the maximum T_m with the alkyl chain length. The binding of these molecules to proteins shows association isotherms, which indicates different steps in the association of the surfactants to the protein. The initial region at the lowest concentrations of surfactants is associated with the binding to specific high-energy sites on the protein, and the interactions are mainly of electrostatic nature. The next region corresponds to noncooperative interactions where hydrophobic interactions are the driving force.[19,20] However, Liu et al.[21] in a previous paper studied the interaction between nucleic acids with cationic surfactants (cetylpyridine and cetyltrimethylammonium bromides) and with anionic surfactants by resonance light scattering spectroscopy, showing that hydrophobic attraction force exists in those interactions even at low concentrations. In another study, Gelamo et al.[19] reveals that the binding constants of surfactants to proteins depend on the alkyl chain length.

Gibbs energies of unfolding, $\Delta G(T)$, have been calculated from equilibrium constants. Plots of Gibbs energies as a function of temperature (Figure 3) show a nonlinear decrease from positive to negative values. The same behavior has been found for all systems under study. It is worth mentioning that $\Delta G(T)$ values, for C8TAB and C10TAB, decrease with concentration whereas, for C12TAB, an increase with concentration can be observed. Obviously, this fact can only be attributed to the alkyl chain length effect. Indeed we have previously described how C12TAB has a minor effect on catalase increasing the T_m. It has been demonstrated that denaturant-induced unfolding and temperature-induced unfolding pathways show different conformational distributions at unfolded and transition states. The free energy of unfolded states far from the native state under temperature-induced unfolding is lower than that for the case under denaturant-induced unfolding. The free energy of the unfolded state close to the native state is higher under temper-

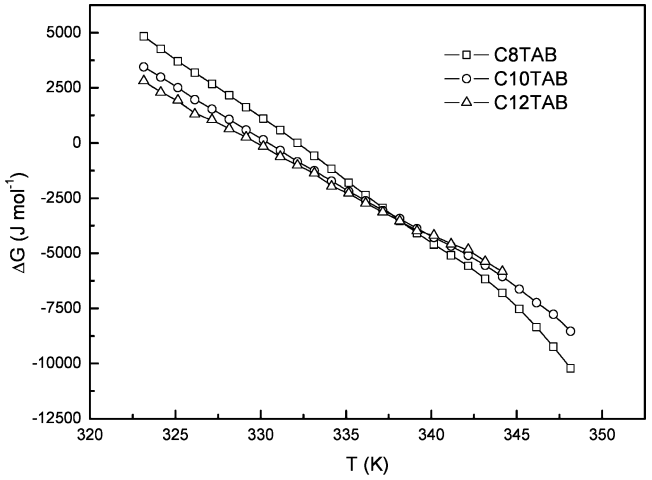

Figure 4. Gibbs energy of unfolding (ΔG) of catalase (0.125 g dm^{-3}) as a function of temperature for concentrations corresponding to the maximum values of unfolding temperature (T_m).

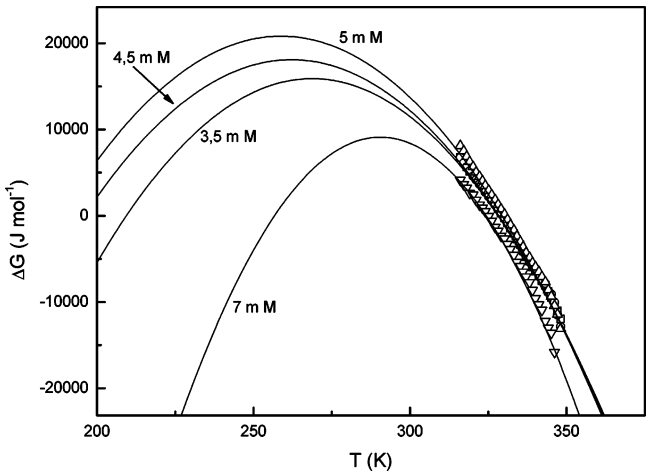

Figure 5. Gibbs energy of unfolding (ΔG) of catalase (0.125 g dm^{-3}) as a function of temperature in the presence of octyltrimethylammonium bromide. The solid lines represent fit of experimental points to eq 2.

ature-induced unfolding than under denaturant-induced unfolding. It is realized that the denaturant-induced unfolding shows a wider conformational distribution than the temperature-induced unfolding because denaturant molecules bound to a protein molecule break contacts between amino acid residues, resulting in higher conformational entropy.[22]

Gibbs energies of unfolding of catalase for concentrations corresponding to the maximum values of the unfolding temperature (4, 0.8, and 0.05 mM for C8TAB, C10TAB, and C12TAB, respectively; Figure 2) are plotted as a function of temperature in Figure 4. We have chosen the maximum of T_m for each surfactant as a reference point to compare the thermodynamics of these processes. At low temperatures, the Gibbs energies increased in the sequence C12TAB < C10TAB < C8TAB. These differences tend to decrease until they reach a common value of -3425 J mol^{-1} at 338 K. Finally, at higher temperatures the Gibbs energies increased in the sequence C8TAB < C10TAB < C12TAB. Again, the differences are explained on the basis of the various pathways of unfolding due to differences in the hydrophobic nature of the surfactants.

