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ORIGINAL CONTRIBUTION

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Effects of L-arginine on aggregates of fatty-acid/potassium soap in the aqueous media

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Abstract In the present study, we investigated the effects of L-arginine on aggregates of fatty acid/fatty soap in the aqueous media as a function of pH, by means of hydrogen ion titration, viscoelastic measurement, cryo-transmission electron microscopy and phase contrast microscopy. We found out that L-arginine effectively inhibits the oil droplet growth of oleic acid or octanoic acid. The effect is explained in terms of the adsorption of arginine at the microscopic drop surface, or at the oil/water microinterface through the hydrophobic effect assisted by the hydrogen bonds between carboxyl group of fatty acid and carboxylate of arginine. As to the crystallization of lauric acid at temperatures below the melting point of the hydrocarbon chain, arginine is not effective. In addition, we also found out that the strong binding of arginine cation to anionic oleate micells induces the dominant micellar growth. L-arginine has been used in many refolding systems to suppress protein aggregation. These effects of L-arginine on the aggregates of fatty acid/fatty soap in the aqueous media observed in the present study is expected to form a basis to the specific function displayed in the protein refolding.

Keywords L-arginine · Arginine oleate · Arginine octanoate · Vesicle · Acid-soap

Introduction

Fatty acids and soaps have the advantage over synthetic detergents with respect to toxicity and biodegradability. In aqueous media, soaps are known to form acid soaps on partial protonation [1]. In spite of its biological and chemical importance, the nature of fatty acid is still unclear compared with that of synthetic detergents. It is known that oleic acids spontaneously form vesicles when pH is close to the pK_a; at higher pH only micelles are formed, while at lower pH oil droplets are formed [2–8]. The vesicle formation due to the partial protonation is thought to be attributed to hydrogen bonding between adjacent protonated and ionized carboxylates and the decrease in the electrostatic repulsion of adjacent head groups [2].

It was reported that the acidification of aqueous solutions of fatty acid soap gives complicated phase behavior as a function of the degree of neutralization (β), composed of a variety of aggregated species such as micelles, vesicles, acid soaps, and solid or oil droplets of fatty acid [9–14]. We reported that hydrogen ion titration profiles of the potassium oleate/oleic acid and the sodium oleate/oleic acid systems as functions of β [12, 14]. At temperatures higher than 5°C, the titration curves of potassium oleate solutions were almost identical to those of sodium oleate solutions at temperatures higher than 20°C, which was characterized by a single constant pH region due to the multiple-phase equilibrium including oleate micelles. At a low temperature of 0°C, on the other hand, the β ranges of the constant-pH region were different: $0.6<\beta<0.95$ for the potassium oleate solution and $0.5<\beta<0.95$ for the sodium oleate solution. These results suggested that the acid soap composition (i.e., oleic acid to oleate soap ratio) was 2:3 for the potassium oleate, while it was 1:1 for the sodium oleate.

In the present study, we have investigated the effects of L-arginine on the aggregates of fatty acid/potassium soap in

the aqueous media. L-arginine has been used in many protein refolding systems to suppress protein aggregation [15]. The function is considered to inhibit the aggregation of unfolded proteins [16]. The characteristic of L-arginine to suppress protein aggregations may influence the aggregate behavior of fatty acid/potassium soap in the aqueous media.

Experimental

Materials

Potassium oleate was prepared by the neutralization of highly pure oleic acid (purity: 99.999%; Research Institute of Biological Materials Japan, Ltd) in ethanol by a 0.5 M KOH ethanol solution. Arginine oleate was prepared by mixing an equimolar solutions of potassium oleate and L-arginine hydrochloride (Peptide Research) in degassed water. Arginine laureate and arginine octanoate were prepared in similar ways to arginine oleate, using lauric and octanoic acid (Aldrich). L-arginine is a univalent cation in the pH range from 5 to 10, as shown in Fig. 1.

Hydrogen ion titration

Hydrogen ion titrations were carried out under nitrogen atmosphere at 25±0.5°C, using a Horiba 6028-T composite pH electrode connected to a Beckman ϕ70 pH meter with an accuracy of 0.01 pH units. Sample solutions were titrated with a 1N HCl solution (Nakarai Tesque). An addition of HCl into the solutions causes the simultaneous protonation of both soaps and arginine. Therefore, the degree of average neutralization β' is defined as follows,

$$\beta' = \frac{1}{2}\left[\frac{[\mathrm{Arg}^+]}{[\mathrm{Arg}]_{\mathrm{tot}}} + \frac{[\mathrm{RH}]}{[R^-]+[\mathrm{RH}]}\right] = \frac{[\mathrm{HCl}]}{2[R]} \quad (1)$$

where $[\mathrm{Arg}]_{\mathrm{tot}}$ and $[\mathrm{Arg}^+]$ are the total concentration of the arginine and the protonated arginine (form B in Fig. 1), respectively. The $[R^-]$ and [RH] are the concentrations of soap and fatty acid, respectively while the [HCl] is the concentration of added HCl. Since arginine salts of fatty acid were prepared by mixing an equimolar of potassium salts of fatty acid and L-arginine hydrochloride, the resultant solutions always contain KCl salts equal to the corresponding surfactant concentration, in addition to arginine salts of fatty acid. As a result, $[\mathrm{Arg}]_{\mathrm{tot}}=[R^-+\mathrm{RH}]=[\mathrm{KCl}]$. Thus, the pH of the solution prepared was about 9, but the initial pH in the hydrogen ion titrations was adjusted to be around pH 11 with KOH. In pH 11, arginine is mostly in form C in Fig. 1. During the titration with HCl, the arginine salts are protonated from form C to B in Fig. 1.

