

# High-Pressure Studies on Aggregation Number of Surfactant Micelles Using the Fluorescence Quenching Method

Naoki Baden,<sup>†</sup> Okitsugu Kajimoto,<sup>†</sup> and Kimihiko Hara\*

Graduate School of Science, Kyoto University, Sakyo-ku Kyoto, 606-8502 Japan, and Research Center for Low Temperature and Materials Sciences, Kyoto University, Sakyo-ku Kyoto 606-8502, Japan

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The aggregation number of a nonionic surfactant micelle, Triton X 100 (TX100), in aqueous solution was determined as a function of pressure by using the method of steady-state fluorescence quenching. The method of this work uses the fluorescence quenching of a probe (pyrene) by a quencher (coumarin 153), which are solubilized within a micelle. With increasing pressure, the aggregation number of TX100 takes a minimum. Namely, it decreases from 250 at atmospheric pressure down to 80 at around 100–150 MPa and then increases up to 230 at 500 MPa, the highest pressure studied. This behavior is closely related to the turnover phenomenon of critical micelle concentration (cmc) against pressure. By taking the pressure effect on the micellar concentration into account, it is demonstrated that in addition to the equilibrium between dispersed state and micellar state, there are equilibria among different-sized micelles.

## 1. Introduction

A variety of methods, such as light scattering, sedimentation, membrane osmometry, small-angle neutron diffraction, fluorescence quenching, etc.,<sup>1</sup> have been used to observe the physical properties of surfactant micelles. In addition to critical micelle concentration (cmc), one of the most fundamental structural parameters of micellar aggregates is aggregation number, i.e., the average number of surfactant molecules in a micellar unit. To determine the aggregation number, the fluorescence quenching method has been developed by Turro and Yekta<sup>2</sup> as a simple and reliable method which measures the fluorescence intensity of a micelle-bound probe as a function of quencher concentration. Originally Ru(bipy)<sub>3</sub><sup>+</sup> as a probe and 9-methylanthracene as a quencher have been employed to calculate the micelle size of a typical anionic surfactant micelle, sodium dodecyl sulfate (SDS). This system has been proposed as appropriate for a micelle having an aggregation number less than ~120.

The purpose of the present work is to reveal the pressure effect on the aggregation number of surfactant micelles on the basis of the steady-state fluorescence quenching method. The experimental system of this paper uses the quenching of the steady-state fluorescence of pyrene by quencher in a typical nonionic surfactant micelle, Triton X 100 (TX100). Here coumarin 153 (C153), which partitions greatly into micelles, is adopted as a quencher. This system has been proven as effective for nonionic micelles with the aggregation numbers larger than 120, whose quenching mechanism has been discussed previously.<sup>3</sup>

High-pressure studies on aqueous solution of surfactant micellar systems are expected to provide significant insights into their molecular aspects.<sup>4–7</sup> Previously we have not only investigated the pressure effect on the cmc of TX100 by using the fluorescence probe method and observed turnover behavior

against pressure<sup>8</sup> but also studied the pressure effect on the solvation dynamics of the TX100 micellar surface by means of time-resolved fluorescence Stokes-shift measurements.<sup>9</sup> Those results are quoted in this paper.

## 2. Experiment

Triton X-100 (TX100)<sup>10</sup> (spectroscopic grade, Nacalai Tesque) and pyrene (optical grade, Nacalai Tesque) were used as received. Coumarin 153 (C153) (laser dye, Lambda Physik) was purified by sublimation. The concentration of TX100 was varied in the range of  $(1.7 \times 10^{-3})$ – $(3.2 \times 10^{-3})$  M. This is approximately 20 times higher than the cmc ( $\sim 2.22 \times 10^{-4}$  M) of TX100. To reduce the fraction of the micelles containing more than one pyrene molecules, the micelle/pyrene concentration ratio was chosen to be greater than 20. The concentrations of probe and quencher are  $1.0 \times 10^{-6}$  M and  $1.8 \times 10^{-5}$  M, respectively.

