Studies on the Dissociation of the Soluble Ovomucin by Sonication

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The soluble ovomucin obtained from the liquid part of thick white by gel filtration on a Sepharose 4B was an aggregated and polymerized molecule (intrinsic viscosity was 365 mg/ml and molecular weight was \(8.3 \times 10^6\)) and it was unable to dissociate the soluble ovomucin into two components without modifications.

Molecular weight and reduced viscosity of the soluble ovomucin decreased markedly with time of sonication. By the sonication for 10 min, it was successful to fractionate it into carbohydrate rich and poor component by density gradient electrophoresis, cellulose acetate electrophoresis and DEAE-cellulose column chromatography.

Concerning carbohydrate and amino acid compositions of two components obtained from the sonicated soluble ovomucin, it was found that the carbohydrate poor component corresponded to the reduced S-component or the reduced \(\alpha\)-ovomucin, and the carbohydrate rich component to the reduced F-component or the reduced \(\beta\)-ovomucin.

It was considered that the sonicated soluble ovomucin was an intermediate of the aggregated, polymerized ovomucin (the soluble ovomucin) and the monomeric ovomucin (the sonicated and reduced soluble ovomucin).

Ovomucin, a high molecular glycoprotein, is present in both thick egg white and thin egg white and is responsible for the viscous property of egg white. In the recent work, it has been shown that ovomucin was composed of carbohydrate rich and poor component,\(^1,2\) and that the carbohydrate rich component was gradually dissociated and solubilized into the liquid part of thick white during the natural thinning.\(^3,4\) Furthermore, it was suggested that alkaline hydrolysis of disulfide bond of ovomucin was responsible for the thinning.\(^6\)

In the previous paper, we suggested that an increase of pH during storage, an decrease of free inorganic ions due to the ion-binding activity of proteins in egg white, and the rise of storing temperature may be a main cause of solubilization of the carbohydrate rich component from insoluble ovomucin.\(^7\)

A number of workers\(^8\) have proposed that interactions between ovomucin and lysozyme molecules are responsible for the gel property of thick white. Kato et al.\(^5\) suggested that either the carbohydrate rich or poor component is linked by disulfide bond and that swollen rigid gelatinous structure of thick white gel might partly come from ovomucin-lysozyme interaction. On the other hand, Tomimatsu and Donovan\(^13\) showed that the egg white thinning was due to the disaggregation of ovomucin, on the basis that a part of an ovomucin chain was composed of several units with mol. wt. of \(2.2 \times 10^5\), which are cross-linked by disulfide bonds. They suggested that aggregated ovomucin (MW \(4.0 \times 10^7\)) might be a bio-polymer associating at some specific portions of the ovomucin chains.

Sonication has widely been used to dissociate aggregated protein molecules.\(^14\) Therefore, sonication was used in this experiment in order to fractionate derived ovomucin into polymers containing only the carbohydrate rich or poor component and to clarify the aggregated and polymerized structure of ovomucin dissociating its interactions with inorganic ions or lysozyme.

**MATERIALS AND METHODS**

**Preparation of the soluble ovomucin.** Soluble ovomucin was prepared by the modification of the methods...
of Young and Gardner\textsuperscript{27} and Kato \textit{et al.}\textsuperscript{28} Egg white was obtained from White Leghorn hens within 6 hr after laying. Thick white was separated from thin white by using a sieve and homogenized in a mechanical blender, at a low speed to avoid foaming. The homogenized thick white was separated into insoluble and liquid parts by ultracentrifugation for 1 hr at 59,000 \times g. The liquid part was diluted with a three-fold volume of water and adjusted to pH 6 with 1 N HCl. The resulting precipitate was collected by decantation (without centrifugation) after standing overnight. The precipitate was washed with water twice and then dissolved in 0.1 M Menzel buffer (pH 9) containing 0.4 M NaCl. About 30 ml of the crude ovomucin solution was applied to a Sepharose 4B column. Fractions eluted at the void volume were pooled. Ovomucin obtained by the above procedure was designated as the soluble ovomucin.

