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ζ-Potential Study on the Interactions between Lysozyme and Sodium *n*-Alkylsulfates

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An experimental investigation on the adsorption of *n*-alkylsulfates with 8, 10, 12, 14, and 16 carbon atoms in the alkyl chain length on lysozyme at pH 3.2, 7.0, and 10.0 by measurements of the zeta potential (ζ-potential) is described. At pH 7.0 and 10.0, the protein surface has a negative electrokinetic charge while at pH 3.2 the alkylsulfate ions affect the ζ-potential causing a change in the neighborhood of the point of zero charge (pzc) from positive to negative values. Values of the Gibbs energies of adsorption, calculated from the pzc of alkylsulfates of carbon chain lengths 8, 10, and 12 agree with Gibbs energies of binding of *n*-alkylsulfates to lysozyme and when compared with the adsorption of *n*-alkyltrimethylammonium ions show that the headgroup interaction is of similar form.

Introduction

The interaction between a surfactant and a protein causes a drastic change in the conformation of protein.[1] Even at a very low surfactant concentration (far below the critical micelle concentration, CMC), the protein is denatured by surfactant molecules. This denaturation is complex and involves ionic forces between the surfactant headgroups and charged amino acid residues of the protein in combination with hydrophobic interactions between the hydrocarbon chains of the surfactants and hydrophobic amino acid residues.[2] The relative importance of these two types of interactions can be partially assessed by investigating the interaction between a protein and a homologous series of surfactants for which a wide range of experimental techniques can be used.

Lysozyme is a small protein (molecular mass 14 603) with 18 cationic amino acid residues (6 lysyl including 1 N-terminal, 11 arginyl, and 1 histidyl) and 12 anionic residues (2 glutamyl, 9 aspartyl, and 1 leucyl C-terminal).[3] X-ray studies have revealed that the lysozyme is a rigid and stable enzyme.[4] In the range of physiological pH values, lysozyme does not show any detectable change in its structure up to 77 °C, and at the physiological temperature, no detectable change in the structure was observed with a pH change from 1.2 to 11.3. The stability of lysozyme has been attributed to the four disulfide bonds besides hydrogen bonds and hydrophobic interactions among the amino acid residues. However, the inactivation of the enzyme is possible when solutions of surfactants are added to the lysozyme, and numerous studies on surfactant interactions have been reported.[5−9] These studies have shown that interactions involve the anionic binding to the cationic sites and further binding by hydrophobic cooperative interactions and that the cationic surfactants inhibit the enzyme as a consequence of the interaction. We have recently reported a study[10] of the interaction of a range of *n*-alkyltrimethylammonium bromides, C_nTAB ($n = 8, 10, 12, 14,$ and 16), with lysozyme at different pH and surfactant concentrations. We considered the protein surface as a surface for surfactant adsorption estimating the energies of the interaction from the zeta-potential (ζ-potential) measurements.

As a continuation of this research we have measured the ζ-potential of lysozyme with *n*-alkylsulfates at pH 3.2, 7.0, and 10.0. We have chosen these media because the conformation and stability of proteins, especially enzymes, are a function of, among others factors, the pH. For each pH, a stable conformation exists. Qualitatively, the pH of a protein-buffered solution affects the net charge on the protein as a consequence of the state of ionization of the ionic side chains of the amino acid residues. Since the initial interaction of surfactants with protein is known to be with the ionic side chains, the initial pH of the protein solution would be expected to influence surfactant binding.[11] Measuring the conformational stability requires determining the Gibbs energy associated with the conformational changes for which we have used the ζ-potential technique that, as we have shown,[10] is very useful in the study of the interaction between proteins and amphipathic ligands.

Experimental Section

Lysozyme (from chicken egg white, product no. L-6876, 48 000 units per mg) was used as supplied by Sigma Chemical Company. Sodium *n*-octyl-, *n*-decyl-, *n*-dodecyl-, *n*-tetradecyl-, and *n*-hexadecylsulfate (product nos. 5787, 5658, 5987, 6305, and 6402, respectively) were obtained from Lancaster MTM Research Chemicals Ltd. Three buffered solutions were used: 50 mM

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glycine plus hydrochloric for a pH of 3.2, 50 mM phosphate for a pH of 7.0, and 50 mM glycine plus sodium hydroxide for a pH of 10.0. All materials were of analytical grade and solutions were made in double distilled water. All measurements were below the literature values of the CMC of the surfactants at each pH.[12,13]

Samples of 2.5 cm^3 of the protein solution of concentration 1.25×10^{-3} kg m^{-3} were equilibrated with 2.5 cm^3 of surfactant solution covering the required range of concentration for over a week at room temperature.

