Heat Capacity Changes Accompanying Micelle Formation upon Burial of Hydrophobic Tail of Nonionic Surfactants

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The heat of micellization ΔH_m for five nonionic surfactants ($C_{10}E_5$, $C_{10}E_6$, $C_{10}E_7$, $C_{10}E_8$, C_8E_5) was measured as a function of temperature by using an isothermal titration calorimeter. The abbreviation C_iE_j stands for the nonionic surfactant $CH_3(CH_2)_{i-1}(OCH_2CH_2)_jOH$. The heat capacity change upon micellization ΔC_p was determined from ΔH_m's. It is found that ΔC_p is constant for four $C_{10}E_j$'s. That is ΔC_p is independent of the chain length of hydrophilic headgroups. Furthermore, ΔC_p is directly proportional to the removal of water accessible nonpolar surface area ΔA_{np}. The ratio $\Delta C_p/\Delta A_{np}$ is equal to -1.28 ± 0.06 J mol^{-1} K^{-1} Å^{-2} for $C_{10}E_j$'s and -1.34 ± 0.06 J mol^{-1} K^{-1} Å^{-2} for C_8E_5, consistent with that observed upon protein folding (-1.34 ± 0.33 J mol^{-1} K^{-1} Å^{-2}) of Spolar et al. (*Biochemistry* **1992**, *31*, 3947.).

When surfactant molecules are dissolved in water up to a certain concentration, known as critical micelle concentration, they can achieve segregation of their hydrophobic portions from water to form micelles. The hydrophobic effect due to the interaction between the hydrocarbon tail of the surfactant and water plays an important role in micelle formation.[1−8] According to the working scheme of Lumry and Rajender,[9] the micellization can be described as consisting of two part processes: the dehydration of the hydrocarbon tail of surfactant molecules and the aggregation of the hydrocarbon tails of surfactant molecules to form a micelle. In addition, the self-organization of surfactants into membranes, protein folding, and nonpolar gas solubility are just a few examples of processes also governed by the hydrophobic interaction.[10−12]

It is well believed that the hydrophobic interaction is a dominant factor that stabilizes the native protein structure. Thermodynamic parameters that describe the hydrophobic interaction of different protein groups with water have been the subject of numerous experimental and theoretical studies.[13−19] Baldwin[13] proposed that the heat capacity change of protein folding could be applied to quantify the contribution of the hydrophobic interaction to the stability of a globular protein. The protein folding process is always accompanied by a large negative heat capacity change.[14,16] The heat capacity change upon protein folding displays a feature similar to those characteristic of pure hydrocarbons as well as surfactants in water, as has been recognized for a long time.[20] Therefore, the heat capacity changes upon protein folding are mainly due to the nonpolar groups of the protein that are buried in the native state and become water exposed in the unfolded state. It was found that the change of heat capacity is directly proportional to the change of water accessible nonpolar surface area upon transferring a hydrocarbon compound to water. Record and coworkers[21] proposed that this proportionality can be straightforwardly applied to extrapolate the experimental thermodynamic data on the heat capacity changes of protein folding. However, in the native protein structure, not only nonpolar but also polar groups are buried upon protein folding.[22,23] Thus the burial of the polar groups from water upon protein folding should also contribute to the heat capacity changes. The heat capacity change upon the decrease of water accessible polar surface area should have a positive heat capacity change, that is, a sign opposite to that of the decrease of water accessible nonpolar surface area. Spolar et al. (1992)[23] concluded an equation to estimate the heat capacity change of protein folding ΔC_p from the changes of water accessible nonpolar and polar surface areas.

$$\Delta C_p\ (\text{J mol}^{-1}\ \text{K}^{-1}) = -1.34\ (\pm 0.33)\ \Delta A_{np}\ (\text{Å}^2) + 0.59\ (\pm 0.17)\ \Delta A_p\ (\text{Å}^2) \quad (1)$$

where ΔA_{np} and ΔA_p stand for the removal of water accessible nonpolar and polar, respectively, surface area upon protein folding.