Turbidity

Turbidity measurements were performed at 25°C with a Jasco Ubest-50 UV/Vis spectrophotometer, equipped with a thermostat cell holder and a magnetic stirring device, using quartz cells of 1 cm path length. Turbidity was measured at 400 nm.

Viscosity

The viscosity was measured at 25±0.02°C using an Ostwald viscometer (flow time of water: 134.5±0.2 s).

Dynamic viscoelastic measurement

Dynamic viscoelastic measurements were performed with a stress-controlled rheometer (Carri-MED CSL-100 England) with a cone-plate (plate diameter of 6 cm, angle of 2°). The storage modulus G' and the loss modulus G" were measured as functions of angular frequency ω from 0.01 to 60 rad s^{-1} at a temperature of 25°C. The dynamic measurements were made at strain amplitude levels where the dynamic moduli are strain independent. The rheometer was equipped with a solvent trap to protect the sample from solvent evaporation. Solutions were kept for at least 3 days before measurements. The temperature was controlled within 0.1°C.

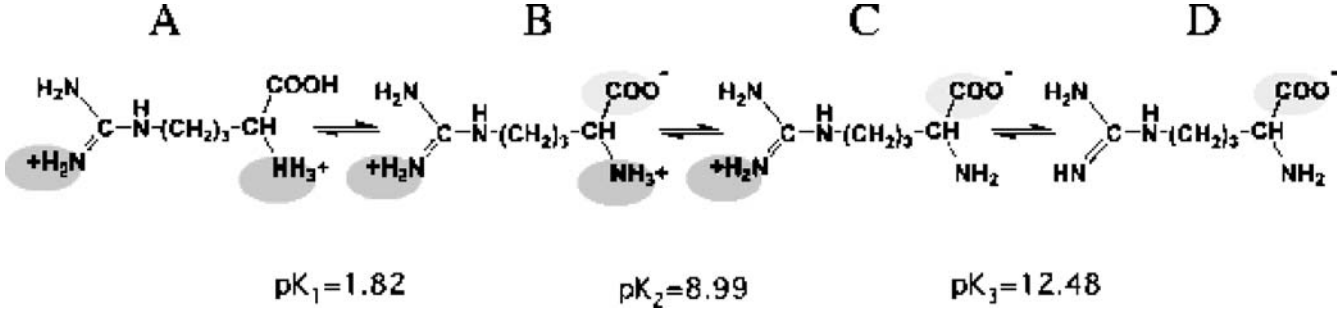

Fig. 1 Various ionization states of L-arginine at different pH values

Cryo-transmission electron microscopy

The cryo-transmission electron microscopy (cryo-TEM) investigations were performed with a Zeiss 902 A instrument, operating at 80 kV. Specimens were prepared by a blotting procedure, performed in a chamber with controlled temperature and humidity. A drop of the sample solution was placed onto an easy mark (EM) grid coated with a perforated polymer film. The excess solution was then removed with a filter paper, leaving a thin film of the solution on the EM grid. Vitrification of the thin film was achieved by rapid plunging of the grid into liquid ethane held at its freezing point. The vitrified specimen was then transferred in the cold state to the microscope and investigated at 108 K.

Phase contrast microscopy

Formations of vesicles and oil droplets were examined using a Nikon microscope OPTIPHOTO-2 at 25°C.

Composition analysis of the precipitates

Precipitated solids of acid soaps were analyzed with an element analysis. Before the element analysis, the precipitated solids were dried overnight under a vacuum.

Results

Hydrogen ion titration curves

The hydrogen ion titration curve for a solution of 50 mM arginine HCl plus 50 mM potassium oleate is shown in Fig. 2a. From the result of the formation of a 1:1 acid–soap ratio with arginine counterions as mentioned below, arginine oleates are considered to be preferentially formed in this mixture. Thus, we call this mixture “arginine oleate”. The titration curve for the 50-mM potassium oleate solution in the presence of 50 mM KCl is also shown for comparison in Fig. 2b. In the case of arginine oleate, the first addition of HCl induces a decrease in pH (region A). In this region, oleate micelles are titrated together with the arginine, hence the pH decreases monotonously. As the pH decreased further, the solution showed the significant extent of viscoelasticity around pH 9.5, suggesting the formation of elongated micelles. Detailed examinations of the elongated micelles will be described later. On further addition of HCl, a constant pH region appears at about pH 9.2 and the solution becomes turbid. The constant pH region (region B) corresponds to a three-phase region: oleate micelles (pseudo phase), lamellar dispersion, and aqueous solution [1]. It is known that vesicles can be distinguished from oil droplets of fatty acid by phase contrast microscopy [3]. The difference in the refractive index between fatty acid and water allowed the discrimination between oil droplets (phase-bright droplets), unilamella vesicles (low-phase-contrast spheres), and multilamellar vesicles (phase-dark spheres). In region B, we observed phase-dark spheres of multilamellar vesicles under a phase contrast microscope (Fig. 3a).

