Studies on the Foaming Property of the Chicken Egg White
Part VI. Spread Monolayer of the Protein Fraction of the Chicken Egg White

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In order to study the relation between the foaming property and the surface chemical property of the egg white proteins, each protein was spread at the A/W interface and the surface pressure of the film was measured. Simultaneously, the life time of the single bubble formed on the surface was measured. According to this experiment, it is concluded that the proteins which foam well are easily surface-denaturated and give on the surface a well-developed network consisting of the side chains of the peptide linkage.

INTRODUCTION
In the previous work\(^1,2\), the foaming power of each egg white protein was measured by the pneumatic method. The results revealed that the foaming power of the egg white proteins decreased in the following order; ovomucin (B), globulin, conalbumin, ovalbumin, ovomucoid and lysozyme. The difference in the foaming property among these proteins is very interesting, yet the reason has not been known. Among the studies concerning the protein foaming, the Cumper’s study\(^3\) deals with the difference of the foaming property between insulin and pepsin by the monolayer technique and the mechanism of a protein foaming phenomenon from the results.

In this report, in order to study the foaming property of the egg white proteins more fundamentally, both the area for life time of a single bubble and the force-area curve were prepared. The results of this study were compared with the result about the other proteins of which the foaming property is already known.

MATERIALS AND METHODS
Materials. Among the egg white proteins, ovalbumin, ovomucoid, lysozyme, conalbumin and globulin were prepared from the fresh chicken egg white by the method\(^1\) described previously. Ovomucin used here was ovomucin (B) prepared by the water-precipitated method\(^4\) described previously. In this case, the protein was dissolved in dil. NaOH without drying and pH of the solution was adjusted to 10.0. To determine the protein concentration of the solution, the total N content of an aliquot was determined by semi-micro Kjeldahl method, and multiplied by 7.75*.

Insulin and pepsin were used as the examples of the proteins of which the foaming property is already reported. Insulin used here was the Japanese pharmacological standard, and pepsin was the commercial preparation (1:10,000, Katayama Chemical Co., Ltd.)

\(^2\) R. Nakamura and Y. Sato, ibid., in press.
* The total N content of ovomucin (B) is 12.9%.*
recrystallized from the ethanol-water by the method of Northrop.\textsuperscript{5}

The Measurement of the Surface Pressure and Life Time of the Single Bubble. The measurement of the surface pressure was carried out with the aid of the Wilhelmy type of balance. A platinum plate (20 mm \times 15 mm \times 0.25 mm) was used as a hanging plate, and a methacryl resin trough coated with paraffin was used. All proteins except ovomucin (B) were dissolved in 50\% n-propanol containing 0.5 m \text{CH}_3\text{COONa} and the protein concentration was adjusted between 0.03\%–0.05\% w/v. The solution was spread on 0.01 n HCl by the method of Turnit,\textsuperscript{6} dropping on a glass rod (5 cm long, 5 mm wide) standing in the aqueous phase. In this case ovomucin (B) was directly spread from the alkali solution by the above method.

As the protein monolayer was compressed slowly with a movable barrier, the surface pressure was measured. Simultaneously, the life time of a single bubble formed by blowing through a glass nozzle (inside diameter 0.2 cm) inserted under the barrier was measured of each surface area. The measurement for each surface area was carried out from 5 to 10 times and all the values were averaged. All measurements were carried out at the room temperatures; namely, the temperature was not controlled especially. The temperature deviation, however, was within \pm 0.5\degree C during a single experiment.

RESULTS

All F-A isotherms (force-area isotherms) shown here represent the average of at least two runs. The life time of a single bubble was plotted at the same figure as the F-A isotherm. The results obtained for each protein were as follows: Fig. 1 for ovalbumin, Fig. 2 for globulin, Fig. 3 for ovomucoid, Fig. 4 for conalbumin, Fig. 5 for lysozyme, Fig. 6 for ovomucin (B), Fig. 7 for pepsin and Fig. 8 for insulin.

