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# Selective Foam Separation of Binary Protein Solution by SDS Complexation Method

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**A fundamental study about the selective foam separation of protein mixture was carried out. A solution containing two proteins, ovalbumin (OA) and lysozyme (LZ), and an anionic surfactant, sodium dodecyl sulfate (SDS), was adjusted to pH 6.0, which referred to an intermediate state between the isoelectric points of the proteins. The solution was processed by continuous foam separation. The results showed that a proper addition of SDS greatly improved the selective recovery of LZ to OA. The experimental data were well explained by a simple model that most of cationic protein molecules (LZ) are associated with SDS and the adsorption of all the species including LZ–SDS complexes are subjected to Langmuir adsorption isotherm. The results also showed that one of the Langmuir parameters, which means a kind of lyophillic property of adsorbed material, of LZ–SDS complexes was extremely large as compared with that of primary protein.** 

*Key Words:* **sodium dodecyl sulfate; lysosome; ovalbumin; foam separation.**

## INTRODUCTION

In downstream processing of bioindustries, the separation of a product component from the nutrient medium containing many subproducts and residual substrates is a very important operation. Foam separation has been widely used in various industrial fields such as ore flotation (1, 2) and wastewater treatment (3). Recently, the application of foam separation to bioindustrial field (4) has emerged as an alternative to traditional separation techniques such as ion exchange, chromatography, and precipitation (5). The most important advantages of foam separation technique are the continuous operation and low running cost.

It is well known that proteins are amphoteric electrolyte and their surface charge is varied with pH environment. Protein molecules are positively charged when the solution's pH is below the isoelectric point. On the other hand, ordinary electrolytes as surface-active agents are charged either positively or negatively by their ionic groups. Sodium dodecyl sulfate (SDS), a kind of anionic surfactant, is always negatively charged in usual pH range. Therefore, when SDS is added to some binary protein solution in intermediate pH between their isoelectric points, it is expected that SDS associates with one positively charged protein to generate a protein–SDS complex which is much more hydrophobic than the raw protein. In the present study, continuous foam separation will be applied to the selective separation of ovalbumin (OA, MW = 45,000; iep = 4.6) and lysozyme (LZ, MW = 14,300; iep = 11) and the experimental results will be discussed in terms of the complexing reaction of LZ with SDS and the adsorption parameters of the complex to bubbles.

## MATERIALS

Mixtures of protein and surfactant solutions were used as feed liquid to a bubble column. Two kinds of proteins, ovalbumin (OA) and lysozyme chloride (LZ), were obtained from Kanto Chemical Co. (Japan) and Tokyo Kasei Kogyo Co. (Japan), respectively. Sodium dodecyl sulfate (SDS) of anionic surfactant was obtained from Wako Pure Chemical Industries (Japan). They were used without further purification. The isoelectric points of OA and LZ are pH 4.6 and pH 11.0, respectively. A NaCl solution (0.005 mol $dm^{-3}$) containing the necessary amounts of OA–SDS, LZ–SDS, and OA–LZ–SDS were prepared. The pH of OA–SDS, LZ–SDS, and OA–LZ–SDS solutions were adjusted with either HCl or NaOH solution (0.1 mol $dm^{-3}$) to 3.5, 6.0, and 6.0, respectively. The protein concentration of solution was determined spectrophotometrically at 280 nm in pH > 11.0.

## EXPERIMENTAL METHODS

An experimental setup is shown in Fig. 1. A bubble column of 0.044 m inside diameter and 0.65 m height was used. The column was made of tranparent acrylic resin to facilitate observation of the motion of bubble swarms and foam. A sintered glass filter, which had pores of 10–15 $\mu$m in mean diameter, was installed as a gas distributor at the bottom of the column.