The Zetamaster Model 5002 (Malvern Instruments, UK) was used to carry out the ζ-potential mesurements. All experiments were made in a 5 mm × 2 mm rectangular quartz capillary and the average of five measurements at stationary level were taken.

Results and Discussion

The calculation of ζ-potential is realized by the Smoluchowski's equation[14]

$$\zeta = \eta\mu_e/\epsilon \quad (1)$$

where η and ϵ are the absolute viscosity and dielectric permittivity of medium, respectively, and μ_e is the electrophoretic mobility. Equation 1 applies to particles with a Debye length, κ^{-1}, much smaller than the mean radius of curvature of the particles and for low values of ζ-potential (ζ < 25 mV).[15−18] The reciprocal Debye length, κ, for charged particles in solution is given by

$$\kappa = 2n_0e^2/\epsilon kT \quad (2)$$

where n_0 is the ionic concentration, e is the charge, k is the Boltzmann constant, and T is the temperature. In our case, $\kappa^{-1} \cong 10^{-4}a$, a being the lysozyme radius obtained from X-ray studies[4] so it is possible to use the Smoluchowski equation.

Figures 1−5 show the ζ-potential values of lysozyme−surfactant systems at different pHs as a function of surfactant concentration. Analysis of these data reveals a number of interesting features. At pH 3.2 the ζ-potential changes in the neighborhood of the point of zero charge (pzc) from positive to negative values except in the case of the *n*-hexadecylsulfate. The results for the *n*-octyl, *n*-decyl, and *n*-dodecyl species suggest that the adsorption is due essentially to an ionic interaction between the cationic residues and the headgroup of the surfactant. At pH 3.2 the lysozyme molecule has a net positive charge of ∼15 with an estimation of 2.7 aspartyl and 0.5 glutamyl residues ionized. When the surfactant concentration increases, more active sites are occupied and the ζ-potential decreases to negative values. In the case of *n*-tetradecyl, a rapid change in ζ-potential is observed. This change can be attributed to the formation in isolated areas on the protein surface of structures called hemimicelles.[19] These clusters are formed at a concentration far below those required for micelle formation in the bulk, and their formation is presumably nucleated by the (opposite) surface charge. The break point in Figure 4 represents the concentration at which the hemimicelles are formed. This rapid increase of negative charge provokes the sudden change in the ζ-potential. In the

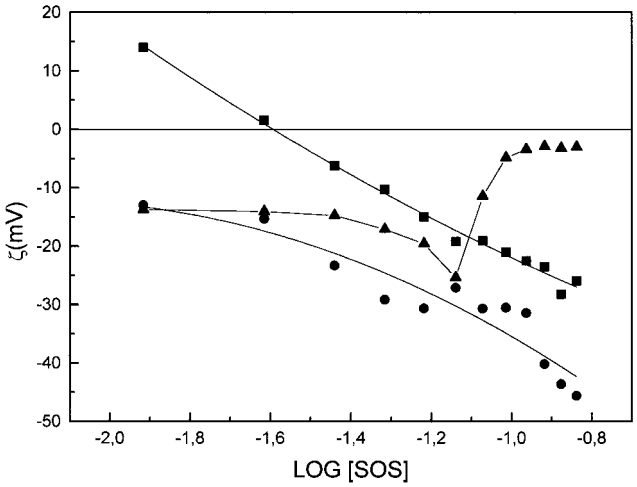

Figure 1. ζ-Potential as a function of log concentration of sodium *n*-octylsulfate (SOS) at different pH: (■) pH 3.20; (▲) pH 7.00; (●) pH 10.00.

