Physicochemical Properties of Acylglutamic Acid-Alkylamine Complexes in Aqueous Media

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Abstract: We studied the physicochemical properties of 1:1 stoichiometric complexes of acylglutamic acids (*C_nGlu*) with tertiary alkylamines (*C_nDMA*) in water at their low and high concentrations. Static surface tensiometry suggested that the critical micelle concentration (cmc) decreased with increasing hydrophobic chain length of the complexes. In addition, *C_nGlu-C_nDMA* yielded lower cmc than the C12Glu single system. In the region of high concentrations, several phase states including isotropic liquid (*L_i*) phase, hexagonal liquid crystal (*H_i*) phase, bicontinuous cubic liquid crystal (*V_i*) phase, and lamellar liquid crystal (*L_a*) phase were observed. Assemblies with lesser positive curvature tend to be formed with increasing complex concentration, increasing temperature, and increasing hydrophobic chain length. Additionally, the complex formation resulted in the molecular assemblies with lesser positive curvature.

Key words: acylglutamic acid, alkylamine, gemini surfactant, surface tension, liquid crystal

1 Introduction

Gemini surfactant is a dimer of monomeric surfactants linked by a space unit, being composed of two hydrophilic headgroups and two hydrophobic chains in a molecule^{1,2)}. Gemini surfactants have been attracted as a new type of surfactants because of their excellent micellization efficiency at low concentrations and good solubility in water^{1,2)}. Thus, gemini surfactants are generally categorized into an eco-friendly surfactant. However, their synthesis and purification processes are usually complicated, and this leads to their high selling price in the market.

Based on these backgrounds, functionally-equivalent materials with gemini surfactants have been investigated, including dicarboxylic acid-alkylamine (or diamine-alkyl carboxylic acid) complexes, as shown in the following references. The stoichiometric complex formation is simply based on the proton transfer from the acid to the amine. This does not lead to an increase in the ionic strength in solution, which is significantly different from systems of catanionic (cationic and anionic) surfactant mixtures. They are sometimes called “gemini-like amphiphile³⁾”, “pseudo-gemini surfactant⁴⁻⁶⁾”, or “counterion-coupled gemini surfactant (cocogem)^{7,8)}”. Such noncovalent-type amphiphiles have been investigated in the aspect of surface tension in water⁷⁾ and rheology based on the formation of wormlike

micelles⁹⁾ or hydrogels¹⁰⁾. Recently, reversible control of stability of emulsion¹¹⁾ was also achieved in the system of citric acid-alkylamidodimethyl amine complex. However, we find a lack of knowledge about their phase behaviors at low and high concentrations in water, which is an important and novel subject for reporting.

In our previous work, we reported that acylglutamic acid-alkylamine complexes (*C_nGlu-C_nDMA*, where *n* is the number of carbon atoms in the hydrophobic chains) form pH-sensitive wormlike micelles and hydrogels¹²⁾. In addition, these complexes can stabilize oil droplets dispersed in water against their coalescence¹³⁾. In this paper, we report the physicochemical property of *C_nGlu-C_nDMA* in their dilute aqueous solutions by static surface tensiometry. We also demonstrate the phase behavior of *C_nGlu-C_nDMA* in water on the basis of polarized optical microscopy (POM) and small angle X-ray scattering (SAXS) results.

2 Experimental Section

2.1 Materials.

Dodecanoylglutamic acid (C12Glu) and *C_nGlu-C_nDMA*, (*n* = 10, 12, and 14) were synthesized according to the same procedure mentioned in our previous paper¹⁴⁾. Figure

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Accepted March 31, 2020 (received for review February 4, 2020)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

Fig. 1 Chemical structures of $C_n\text{Glu}-C_n\text{DMA}$ ($n = 10, 12$, and 14) neutralized by NaOH. $C_n\text{Glu}-C_n\text{DMA}$ was always neutralized by NaOH at a fixed mole ratio, $C_n\text{Glu}-C_n\text{DMA}:\text{NaOH} = 1:1$.

1 shows chemical structures of $C_n\text{Glu}-C_n\text{DMA}$ neutralized by NaOH. 1 mol/L NaOH aqueous solution was purchased from Fujifilm Wako Pure Chemical Co. The water used in this study was deionized with a Barnstead NANO Pure Diamond UV system and filtered with a Millipore membrane filter ($0.22\ \mu\text{m}$ pore size).

2.2 Preparations and instrumentations

In our previous papers^{12, 14}, we reported the solubility of $C_n\text{Glu}-C_n\text{DMA}$ in their aqueous solutions as a function of pH. In our present study, $C_n\text{Glu}-C_n\text{DMA}$ was always neutralized by NaOH at a fixed mole ratio, $C_n\text{Glu}-C_n\text{DMA}:\text{NaOH} = 1:1$.