All experiments were conducted as continuous runs with respect to liquid and gas. Nitrogen gas was supplied to the column through the gas distributor at the superficial velocity of 0.000537 m $s^{-1}$. Liquid solution was fed into the column at the flow rate of $5 \times 10^{-4}$ $dm^3$ $s^{-1}$ from a feed tank. The feed position was 30 cm above the gas distributor. After starting experiment, bulk solution was sampled at the drain in the bottom of column

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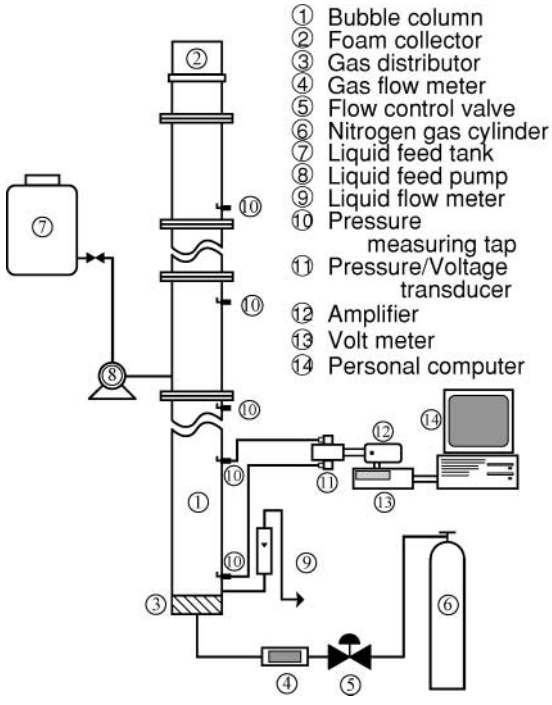


**FIG. 1.** Schematic drawing of experimental setup for continuous foam separation.

and the protein concentration was measured. Gas holdup was determined by the difference in static pressure between the clear and the aerated liquid using a differential pressure transducer. All experiments were carried out at room temperature and under atomospheric pressure.

## RESULTS AND DISCUSSION

### *Adsorption Equilibrium in Continuous Foam Separation*

The mass balances of liquid and objective substance in continuous foam separation at the steady state can be expressed as

$$W_0 = W + W_\mathrm{f}, \qquad [1]$$

$$W_0 C_0 = WC + W_\mathrm{f} C_\mathrm{f}, \qquad [2]$$

where $C$ and $W$ represent the concentration of objective substance and the volumetric flow rate, respectively, and the subscripts 0, f, and blank denote the feed liquid, the foam layer discharged from the upper end of bubble column, and the drained liquid, respectively. Taking into account that the objective substance contained in foam phase arises from the adsorbed one on the bubble–liquid interface and the dissolved-in-bulk liquid entrained with the foam, the following equation holds:

$$W_\mathrm{f} C_\mathrm{f} = S_\mathrm{f} X + W_\mathrm{f} C, \qquad [3]$$

where $S_\mathrm{f}$ and $X$ represent the production rate of foam surface and the surface density of the objective substance on the bubble surface, respectively. Equations [1]–[3] give

$$W_0(C_0 - C) = W_\mathrm{f}(C_\mathrm{f} - C) = S_\mathrm{f} X \qquad [4]$$

or

$$X = \frac{W_0(C_0 - C)}{S_\mathrm{f}}. \qquad [5]$$

Assuming that $S_\mathrm{f}$ is equal to the production rate of bubble surface, $S_\mathrm{b}$, in the column (6–12), the adsorption density (Eq. [5]) can be rewritten as (13)

$$X = \frac{W_0(C_0 - C)}{S_\mathrm{b}}. \qquad [6]$$

On the other hand, the adsorption equilibrium of most substances between the bulk liquid and the gas interface is subjected to the Langmuir adsorption isotherm expressed as

$$X = \frac{\gamma K C}{KC + 1}. \qquad [7]$$

In this equation, $\gamma$ and $K$ represent the saturated surface density and the adsorption equilibrium constant, respectively. Combining Eqs. [6] and [7], we get

$$\frac{S_\mathrm{b}}{W_0(C_0 - C)} = \frac{1}{\gamma K} \cdot \frac{1}{C} + \frac{1}{\gamma}. \qquad [8]$$