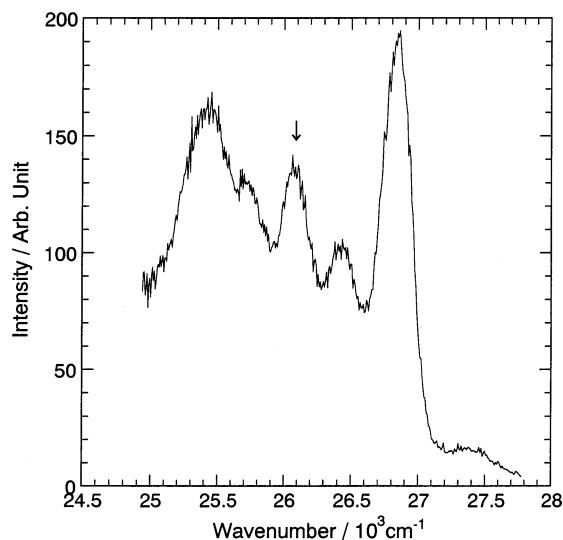
Steady-state fluorescence of pyrene in TX100 was measured at high pressures up to 500 MPa. Spectra at atmospheric pressure ( $\approx 0.1$  MPa) were recorded on a fluorophotometer (Shimadzu RF-5300PC), and those at high pressures were recorded on an originally designed fluorophotometer.<sup>11</sup> The high-pressure cell equipped with four sapphire windows, the pressure generating system, and the procedure of data analysis used for the present measurements have been described elsewhere.<sup>12</sup> Sample solution was provided in a quartz inner cell. The pressure effect on each concentration was corrected by using the compressibility data of water.<sup>13</sup> All the steady-state fluorescence measurements at high pressures were made at 298 K. Pyrene was excited at 336 nm where there is no absorption of TX-100. In each experiment the whole fluorescence spectrum of pyrene was measured and its third vibronic peak (381 nm) was used for the analysis. The fluorescence spectra were converted from a linear wavelength to a linear frequency representation prior to analysis.

## 3. Results and Discussions

By assuming that the distribution of probe and quencher in micelles is described by Poisson statistics and that the probe

\* Corresponding author: Research Center for Low Temperature and Materials Sciences, Kyoto University. E-mail: hara@kuchem.kyoto-u.ac.jp. Fax: +81 75 753 3975.

<sup>†</sup> Graduate School of Science, Kyoto University.



**Figure 1.** Fluorescence spectrum of pyrene ( $1 \times 10^{-6}$  M) in aqueous solution of TX100. The vibronic peak noted by an arrow is used for quenching analysis.

emits only when it occupies an empty micelle, the measured ratio of fluorescence intensity ( $I/I_0$ ) in the presence of quencher to that in the absence of quencher can be expressed by the relation:<sup>14</sup>

$$I/I_0 = \exp(-[Q]/[M]) \quad (1)$$

The micelle concentration ( $[M]$ ) is related to surfactant concentration ( $[S]$ ) and mean aggregation number ( $\bar{n}$ ) as given by

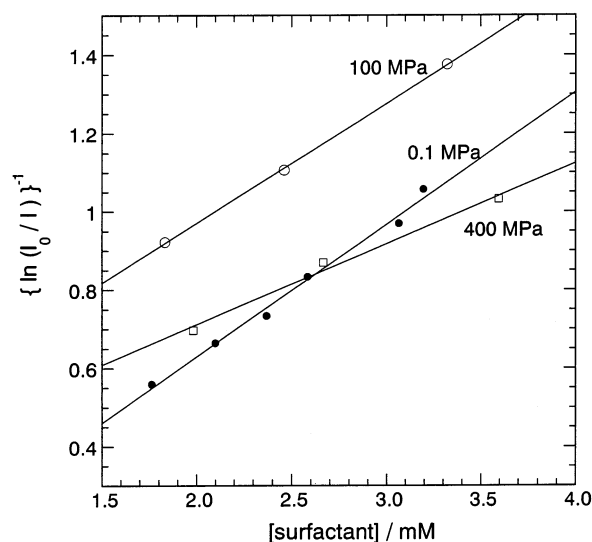
$$[M] = \{[S] - [\text{free monomer}]\}/\bar{n} \quad (2)$$