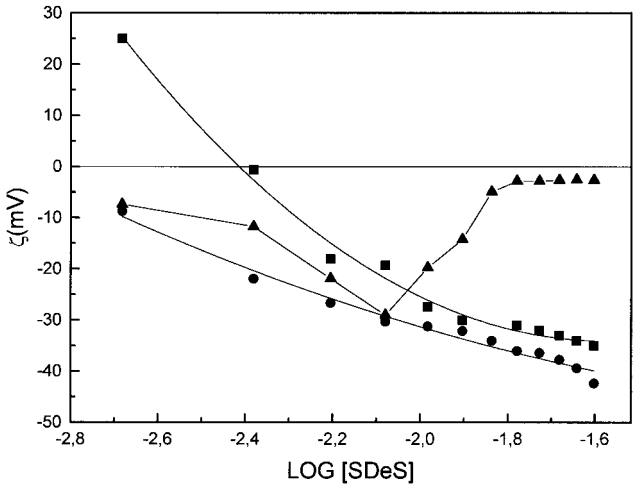

Figure 2. ζ-Potential as a function of log concentration of sodium *n*-decylsulfate (SDeS) at different pH: (■) pH 3.20; (▲) pH 7.00; (●) pH 10.00.

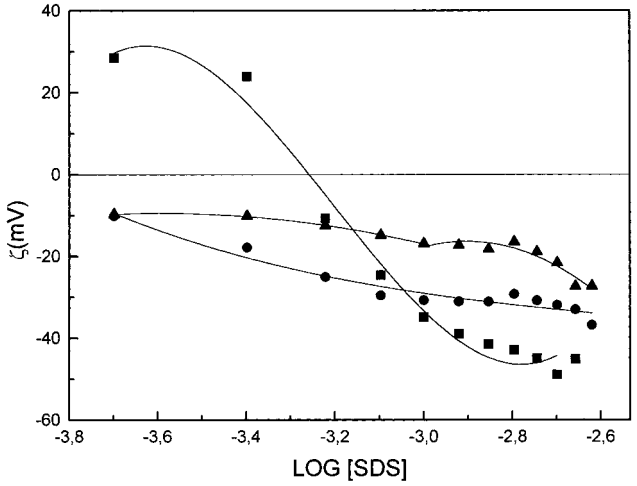

Figure 3. ζ-Potential as a function of log concentration of sodium *n*-dodecylsulfate (SDS) at different pH: (■) pH 3.20; (▲) pH 7.00; (●) pH 10.00.

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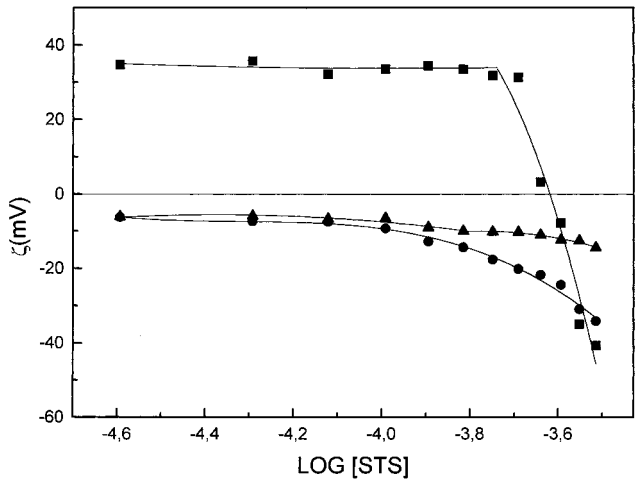

Figure 4. ζ-Potential as a function of log concentration of sodium *n*-tetradecylsulfate (STS) at different pH: (■) pH 3.20; (▲) pH 7.00; (●) pH 10.00.

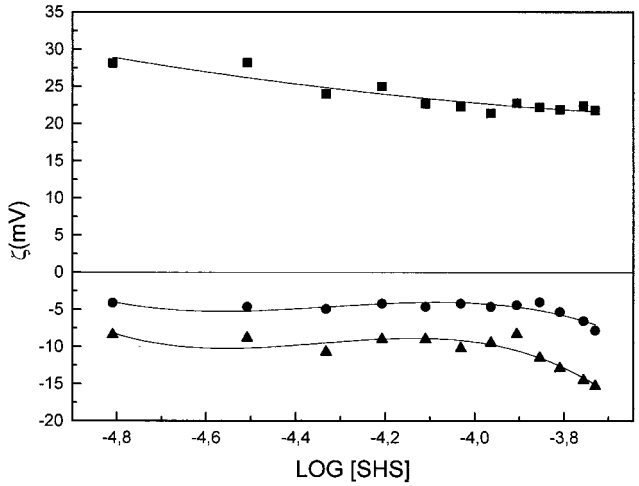

Figure 5. ζ-Potential as a function of log concentration of sodium *n*-hexadecylsulfate (SHS) at different pH: (■) pH 3.20; (▲) pH 7.00; (●) pH 10.00.