In region C, all micelles have been converted into lamellas, resulting in the decrease of the pH again. The solutions become highly turbid. A solid-like unidentified phase has been reported for the potassium oleate system in region C [1], but the existence of the unidentified phase was not clear for arginine oleate. In region F, the system is again nearly invariant, suggesting three phases are coexisting: oil droplets, the lamellar droplets (or an unidentified phase), and the aqueous phase. At the end of the

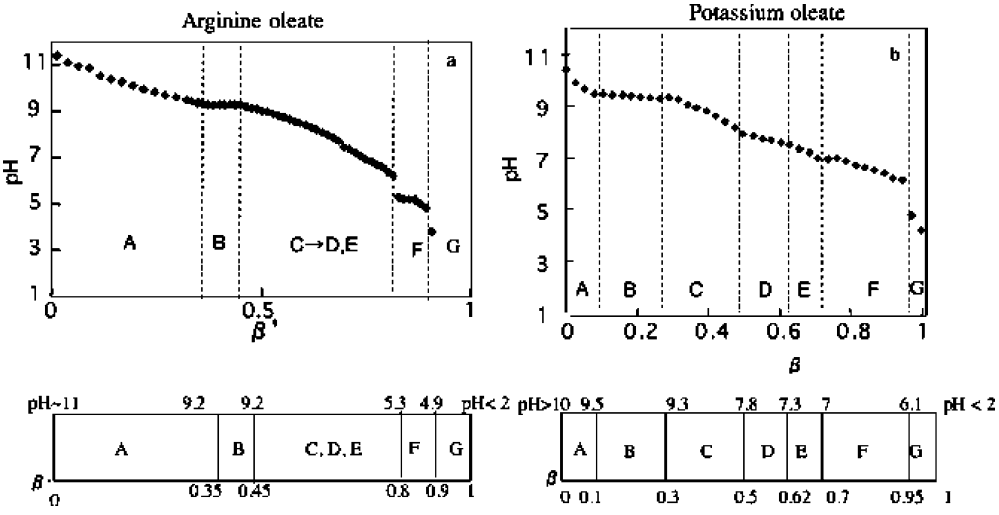

Fig. 2 Titration curves at 25°C in the presence of 50 mM KCl. **a** 50 mM arginine oleate solution. **b** 50 mM potassium oleate solution. *A*: micelles, *B*: micelles + lamellar dispersion, *C*: lamellar dispersion, *D*: lamellar dispersion + solid-like unidentified particles, *E*: solid-like unidentified particles, *F*: solid-like unidentified particles + oil droplets and *G*: oil droplets of fatty acid. These phase sequences as a function of pH are summarized in the figure