\textbf{FIG. 2.} Globulin.

\textbf{FIG. 3.} Ovomucoid.

\textbf{FIG. 4.} Conalbumin.
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In these F-A isotherms, the unusually long straight portion of the curve allowed an extrapolation to be made to zero pressure. This gave an "area of close-packing", and the values for all the proteins were shown in Table I. But, as for the value of lysozyme in this table, the isotherm was so expanded that this drawing could not be made. So, the compressibility coefficient (δ=−dA/AdF) was calculated and the area which gave the minimum value was adopted as the "area of close-packing".

**DISCUSSIONS**

1. **F-A Isotherm.** As for all proteins used here except lysozyme, ovomucoid and pepsin, the shape of their F-A isotherm had the same inclination, and the "area of close-packing" was within the range of 0.95~1.02 m²/mg protein (Table I). The value coincides with that of many well-spreading proteins. On the other hand, in the cases of lysozyme, ovomucoid and pepsin, the F-A isotherm was very expanded and the "area of close-packing" was very small compared with that of the other proteins used here. These results show that these three proteins are resistant to the surface denaturation though all the proteins except them are easily surface-denaturated.
Why these three proteins are resistant to the surface denaturation cannot be explained fully, although this depends upon the difference of the intramolecular structure; the complete study on the structure of these three proteins has not been carried out yet. So, the study on lysozyme by Hamaguchi\textsuperscript{7}) is very interesting. As lysozyme was not considered to spread easily on the ordinary aqueous phase, he spread it on the dil. alkali solution of pH 10.5. In this case, the "area of close-packing" was small, but increased to the S-S bond, which is resistant to the surface denaturation. And thus the protein molecule is not considered to unfold fully.

2. The Life Time of the Single Bubble. In regard to the results of the life time measurement, a common feature for all proteins was to form a stable foam in a very wide area of which the surface pressure could not be measured with the surface balance used here (Figs. 1~8). The values of the maximum area for forming a stable foam for all proteins were shown in Table II.

\begin{table}[h]
\centering
\caption{The Maximum Area for Forming a Stable Foam (m²/mg)}
\begin{tabular}{cccc}
Ovalbumin & Globulin & Ovomucoid & Conalbumin \\
2.51 & 3.34 & 2.49 & 3.58 \\
Lysozyme & Ovomucin (B) & Insulin & Pepsin \\
1.29 & 3.31 & 3.62 & 1.28 \\
\end{tabular}
\end{table}

Moreover, the life time of a single bubble was constant in a comparatively wide area and began to decrease as the monolayer was saturated. As for all proteins, there was a difference among the values of the life time which became constant, but this difference seems to be very little, considering the great difference of the foaming power of the proteins reported previously\textsuperscript{1,2}). So, the life time of the single bubble is not considered to correlate directly to the foaming property of the proteins.

Cumper\textsuperscript{3}) also studied the life time of a single bubble formed over the monolayer of the two proteins, pepsin and insulin. His results, contrary to that of author, showed that life time of a single bubble increased infinitesimally as the monolayer was saturated. As the exact method was not described in Cumper's report, complete comparison could not be made; yet, there are also many reports\textsuperscript{10}) indicating that the life time of the single bubble is maximum when the monolayer is not saturated fully. Moreover, the results of the experiments concerning fatty

\textsuperscript{7}) K. Hamaguchi, \textit{J. Biochem.}, 42, 449, 705 (1955); \textit{ibid.}, 43, 83, 335 (1956).
\textsuperscript{8}) J.C. Lewis et al., \textit{J. Biol. Chem.}, 186, 23 (1950).
acids\textsuperscript{11}) are almost the same as that of author. Considering these experiments described above, the author’s result seems to be a correct one.

3. The Property of the Monlayer and the Foaming Property of the Egg White Proteins. The foaming power of egg white proteins measured in the previous report was shown in Table III.