case of *n*-hexadecyl, the ζ-potential is practically constant and no formation of hemimicelles have been found. This observation may suggest that the length of surfactant's alkyl chain impedes a counteraction of all of the active sites of the lysozyme and that greater amounts of surfactant will be necessary to obtain the complete adsorption. This also explains the absence of hemimicelles in the range of concentrations studied, always below the CMCs of the surfactants.

At pHs 7 and 10 the ζ-potential of the particles indicates a negative electrokinetic charge. At pH 10 only the hystidyl cationic residue is desprotonated but the carboxylic groups (aspartyl and glutamyl) are negatively charged, giving the lysozyme molecule a net positive charge of ∼7. Then small amounts of surfactant are sufficient to give a negative ζ-potential observed which becomes more negative when the surfactant concentration increases.

Figures 1 and 2 show a minimum singular point at pH 7 which was not found for other surfactants. As a possible explanation, we suggest that from these points adsorption of counterions is produced which changes the ζ-potential toward more positive values. This is due to the decrease of negative changes in the complex protein−surfactant. The absence of these singular points for *n*-decyl, *n*-tetradecyl, and *n*-hexadecyl could be due to the alkyl chain length which impedes the adsorption of counterions.

Table 1. Data for Adsorption of *n*-Alkylsulfates on Lysozyme at pH 3.2

	c_0 (mmol L^{-1})	(d ζ/d log c)$_{\zeta=0}$ (V)	k_2 (L mol^{-1})	$-\Delta G^0_{ads}$ (kJ mol^{-1})
n-octyl	15.97	−0.0415	16.40	14.99
n-decyl	3.98	−0.0085	31.92	16.46
n-dodecyl	1.07	−0.1692	62.08	17.92

Anionic Surfactant Adsorption on Lysozyme. The system was analyzed as we have previously reported.[10] We distinguish between the surface charges before addition of the surfactant

$$\sigma_0^0 + \sigma_i^0 + \sigma_d^0 = 0 \tag{3}$$

where σ_0^0 is the charge per unit area on the surface, σ_i^0 is the charge density of the ion, and σ_d^0 is the charge density in the diffuse layer and the surface charges (σ_0, σ_i, σ_d) after adsorption of the surfactant

$$\sigma_0 + \sigma_i + \sigma_d = 0 \tag{4}$$

where eqs 3 and 4 have been written using the electroneutrality condition.

Assuming that the surfactant adsorption did not affect the potential determining ions (so that $\sigma_0 = \sigma_0^0$)

$$\sigma_i - \sigma_i^0 = \sigma_d^0 - \sigma_d = \Delta\sigma_d \tag{5}$$

the Stern equation[20] of the adsorption can be used in the form

$$-\Delta\sigma_d = \Delta\sigma_i = \frac{k_1 c}{1 + k_2 c} \tag{6}$$

where

$$k_1 = ZeN_1k_2 \tag{7}$$

$$k_2 = \exp(-\Delta G^0_{ads}/kT)/55.6 \tag{8}$$

(The factor 55.6 converts concentration in mole liter^{-1} to mole fraction for aqueous solution) where Z is the valence, N_1 the number of sites of adsorption, and ΔG^0_{ads} is the adsorption energy. From the ζ vs log c curves and using the Ottewill and Watanabe equation,[21] at the pzc we have

$$\left(\frac{\mathrm{d}\,\zeta}{\mathrm{d}\log c}\right)_{\zeta=0} = 2.303\zeta^0\left[\frac{\epsilon_0 D(1+\kappa a)\zeta^0}{aN_1Ze} - 1\right] \tag{9}$$

$$\frac{1}{c_0} = k_2\left[\frac{aZeN_1}{\epsilon_0 D\zeta^0(1+\kappa a)} - 1\right] \tag{10}$$

where c_0 is the surfactant concentration at the pzc, ζ^0 the ζ-potential in the absence of surfactant, and D the dielectric constant ($D = 4\pi\epsilon_r\epsilon_0$ where $\epsilon_r\epsilon_0$ is the product of the relative permittivity of the medium and the permittivity of free space).