Equation [8] refers to the Langmuir plot in the present continuous bubble separation system, and the slope and the intercept of the straight line give the values of $\gamma$ and $K$. Solving Eq. [8] with respect to $C$, we obtain

$$C = \frac{-\{S_\mathrm{b} K_\gamma - W_0(KC_0 - 1)\} + \sqrt{\{S_\mathrm{b} K_\gamma - W_0(KC_0 - 1)\}^2 + 4W_0^2 KC_0}}{2KW_0}. \qquad [9]$$

This equation gives the theoretical value of the protein concentration of bulk liquid in foam separation column, provided that the operating condition ($W_0$ and $C_0$) and the material properties ($K$ and $\gamma$) are given. The production rate of bubble surface, $S_\mathrm{b}$, was determined from the relationship appearing in the previous paper (13, 14):

$$S_\mathrm{b} = 6A\varepsilon(1 - \varepsilon)^{4.65} \left\{ \frac{4}{225} \cdot \frac{(\rho_\mathrm{L} - \rho_\mathrm{g})^2 g^2}{\mu_\mathrm{L} \rho_\mathrm{L}} \right\}^{\frac{1}{3}}, \qquad [10]$$

where, $A$, $\varepsilon$, and $g$ represent the cross-sectional area of the column, the gas holdup, and the gravitational acceleration,

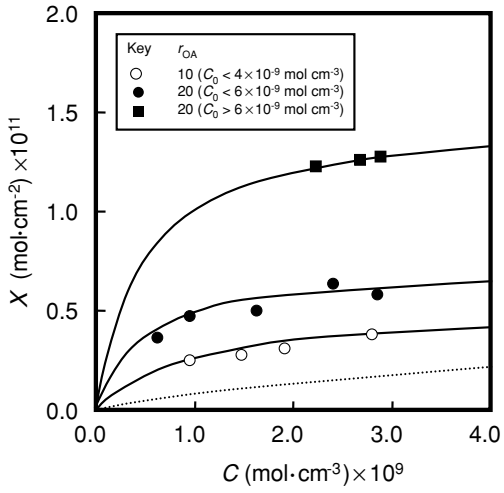


**FIG. 2.** Adsorption isotherms of OA–SDS complex on a bubble surface at pH 3.5. The solid lines represent the theoretical curves calculated from Eq. [7]. The dotted line represents OA adsorption without SDS calculated from Eq. [7] with the adsorption parameters obtained by Shirahama (15).

respectively, and $\rho_L$, $\rho_g$, and $\mu_L$ represent the densities of liquid and gas and the viscosity of liquid, respectively.

*Adsorption of OA and LZ onto Bubble Surface in the Presence of SDS*

Figures 2 and 3 show the adsorption isotherms of OA at pH 3.5 and LZ at pH 6.0 in the presence of SDS, respectively. The mixing ratio, $r$, denotes the mole ratio of SDS to protein in feed liquid. The amount of adsorbed protein was obtained through Eq. [6]. The solid lines represent the theoretical curves calculated from Eq. [7] using the adsorption parameters, $K$ and $\gamma$,

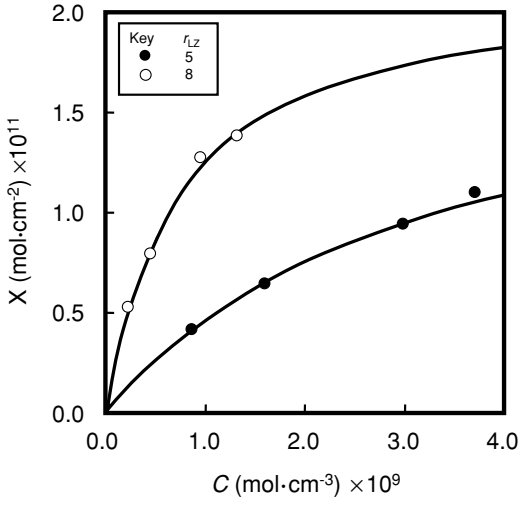


**FIG. 3.** Adsorption isotherms of a LZ–SDS complex on a bubble surface at pH 6.0. The solid lines represent the theoretical curves calculated from Eq. [7] with the adsorption parameters listed in Table 3.