In Figure 5, the experimental data from the Gibbs energies of unfolding of catalase in the presence of C8TAB are shown along with theoretical curves calculated by fitting experimental points to eq 2. Similar plots have been obtained for the rest of the concentrations and for the other two surfactants. These kinds

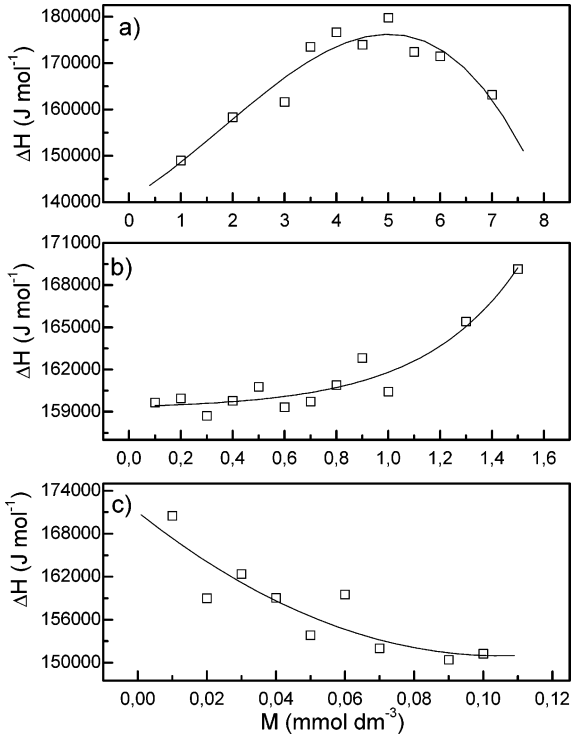

Figure 6. Enthalpies of denaturation (ΔH_m) of catalase (0.125 g dm^{-3}) as a function of concentration of (a) octyltrimethylammonium bromide, (b) decyltrimethylammonium bromide and (c) dodecyltrimethylammonium bromide.

of plots are known as protein stability curves[23] and show that the temperature of maximum stability, T_s, where $\Delta S = 0$, can be calculated by using eq 11. The T_s values of proteins generally fall between 263 K for a relatively hydrophilic protein such as RNase A and 308 K for a relatively hydrophobic protein such as β-lactoglobulin.[13] The calculated values of T_s for the four curves in Figure 5 are 258, 262, 269, and 290 K. For the rest of the systems, we have obtained values in the same range, although it was not possible to determine any guideline. Thus, the presence of surfactants can affect even the cold denaturation of the proteins.[24,25]

Figure 6 shows the values obtained for the enthalpies of denaturation of catalase in the presence of the three surfactants. At low surfactant concentrations, ΔH_m increased in the sequence C8TAB < C10TAB < C12TAB. This nearly follows the sequence for the increase in the denaturation temperature, which indicates that C12TAB binds to catalase more strongly than to surfactants. This emphasizes the importance of the hydrophobic part of surfactants in binding with catalase. Deep et al.[14] found this behavior for interactions between bovine serum albumin and anionic surfactants. With increasing concentration, ΔH_m increases for C8TAB and C10TAB (except at the highest C8TAB concentrations, where ΔH_m decreases slightly), in contrast to C12TAB. Again, this result could be explained on the basis of different pathway unfoldings.