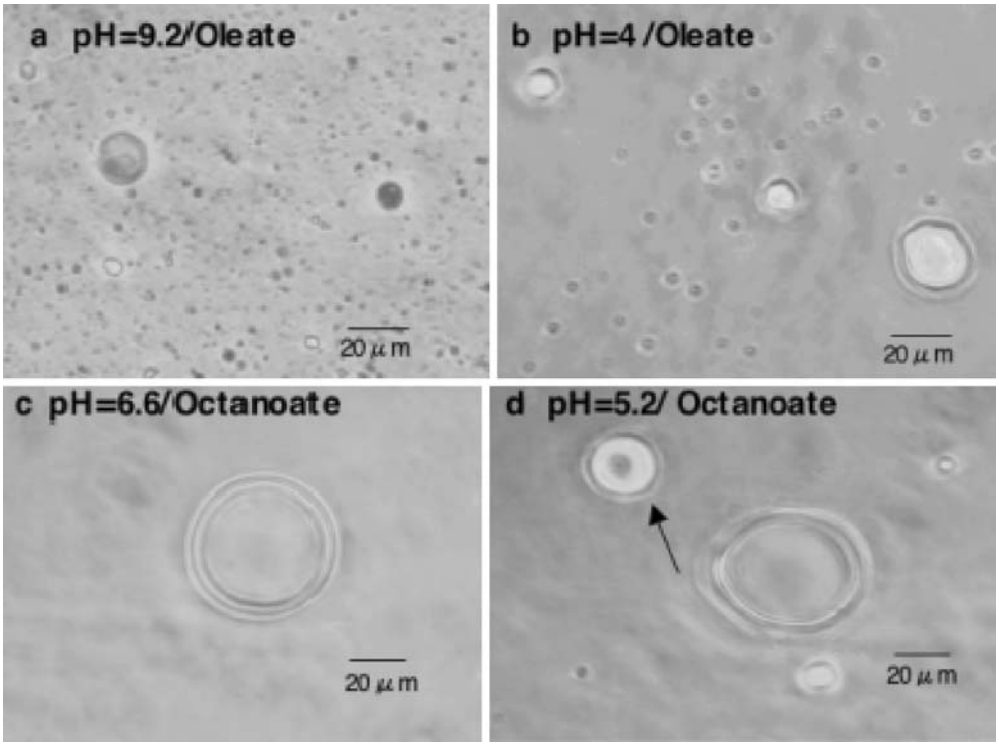

Fig. 3 Phase-contrast microscopy of arginine oleate vesicles and emulsions at 25°C. **a** Arginine oleate at pH 9.2, forms multilamellar vesicles characterized by *phase-dark spheres* under a phase contrast microscope. **b** Arginine oleate at pH 4, forms oil droplets. **c** Arginine octanoate at pH 6.6, forms oligolamellar vesicles characterized by *low-phase-contrast spheres* under a phase contrast microscope. **d** Arginine octanoate at pH 5.2, forms both oil droplets (denoted by an *arrow*) and oligolamellar vesicles. The phase contrast microscope demonstrates that refractive index differences between the oleate and water that cause the oil droplets to appear bright by phase contrast

titration only two phases are present: oil droplets of oleic acid and the aqueous solution saturated with monomeric oleic acid. In this region, the oil droplets of fatty acids were clearly identified under a phase contrast microscope (phase-bright droplets) as shown in Fig. 3b.

A similar titration curve was obtained for the potassium oleate solution (Fig. 2b), but the pH value at which the oil phase of oleic acid is formed, pH (Oil) (i.e., region F) is lower for arginine oleate (pH~5.3) than potassium oleate (pH~7). The pH value at which the lamellar phase is formed, pH (L) (i.e., region B) is also slightly lower for arginine oleate: 9.5 for potassium oleate and 9.2 for arginine oleate. These results indicate that a change of the counter ion from potassium to arginine decreases both pH (Oil) and pH (L). The phase sequences of arginine oleate and potassium oleate as a function of pH are summarized in Fig. 2.

The hydrogen ion titration curve for the solution of a 500-mM arginine HCl plus a 500-mM potassium octanoate (i.e., arginine octanoate) is shown in Fig. 4a. The titration curve for the 500-mM potassium octanoate solution in the presence of 500 mM KCl is also shown for comparison in Fig. 4b. The titration profile of the arginine octanoate solution is similar to that of the arginine oleate system except for the absence of a solid-like unidentified phase. Two constant pH regions were observed in region B and region D. The three-phase system in region B consists of micelles, lamellar dispersion, and the aqueous phase, while region D consists of lamellar dispersion, oil droplets of fatty acid and the aqueous phase. This was supported by the phase contrast microscopy. Low-phase-contrast spheres of the oligolamella vesicles were observed in region B (Fig. 3c), while both the phase-bright spheres of oil droplets (denoted by an arrow in the figure) and the low-phase-contrast spheres of the oligolamella vesicles are observed (Fig. 3d), suggesting the coexistence of oligolamella vesicles and the oil droplets. The phase sequences of arginine octanoate and potassium octanoate as a function of pH are summarized in Fig. 4. As what we found in the oleate/oleic acid system, both pH (Oil) and pH (L) are lower for the arginine octanoate system than for the potassium octanoate system.

The hydrogen ion titration curve for the solution of a 50-mM arginine HCl plus a 50-mM potassium laurate (i.e., arginine laurate) is shown in Fig. 5a. The titration curve for the 50-mM potassium laurate solution in the presence of 50 mM KCl is also shown for comparison in Fig. 5b. The system is titrated at a temperature of 25°C, which is below the chain-melting temperature T_{ch} of both the acid and the acid-soap [1]. Thus, the shape of the titration curve essentially differs from those of arginine octanoate and arginine oleate obtained at above T_{ch}. The first addition of HCl induces a monotonous decrease in the pH (region A) due to the titration of both the micelles and arginine. On