**TABLE 1**
**Variation of Adsorption Parameters for OA with Mixing Mole Ratio in OA–SDS System at pH 3.5**

| $r_{OA}$ | $K$ (cm$^3$ mol$^{-1}$) × $10^{-9}$ | $\gamma$ (mol cm$^{-2}$) × $10^{11}$ |
|---|---|---|
| 10 | 0.95 | 0.53 |
| 20 | 2.04[a] | 0.73[a] |
| 20 | 2.08[b] | 1.49[b] |

[a] $C_0 < 4.5 \times 10^{-9}$ mol/cm$^3$.
[b] $C_0 > 4.5 \times 10^{-9}$ mol/cm$^3$.

listed in Table 1. All the experimental data were in good accordance with the Langmuir isotherm. The dotted line in Fig. 2 depicts the adsorption isotherm of OA without SDS at pH 3.5 and it was calculated from Eq. [7] with the adsorption patameters obtained by Shirahama listed in Table 2 (15). It should be noted that an addition of SDS to OA solution greatly improved OA adsorption on bubble surface, as compared with raw OA. On the other hand, LZ solution at pH 6.0 without SDS did not generate any foam layer in all the concentration range. However, as shown in Fig. 3, an addition of SDS to LZ solution enabled the foam formation and the adsorption of LZ to bubble surface was remarkably improved. These results strongly suggest that the protein and SDS combined to form more hydrophobic complex than primary raw protein. As SDS is an anionic surfactant and OA (iep = 4.6) and LZ (iep = 11) are cationic under the present experimental condition (pH 6.0), the formation of a protein–SDS complex is understandable.

The replotted result for the data in Fig. 2 is shown on Fig. 4. The OA concentration in bulk liquid, $C$, simply increased with the increase of OA concentration in feed liquid, $C_0$, for the mixing ratio of $r_{OA} = 10$, but in high concentration region of feed liquid the decreace of OA concentration in bulk liquid was observed for $r_{OA} = 20$. This fact apparently demonstrates that different kinds of adsorptions took place in low and high concentration regions. A Langmuir plot of Eq. [7] for an OA–SDS solution at pH 3.5 is shown in Fig. 5. The straight lines were obtained at $r_{OA} = 10$, and also for both the low-concentration region in the feed liquid and the high-concentration region at $r_{OA} = 20$. It is seen that the OA–SDS complex is in good agreement with the Langmuir type adsorption isotherm expressed by Eq. [8]. Table 1 shows the adsorption parameters determined from the intercept and the slope of each line in Fig. 5.

**TABLE 2**
**Variation of Adsorption Parameters of OA, Obtained by Shirahama (15), with Solution's pH**

| pH | $K$ (cm$^3$ mol$^{-1}$) × $10^{-9}$ | $\gamma$ (mol cm$^{-2}$) × $10^{11}$ |
|---|---|---|
| 3.5 | 0.18 | 0.51 |
| 4.6 | 0.46 | 0.75 |
| 6.0 | 0.39 | 0.43 |

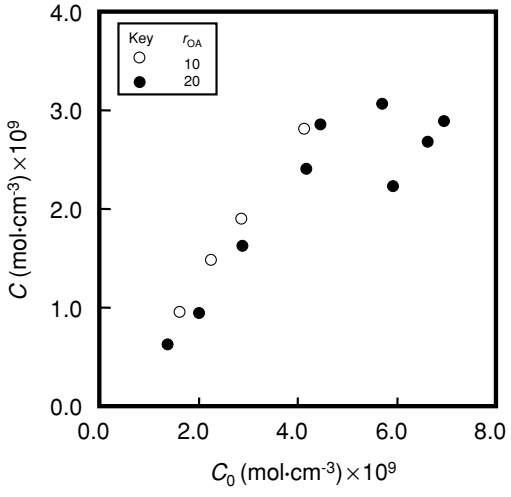


**FIG. 4.** Relationship between OA concentrations in feed liquid and in bulk liquid in column for an OA–SDS system at pH 3.5.

