Selective Foam Separation of Binary Protein Solution

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ABSTRACT

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 Dodecyle 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.
INTRODUCTION

In downstream processing of bioindustries, the separation of a product component from the nutrient medium containing many subproducts and residual substrates is very important operation. Foam separation has been widely used in various industrial fields such as ore flotation [Fuerstenau et al (1972), Hornsby et al (1982)] and wastewater treatment [Jenkins et al (1972)]. Recently, the application of foam separation to bioindustrial field [Saker et al (1987)] has emerged as an alternative to traditional separation techniques such as ion exchange, chromatography and precipitation [Coen et al (1997)]. 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 Dodecyle 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 of protein charged positively to generate 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) and Lysozyme (LZ) 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 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 Dodecyle 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. 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 280nm in pH > 11.0.

EXPERIMENTAL METHODS

An experimental setup is shown in Fig. 1. A bubble column of 0.044 m in inside diameter and 0.65 m in height was used. The column was made of transparent 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 the 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 solutions were fed at the flow rate of 5×10$^{-4}$ dm$^{3}$ s$^{-1}$ from a feed tank to the column. After starting experiment, bulk solution was sampled at the drained mouth in the bottom of column and the protein concentration was measured. Gas holdup was determined by the difference in static pressure between the clear and aerated liquid using a differential pressure transducer. All experiments were carried out at room temperature and
under atmospheric pressure.
RESULTS AND DISCUSSION

Adsorption Equilibrium in Continuous Foam Separation

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

$$W_0 C_0 = WC + W_f C_f$$  \[1\]

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 the bubble column and the drained liquid, respectively. Taking into account that the objective substance contained in foam phase arises from the adsorbed one on bubble-liquid interface and the dissolved in bulk liquid entrained with the foam, the following equation holds.

$$W_f C_f = S_f X + W_f C$$  \[2\]

where $S_f$ and $X$ represent the production rate of foam surface and the surface density of the objective substance on bubble surface, respectively. Eqs. [1] and [2] give

$$W_0 (C_0 - C) = W_f (C_f - C) = S_f X$$  \[3\]

Considering that $S_f$ is equal to the production rate of bubble surface, $S_b$, in the column, the adsorption density, $X$, can be expressed as

$$X = \frac{W_0 (C_0 - C)}{S_b}$$  \[4\]

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

$$X = \frac{\gamma KC}{KC + 1}$$  \[5\]

In this equation, $\gamma$ and $K$ represent the saturated surface density and the adsorption equilibrium constant, respectively. Combining Eqs. [4] and [5], we get
\[
\frac{S_b}{W_0(C_0 - C)} = \frac{1}{\gamma K} \cdot \frac{1}{C} + \frac{1}{C}
\]  

[6]

This equation shows 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 \(K\). The production rate of bubble surface, \(S_b\), was determined from the relationship appearing in the previous paper [Suzuki et al. (1995)].

\[
S_b = 6A \varepsilon (1 - \varepsilon)^{4.65} \left\{ \frac{4}{225} \cdot \frac{(\rho_L - \rho_g)^2 g^2}{\mu_L \rho_L} \right\}^{\frac{1}{3}}
\]  

[7]

where, \(A\), \(\varepsilon\) and \(g\) represent the cross sectional area of the column, the gas hold up and the gravitational acceleration, 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 pH3.5 and LZ at pH6.0 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. [4]. The solid lines represent the theoretical curves calculated from Eq. [5] using the adsorption parameters, \(K\) and \(\gamma\), listed in Tables 1 and 2. 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 pH3.5 [Shirahama (1994)]. It should be noted that an addition of SDS to OA solution greatly improves the OA adsorption on bubble surface, as compared with the raw OA. On the other hand, LZ solution at pH6.0 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 were combined
to form more hydrophobic complex than primary raw protein. As SDS is an anionic surfactant and OA (i.e.p. = 4.6) and LZ (i.e.p. = 11) are cationic in the present experimental condition, the formation of SDS-protein complex is understandable.

The replotted results for the data in Fig. 2 are shown in 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 decrease 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. Langmuir plot of equation 6 for SDS-OA solution at pH3.5 are shown in Fig. 5. The straight lines were obtained at $r_{OA} = 10$, and also both low concentration region in feed liquid and high concentration region at $r_{OA} = 20$. It is seen that SDS-OA complex is in good agreement with the Langmuir type adsorption isotherm expressed by Eq. [6]. Table1 shows the adsorption parameters determined from the intercept and the slope of each line in Fig. [5]. Table 2 shows also the adsorption parameters of OA without SDS [Shirahama (1994)].

Langmuir plot of Eq. [7] for SDS-LZ complex at pH6.0 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 SDS-LZ complex onto 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 SDS-OA system (Table 1) with in OA single component system (Table 2), K of SDS-OA complexes for $r_{OA} = 10$ and $r_{OA} = 20$ were about 5 and 11 times as large as that of OA at pH3.5, and about 2 and 4 times as large as in OA at pH4.6 (i.e.p.), 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, $\gamma$.