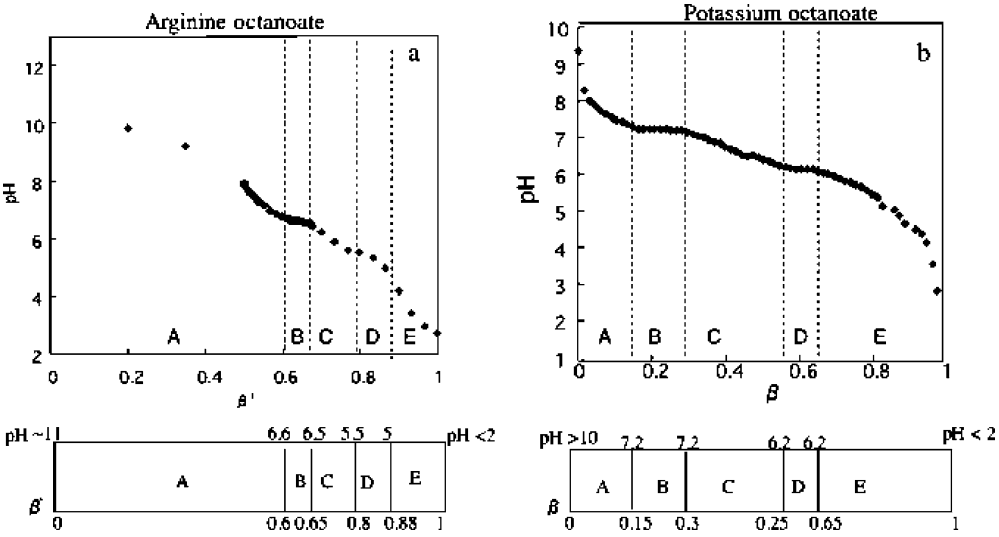

Fig. 4 Titration curves at 25°C in the presence of 500 mM KCl. **a** 500 mM arginine octanoate solution. **b** 500 mM potassium octanoate solution. *A*: micelles, *B*: micelles + lamellar dispersion, *C*: lamellar dispersion, *D*: lamellar dispersion + oil droplets and *E*: oil droplets. These phase sequences as a function of pH are summarized in the figure

further addition of HCl, the constant pH region appears at about pH 9 and the solution becomes slightly turbid. The constant pH region (region B) corresponds to a three-phase region: laurate micelles, acid-soap crystalline, and the aqueous phase. Microscopic observations in region B verified crystalline materials of the acid-soap (not shown). Element analysis of the precipitated solid in region B demonstrated the 1:1 acid soaps having the counter ion arginine (Calculated for the 1:1 acid soaps with the arginine: C, 62.68; H, 10.87; N, 9.75. Found: C, 61.82; H, 10.85; N, 9.68). This indicates the preferential binding of arginine cation over potassium cation in the acid soap formation of lauric acid/laurate. The specific attractive interaction between the nonionic and the anionic species in the 1:1 acid soaps is most likely because hydrogen bonds between acid-anions [2]. In region D, the system is nearly invariant, suggesting that three phases are coexisting: the acid crystal, the 1:1 acid-soap crystal, and the aqueous phase. At the end of the titration, only two phases are present: the acid crystal and the aqueous solution saturated with monomeric lauric acid. A similar titration curve is obtained for the potassium laurate solution (Fig. 5b). The phase sequences of arginine laurate and potassium laurate as a function of pH are summarized in Fig. 5. Argine was not effective in inhibiting the crystallization of lauric acid, contrary to its inhibitory action for acid oil separation of both oleic acid and octanoic acid.

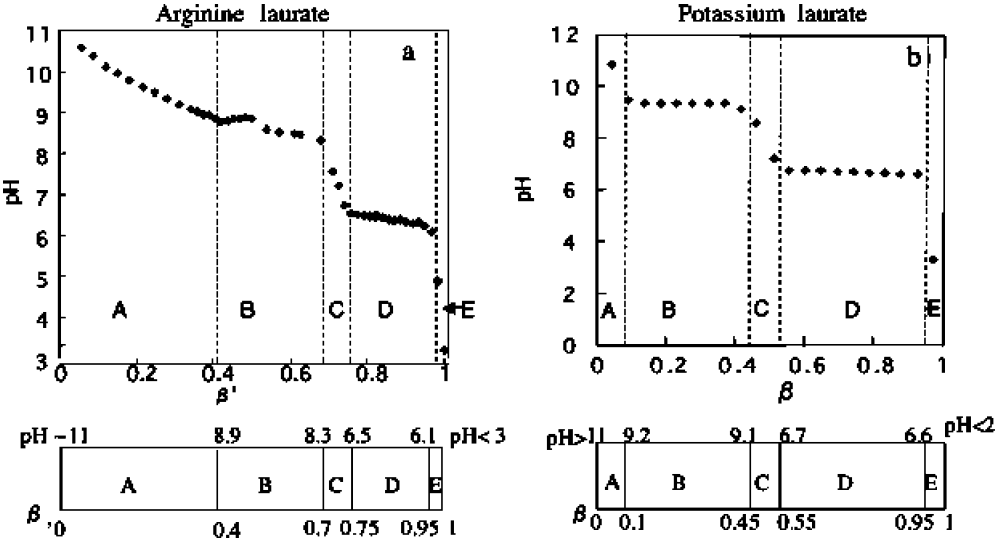

Fig. 5 Titration curves at 25°C in the presence of 50 mM KCl. **a** 50 mM arginine laurate solution. **b** 50 mM potassium laurate solution. *A*: micelles, *B*: micelles + acid-soap crystalline, *C*: acid-soap crystalline, *D*: acid-soap crystalline + acid crystals and *E*: acid crystals. These phase sequences as a function of pH are summarized in the figure