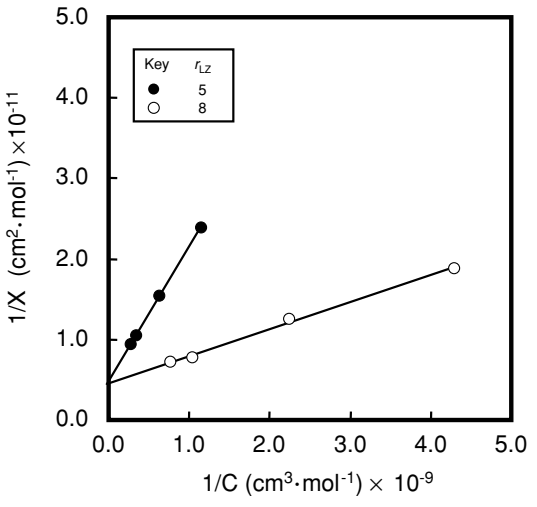


**FIG. 6.** Langmuir plot of the adsorption of a LZ–SDS complex on a bubble surface at pH 6.0. The solid lines denote the fitting by Eq. [8].

Table 2 shows also the adsorption parameters of OA without SDS (15).

The Langmuir plot of Eq. [8] for the LZ–SDS complex at pH 6.0 is shown in Fig. 6. The straight line was obtained at the mixing ratios of both $r_{LZ} = 5$ and 8. Judging from these results, the adsorption of the LZ–SDS complex onto the bubble surface is also Langmuir type. Table 3 shows the adsorption parameters determined from the intercept and the slope of each line in Fig. 6. Tables 1 and 3 reveal that the equilibrium constant, $K$, increases with the increase of the mixing ratio, $r$. Comparing $K$ in the OA–SDS system (Table 1) with OA in a single-component system (Table 2), $K$s of the OA–SDS complexes for $r_{OA} = 10$ and $r_{OA} = 20$ were about 5 and 11 times as large as those of OA at pH 3.5, and about 2 and 4 times as large as in OA at pH 4.6 (iep), respectively. These results mean that protein–SDS complexes are sufficiently hydrophobic. Furthermore, Table 1 shows that $K$ value is not so different between high and low concentration regions, but a significant difference is observed in the saturated surface density, $\gamma$.

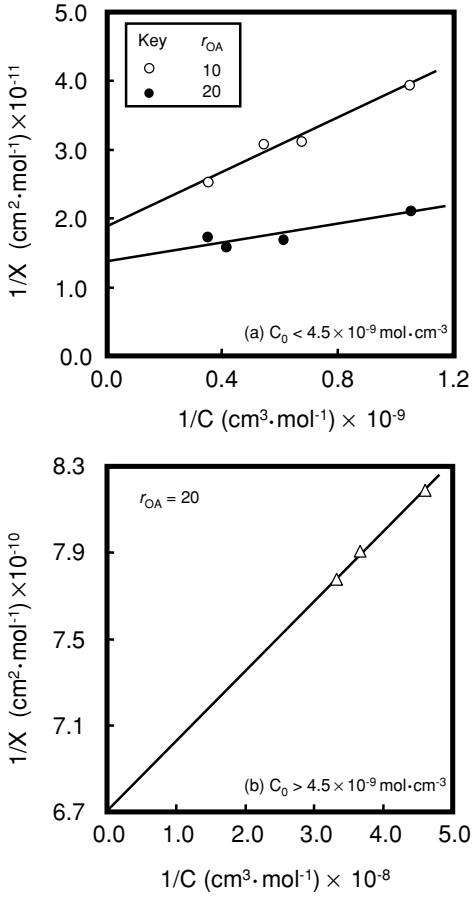


**FIG. 5.** Langmuir plot of the adsorption of an OA–SDS complex on a bubble surface at pH 3.5. The solid lines denotes the fitting by Eq. [8].

