Surface Potentials of Mixed Micelles Composed of Arachidonic Acid and Heptaethylene Glycol Dodecyl Ether

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Abstract: The surface potentials (Δψ) of the mixed micelles composed of arachidonic acid (AA) and nonionic heptaethylene glycol dodecyl ether (HED) were estimated at 25 and 60°C by measuring the fluorescence intensity of 8-anilino-1-naphthalenesulfonate (ANS) as a fluorescent probe. The surface potentials of AA-HED mixed micelles, |Δψ|, increased with increasing mole fraction of AA (XAA), and decreased with increasing ionic strength (I). These changes are due to the effects of the negative charge of AA and of the ionic atmosphere of Na+, respectively. The surface charge density (σ) was nearly independent of I and proportional to XAA, indicating that the surface state of AA-HED mixed micelles is represented by the Gouy-Chapman theory. The absolute value of σ for AA-HED mixed micelles at 60°C was slightly decreased as compared with that at 25°C.

Key words: arachidonic acid, surface potential, micelle, Gouy-Chapman theory, heptaethylene glycol dodecyl ether

1 Introduction

Arachidonic acid (AA), [icoso-cis-5,8,11,14-tetraenoic acid], is well known as a precursor of prostaglandins. Regarding the surface properties of AA, the surface pressure for the insoluble monomolecular film of AA has been measured at various temperatures, and the relationship between the limiting area of an AA molecule and temperature has been reported. We have measured the surface tension of aqueous solution of AA at pH 7.80 and 25 ~ 60°C, and the adsorption of AA at the air-water interface and the micellization of AA were discussed from the thermodynamic viewpoint. However, the micellar surface potential of AA has not yet been measured.

On the other hand, we have recently reported that the base-catalyzed degradation of prostaglandin E2 (PGE2) is suppressed by being incorporated in AA micelles. It is necessary to know the micellar surface potential of AA to deeply discuss the stabilization effect of AA on PGE2.

From these points of view, the surface potentials of mixed micelles composed of AA and nonionic surfactant were measured at 25 and 60°C, and the effect of temperature on the surface potential of AA micelle was investigated. Furthermore, the value of micellar surface potential of AA was compared with that of sodium dodecyl sulfate and so on.

2 Experimental

2.1 Materials

Arachidonic acid (AA) purchased from Sigma Chemical Co. was of guaranteed reagent grade and...
used without further purification. Heptaethylene glycol dodecyl ether (HED) as a nonionic surfactant and ammonium 8-anilino-1-naphthalene-sulfonate (ANS) as a fluorescent probe were purchased from Wako Pure Chemical Industries. Mixed micelles composed of AA and HED were prepared as previously described, and dissolved in a phosphate buffer containing NaH₂PO₄ and Na₂HPO₄ (pH 7.0 with ionic strength 0.02 mol dm⁻³) when fluorescence intensity was measured. All AA molecules were able to contribute to the formation of mixed micelles whose mole fraction of AA is less than 0.6, which was confirmed by the surface tension measurement of aqueous solution of 5 × 10⁻⁴ mol dm⁻³ of HED containing various concentrations of AA. Above 0.7 mole fraction of AA, all AA molecules were not able to contribute to the formation of mixed micelles. Accordingly, the mixed micelles whose mole fraction of AA is above 0.7 were not subjected to the measurement of surface potential. The oxidation of AA by air is not seriously produced at least during the experimental period.

2-2 Measurement of Fluorescence Intensity
Mixed micelles were dissolved in a phosphate buffer (pH 7.0 with ionic strength 0.02 mol dm⁻³). The total concentration of AA and HED was kept constant at 1 × 10⁻³ mol dm⁻³. A solution of 1 × 5 × 10⁻⁵ mol dm⁻³ of ANS as a fluorescent probe was added to the solutions of AA and HED. The fluorescence intensity was measured by use of a Hitachi F-4000 spectrofluorometer equipped with a pump-circulating water at a constant temperature through an outer tube. The temperature of thermostat was maintained at 25 ± 0.2°C or 60 ± 0.2°C. The wavelength of excitation and emission are 360 and 480 nm, respectively. Sodium chloride was used to control the ionic strength of buffer solution.

3 Results
3-1 Fluorescence Intensity
The relationship between the fluorescence intensity (F) and the concentration of ANS (D) at ionic strength 0.02 mol dm⁻³ was shown in Fig. 1. The fluorescence intensity was increased with increasing concentration of ANS, and decreased with increasing the mole fraction of AA.

Fig. 1 Plots of Fluorescence Intensity vs. Concentration of ANS.
Mole fraction of AA: ○, 0 (HED alone); ●, 0.1; ▲, 0.2; ■, 0.4; ▼, 0.6.
Total concentration of AA and HED: 1 × 10⁻³ mol dm⁻³.
Ionic strength: 0.02 mol dm⁻³.