Micellar solutions of arginine oleate and arginine laurate

In the hydrogen ion titration of the arginine oleate micellar solution, we observed that the solution viscosity increased around pH 9.5, suggesting the formation of elongated micelles. To examine the micelle structure and the micelle growth, viscoelasitic measurements and cryo-TEM observations were carried out. Figure 6a shows a plot of the reduced viscosity (η_{sp}/C) of the arginine oleate solution at pH 9.5 at 25°C as a function of the surfactant concentration, C. The viscosity for arginine laurate solution is also shown for comparison. The solutions contain KCl to the same concentration as that of surfactant, oleate and laurate in these measurements. The viscosity of arginine oleate solution increased sharply for a range of C>~5 mM, suggesting the formation of the elongated micelles, while the increase in viscosity is negligible for the arginine laurate solution in the examined C range. This indicates the more pronounced micellar growth for arginine oleate with a longer hydrocarbon chain. However, the viscosity of the potassium oleate solution (C=50 mM with 100 mM KCl) was reported to be ~1 mPa [17]. This in turn suggests that the preferential binding of arginine cation over potassium cation to oleate anion also plays an important role in the micellar growth of the arginine oleate micelles. The strong binding of arginine cation would facilitate micellar growth by screening the electrostatic repulsions between the negatively charged surfactant head groups. As a result, long, flexible anionic elongated micelles were formed in the solution, which in turn, became entangled into a transient network and thereby imparted a high viscosity to the sample.

Figure 6b shows the shear rate dependence of the viscosity η obtained from the flow measurements for arginine oleate solutions at C=50 and 100 mM at pH 9.5. The arginine oleate solutions indicate a shear-thinning behavior at low shear rates, followed by a shear-thickening behavior at a high shear rate $\dot{\gamma}$ (0.6~0.9 s^{-1}). The shear-thinning behavior was fitted through the Cross model (Eq. (2)) [18],

$$\eta\left(\dot{\gamma}\right) = \eta_\infty + \left(\frac{\eta_0 - \eta_\infty}{1 + \alpha\dot{\gamma}^{2/3}}\right) \tag{2}$$

where η_0 and η_∞ are the low and high shear viscosities, respectively. As shown, the fitting is quite good except for high shear rate where the shear-thickening occurs. The constant α is almost independent of the surfactant concentration and it is about 3.2–3.3. The η_0 values are 26 and 108 Pa and the η_∞ values are 0.7 and 3.1 Pa for C=50 mM and C=100 mM, respectively. The shear-thinning behavior at low shear rates may be regarded as arising from the disruption of entanglement of the threadlike micelles and the alignment of the threadlike micelles by imposed shear. The critical shear rate ($\dot{\gamma}_c$) for the shear-thickening weakly decreases with the surfactant concentration ($\dot{\gamma}_c$ =0.8 s^{-1} at C=50 mM and $\dot{\gamma}_c$ =0.6 s^{-1} at C=100 mM). The shear-thickening behavior at high shear rates may be regarded as arising from the interactions of the threadlike micelles

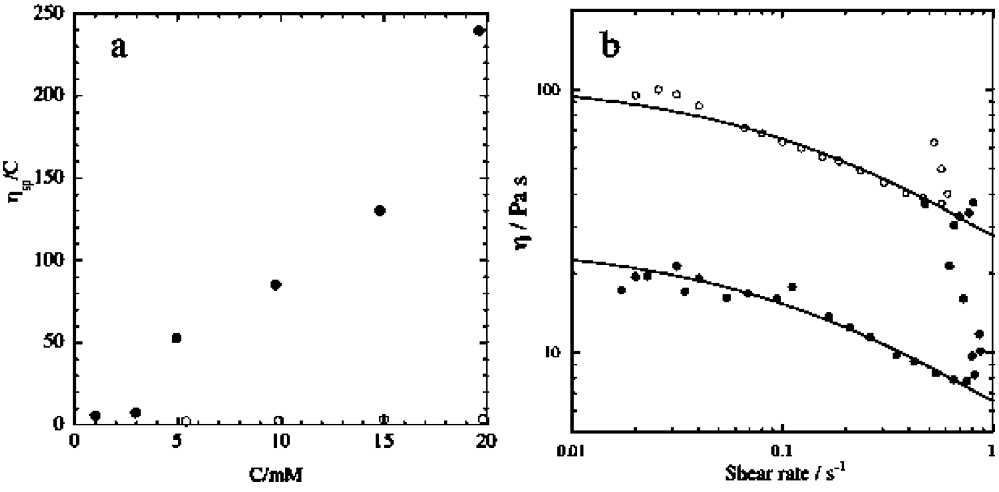

Fig. 6 **a** The viscosity (η_{sp}/C) at pH 9.5 at 25°C as a function of the surfactant concentration, C/mM. Here, η_{sp} is a specific viscosity. *Closed circles*: arginine oleate and *open circles*: arginine laurate. All surfactant solutions contain KCl salts of the concentration equal to the corresponding surfactant concentration. **b** The shear rate dependence of the viscosity η obtained from the flow measurements for arginine oleate solutions. *Closed circles*: surfactant concentration, C=50 mM in the presence of 50 mM KCl. *Open circles*: C=100 mM in the presence of 100 mM KCl. A *solid line* is fitted through the Cross model (Eq. 2)