Figure 7 shows the influence of OA concentration in feed liquid on turbidity in OA–SDS system at the mixing ratio of $r_{OA} = 20$. The turbidity was measured by absorbance at 600 nm using a spectrophotometer. The turbidity hardly increased in the low concentration region, but increased rapidly in concentration region ranging from $4.5 \times 10^{-9}$ to $6.0 \times 10^{-9}$ mol cm$^{-3}$ (gray-painted region). It should be noted that OA–SDS complexes generated in the low and high concentration regions have the same adsorbability (surface characteristic) to bubbles, but their size is different.

**TABLE 3**
**Variation of Adsorption Parameters for LZ with Mixing Mole Ratio in LZ–SDS System at pH 6.0**

| $r_{LZ}$ | $K$ (cm$^3$ mol$^{-1}$) × 10$^{-9}$ | $\gamma$ (mol cm$^{-2}$) × 10$^{11}$ |
|---|---|---|
| 5 | 0.31 | 1.98 |
| 8 | 1.38 | 2.16 |

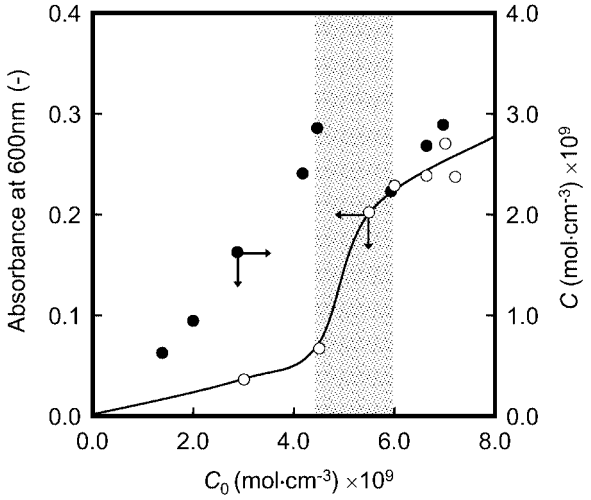


**FIG. 7.** Influence of OA concentration on the turbidity of an OA–SDS system at the condition of mixing ratio of 20 : 1 ($r_{OA} = 20$) and pH 3.5. The turbidity was measured by absorbance at 600 nm. OA concentration in bulk liquid in column is plotted together.

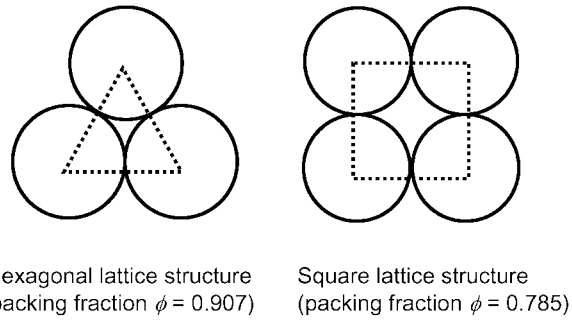


**FIG. 8.** Schematic drawing of typical packing structure of sphere molecules adsorbed on bubble surface.

*Saturated Density of Adsorbed Protein on Bubble Surface*

As shown in Tables 1 and 3, $\gamma$ value increased with increasing SDS : protein mixing ratio, and this suggests that protein molecule–SDS complexes can be packed more closely on a bubble surface. Assuming that protein molecules and the complexes with SDS are spherical in shape and have same size, the packing diameter of adsorbed protein on a bubble surface can be expressed as

$$d = 2\sqrt{\frac{\phi}{\pi N_A \gamma}}, \qquad [11]$$

where $d$ and $\phi$ represent the packing diameter and the packing fraction of adsorbed molecules, respectively, and $N_A$ denotes Avogadoro's constant.

The diameters of adsorbed molecules calculated from Eq. [11] are listed in Table 4. At this calculation two typical packing structures (hexagonal and simple lattice structures) were assumed as shown in Fig. 8. The reported values of molecular diameter for OA and LZ are 5.0 nm (16) and 3.4 nm (17), respectively, and they were in good accordance with the values obtained in this work (Table 4). This fact demonstrates that Eq. [8] is valid for the present system and the protein size is hardly influenced by complexation with SDS.