Table 1 summarizes the values obtained for the entropy and heat capacity of catalase unfolding in the different systems. Since surfactant cations are being added to a flexible macroanion, an expansion should occur, not a contraction. Hence, it seems fairly certain that the hydrophobic section of the surfactants is not only somehow causing shrinkage to ensue, but makes up for entropy terms. Thus, at the lowest concentrations, the entropy is higher for C12TAB; meanwhile, at the highest ones, this surfactant presents the lowest entropy values. Presumably the

TABLE 1: Parameters Characterizing the Thermal Unfolding of Catalase−Surfactant System

M (mmol dm^{-3})	ΔS (J mol^{-1} K^{-1})	ΔC_p (J mol^{-1} K^{-1})
	C8TAB	
1.0	451	6522
2.0	478	4302
3.0	493	5784
3.5	529	5382
4.0	538	6119
4.5	529	4506
5.0	551	3471
5.5	526	2676
6.0	519	3755
7.0	494	6522
	C10TAB	
0.1	484	9708
0.2	486	5458
0.3	478	5988
0.4	482	5812
0.5	486	5403
0.6	479	3023
0.7	481	3000
0.8	485	3094
0.9	492	2887
1.0	482	5013
1.3	495	2678
1.5	512	2989
	C12TAB	
0.01	533	4860
0.02	479	4466
0.03	496	4389
0.04	481	4033
0.05	464	4245
0.06	482	5782
0.07	459	6202
0.09	453	5939
0.10	460	4544

alkyl chain of the surfactant loses several degrees of freedom. Also, their length yields a more compact macromolecule.

Experimentally, one finds that the hydration contribution to the heat capacity is positive for apolar surfaces, whereas it becomes negative for polar surfaces. By the term "apolar" we mean that the surface has no permanent dipoles, whereas a polar surface consists of permanent dipoles and charges.[26] Thus, an increase in heat capacity upon the unfolding of proteins is caused by the hydration change of proteins, and it is closely related to the change in solvent accessible and nonaccessible surface area. We have previously indicated that the unfolding process of catalase has different pathways as a function of the surfactant and the concentration. The different values of heat capacity listed in Table 1 corroborate this fact. Although it is not easy to extract conclusions due to the dispersion of the points, a general feature of this process can be extrapolated. The heat capacity values for C12TAB exhibit less dispersion than the other two surfactants. This could be related to the length of the alkyl chain covering a greater number of apolar residues, which avoid their hydration.

In principle, the sharpness of a phase transition can be quantified by the coefficient α:[1]

$$\alpha = \frac{\langle H_D - H_N \rangle^2}{\langle H^2 \rangle - \langle H \rangle^2} \tag{12}$$

where H_N and H_D are the enthalpies for the native and unfolded states, respectively. Thus, the smaller α is, the sharper the transition. This possible transition is a two-state system, where there are no intermediate or molten states. In that case, at the midpoint of the transition, $\alpha = 4$. In real proteins and based on

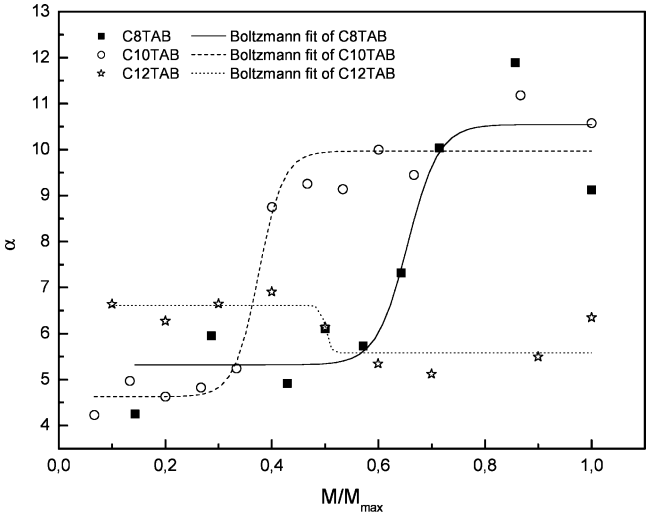

Figure 7. Coefficient α versus normalized concentration (M/M_{max}) for the thermal unfolding of catalase.

data from calorimetry, the heat capacity, C_p, determines the denominator in eq 12. Meanwhile, the numerator can be determined by integrating the excess heat capacity:

$$\Delta H = \Delta H_{cal} = Q \tag{13}$$

This latter quantity is denoted as calorimetric enthalpy in the protein literature. Then

$$\alpha = \frac{\Delta H_{cal}^{\ 2}}{k_B T_m^{\ 2} C_p} \tag{14}$$

Obtained values of α for all our systems are plotted in Figure 7. For systems containing C8TAB and C10TAB, at low concentrations, values are close to 4, indicating a perfect transition between two states (folded and unfolded) as we had initially postulated. When the concentration is increased, α presents a stepped change to values around 10, a broader transition but without intermediate states. For C12TAB, lower values have been obtained, which decrease with concentration.