Consider the heat capacity change upon micellization of surfactant C_iE_j's. The heat capacity change ΔC_p can be empirically related to changes in hydrophobic and hydrophilic solvation upon protein folding, as shown in eq 1. Because the hydrophilic headgroups of nonionic surfactants remain hydrated upon micelle formation, ΔC_p can be assumed to solely reflect the change in the exposure of hydrophobic tails to water. If the chain length of the hydrophobic tail is fixed, then the heat capacity change in micellization ΔC_p should be constant and independent of the chain length of the hydrophilic headgroup. The purpose of this study is to provide experimental data to verify this point. In addition, the validity of eq 1 is also examined upon the micellization process.

Nonionic surfactants $C_{10}E_5$, $C_{10}E_6$, $C_{10}E_7$, and $C_{10}E_8$ were purchased from Nikko Chemicals Co. and C_8E_5 from Bachem AG. All these surfactants were used as received without further purification. The abbreviation C_iE_j stands for the nonionic surfactant $CH_3(CH_2)_{i-1}(OCH_2CH_2)_jOH$. Water was purified by a Milli-RO plus (Millipore) and Milli-Q (Millipore) in series with the resistance better than 18 Mohm-cm.

The isothermal titration calorimeter of Thermometric (TAM 2277) was applied to perform the heat of mixing. In an experiment, 3 g of water was filled in a 4 mL stainless steel ampule. The injection syringe (Hamilton) was filled with a concentrated solution of a surfactant, which was injected 5−15

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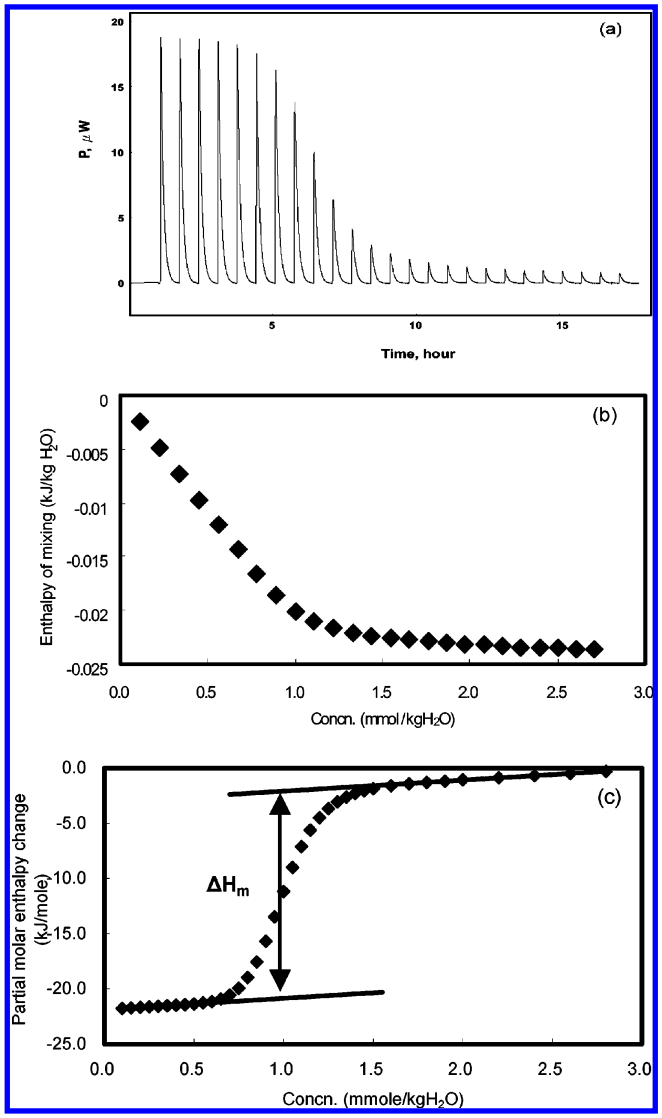

Figure 1. (a) A typical thermogram of titration curve (heat flow vs time). (b) Heat of mixing as a function of molality. (c) Partial molar enthalpy of $C_{10}E_j$ as a function of molality.