Figure 7 shows the influence of OA concentration in feed liquid on turbidity in SDS-OA system at the mixing ratio of $r_{OA} = 20$. The turbidity was measured by absorbance at 600nm using spectrophotometer. The turbidity hardly increased in 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 same adsorbability (surface characteristic) to bubbles, but their size is different.

**Packing State of Adsorbed Protein on Bubble Surface**

As shown in Tables 1 and 3, $\gamma$ value increased with the increase of SDS-protein mixing ratio, and this suggests that protein molecules complexes with more SDS can be packed more closely on 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 bubble surface can be expressed as

$$d = 2 \sqrt{\frac{\phi}{\pi N_A \gamma}} \tag{8}$$

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. [8] are listed in Table 4. At this calculation two typical packing structures (hexagonal and simple lattice structures) were assumed as shown in Fig. 10. The reported values of molecular diameter for OA and LZ are 5.0 nm [Matsumoto et al (1993)] and 3.4 nm [Schnabel et al (1988)], respectively. The molecular diameters obtained in this work (Table 4) agree with the literature values.
Selective Foam Separation

Figure 9 shows the effect of SDS addition on the removal of LZ at pH6.0. In case of higher mixing ratios \( r_{LZ}=10 \) and 15, LZ was almost completely removed by foam separation operation. \( C \) and \( C_0 \) are simply correlated by straight lines, and the slope of line denotes the residual fraction of LZ. The slopes for \( r_{LZ} = 5 \) and 8 refers to 0.5 and 0.2 (0.5 and 0.8 in removal fraction), respectively, and the following terms are derived; a LZ molecule combines to 10 SDS molecules and the LZ-SDS complex is completely removed by foam separation. The solid lines in 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 OA-LZ binary mixture was attempted. The feed liquid was adjusted to the ionic strength of 0.005M NaCl and pH6.0. Figure 10 shows the relationship between the total protein concentrations in feed liquid and in 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 \( (r_{OA} = 5, \ r_{LZ} = 10) \) in mole ratio, respectively.

To analyze the experimental results, the following assumptions were made:

1) SDS associates with LZ only,
2) SDS-LZ complex is completely removed from bulk liquid,
3) the removal of OA is subjected to the Langmuir type adsorption isotherm.

Then, the total protein concentration in bulk liquid can be written as

\[
C = \frac{-\{S_b K_{OA} \gamma_{OA} - W_0 (K_{OA} C_0 - OA - 1)\}}{2K_{OA} W_0} \left[ + \frac{\sqrt{\{S_b K_{OA} \gamma_{OA} - W_0 (K_{OA} C_0 - OA - 1)\}^2 + 4W_0^2 K_{OA} C_0 - OA}}{2K_{OA} W_0} \right] \tag{9}
\]

where \( K_{OA} \) and \( \gamma_{OA} \) represent the adsorption equilibrium constant and the saturated surface density of OA on bubble surface at pH3.5, respectively, and \( C \) represents total protein concentration in bulk liquid.
The solid lines in Fig. 10 represent the theoretical curves calculated from Eq. [9]. The experimental data were in good accordance with the theoretical. Figure 11 shows the mole composition of proteins contained in discharged foam liquid. Figure 11 (a) and (b) correspond to the open and solid circle data in Fig. 10 and calculated from Eq. [9] 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 SDS-OA and SDS-LZ solution systems at pH < the isoelectric point, and the followings were concluded: 1) A proper addition of SDS greatly improved the selective recovery of LZ to OA. 2) 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. 3) 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.
Nomenclature

\[ A = \text{cross sectional area of bubble column, cm}^2 \]
\[ C_f = \text{protein concentration in drained foam, mol cm}^{-3} \]
\[ C_0 = \text{protein concentration of in feed liquid, mol cm}^{-3} \]
\[ C = \text{protein concentration in bulk liquid in equilibrium, mol cm}^{-3} \]
\[ g = \text{gravitation acceleration, cm}^2 \text{s}^{-1} \]
\[ K = \text{the adsorption equilibrium constant, cm}^3 \text{ mol}^{-1} \]
\[ N_A = \text{Avogadoro's constant} \]
\[ r = \text{the packing radius of adsorbed protein on bubble surface, cm} \]
\[ S_b = \text{the production rate of bubble surface, cm}^2 \text{s}^{-1} \]
\[ S_f = \text{the production rate of foam surface, cm}^2 \text{s}^{-1} \]
\[ V = \text{volume of bulk liquid, cm}^3 \]
\[ W_f = \text{volumetric flow rate of drained foam, cm}^3 \text{s}^{-1} \]
\[ W_0 = \text{volumetric flow rate of feed liquid, cm}^3 \text{s}^{-1} \]
\[ W = \text{volumetric flow rate of bulk liquid, cm}^3 \text{s}^{-1} \]
\[ X = \text{the amount of adsorbed protein on bubble surface, mol cm}^{-2} \]