Equations 9 and 10 can be simultaneously solved using the experimental values of (dζ/d log c)$_{\zeta=0}$ and c_0 to obtain values of N_1 and k_2. The results obtained are listed in Table 1. ΔG^0_{ads} becomes more negative with an increas-

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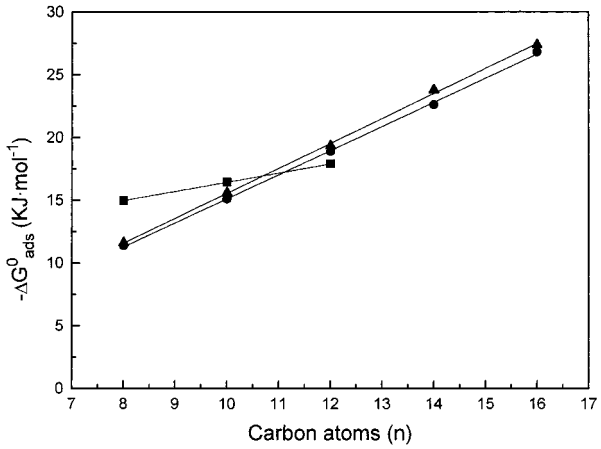

Figure 6. Variation of the Gibbs energies of adsorption on lysozyme of *n*-alkylsulfates at pH 3.20 (■) and *n*-alkyltrimethylammonium at pH 7.0 (Δ) and pH 10.0 (●) as a function of alkyl chain length.

Table 2. Gibbs Energies of Adsorption of *n*-Alkyltrimethylammonium Bromides (C_nTAB) on Lysozyme at Different pH

	$-\Delta G^0_{ads}$ (kJ mol^{-1})	
	pH 7.00	pH 10.00
C_8TAB	11.3	12.4
C_{10}TAB	15.0	15.0
C_{12}TAB	18.7	19.1

ing surfactant chain length. The magnitude of these Gibbs energies of adsorption may be compared with the Gibbs energies of adsorption of *n*-alkytrimethylammonium bromides on lysozyme. In Table 2 data for *n*-octyl, *n*-decyl, and *n*-dodecyl residues at pHs 7.00 and 10.00 published by Mosquera et al.[10] are shown. These results agree with the values in Table 1, i.e., the ionic interaction between the tetramethylammonium headgroup and the lysozyme is similar to that of *n*-alkylsuphate-lysozyme. Both ionic headgroups have a similar capacity to interact with the charged groups on the enzyme, and the pH does not affect this type of electrostatic interaction.

Gibbs energies of binding, $\Delta G_{\bar{\nu}}$, of these surfactants to lysozyme at pH 3.2, measured by equilibrium dialysis at 25 °C have been reported by Jones and Manley.[19] For a given chain length $\Delta G_{\bar{\nu}}$ becomes less negative with increasing bound surfactant ions, $\bar{\nu}$, and tends to limiting values of approximately −15, −16, and −18 kJ mol^{-1} for *n*-octyl, *n*-decyl, and *n*-dodecyl respectively at high values of $\bar{\nu}$. The agreement with our results suggest that adsorption and binding have analogous features and, after an initial adsorption to "higher energy" sites, subsequent adsorption is weaker.

Figure 6 shows the variation of $-\Delta G^0_{ads}$ with the number of carbon atoms in the alkyl chain, *n*, of the surfactants. In the case of the *n*-alkylsulfates, only values corresponding to 8, 10, and 12 carbon atoms have been used, representing this change by the equation $-\Delta G^0_{ads} = (9.13 \pm 0.01) + (0.73 \pm 0.01)n$ with a correlation coefficient of 0.9997, the slope being the Gibbs energies of adsorption per CH_2 group. We also have included in this figure literature values[10] corresponding to adsorption of *n*-alkyltrimethylammonium bromides on lysozyme. It is interesting to observe that the contribution of CH_2 group to Gibbs energies of adsorption increase more rapidly for the alkyltrimethylammonium ions than for alkylsulfates, which suggest that the hydrophobic contribution to interaction is more important for the alkyltrimethylammonium possibly due to a contribution from the hydrophobicity of the headgroup.