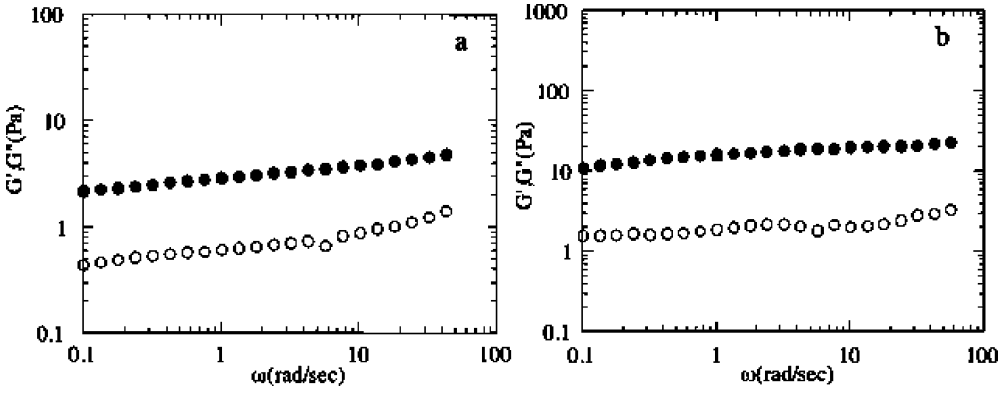

Fig. 7 The angular frequency ω dependence of the storage G' (*closed circles*) and the loss shear modulus G" (*open circles*) of arginine oleate solutions at pH 9.5 at 25°C. **a** Surfactant concentration, *C*=50 mM in the presence of 50 mM KCl. **b***C*=100 mM in the presence of 100 mM KCl

induced by imposed shear [19–22]. The cationic part of arginine counter ions is strongly bound to the anionic micelle, and another part with a dipole (COO^- and NH_3^+) would protrude into water. In the attractive interaction mediated by the bound counter ions, we think that the dipole interaction may enhance the interactions of the threadlike micelles induced by imposed shear. The detailed mechanism of the shear-thickening behavior in the arginine oleate solution was not clear and, this issue should be clarified in the future.

Figure 7 shows the angular frequency ω dependence of the storage G' and the loss shear modulus G" of arginine oleate solutions at *C*=50 and 100 mM at pH 9.5. Both G' and G" show weak dependencies on the angular frequency with a relation G' >G" in the frequency range, indicating a kind of behavior typical to gels or concentrated suspensions. The values of G' (1 rad/sec) increase with *C* by six times (2.5 and 15 Pa), which follows the relation $G' \sim C^{2-2.5}$ for the network of flexible chains [23].

Cryo-TEM observations of arginine oleate solutions at *C*=50 mM at pH 9.5 supported the formation of threadlike micelles suggested by the viscoelasitic measurements (Fig. 8). Many of the threadlike micelles extend over 1 μm without any branching. The small thickness of the film and the shear applied on the blotting of the samples explains the orientation of the threadlike micelles. However, besides threadlike micelles, unilamellar vesicles can be also seen. The vesicle sizes are mainly in the range of 10–50 nm radius. It should be noted that the titration curve showed no sign of coexistence of micelles and vesicles at pH 9.5. This inconsistent result may originate from the factor that the vesicles at pH 9.5 are not large enough to be approximated as a pseudophase. We observed that the solution became turbid at pH 9.2 due to the existence of multilamellar vesicles, while the solution was transparent at pH 9.5. We also did not observe any multilamellar vesicles in cryo-TEM. These observations indicate no formation of the multilamellar vesicles at pH 9.5.

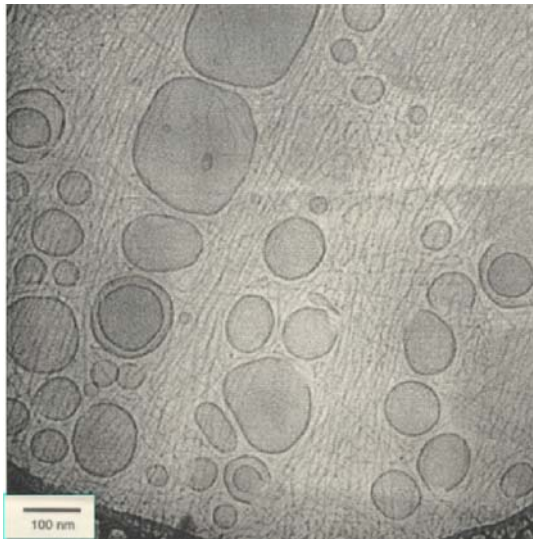

Fig. 8 Cryoscopic transmission electron micrograph of 50 mM arginine oleate solution at pH 9.5 in the presence of 50 mM KCl

