J. Chem. Thermodynamics 40 (2008) 1445–1450

Contents lists available at ScienceDirect

J. Chem. Thermodynamics

journal homepage: www.elsevier.com/locate/jct

A study on the protein concentration dependence of the thermodynamics of micellization

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ARTICLE INFO

Article history:
Received 29 February 2008
Received in revised form 18 April 2008
Accepted 23 April 2008
Available online 30 April 2008

Keywords:
Hydrogenated
Fluorinated
HSA
Thermodynamics
UV-CD

ABSTRACT

An investigation on the dependence of the thermodynamics of micellization of different surfactants such as sodium dodecyl sulfate (SDS), sodium octanoate (C8HONa), and sodium perfluorooctanoate (C8FONa) on the concentration of human serum albumin (HSA) has been realized. The critical micelle concentration (cmc) and ionisation degree of micellization, β, as a function of temperature (T), in solutions containing 0.125% and 0.250% in v/w of HSA, were estimated from conductivity data. From these results, the average number of surfactant monomers per protein molecule was calculated: higher values were found for C8HONa, the lowest value corresponded to SDS. For all the systems under study, electrostatic forces mainly drive the interaction between the surfactants and the proteins. Plots of cmc against temperature appear to follow the typical U-shaped curve with a minimum T_{min}. Thermodynamic functions of micellization were obtained by applying the theoretical models that best fit our experimental data, showing that the addition of HSA shows different patterns depending on the surfactant and thermodynamic quantity. Changes in the protein conformation due to the adsorption of surfactant molecules have been monitored by using UV-CD spectra. Greater changes in α-helical contents correspond with the concentrations over cmc, indicating that at low concentrations surfactants act as a structure stabilizer; meanwhile they act as a destabilizer at higher concentrations. C8HONa is the most effective reducing α-helical content, SDS is the less effective content.

1. Introduction

The association of surfactants with proteins has been widely studied due to their importance in food processing, pharmaceutical production, and detergent products. Complexation between proteins and surfactants, especially ionic surfactants, occurs in response to several different thermodynamic driving forces. As a result, the mode of binding and the supramolecular structure of the resulting complexes are quite sensitive to solution composition and temperature [1]. On the other hand, the properties of aggregates or the micelles of surfactants are strongly influenced by the kinds of surfactant ions, or temperature, or counterions present [2]. For this reason, the knowledge of the thermodynamics parameters of micellization is of utmost importance for a complete explanation of the effect of structural and environmental factors on the critical micellar concentration. It is relatively to find literature references concerned with the effects that electrolyte [3], or alkyl chain [4], or biomacromolecules [5] have on the micellization of surfactants. Extensive studies on the interactions between proteins and surfactants have been under close scrutiny for many years [6–10]. However despite these, broad and intensive production studies on the effects of thermodynamics of micellization in the presence of proteins are relatively few.

The work presented in this paper is aimed at obtaining a better understanding of the role of the presence of proteins in the thermodynamic quantities of micellization. For this purpose human serum albumin (HSA), sodium perfluorooctanoate, sodium octanoate, and sodium dodecyl sulfate have been chosen.

HSA is a globular protein, consisting of 583 amino acids in a single polypeptide chain with a molar mass of $66.500 \cdot 10^3$ g · mol^{-1}, and is widely used as a model protein in the study of such interactions [11]. X-ray crystallography [12] has shown an asymmetric heart-shaped molecule with sides of 8 nm and thickness of 3 nm that can be roughly described as an equilateral triangle with a height of 6.9 nm. The two heart lobes contain the molecule's hydrophobic binding sites, while the outside of the molecule contains most of the polar groups. Its secondary structure is dominated by a high content of α-helices (67%) HAS, which constitute approximately half of the total blood protein. Due to its high diffusion coefficient, HAS is the first protein that is adsorbed on foreign surfaces acting as a carrier for fatty acids and several amphiphiles from the bloodstream to tissues [13]. Obviously, it is an appropriate choice of protein for use in a study of interaction with amphiphilic compounds.

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doi:10.1016/j.jct.2008.04.014

Sodium perfluorooctanoate, sodium octanoate, and sodium dodecyl sulfate have been chosen because their solution properties have been widely characterized in previous works and because these surfactants allow us to compare the differences between (hydrocarbon + fluorocarbon) with the same alkyl chain with mixtures where the hydrocarbon chain is 1.5 times longer than the fluorocarbon chain. It is well known that in this relation both the surfactants have the same critical micellar concentration [6].

Electrical conductivities and circular dichroism have been used to achieve the desirable objectives. Conductivity measurements of ionic micellar solutions have long been used to determine a critical micelle concentration. These measurements will help us to determine the commencement of aggregate formation in the solution and how this is affected by the presence of different concentrations of protein. Based on the shape of the plots of cmc against temperature, thermodynamic quantities will be obtained by the application of the appropriate theoretical model. In addition, far ultraviolet circular dichroism measurements have been used to monitor the changes in the protein structure due to surfactant adsorption.

2. Materials and methods

Sodium octanoate and sodium perfluorooctanoate of at least 0.97 mass fraction purity were obtained from Lancaster Synthesis Ltd. Sodium dodecanoate, with mass fraction purity greater than 0.99 was obtained from Sigma Chemical Co. All these products were used as received. All the measurements were performed using distilled water with conductivity below 3 μS cm^{-1} at T = 298.15 K.