3-2 Adsorption of ANS at Micelle Surface
It is considered that fluorescence intensity is proportional to the amount of ANS adsorbed at the micelle surface because ANS fluoresces when adsorbed at the micelle surface. The Langmuir adsorption isotherm can, therefore, be applied. The equation is written as

\[ \Gamma = \frac{D_m}{C} = \frac{\Gamma_s k D_t}{1 + k D_t} \]  (1)

where \( \Gamma \) is the adsorbed amount of ANS per mole of micelle, \( \Gamma_s \) is the saturated adsorbed amount, \( k \) is the binding constant related to the strength of adsorption, \( C \) is the total concentration of AA and HED (in this study \( C = 1 \times 10^{-3} \) mol dm⁻³), which are in the form of micelles, \( D_m \) is the concentration of bound ANS, and \( D_t \) is the concentration of free ANS; \( D = D_m + D_t \). Fluorescence intensity \( F \) is related to \( D_m \) by

\[ F = f D_m \]  (2)

where \( f \) is the fluorescence coefficient of ANS. \( F \) is defined by

\[ F_m = \lim_{D \to m} F = C/\Gamma_s \]  (3)

where \( F_m \) is the fluorescence intensity at \( D \to \infty \).

Equation 4 is derived from Eqs. 1–3:

\[ \frac{1}{F_m - F} = k \left( \frac{D}{F} - \frac{1}{f} \right) \]  (4)
Under the experimental conditions, $D$ is sufficiently large. Therefore, Eq. 4 is reduced to Eq. 5.

$$\frac{F}{D} = k(F - F)$$  \hspace{1cm} (5)

Plots of $F/D$ against $F$ based on the data shown in Fig. 1 were presented in Fig. 2, which show a good linear relationship in accordance with Eq. 5. From the value of slope, $k$ is evaluated.

### 3-3 Values of $k$

The values of $k$ obtained from Fig. 2 were plotted against the mole fraction of AA and were shown in Fig. 3 by closed circles. The value of $k$ decreased with increasing the mole fraction of AA.

The decrease in $k$ is considered to be due to the electrostatic repulsion between AA and ANS on the micelle surface. To investigate the effect of ionic strength on $k$, experiments were carried out with the buffer solutions containing sodium chloride so that their ionic strengths are 0.10 and 0.20 mol dm$^{-3}$ as well as 0.02 mol dm$^{-3}$, plots of $k$ against the mole fraction of AA being shown by symbols of closed squares and closed triangles in Fig. 3. Furthermore, the values of $k$ at 60°C and ionic strength 0.02 mol dm$^{-3}$ were similarly obtained and shown by open circles in Fig. 3. The values of $k$ for AA-HED mixed micelles increased with increasing ionic strength. The value of $k$ decreased when the temperature was raised.

### 4 Discussion

#### 4-1 Surface Potential of Micelles

On the assumption described in a previous paper, the binding constant of ANS to AA-HED mixed micelles, $k$, depends on the surface potential of the micelle, $\Delta \psi$. The relation is written as

$$k = k_0 \exp \left(-\frac{zF_A \Delta \psi}{RT}\right)$$  \hspace{1cm} (6)

where $z$ is the valency of ANS ($z = -1$), $F_A$ is the Faraday constant, $R$ is the gas constant, $T$ is the absolute temperature, and $k_0$ is the $k$ for nonionic HED micelle. $\Delta \psi$ is calculated by using Eq. 7.

$$\Delta \psi = \frac{RT}{zF_A} \ln \left(\frac{k_0}{k}\right)$$  \hspace{1cm} (7)

The surface potential of the micelle, $\Delta \psi$, as calculated from the values of $k$ shown in Fig. 3,
was plotted against the mole fraction of AA in Fig. 4. The surface potential of the mixed micelle became more negative with increasing the mole fraction of AA, and the surface potential of the mixed micelle, $|\Delta \phi|$, became smaller as the ionic strength was increased by adding sodium chloride. These changes may be considered to be due to the effects of the negative charge of AA and of the ionic atmosphere of Na', respectively. The surface potential of AA at 60°C was not largely differ from that at 25°C.

The surface potential of AA micelle can be estimated by extrapolation of the theoretical curve based on the Gouy-Chapman theory, which is described in the next section. The surface potential of AA micelle thus obtained at ionic strength 0.02 mol dm$^{-3}$ is $-55.0$ mV. This value of $|\Delta \phi|$ for AA micelle is larger than the value $|-33$ mV$|$ for prostaglandin B$_2$ (PGB$_2$) micelle and is smaller than the value $|-73$ mV$|$ for sodium dodecyl sulfate (SDS) micelle as a typical anionic surfactant at the same ionic strength. These values suggest that AA forms a loosely packed micelle as compared with SDS micelle but forms a tightly packed micelle as compared with PGB$_2$ micelle.