μL in each titration into the pure water (or solution) in the ampule by using a computer-controlled syringe pump (Lund, Sweden) at 45 min intervals under constant stirring condition at a prescribed temperature. The heat flows through highly sensitive thermopiles surrounded by a heat sink, stabilized within $\pm 2 \times 10^{-4}$ °C, were recorded. The weight of injected solution was evaluated from the density of the concentrated surfactant solution, which was measured by using a vibrating-tube densiometer (Paar DAM 58, Austria). The experiments were performed in at least three individual runs at a given temperature. The determination of the heat of micellization ΔH_m closely followed the method proposed by Paula et al. (1995).[24] Figure 1a shows a typical experimental titration thermogram from consecutively injecting concentrated $C_{10}E_j$ solution into the ampule at 298.15 K. The heat of mixing for each titration was determined by its peak area. The heat of mixing at a given surfactant concentration was evaluated by summing up the area from the first peak to the one of the prescribed concentration. Figure 1b illustrates the heat of mixing vs the surfactant concentration in the ampule. The derivative of the heat of mixing with respect to the surfactant concentration yields the partial molar enthalpy change of $C_{10}E_j$,[4] as shown in Figure 1c. The experimental data in Figure 1c were then correlated by a

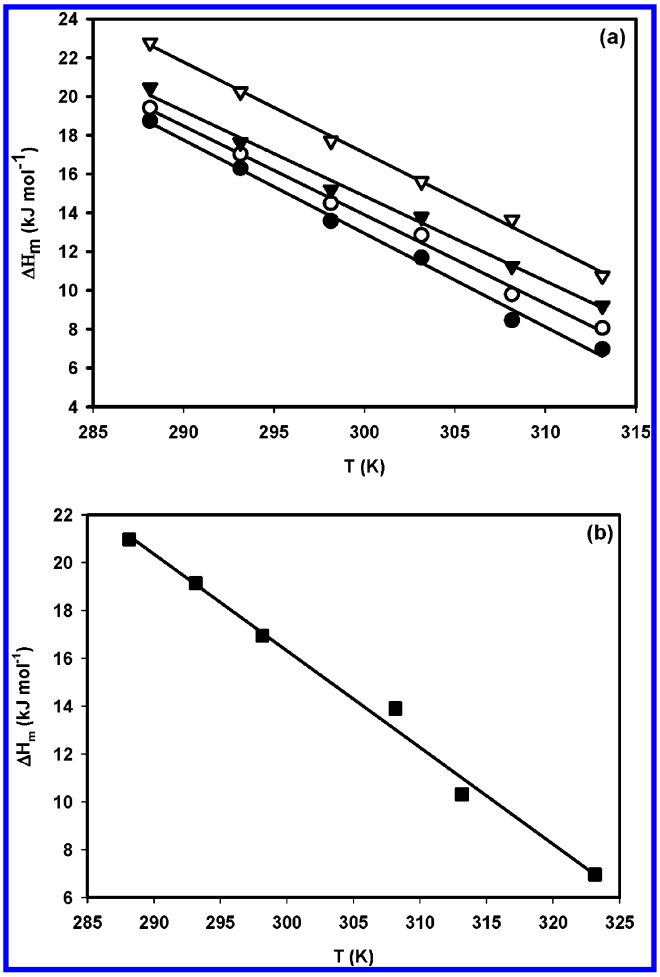

Figure 2. The experimental results of the heat of micellization as a function of temperature. (a) $C_{10}E_5$ (●), $C_{10}E_6$ (○), $C_{10}E_7$ (▼) and $C_{10}E_8$ (▽); (b) C_8E_5 (■).

sigmoidal curve. The inflection point of this sigmoidal curve gives the critical micelle concentration (cmc). The heat of micellization ΔH_m is equal to the enthalpy difference between two extrapolated lines right at cmc, as illustrated in Figure 1c. Replicate measurements of ΔH_m indicated a precision of less than 5% deviation.

The experimental results of the heat of micellization ΔH_m as a function of temperature are illustrated in Figure 2a for four nonionic surfactants: $C_{10}E_5$, $C_{10}E_6$, $C_{10}E_7$, and $C_{10}E_8$, and Figure 2b for C_8E_5. On average, ΔH_m increases by 1.28 ± 0.03 kJ mol^{-1} when the hydrophilic headgroup increases by one oxyethylene group. This enthalpy difference per oxyethylene group is simply due to the interactions between the hydrophilic headgroups stretching out of the core (formed by the hydrophobic tails) of micelles. The increase of hydrophilic chain length would certainly enhance the interactions between the hydrophilic chains.