Conductivities were measured using a Kyoto Electronics conductometer model CM-117 with a K-121 cell type. The cell constant was determined using KCl solutions following the procedure suggested by Monk [14]. All the measurements were taken in a PolyScience Model PS9105 thermostatted water bath, at a constant temperature within ±0.05 K. The determination of the isotherms of conductivity was carried out by a continuous dilution of a concentrated sample prepared by apparent mass. The duration of dynamics processes may vary from 10^{-8} s (which is the time it takes for a surfactant to leave or enter a micelle) to 10^{-2} s (the time scale of the fusion of micelles), so the equilibrium process is guaranteed in just a few seconds after dilution when the measure is stored [15].

Far-UV circular dichroism (CD) spectra were obtained using a JASCO-715 automatic recording spectropolarimeter (Japan) with a JASCO PTC-343 Peltier-type thermostated cell holder. Quartz cuvettes with 0.2-cm path length were used. The CD spectra of pure HSA and HSA-surfactants dilute solutions were recorded from (195 to 380) nm. Protein concentration was 8 μM and the surfactant concentrations varied from (4.20 to 8.50) mM. The following settings were used: resolution, 1 nm; bandwidth, 1 nm; sensitivity, 50 mdeg; response time, 8 s; accumulation, 3; and scan rate 50 nm · min^{-1}. The corresponding absorbance contributions of buffer solution and water were subtracted with the same instrumental parameters. Data are reported as molar ellipticity and determined as

$$[\theta]_\lambda = \frac{\theta_\lambda M_r}{ncl}, \tag{1}$$

where c is the protein concentration, l is the path length of the cell, $[\theta]_\lambda$ is the measured ellipticity at a wavelength λ, M_r is the molar mass of the protein, and n is the number of residues. Temperature denaturation was followed by the CD responses at 222 nm from T = (278 to 353) K at a scanning rate of 18 K · min^{-1}; bandwidth, 1 nm; sensitivity, 50 mK; and response time, 8 s.

3. Results and discussion

The isotherms of molality dependence of electrical conductivity for the systems under study have been measured at different temperatures. For all the temperatures, the concentration dependence of the electrical conductivity shows a monotonic increase with a gradual decrease in slope. This fact observed in all the curves at a certain concentration of surfactants is considered to be the cmc of the micelles in the presence of different protein concentrations. To calculate the critical micellar concentration, we have used a method based on the fit of the experimental raw data to a simple non-linear function obtained by the direct integration of a Boltzmann-type sigmoid function having the following analytical expression [16,17]:

$$f(m) = \frac{A_1 - A_2}{1 + \exp((m - \mathrm{cmc})/\Delta m)} + A_2, \tag{2}$$

where m is the amphiphilic concentration, A_1 (A_2) represents the asymptotic value for small (large) values of m, cmc represents the central point of the transition and Δm deals with the width of the transition. If the derivative of the original data behaves as a sigmoid, then the original data behave as the integral of the sigmoid. A direct integration of equation (2) yields

$$F(m) = F(0) + A_1 m + \Delta m(A_2 - A_1) \ln\left\{\frac{1 + \exp((m - \mathrm{cmc})/\Delta m)}{1 + \exp(-\mathrm{cmc}/\Delta m)}\right\}, \tag{3}$$

where $F(0)$ represents the value of $F(m)$ at m = 0. The cmc values obtained by this method for our systems over the temperature range studied are plotted in figures 1 to 3. Each plot appears to follow a U-shaped curve with a minimum at a certain temperature T_{min}. The values of T_{min}, calculated by the least-squares fitting of the experimental values of the cmc, were (316.18, 314.90, 316.78, 333.35, 333.32, 334.16, 298.21, 298.51, 300.22) K for C8FONa, (C8FONa + HAS) (0.125%), (C8FONa + HAS) (0.250%), C8HONa, (C8HONa + HAS) (0.125%), (C8HONa + HAS) (0.250%), SDS, (SDS + HAS) (0.125%), (SDS + HAS) (0.250%), respectively.

Zielinski *et al.* [18,19] have postulated that the occurrence of a minimum on these plots suggests the existence of at least two factors affecting the cmc value in an aqueous solution: hydrophilic hydration around the surfactant in the aggregate state and two types of hydration around surfactant molecules in the monomer state, hydrophobic around the alkyl chain and hydrophilic around the polar head group. Thus, the observed minimum reflects the effect of raising the temperature in a balance between a gradual

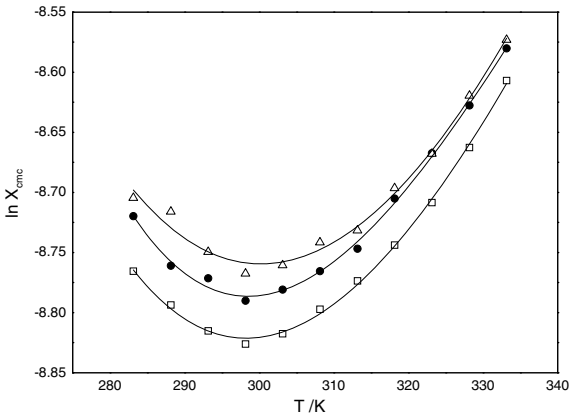

FIGURE 1. Plot of the logarithm of the mole fraction of the cmc *versus* temperature for (□) SDS, (●) SDS plus HSA (0.125%) and (△) SDS plus HSA (0.250%). The solid lines correspond to the fits of the temperature dependence of $\ln x_{cmc}$ using equation (5).