The Krafft point of C12Glu monosodium salt is reported as 38.1°C ¹⁵, being higher than room temperature (25°C). This means that the surfactant precipitates in water at 25°C . Therefore, C12Glu was neutralized by NaOH at a mole ratio, $\text{C12Glu}:\text{NaOH} = 1:2$.

Static surface tension measurements were performed using a Krüss K100C Wilhelmy auto surface tensiometer with a platinum plate. The surface tension was assumed to be equilibrated when the change in the surface tension became less than $0.01\ \text{mN m}^{-1}$ per 90 s. The measurement temperature was set at 25°C .

Mixtures composed of $C_n\text{Glu}-C_n\text{DMA}$ and water were prepared as follows. The mixtures were sealed in a glass test tube. The system was heated up to 80°C using a temperature-controlled water bath for 30 min, mixed at a constant rotation speed of 3,000 rpm for 3 min using a vortex mixer, centrifuged for 30 min at a constant rotation speed of 3,500 rpm using a Kokusan H11-NB centrifuge, and finally the equilibration cycle was repeated 3 times. Then, the system was left for 2 weeks in an incubator set at a constant temperature of 25°C . The phase state was examined after this equilibration period.

Fig. 2 Surface tension – concentration plots for the $C_n\text{Glu}-C_n\text{DMA}$ and C12Glu systems.

POM observations were performed at 25°C using an Olympus IX73 microscope.

SAXS measurements were carried out using an Anton Paar SAXSess camera equipped with a PANalytical PW3830 laboratory X-ray generator, a multilayer film Goebel mirror, a block collimator, a semi-transmissible beam stop, a TCS120 temperature controller, and an imaging plate detector. The apparatus was operated at 40 kV and 50 mA using Cu-K α X-rays (wavelength of $0.154\ \text{nm}$). The measurement time was fixed at 10 min. The measurement temperatures were in the range $25 - 65^\circ\text{C}$.

3 Results and Discussion

3.1 Aqueous solution properties at low concentrations

The $C_n\text{Glu}-C_n\text{DMA}$ complex is a mixture of the two possible isomers shown in Fig. 1. Furthermore, the complex is a dynamic amphiphile. This means that the complex formation/deformation (in other words, the complex-monomer transition) would continually occur not only in the solution phase but also at the air/solution interface. Figure 2 shows the surface tension results for the aqueous $C_n\text{Glu}-C_n\text{DMA}$ and C12Glu solutions as a function of their concentrations. For all of the analogs, the surface tension decreased with increasing concentration, and attained a plateau level at a given concentration (critical micelle concentration, cmc). Table 1 shows the interfacial parameters calculated from the results; cmc, surface tension measured at the cmc (γ_{cmc}), surface excess concentration estimated at the cmc (Γ_{cmc}), and occupied area per $C_n\text{Glu}-C_n\text{DMA}$ (or C12Glu) adsorbed at the air/aqueous solution interface (A_{cmc}). Γ_{cmc} and A_{cmc} values were calculated according to the following equations;

$$\Gamma_{\text{cmc}} = -\frac{1}{2.30iRT} \left(\frac{d\gamma}{d \log C} \right)_\tau \quad (1)$$

Table 1 Interfacial parameters calculated from the surface tension data.

System	cmc [mmol dm ⁻³]	γ_{cmc} [mN m ⁻¹]	Γ_{cmc} [$\mu\text{mol m}^{-2}$]	A_{cmc} [nm ²]
C10Glu-C10DMA	2.7	25	1.3	1.3
C12Glu-C12DMA	0.15	26	1.5	1.1
C14Glu-C14DMA	0.014	25	1.6	1.1
C12Glu	9.7	24	0.75	2.2

$$A_{\text{cmc}} = \frac{1}{N_A \Gamma_{\text{cmc}}} \quad (2)$$

where, R is the gas constant, T is the absolute temperature, and N_A is the Avogadro number. Additionally, i is the number of adsorption species. In this study, Γ_{cmc} and A_{cmc} values were calculated under the assumption $i = 3$, although the dissociation constant of CnGlu-CnDMA in the solution phase has not been known, yet.