\textbf{TABLE III. FOAMING POWER OF EGG WHITE PROTEINS}

<table>
<thead>
<tr>
<th>Ovalbumin</th>
<th>Globulin</th>
<th>Ovomucoid</th>
<th>Conalbumin</th>
<th>Lysozyme</th>
<th>Ovomucin (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>19.8</td>
<td>9.0</td>
<td>15.0</td>
<td>1.5</td>
<td>19.6</td>
</tr>
</tbody>
</table>

\* These values were measured by the pneumatic method for the protein concentration 0.2%.

\*\* Measurements for all proteins except ovomucin (B) and lysozyme were carried at the pH near their isoelectric points. The pH of ovomucin (B) was adjusted to 10.0 and the pH of lysozyme was adjusted to 4.8.

First, ovomucoid and lysozyme which have small values in Table III have small values in Table I. On the other hand, globulin, conalbumin and ovomucin (B), which have large values in Table III, have large values in Table I. The values of Table I show the degree of the surface denaturation of the proteins. So, there seems to be correlation between the surface denaturation and the foaming power of the proteins. This thought is supported by the following fact; pepsin whose foaming power is considered to be small shows a small value in Table I, and insulin whose foaming power is considered to be large is also gives a large value in Table I. The value for ovalbumin is large in Table I, although the value for it is small in Table III; the case of ovalbumin will be discussed later.

Next, there appears to be the same correlation described above among the values of Table III and Table II; namely, the proteins whose values are large in Table II have also large values in Table III, and the proteins whose values are small in Table II have also small values in Table III. In the case of ovalbumin, this correlation is also recognized. Isemura\textsuperscript{11}) studied about the monolayer of fatty acid by the same method as was carried in this report. In the case of fatty acid, he found that a foam was formed at the greater area than the close-packing area, and he expressed that the molecules of fatty acid did not cover the surface of liquid phase fully and composed a network one another, and this supposed network will contribute to the foam formation. Also, Tachibana and Inokuchi\textsuperscript{12}) studied about the monolayer of ovalbumin and found that the surface viscosity was measured at the greater area than the close-packing area. They considered this phenomenon was caused by the same network as was suggested by Isemura. By these experiments, it seems to be certain that there is a network of the denaturated protein molecule for the monolayer of every protein studied here. Ovalbumin whose value is small in Table II seems not to form such a network well. It may be suggested that, the more easily the network of denaturated is formed, the larger the foaming power of the proteins is.

The development of the network is considered to depend chiefly upon the primary structure of the protein. The structure of all the proteins used here, however, is not known enough, so that the case of the organic substance which has the comparatively lower molecular weight was considered. In this case, the one which foams well has inside its own molecule polar and non-polar groups, which are in good proportion\textsuperscript{13}). The results obtained by calculating the non-polar/polar side chain ratio for the protein of which the amino acid composition is already known are shown in Table IV.

Table IV, shows that the ratio for ovomuc-
TABLE IV. THE NON-POLAR/POLAR SIDE CHAIN RATIO FOR THE PROTEINS

<table>
<thead>
<tr>
<th>Names of proteins</th>
<th>Non-polar/polar side chain ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>1.35</td>
</tr>
<tr>
<td>Pepsin</td>
<td>0.75</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>0.84</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0.74</td>
</tr>
<tr>
<td>Ovomucoid</td>
<td>0.67</td>
</tr>
<tr>
<td>Conalbumin</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Calculations were made for the ratio of the number of amino acids which have a non-polar side chain to the number of amino acids which have a polar side chain. All these proteins seem to show that there is a close relation between the foaming power and the ratio of the non-polar/polar side chain. But, in Table IV, conalbumin is the only protein that does not coincide with the above relation. The cause is considered to depend upon the nature of the sample used here; it was not crystallized, though purified by using the carboxymethyl cellulose. Whether this difference depends upon the small impurity or the own property of conalbumin, however, is uncertain; further study is necessary.

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