Discussion

It is shown in the present study that arginine effectively inhibits the growth of fatty acid oil drops. The action is supposed to be due to the adsorption of arginine at microscopic drop surfaces or at oil/water interface, probably by inserting methylene groups into the hydrocarbon core of the oil droplets and lower the 'interfacial tension' by covering the surface with hydrophilic groups of arginine. We con-

sidered that the adsorption is driven by this hydrophobic effect assisted by the hydrogen bonds between carboxyl group of fatty acid and carboxylate of arginine. As to the crystallization of a fatty acid at temperatures below the chain melting point, arginine is not effective to interfere with the crystal growth, probably because methylene groups of arginine cannot be dissolved into crystalline hydrocarbon, hence the affinity will be reduced. Without the hydrophobic effect, the adsorption driven only by the hydrogen bond will not be effective enough to overcome the electric repulsion among adsorbed arginines. Preferential binding of arginine cation over potassium cation in the acid soap formation of lauric acid/laurate is contributed from the supposed hydrogen bond because electric attraction is operating for both counter ion species.

The proposed mechanism of the effects of arginine observed in the present study is expected to be related with the function of arginine in the refolding of proteins. The role of arginine has been considered to inhibit the aggregation of unfolded proteins, partially or fully, rather than to enhance the stability of the native or correctly folded states of proteins. For example, heat denaturation temperature of lysozyme at neutral pH does not change significantly by the presence of arginine but the aggregation of the denatured protein is largely inhibited [16]. Since the isoelectric point of lysozyme is 10.5–11, the adsorption of arginine to denatured lysozyme at neutral pH will be driven by the hydrogen bonds and the hydrophobic effect. The several kinds of hydrogen bond that are likely to drive the adsorption of arginine are as follows: (1) NH_3^+ groups (lysozyme)/carboxylate group (arginine) and (2) carboxylate (lysozyme)/NH_3^+ group (arginine). According to the suggested mechanism in the present study, the binding of arginine to lysozyme seems to be a prerequisite for its action, although evidence of arginine binding to lysozyme is poor [24]. It is not easy to infer any stabilizing medium effect (without binding) at relatively low arginine concentrations (50–100 mM). One possibility will be the binding of nearly equal amount of arginine to both native and denatured states of lysozyme, leading to the heat denaturation temperature independent of the binding.

Some nonionic surfactants like lauryl maltoside have been used to facilitate the solubilization of the inclusion bodies. This function resembles that of arginine and hence we briefly discuss the interaction of surfactants with proteins. Since the nonpolar groups of amino acid side chains are relatively short in comparison with the nonpolar chain of surfactants, the hydrophobic interaction of surfactant tails with proteins is expected not to be strong. This is shown by the shape of the binding isotherm of nonionic surfactants onto reduced or alkylated lysozyme, which is Freundlich type rather than Langmuir type [25, 26]. This weak direct interaction which differs from the solubilization by micelles is effective as shown in the following two observations:

1. Conformational changes of reduced lysozyme were observed at the bound amounts of 0.3–0.5 mol per mole amino acid residue [26].
2. At neutral pH of 7.4, native lysozyme forms aggregates at 25°C, not extensively but significantly, and this aggregation was shown to be suppressed by the binding of nonionic surfactants [25].

Although arginine is not a surfactant in the sense it does not form micelles, it is likely that the nonpolar group of arginine interacts with short nonpolar groups of amino acid side chains through the hydrophobic effect.

Table 1 pH values at which the oil droplets or crystals of fatty acid are formed

Counter ions	Oleate	Laurate[a]	Octanoate
Potassium	7.0	6.7	6.2
Arginine	5.3	6.5	5.5

[a]Crystals

Conclusion

In this paper, we investigated the effects of L-arginine on aggregates of fatty acid/potassium soap in the aqueous media. We have shown that L-arginine decreases pH (Oil), as shown in Table 1. This indicates that L-arginine effectively inhibits the growth of fatty acid oil droplets. This inhibitory action is attributed to the adsorption of arginine at the microscopic drop surface, or at the oil/water microinterface through the hydrophobic effect assisted by the hydrogen bonds between the carboxyl group of fatty acid and carboxylate of arginine. As to the crystallization of a fatty acid at temperatures below the chain melting point, however, arginine is not effective. In the acid soap of lauric acid/laurate, the counterions were exclusively arginate. Thus, preferential binding of arginate over potassium cation is concluded. It is also shown that the addition of arginine enhances the micelle growth of anionic oleate micelles. Strong binding of arginine counterions is postulated to be responsible for the micelle growth.

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