As a consequence, combination of eqs 1 and 2 leads to

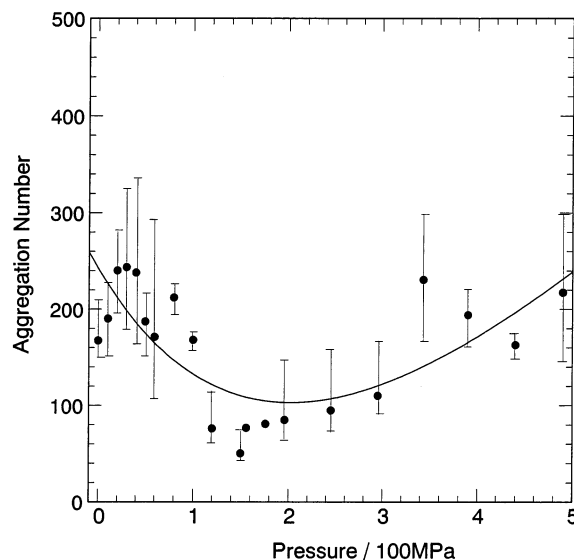
$$\ln(I_0/I) = \frac{[Q]\bar{n}}{[S] - [\text{free monomer}]} \quad (3)$$

where the free monomer concentration in equilibrium with micellar aggregation can be equated to the critical micelle concentration (cmc). Here it should be emphasized that this expression was derived under the assumption that the excited pyrene is completely quenched when it occupies a micelle which contains at least one quencher molecule. Namely, it is only the excited pyrene molecule in the micelles which contains no quencher that exhibits fluorescence.

Figure 1 shows the fluorescence spectrum of pyrene in aqueous solution of TX100. The intensity of the third vibronic peak ( $26.2 \times 10^3 \text{ cm}^{-1}$ ) of pyrene was monitored as a function of surfactant concentration at various pressures, keeping the quencher concentration fixed at  $1.8 \times 10^{-5}$  M. It should be noted that the first strong peak at around  $26.9 \times 10^3 \text{ cm}^{-1}$  is inappropriate for the present analysis, since it is sensitive to other solvent properties such as polarity and density.<sup>7</sup> Figure 2 shows the representative plot of the inverse values of  $\ln(I_0/I)$  as a function of the surfactant concentration. As expected from eq 3, the fluorescence intensity increases with increasing surfactant concentration. The slope of the line indicates  $\{[Q]\bar{n}\}^{-1}$ . Averaging several data of different runs, we thus obtained the mean aggregation number ( $\bar{n}$ ) to be 162 at atmospheric pressure ( $=0.1$  MPa). This value agrees reasonably well with those reported in the literatures determined by the same method ( $\bar{n} = 121$ )<sup>3</sup> as well as by other methods ( $\bar{n} = 156$ ).<sup>15</sup>



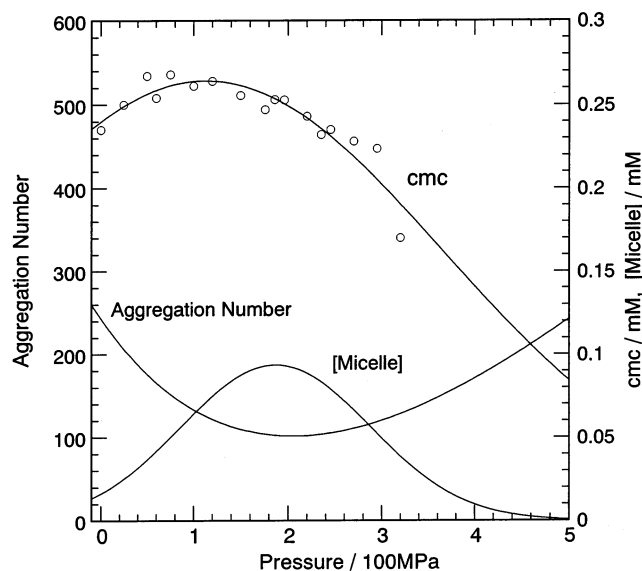
**Figure 2.** Plot of  $\{\ln(I_0/I)\}^{-1}$  as a function of surfactant concentration at 0.1, 100, and 400 MPa keeping  $[Q] = 1.8 \times 10^{-5}$  M.