Greek Symbols

\[ \varepsilon = \text{gas hold up} \]
\[ \phi = \text{packing ratio of adsorbed protein} \]
\[ \gamma = \text{the saturated surface density of protein on bubble surface, mol cm}^{-2} \]
\[ \rho_L = \text{liquid density, g cm}^{-1} \text{s}^{-1} \]
\[ \rho_g = \text{gas density, g cm}^{-3} \]

Subscripts
LZ = Lysozyme

OA = Ovalbumin
**Literature Cited**


**Figure Captions**

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

**Figure 2.** Adsorption isotherms of OA-SDS complex on bubble surface at pH3.5. The solid lines represent the theoretical curves calculated from eq 6. The dotted line represents OA adsorption without SDS calculated from eq 6 and the adsorption parameters obtained by Shirahama.

**Figure 3.** Adsorption isotherms of LZ-SDS complex on bubble surface at pH6.0. The solid lines represent the theoretical curves calculated from eq 6.

**Figure 4.** Relationship between OA concentrations in feed liquid and in bulk liquid in column for SDS-OA system at pH3.5.

**Figure 5.** Langmuir plot of the adsorption of OA-SDS complex on bubble surface at pH3.5. The solid lines denotes the fitting by eq 7.

**Figure 6.** Langmuir plot of the adsorption LSZ-SDS complex on bubble surface at pH6.0. The solid lines denotes the fitting by eq 7.

**Figure 7.** Influence of OA concentration in feed liquid on turbidity of SDS-OA system at the condition of mixing ratio of 20:1 (r_{OA}=20) and pH3.5. The turbidity was measured by absorbance at 600nm. OA concentration in bulk liquid in column is plotted together.
Figure 8. Schematic drawing of typical packing structure of sphere molecules adsorbed on bubble surface.

Figure 9. Influence of LZ concentration in feed liquid on residual concentration of LZ in bulk liquid for various mixing ratios of LZ-SDS system at pH6.0.

Figure 10. Relationship between total protein concentrations in feed liquid and in bulk liquid for SDS-OA-LZ system at pH6.0. The solid lines represent the theoretical curves calculated from eq 11.

Figure 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.
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Figure 5. Langmuir plot of the adsorption of OA-SDS complex on bubble surface at pH 3.5. The solid lines denotes the fitting by eq 7.
Figure 6. Langmuir plot of the adsorption LSZ-SDS complex on bubble surface at pH6.0. The solid lines denotes the fitting by eq 7.
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### Table 1. Variation of adsorption parameters of OA on bubble surface with the mixing mole ratio (SDS/OA) at pH3.5.

<table>
<thead>
<tr>
<th>$r_{OA}$</th>
<th>$K$ (cm$^3$·mol$^{-1}$) $\times 10^{-9}$</th>
<th>$\Gamma$ (mol·cm$^{-2}$) $\times 10^{11}$</th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>0.95</td>
<td>0.53</td>
</tr>
<tr>
<td>20</td>
<td>2.04&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>0.73&lt;sup&gt;a&lt;/sup&gt;)</td>
</tr>
<tr>
<td>20</td>
<td>2.08&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>1.49&lt;sup&gt;b&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

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

### Table 2. Variation of adsorption parameters of OA on bubble surface with the solution’s pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>$K$ (cm$^3$·mol$^{-1}$) $\times 10^{-9}$</th>
<th>$\Gamma$ (mol·cm$^{-2}$) $\times 10^{11}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>0.18</td>
<td>0.51</td>
</tr>
<tr>
<td>4.6</td>
<td>0.46</td>
<td>0.75</td>
</tr>
<tr>
<td>6.0</td>
<td>0.39</td>
<td>0.43</td>
</tr>
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</table>
Table 3. Variation of adsorption parameters for LSZ on bubble surface for SDS-LSZ system with mixing rations at pH6.0.

<table>
<thead>
<tr>
<th>$r_{LZ}$</th>
<th>$K$ (cm$^3$·mol$^{-1}$) $\times 10^{-9}$</th>
<th>$\theta$ (mol·cm$^{-2}$) $\times 10^{11}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.31</td>
<td>1.98</td>
</tr>
<tr>
<td>8</td>
<td>1.38</td>
<td>2.16</td>
</tr>
</tbody>
</table>
Table 4. Sphere equivalent packing radii of OA and LZ adsorbed on bubble surface calculated from eq 10.

<table>
<thead>
<tr>
<th></th>
<th>( d ) [nm]</th>
<th>( \theta = 0.907 )(^{a)})</th>
<th>( \theta = 0.785 )(^{b)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA 10</td>
<td>6.02</td>
<td>5.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.14</td>
<td>4.78</td>
<td></td>
</tr>
<tr>
<td>LZ 5</td>
<td>3.12</td>
<td>2.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.98</td>
<td>2.78</td>
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</tr>
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</table>

\(^{a)}\) hexagonal lattice structure
\(^{b)}\) square lattice structure