The cmc of CnGlu-CnDMA decreased with increasing chain length, as expected. The increased chain length resulted in the increased hydrophobicity of CnGlu-CnDMA , leading to the decrease in cmc. This behavior is generally observed in many surfactant systems¹⁶⁾, including the CnGlu analogs¹⁵⁾ and the carboxylic acid-alkylamine complex systems^{5, 7)}. In general, the linear relationship between n and cmc is present for surfactant systems¹⁷⁾.

$$\log \text{cmc} = A - Bn \quad (3)$$

where A and B are constant. **Figure 3** show the $\log \text{cmc} - n$ plot for the CnGlu-CnDMA and CnGlu disodium salts¹⁵⁾ systems. Here, we plotted the $\log \text{cmc}$ as a function of n (CnGlu) or $2n$ (CnGlu-CnDMA), based on the expectation that the chain length of CnDMA also affects the cmc, even if CnDMA merely acts as a counterion and dissociates from CnGlu . The cmc decreased linearly with increasing n or $2n$, as expected. The slope was greater for CnGlu-CnDMA than for CnGlu , suggesting the larger impact of hydrophobic chain length on the cmc for the CnGlu-CnDMA analogs.

As shown in **Table 1**, the A_{cmc} values estimated for CnGlu-CnDMA were much smaller than that for C12Glu . CnDMA acts as not only a counterion of CnGlu but also a hydrophobicity enhancer of the complex. As a result of these effects, the packing density of the complexes became larger at the air/solution interface than that of C12Glu .

3.2 Phase behavior at high concentrations

As stated in the Experimental section, the aqueous phase behavior was studied through POM and SAXS measurements. The temperature-concentration phase diagrams of CnGlu-CnDMA are summarized in **Fig. 4**. We discussed their phase behaviors as a function of (i) concentration, (ii) temperature, (iii) hydrophobic chain length, and (iv) complex formation with comparison for the reported C12Glu system¹⁸⁾.

Fig. 3 Relationship between cmc and n or $2n$ (total carbon numbers in hydrophobic chains).

(i) **Figure 5** shows typical SAXS and POM results obtained for the (a) C10Glu-C10DMA , (b) C12Glu-C12DMA , and (c) C14Glu-C14DMA systems at various concentrations at a constant temperature of 25°C. In the case of C10Glu-C10DMA (**Fig. 5a**), the phase states were determined as follows: hexagonal liquid crystal (H_1) phase at 36 wt% (indicated by the ratio of q values = $1 : \sqrt{3} : 2$, the fan-like texture observed in the POM image, and its optically anisotropic nature), H_1 and bicontinuous cubic liquid crystal (V_1) phases at 55 wt% (indicated by the ratio of q values = $\sqrt{6} : \sqrt{8}$, the fan-like texture observed in the POM image, and its optically anisotropic nature), V_1 phase at 70 wt% (indicated by the ratio of q values = $\sqrt{6} : \sqrt{8}$, and its optically isotropic nature), and lamellar liquid crystal (L_a) phase at 73 wt% (indicated by the ratio of q values = $1 : 2$, the Maltese-cross texture observed in the POM image, and its optically anisotropic nature).

Similarly, the H_1 phase was identified in the cases of C12Glu-C12DMA (**Fig. 5b**) and C14Glu-C14DMA (**Fig. 5c**). However, the SAXS pattern yielded splitting of the peaks above the concentration of 54 wt% (C12Glu-C12DMA) and 48 wt% (C14Glu-C14DMA). This means the coexistence of two lyotropic liquid crystals. These complexes have a gel-liquid crystal phase transition temperature, and the temperature increases with hydrophobic chain length¹³⁾. This causes a difficulty in preparing a homogenous sample at high concentrations and at low temperatures, under a given homogenization process.

In summary, the increased concentration resulted in the

Fig. 4 Temperature-concentration phase diagrams of (a) C10Glu-C10DMA, (b) C12Glu-C12DMA, and (c) C14Glu-C14DMA. The complex samples were neutralized by 1 eq. NaOH.

Fig. 5 POM images and SAXS patterns obtained for the (a) C10Glu-C10DMA, (b) C12Glu-C12DMA, and (c) C14Glu-C14DMA systems at various concentrations at a constant temperature of 25°C. The characteristic ratios of q values and POM images are as follows: (b) H_1 phase at 30 wt% ($1 : \sqrt{3} : 2$, the fan-like texture), $H_1 + V_1$ phases at 55 wt% ($1 : \sqrt{3} : 2$ and $\sqrt{2} : \sqrt{3}$, the fan-like texture), and $V_1 + L_\alpha$ phases at 60 wt% ($\sqrt{2} : \sqrt{3} : \sqrt{4}$ and $1 : 2$, the Maltese-cross texture); and (c) H_1 phase at 20 wt% ($1 : \sqrt{3} : 2$, the fan-like texture), and $V_1 + L_\alpha$ phases at 54 wt% ($\sqrt{6} : \sqrt{20} : \sqrt{26}$ and $1 : 2$). The characterization information of (a) is shown in the text.