**TABLE 4**
**Sphere Equivalent Packing Diameters, Calculated from Eq. [11], of OA–SDS and LZ–SDS Complexes Adsorbed on Bubble Surface**

| | | $d$ [nm] | |
|---|---|---|---|
| | $r$ | $\phi = 0.907^a$ | $\phi = 0.785^b$ |
| OA | 10 | 6.02 | 5.60 |
| | 20 | 5.14 | 4.78 |
| LZ | 5 | 3.12 | 2.90 |
| | 8 | 2.98 | 2.78 |

[a] Hexagonal lattice structure.
[b] Square lattice structure.

*Selective Foam Separation*

Figure 9 shows the effect of SDS addition on the removal of LZ at pH 6.0. In the case of higher mixing ratios ($r_{LZ} = 10$ and 15), LZ was almost completely removed by foam separation. $C$ and $C_0$ are simply correlated by straight lines, and the slope of the line denotes the residual fraction of LZ in bulk liquid to the feed. The slopes for $r_{LZ} = 5$ and 8 refer nearly to 0.5 and 0.2, which correspond to $1 - r_{LZ}/10$. In other words, a LZ molecule should be associated with 10 SDS molecules to form a LZ–SDS complex that has strong hydrophobicity and is almost

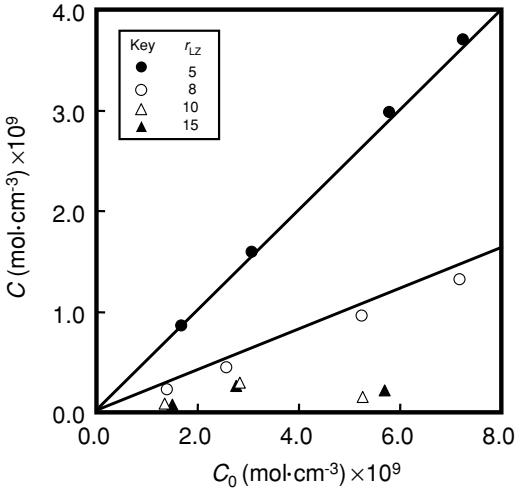


**FIG. 9.** Influence of LZ concentration in feed liquid on residual concentration of LZ in bulk liquid for various mixing ratios of a LZ–SDS system at pH 6.0.

completely removed by foam separation. The solid lines in the figure were drawn to have the slopes of 0.5 and 0.2.

Based on the experimental results obtained so far, the selective separation of LZ from the OA–LZ binary mixture was attempted. The feed liquid was adjusted to the ionic strength of 0.005 M NaCl and pH 6.0. Figure 10 shows the relationship between the total protein concentrations in feed and bulk liquid. Open and solid circles refer to the mixture of SDS : OA : LZ = 10 : 1 : 1 ($r_{OA} = 10$; $r_{LZ} = 10$) and 10 : 5 : 1 ($r_{OA} = 2$; $r_{LZ} = 10$) in mole ratio, respectively.

From the experiment shown in Fig. 9 it was suggested that $r_{LZ} = 10$ is the minimum condition to make all the LZ molecules convert to hydrophobic LZ–SDS complexes (1 : 10 in mole ratio) and such complexes were almost completely removed from bulk liquid. Provided that the above consideration is also hold in LZ–OA–SDS system, the bulk liquid should comprise approximately OA only as protein. As OA is anionic at pH 6 and it cannot combine with SDS, it should remain in native state. Then, the residual concentration of OA in bulk liquid, $C$, is subjected to the Langmuir-type adsorption isotherm expressed by Eq. [9] and it can be written as

$$C = \frac{-\{S_b K_{OA}\gamma_{OA} - W_0(K_{OA}C_{0-OA} - 1)\}}{2K_{OA}W_0} + \frac{\sqrt{\{S_b K_{OA}\gamma_{OA} - W_0(K_{OA}C_{0-OA} - 1)\}^2 + 4W_0^2 K_{OA}C_{0-OA}}}{2K_{OA}W_0}, \quad [12]$$

where $K_{OA}$ and $\gamma_{OA}$ represent the adsorption equilibrium constant and the saturated surface density of OA on bubble surface at pH 6, respectively.