Linear regression was applied to describe the temperature dependence of ΔH_m for each surfactant, and the slope of each line, shown in Figure 2, gives the heat capacity change upon micellization ΔC_p. The values of ΔC_p's for four surfactants $C_{10}E_5$, $C_{10}E_6$, $C_{10}E_7$, and $C_{10}E_8$ were found out to be -0.48 ± 0.02, -0.46 ± 0.02, -0.44 ± 0.02, and -0.47 ± 0.01 kJ mol^{-1} K^{-1}, respectively. Indeed, it is found that all four surfactants of the same hydrophobic chain length but with different hydrophilic chain lengths ($C_{10}E_j$ and $j = 5-8$) have a constant ΔC_p, as expected. This confirms our conjecture that the heat capacity change ΔC_p is constant for four $C_{10}E_j$ surfactants. The

average value of ΔC_p for these four $C_{10}E_j$ surfactants is -0.46 ± 0.02 kJ mol^{-1} K^{-1}. Note that ΔC_p of micellization is solely attributed to the change in the exposure of hydrophobic tails to water, and independent of the hydrophilic chain length. Although for C_8E_5, $\Delta C_p = -0.40 \pm 0.02$ kJ mol^{-1} K^{-1}, the heat capacity change per methylene upon micellization can be estimated to be -40 ± 20 J mol^{-1} K^{-1} from ΔC_p's of C_8E_5 and $C_{10}E_5$. That is close to, at least within experimental uncertainty of, the literature value for the heat capacity change per methylene for transferring of an alkyl chain from an aqueous to a hydrocarbon environment -49.2 J mol^{-1} K^{-1}.[3]

A more crucial examination is to check the validity of eq 1 to the micellization process. There is no removal of water accessible polar surface area upon micelle formation, because the hydrophilic headgroups of C_iE_j surfactants remain hydrated. That is, $\Delta A_p = 0$. Richards and co-workers[25] developed an algorithm to estimate the water accessible surface area of a protein. According to Richards,[26] the water accessible surface areas of one methylene group ($-CH_2-$) and of one methyl group ($-CH_3$) are 30 and 88 Å^2, respectively. The hydrophobic tail of a surfactant $C_{10}E_j$ is composed of nine methylene groups and one methyl group. Thus, the water accessible surface area of the hydrophobic tail of a surfactant $C_{10}E_j$ molecule is 358 Å^2 ($=9 \times 30 + 88$), and that of C_8E_5 is 298 Å^2. For micelle formation of a surfactant C_iE_j, the whole hydrophobic tail is assumed to bury inside to form the core of a micelle. That is, for micelle formation, $\Delta A_{np} = 358$ Å^2 for $C_{10}E_j$ and $\Delta A_{np} = 298$ Å^2 for C_8E_5. Furthermore, the ratio of ΔC_p and ΔA_{np} for micellization was calculated, $\Delta C_p/\Delta A_{np} = -1.28 \pm 0.06$ J mol^{-1} K^{-1} Å^{-2} for $C_{10}E_j$ and -1.34 ± 0.06 J mol^{-1} K^{-1} Å^{-2} for C_8E_5, that is in excellent agreement with the value -1.34 ± 0.17 J mol^{-1} K^{-1} Å^{-2} deduced from protein folding, appearing in eq 1.[23] That is, eq 1 is still valid to the heat capacity change for nonionic surfactants.

In conclusion, we present here the first experimental evidence that the anomalous heat capacity change upon micellization for a nonionic surfactant C_iE_j is mainly due to the hydrophobic interaction of the hydrophobic tail solely. In addition, there is no contribution to the heat capacity change from the hydrophilic headgroup. It is much more interesting to demonstrate that the heat capacity change upon micellization is directly proportional to the removal of water accessible nonpolar surface area, as described by eq 1, that is a relationship deduced from protein folding. Currently, we are in the process of further verifying the validity of eq 1 to other types of surfactants.

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