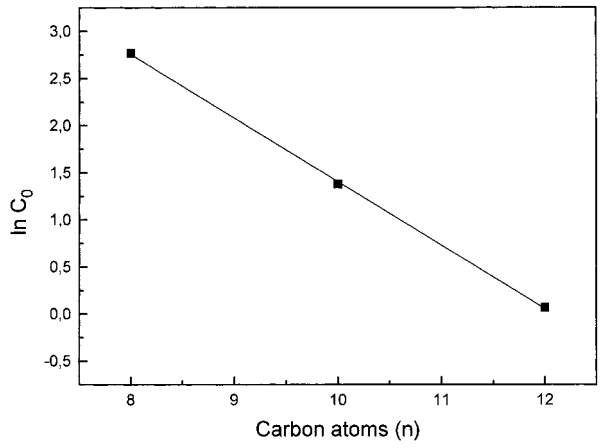

Figure 7. Variation of ln(concentration of sodium *n*-alkylsulfates at zero ζ-potential) as a function of alkyl chain length.

Effect of the Surfactants on the Lysozyme ζ-Potential. To study the effect of *n*-alkylsulfates on the ζ-potential of lysozyme, we have considered the Grahame model,[22] based on an original concept by Stern.[20] The adsorption density of surfactants, Γ_+, can be written as

$$\Gamma_+ = 2rc \exp(-\Delta G^0_{ads}/kT) \qquad (11)$$

where *r* is the radius of the adsorbed ion and *c* is the surfactant concentration, and the adsorption energy is expressed by

$$\Delta G^0_{ads} = Ze\psi_i + \theta_{HG} + n\theta' \qquad (12)$$

where $Ze\psi_i$ is the electrostatic term, ψ_i is being the ion potential, θ_{HG} represent the interaction of the headgroup with the surface, and θ' measures the adsorption energy of each of the $n$$CH_2$ groups in the surfactant chain. Differentiation of eq 11 with respect to ln *c* yields

$$\frac{d \ln \Gamma_+}{d \ln c} = 1 - Z \frac{d \tilde{\psi}_i}{d \ln c} \frac{-\theta'}{kT} \frac{d n}{d \ln c} \qquad (13)$$

where $\tilde{\psi}_i = e\psi_i/kT$ is the reduced potential. The differential d *n*/d ln *c* refers to the fact that the adsorption energy θ' involves the interaction between adsorbed $-CH_2$ groups. When adsorption of the surfactant has reduced the ζ-potential to zero, we may set $\psi_i = 0$ and from eq 11

$$\ln c_0 = \left(\ln \Gamma_+ - \ln 2r + \frac{\theta_{HG}}{kT}\right) + n \frac{\theta'}{kT} \qquad (14)$$

The term in parentheses is often constant at the pzc, and the plot of ln c_0 (where c_0 is the surfactant concentration at the pzc) against chain length, *n*, is linear (Figure 7). A value of $\theta' = 0.68kT$ from the slope of this line was obtained, which is in excellent agreement with the values obtained for the alkyltrimethylammonium ions adsorption on lysozyme,[10] $0.68kT$ for pH 7.0 and $0.73kT$ for pH 10.0, and with values of the van der Waals cohesive energy per CH_2 group determined by Somasundaran et al.[19]

At the concentration equal to the CMC, the lysozyme−surfactant complex and the surfactant micelles should be equally stable. For example, the Gibbs energy of micelle formation (ΔG^0_m) can be calculated from the equation[23]

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$$\Delta G_m^0 = 2.303RT[(2-\alpha)\log \mathrm{CMC}] \qquad (15)$$

where α is the degree of dissociation of the micelles. The value of ΔG_m^0 calculated from eq 15 taking $\alpha = 0.45$[12] for SDS was −18.5 kJ mol^{-1}. The agreement between ΔG_m^0 and ΔG_{ads}^0 (Table 1) is good and suggests that the amino acid residues are in the surface of native protein and hence accessible to the surfactant.

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