The solid lines in Fig. 10 represent the theoretical curves calculated from Eq. [12]. The experimental data were in good accordance with the theoretical. This result strongly demonstrates that the above-mentioned assumptions were confirmed by the experiment.

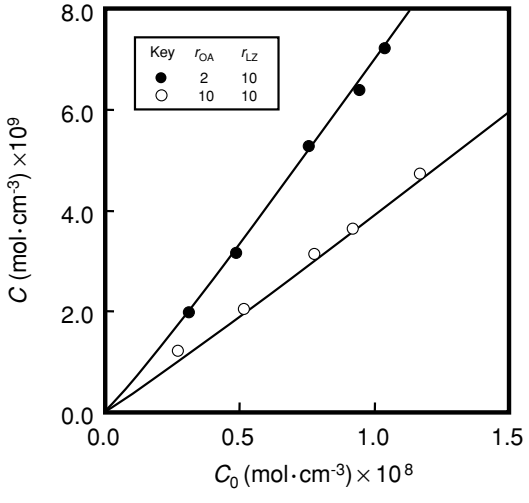


**FIG. 10.** Relationship between total protein concentrations in feed liquid and in bulk liquid for an OA–LZ–SDS system at pH 6.0. The solid lines represent the theoretical curves calculated from Eq. [12].

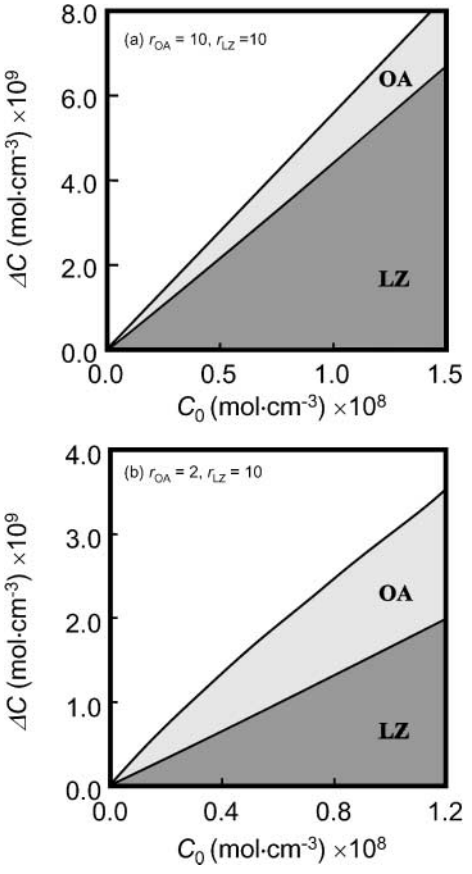


**FIG. 11.** Calculated concentrations of removed OA and LZ in binary protein system at pH 6.0. $\Delta C$ represents the difference of protein concentrations between feed and outlet solutions (bulk liquid in column).

Figure 11 shows the mole composition of proteins contained in discharged foam liquid. Figures 11a and 11b correspond to the open and solid circle data in Fig. 10 and calculated from Eq. [10] and the assumption stated before. In solutions of (a) OA : LZ = 1 : 1 and (b) OA : LZ = 5 : 1, the percentages of LZ in total protein in foam liquid were ca. 85 and 50%.

## CONCLUSIONS

In this work, a new method was proposed for the selective separation of binary protein solution by continuous foam separation technique. The experiments were conducted as to OA–SDS and LZ–SDS solution systems at pH less than the isoelectric point, and the following were concluded: (i) A proper addition of SDS greatly improved the selective recovery of LZ to OA. (ii) The experimental data were well explained by a simple model that most of cationic protein molecules (LZ) are associated with SDS and the adsorption of all the species including LZ–SDS complexes are subjected to Langmuir adsorption isotherm. (iii) One of the

Langmuir parameters, which means a kind of lyophillic property of adsorbed material, of LZ–SDS complexes was extremely large compared with that of primary protein.

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