Conclusions

Spectroscopic techniques have been demonstrated to be useful to determine the thermodynamics of protein unfolding even in the presence of different surfactants. One of the main advantages of these techniques is that much less protein is needed in the experiments. The critical role that pre- and posttransition baselines play in the obtaining of the thermodynamic parameters has been solved by including experimental data corresponding to the pre- and posttransitions in the observable transition.

n-Alkyltrimethylammonium bromides play two opposite roles in the folding and stability of catalase. They act as a structure stabilizer at low molar concentrations (enhancing T_m) and as a destabilizer at higher concentrations (diminishing T_m). The maximum in unfolded temperature has been found to decrease with the alkyl chain length. Various pathways of unfolding due to differences in the hydrophobic nature of the surfactants have been suggested. Because of the fact that surfactants bound to a protein molecule break contacts between amino acid residues, higher values of conformational entropy have been obtained. Finally, it has been demonstrated that catalase in the presence of *n*-alkyltrimethylammonium bromides exhibit a simple two-state thermal denaturation, which becomes narrower when the

C12TAB concentration is increased, and broader with C8TAB and C10TAB.

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References and Notes

(1) Sneppen, K.; Zocchi, G. *Physics in Molecular Biology*; Cambridge University Press: Cambridge, U.K., 2006.
(2) Kuwahara, J.; Akisada, H.; Kato, T.; Nishino, N. *Colloid Polym. Sci.* **2005**, *283*, 747.
(3) Gao, H-W.; Hu, Z-J.; Zhao, J-F. *Chem. Phys. Lett.* **2003**, *376*, 251.
(4) Mosquera, V.; Ruso, J. M.; Prieto, G.; Sarmiento, F. *J. Phys. Chem.* **1996**, *100*, 16749.
(5) Sarmiento, F.; Ruso, J. M.; Prieto, G.; Mosquera, V. *Langmuir* **1998**, *14*, 5725.
(6) Ruso, J. M.; Taboada, P.; Martínez-Landeira, P.; Prieto, G.; Sarmiento, F. *J. Phys. Chem. B* **2001**, *105*, 2644.
(7) González-Pérez, A.; Ruso, J. M.; Prieto, G.; Sarmiento, F. *Colloid Polym. Sci.* **2004**, *282*, 351.
(8) Ruso, J. M.; Deo, N.; Somasundaran, P. *Langmuir* **2004**, *20*, 8988.
(9) Blanco, E.; Messina, P.; Ruso, J. M.; Prieto, G.; Sarmiento, F. *J Phys. Chem. B* **2006**, *110*, 11369.
(10) Privalov, P. L. *Thermochim. Acta* **1990**, *163*, 33.
(11) Robertson, A. D.; Murphy, K. P. *Chem. Rev.* **1997**, *97*, 1251.
(12) Kaushik, J. K.; Bhat, R. *J. Phys. Chem. B* **1998**, *102*, 7058.
(13) Pace, C. N.; Laurents, D. V. *Biochemistry* **1989**, *28*, 2520.
(14) Deep, S.; Ahluwalia, J. C. *Phys. Chem. Chem. Phys.* **2001**, *3*, 4583.
(15) Moriyama, Y.; Kawasaka, Y.; Takeda, K. *J. Colloid Interface Sci.* **2003**, *257*, 41.
(16) Otzen, D. E. *Biophys. J.* **2002**, *83*, 2219.
(17) Vieira, E. P.; Hermes, H.; Möhwald, H. *Biochim. Biophys. Acta* **2003**, *1645*, 6.
(18) Moriyama, Y.; Takeda, K. *Langmuir* **1999**, *15*, 2003.
(19) Gelamo, E. L.; Tabak, M. *Spectrochim. Acta, Part A* **2000**, *56*, 2255.
(20) (a) Jones, M. N. *Chem Soc. Rev.* **1992**, *127*. (b) Jones, M. N.; Manley, P.; Wilkinson, A. E. *Biochem. J.* **1982**, *203*, 285.
(21) Liu, R.; Yang, J.; Sun, C.; Wu, X.; Li, L.; Su, B. *Colloids Surf., B: Biointerfaces* **2004**, *34*, 59.
(22) Choi, S.; Huh, J.; Jo, W. H. *Biomacromolecules* **2004**, *5*, 2289.
(23) Becktel, W. J.; Schellman, J. A. *Biopolymers* **1987**, *26*, 1859.
(24) Pace, C. N.; Tanford, C. *Biochemistry* **1968**, *7*, 198.
(25) Privalov, P. L.; Griko, Y. V.; Venyaminov, S. Y.; Kutyshenko, V. P. *J. Mol. Biol.* **1986**, *190*, 487.
(26) Bakk, A.; Hoye, J. S.; Hansen, A. *Biophys. J.* **2002**, *82*, 713.