4.2 Surface Charge Density of Micelles

According to the Gouy-Chapman theory, the relation between surface potential, $\Delta \phi$, and surface charge density, $\sigma$, is expressed as Eq. 8,

$$\sigma = \frac{RT\varepsilon}{2\pi} \sum_i C_i \left( \exp\left(-\frac{eF_i\Delta \phi}{RT}\right) - 1 \right)^{1/2}$$  \hspace{1cm} (8)

where $\varepsilon$ is the relative dielectric constant of water. The values$^8$ of 78.54 and 66.94 were used as the values of $\varepsilon$ for water at 25 and 60°C, respectively. The values of $\sigma$ as calculated from the values of $\Delta \phi$ shown in Fig. 4 by using Eq. 8 were plotted against the mole fraction of AA in Fig. 5. The surface charge density, $\sigma$, was found to be nearly independent of ionic strength and nearly proportional to $X_{AA}$. These results indicate that the state of the diffuse layer surrounding the Stern layer at the micelle surface is represented well by the Gouy-Chapman theory. The surface charge density $|\sigma|$ was slightly decreased when the temperature was raised from 25°C to 60°C. The relationships between $\sigma$ and $X_{AA}$ can be written as Eqs. 9 and 10 at 25°C and 60°C, respectively.

$$\sigma = -1.942 \times 10^{-6} \cdot X_{AA} \hspace{1cm} \text{(at 25°C)} \hspace{1cm} (9)$$

$$\sigma = -1.642 \times 10^{-6} \cdot X_{AA} \hspace{1cm} \text{(at 60°C)} \hspace{1cm} (10)$$

By using Eqs. 7~10, the theoretical values of the surface potential and the binding constant for AA-HED mixed micelles were calculated, the results being shown in Figs. 4 and 5 by solid lines, respectively.

5 Conclusion

The surface potentials of AA-HED mixed micelles, $|\Delta \phi|$, increased with increasing the mole fraction of AA, and decreased with increasing ionic strength. The surface state of AA-HED mixed micelles was represented well by the Gouy-Chapman theory. The value of $|\Delta \phi|$ for AA micelle was larger than that for prostaglandin B$_2$ micelle at the same ionic strength. The absolute value of $\sigma$ for AA-HED mixed micelle at 60°C was slightly smaller than that at 25°C.

References

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日本油化学会誌本号掲載 論文要旨

【総説】 環境調和型バイオポリエステル研究の新展開：
代謝制御工学から分子生理まで
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多くの微生物は、ある種の栄養制限下でポリヒドロキシアルカン酸（PHA）をエネルギー貯蔵物質として蓄積する。PHAは合成プラスチックと同様に熱可塑性を有する生分解性ポリエステル（バイオポリエステル）であることから、新しい環境調和型の高分子材料として期待される。典型的な微生物ポリエステルであるポリ[(R)-3-ヒドロキシブタン酸]（P(3HB)あるいはPHB）は硬くて脆いため、その用途が制限される。しかしながら、鎖長のより長い3-ヒドロキシアルカン酸ユニットとの共重合ポリエステルを合成することで物性の優れた高分子材料を得ることができる。それによっては、PHA合成について詳細な研究を行うことが重要である。このようなにして得られた知見を基に、遺伝子工学的・代謝制御工学的手法を用いて、PHAのモノマー組成を自由にコントロールできれば、物性の優れたPHAの発酵生産と用途開発が可能となる。

また、上記のようなエネルギー貯蔵物質や高分子材料としてPHBとは異なり、約150ユニットサイズの低分子量3HBオリゴマー（OHMB）が、微生物から動・植物にいたるまでは特に存在することが知られている。実質、このOHMBは無機のポリリン酸と複合体を形成してカルシウムイオンチャネルを膜中に構築していることが明らかにされ、細胞内へのDNA取り込み機構との関連性も注目を集めている。エステルオリゴマーの生理機能についてはまだ未知の部分が多いが、このOHMBの細胞内での分子生理が徐々に解き明かされていくことで第5の生体高分子という概念が生まれるかもしれない。

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【報文】 アラキドン酸とヘプタエチレングリコール
ドデシルエーテルから成る混合ミセルの表面電位
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アラキドン酸（AA）とノニオン性界面活性剤ヘプタエチレングリコールドデシルエーテル（HED）から成る混合ミセルの表面電位を25℃と60℃において蛍光プローブである8-アミリノ-1-ナフタレンスルホン酸アンモニウム（ANS）を作用させ蛍光強度を測定することにより求めた。AA-HED混合ミセルの表面電位（δ）はAAのモル分率（xmin）が増加するにつれて増大し、イオン強度（I）が増加するとついても減少する。これらの現象はそれぞれAAの負の電荷とNa⁺のイオン濃度に起因した。表面電荷密度（δ）はIにはほとんど依存せずxminに比例した。このことからAA-HED混合ミセルの表面状態はCoulomb-Chapmanの理論で表されることが示された。AA-HED混合ミセルの60℃におけるδの絶対値は25℃の値に比べてわずかに減少した。

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