Fig. 6 SAXS patterns obtained for (a) C10Glu-C10DMA at a fixed concentration of 55 wt%, (b) C12Glu-C12DMA at a fixed concentration of 60 wt%, and (c) C14Glu-C14DMA at a fixed concentration of 44 wt% at various temperatures. The characteristic ratios of q values are as follows: (a) $H_1 + V_1$ phases at 25 °C ($1 : \sqrt{3}$ and $\sqrt{6} : \sqrt{8}$), V_1 phase at 45 °C ($\sqrt{6} : \sqrt{8} : \sqrt{24}$), and L_α phase at 65 °C; (b) $V_1 + L_\alpha$ phases at 25 °C ($\sqrt{2} : \sqrt{3} : \sqrt{4}$ and $1 : 2$), and L_α phase at 35 °C ($1 : 2 : 3$); and (c) H_1 phase at 25 °C ($1 : \sqrt{3}$), $H_1 + L_\alpha$ phases at 55 °C ($1 : \sqrt{3}$ and $1 : 2$), and L_α phase at 65 °C ($1 : 2$).

phase transitions as L_1 - H_1 - V_1 - L_α for all of the complexes. This means that the increased complex concentration yields molecular assemblies with lesser positive curvature, resulting from a decreased cross-sectional area of the headgroups and hence an increased critical packing parameter (CPP) of the complexes.

(ii) **Figure 6** shows typical SAXS patterns obtained for the (a) C10Glu-C10DMA, (b) C12Glu-C12DMA, and (c) C14Glu-C14DMA systems at various temperatures at a given concentration. For example, we observed the ($H_1 + V_1$)- V_1 - L_α phase transition with increasing temperature in the C10Glu-C10DMA system at a fixed concentration of 55 wt%. These phase transitions reflect the decreased hydration around the headgroups and the amide bond, leading to the decreased cross-sectional area of the headgroups. Hence, the positive curvature becomes lower at high temperatures. We confirmed the similar behavior in the C_n Glu single system¹⁸⁾, the amino acid-based gemini surfactant system¹⁸⁾, and the oleic acid-based gemini surfactant system¹⁹⁾.

(iii) For understanding the effects of hydrophobic chain length, the phase diagrams in **Fig. 4** were compared. First, the single V_1 phase is present in the C10Glu-C10DMA system, whereas this phase appears as mixture with other phases in the C12Glu-C12DMA and C14Glu-C14DMA systems. Second, the phase transitions occur at lower con-

centrations for longer hydrophobic chain analogs. This behavior is predicted by the CPP; the shorter hydrophobic chain analog tends to form assemblies with greater positive curvature, whereas the longer hydrophobic chain analog tends to form assemblies with lesser positive curvature. Third, the d -spacing values of the H_1 phase are shown in **Table 2**. The values become larger with increasing chain length. Fourth, the increased chain length also resulted in the narrower L_α region and the wider solid-present region. This results from the increased hydrophobicity and hence the decreased solubility in water for the longer chain analogs.

(iv) Finally, the phase diagrams of the C12Glu-C12DMA and C12Glu systems¹⁸⁾ were compared. In the case of the C12Glu-C12DMA system, the L_1 - H_1 phase transition occurs and the H_1 region appears in a wide range, whereas in the case of the C12Glu system, the discontinuous cubic liquid crystal (I_1) phase appeared and L_1 - I_1 phase transition occurred. Here, C12Glu-C12DMA consists of double-chains and a single-head, whereas C12Glu consists of a single-chain and double-heads. Clearly, the CPP of C12Glu-C12DMA is expected to be larger than that of C12Glu, and hence the assemblies having lesser positive curvature (such as H_1 phase) are preferentially formed for C12Glu-C12DMA.

Table 2 *d*-spacing values of the H₁ phase at a fixed concentration of 40 wt%.

Complex	<i>d</i> (nm)
C10Glu-C10DMA	4.08
C12Glu-C12DMA	4.80
C14Glu-C14DMA	5.57

4 Conclusions

In this study, we characterized the physicochemical properties of *Cn*Glu-*Cn*DMA in water at low and high concentrations. The static surface tensiometry revealed that the increased chain length of *Cn*Glu-*Cn*DMA resulted in the decreased cmc, as generally observed in surfactant systems. The alkylamine (*Cn*DMA) plays a role as a counterion of *Cn*Glu as well as a hydrophobicity enhancer. This leads to the excellent surface activity, being comparable to gemini surfactant. In the aspect of the phase behavior at their high concentrations, we observed the L₁-H₁-V₁-L_α phase transition with increasing concentration. The increased temperature also yielded the assemblies with lesser positive curvature. The complex formation of *Cn*Glu with *Cn*DMA induced the formation of assemblies with lesser positive curvature, and hence *Cn*DMA also acts as a curvature modifier of *Cn*Glu.

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