**Figure 3.** Plot of aggregation number ( $\bar{n}$ ) of TX100 as a function of pressure. The solid line represents the nonlinear least-squares fitting.

The limit of total quenching, that is the intercept at  $\ln(I_0/I) = 0$ , should give the free monomer concentration ( $[M]$ ), which reduces to  $\sim 0.22 \times 10^{-3}$  M for 0.1 MPa. Comparing with the best reported value, the cmc value for TX100 is  $(0.24 \times 10^{-3}) - (0.268 \times 10^{-3})$  M.<sup>8,16</sup> This agreement demonstrates the validity of the usual conjecture that the free monomer concentration remains relatively constant above the cmc. For the data at high pressures, however, the error bar is too large to determine the cmc value from such an extrapolation.

The  $\bar{n}$  values at high pressures were calculated on the basis of eq. 3. All  $\bar{n}$  values thus obtained were plotted in Figure 3 as a function of pressure. The solid line represents the nonlinear least-squares fit.<sup>17</sup> We find that  $\bar{n}$  decreases from 250 at 0.1 MPa down to 80 at 100–200 MPa and then increases up to 230 at 500 MPa, the highest pressure studied. Namely, it takes a minimum at around 150 MPa. This turnover behavior of  $\bar{n}$  against pressure has not been well established so far, although it has previously been reported only for a typical anionic micelle, SDS, by light scattering measurements.<sup>18</sup> However, it has not been detected for SDS<sup>19</sup> and a nonionic micelle, hexaoxyethylene dodecyl ether, ( $C_{12}E_6$ )<sup>18</sup> by means of fluorescence lifetime measurements.



**Figure 4.** Pressure effect on aggregation number, cmc, and micelle concentration.

In Figure 4, the high-pressure data of cmc and micelle concentration are plotted together with  $\bar{n}$  for comparison. The micelle concentration was calculated by using the high-pressure cmc data which were determined previously.<sup>8</sup> From this figure we find that the minimum behavior of  $\bar{n}$  with pressure is closely correlated with the turnover behavior of the cmc.

The turnover behavior of the cmc against pressure can be explained on the basis of the “hydrophobic interaction”.<sup>20–23</sup> When the solvent of a hydrophobic molecule or hydrophobic group is transferred from a nonpolar solvent to water at atmospheric pressure, the partial molar volume decreases. The hydrophobic molecule induces first-shell waters to contract around it, which is called “hydrophobic hydration”. For example, the partial molar volume of methane decreases from 60 to 37.3 mL/mol upon transferring the solvent from hexane to water.<sup>24</sup> When we consider the solubility ( $X$ ) of a hydrophobic molecule or hydrophobic group in water, pressure dependence is written as

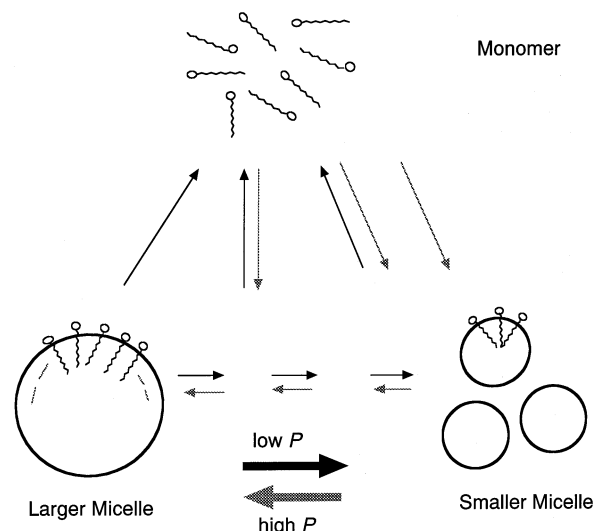
$$(d \ln X/dP)_T = -\Delta V/k_B T \quad (4)$$

where  $\Delta V$  is the difference between the partial molar volume ( $\bar{V}$ ) and the molar volume ( $V$ ) of solute. According to the hydrophobic hydration as noted above, it follows that  $\Delta V < 0$ . This indicates that the application of pressure causes hydrophobic hydration which drives a hydrophobic molecule into the water phase.

It has been observed that the solubility of a hydrophobic molecule or a hydrophobic group shows a maximum against pressure.<sup>25,26</sup> This is a specific feature of hydrophobic hydration at high pressures. Namely, at the higher pressures the sign of  $\Delta V$  becomes positive. This means that  $\bar{V}$  becomes smaller than  $V$ . This fact has been explained as due to the network structure (or iceberg structure) of water.

At pressures higher than around 100–200 MPa, the increase in pressure serves to decrease the hydrophobic hydration and drive the hydrophobic molecule out of the water phase.<sup>27</sup> As a result at lower pressures the compression causes an increase in the cmc, that is, on increase in free monomer concentration. Conversely, at higher pressures the monomer concentration decreases.

We find in Figure 4 that in the lower pressure range, less than around 100–150 MPa,  $\bar{n}$  decreases with increasing



**Figure 5.** Schematic view representing the pressure effect on micellar aggregation. The solid arrows indicate the direction of pressure shift in the lower pressure region. The shaded arrows indicate the direction of pressure shift in the high-pressure region.

pressure, while both the cmc and micellar concentration increase. As noted above, the increase in the cmc means an increase in monomer concentration. The fact that the monomer concentration increases due to an increase in the cmc indicates that the surfactant molecules transfer from the micellar state to the free monomer state. This leads to a decrease in  $\bar{n}$ , which reduces to shrinking of micelles. Furthermore, such a process will be accompanied by redistribution of surfactant molecules within a micelle. Throughout this redistribution process, the dissociation of a micelle to give smaller micelles occur. In this pressure range, the hydrophobic hydration is accelerated with pressure.

In the pressure range higher than ca. 200 MPa, on the other hand, the pressure effect is completely reversed. Namely,  $\bar{n}$  increases with increasing pressure, while both the cmc and micellar concentration decrease with pressure. The transfer of free monomer to micellar state leads to an increase in  $\bar{n}$ , which indicates swelling of micelles. In this pressure range, the application of pressure leads to an increase in hydrophobic dehydration, which may cause the association of hydrophobic molecules.

The aspect of describing the pressure effect is schematically depicted in Figure 5. It should be stressed that the application of pressure causes not only the change in cmc, which follows the change in free-monomer concentration, but also the change in the total micellar concentration. This indicates that the dissociation or association of micelles should take place.

#### 4. Concluding Remarks

It is concluded that the fluorescence quenching method for obtaining the mean aggregation number of nonionic micelle, TX100, using C153 as a quencher and pyrene as a fluorescent probe offers a useful method when it must be measured as a function of pressure.

In this paper it has been established that the aggregation number exhibits turnover behavior against pressure, whose minimum is located around 100–150 MPa. This behavior is closely correlated with the turnover behavior of the cmc.

For the most part, micelle formation has been explained on the basis of a simple equilibrium model between monomer state and micellar state. In this paper, however, taking the pressure effects on both micelle and free-monomer concentrations into

account, we demonstrated that there should exist equilibria among different-sized micelles. Namely, closely correlated with the change in  $\tilde{n}$  and cmc with pressure, the association or dissociation of micelles should be brought about by changing the pressure.

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