\textbf{Sonication of the soluble ovomucin.} The soluble ovomucin was dialyzed against Menzel buffer (pH 9.5, \textit{i}=0.01) for 24 hr and subsequently sonicated for 10 min at 2°C (100 W and 9 kHz).

\textbf{Reduction of the sonicated soluble ovomucin.} 0.5 M Tris-HCl buffer, pH 8.2, containing 0.1 M dithiothreitol and 6 M guanidine hydrochloride was used to reduce disulfide bonds of the sonicated soluble ovomucin at 20°C for 16 hr. The thiol groups were alkylated by the addition of 1 M Tris solution containing 4 M guanidine hydrochloride and 0.24 M iodoacetic acid for 1 hr, according to the Small and Lamm’s procedure.\textsuperscript{10}

\textbf{Gel filtration.} Gel filtration was performed on a Sepharose 4B column (2.5 \times 40 cm) equipped with a reverse flow adaptor. The elution was made with 0.1 M Menzel buffer, pH 9, containing 0.4 M NaCl. Each protein fraction was monitored by the extinction at 280 nm and by the lysozyme activity.

\textbf{Lysozyme assay.} Lysozyme activity in each of the protein fraction was determined by the method of Gàumann and Böhn\textsuperscript{20}. The standard lysozyme was prepared from the hen egg white by the method of Alderton and Fervold.\textsuperscript{21} The substrate of \textit{Micrococcus lysisodeiktyicus} cells was purchased from Worthington Biochemical Corporation, Freehold, New Jersey.

\textbf{Light scattering.} Light scattering measurements for estimation of molecular weight of the soluble, the sonicated soluble and the sonicated and reduced soluble ovomucin in Menzel buffer (pH 9.5, \textit{i}=0.01) were made with a photoelectric light scattering photometer (Shimadzu Co. Ltd., Type PG-21) at a wave length of 4360 Å, and the angular distribution of the scattered light was measured between 30° and 135°. Millipore filters were used to clarify the solutions. The experiment was performed only at a low concentration of ovomucin (0.2 mg/ml), according to the suggestion of Kamata and Nakahara.\textsuperscript{23} A value of refractive increment, \(dn/dc=0.182\), was used according to Tomimatsu and Donovan.\textsuperscript{13} Ovomucin concentration was calculated basing on the nitrogen factor of 8.77.

\textbf{Viscosity measurement.} Reduced viscosities (\(\eta_{	ext{red}}\)) of solution were calculated: \(\eta_{	ext{red}}=\eta_{	ext{solut}}/\eta_{	ext{solvent}}-1\) and \(c\) is ovomucin concentration. Intrinsic viscosity was determined by measuring \(\eta_{	ext{red}}\) at various concentrations and extrapolating to \(c=0\). Viscosities of ovomucin solutions were measured with an Ostwald viscometer, which had a flow time for water of about 100 sec at 25°C. The temperature of the viscosity bath was controlled to vary within ±0.1°C. Ovomucin concentration (0.2~7 mg/ml in Menzel buffer: pH 9.3, \(i=0.1\)) was determined by the Kjeldhal method.

\textbf{Density gradient electrophoresis.} Density gradient electrophoresis was carried out using an LKB 7900 Uniphor column (2.5 \times 50 cm) according to the method of Kato and Sato.\textsuperscript{13} A linear density gradient was formed in a column with decreasing concentration, from bottom (40% (w/w) sucrose) to top in 0.3 M borate buffer, pH 8.2. Sample was added onto the column. All runs were performed for 24 hr at a constant voltage of 300 V at 4°C. After the electrophoresis, the effluent was fractionated into 5 ml portions in test tubes. Each fraction was dialyzed against 0.3 M borate buffer, pH 8.2, to remove sucrose, then filled up to 10 ml with water and its hexose content was determined by phenolsulfuric acid method\textsuperscript{23} reading the extinction at 490 nm.

\textbf{Cellulose acetate electrophoresis.} Cellulose acetate electrophoresis was carried out on a cellulose acetate strip (Separax, 6 cm length, purchased from Joko Sangyo Co. Ltd., Tokyo) at a constant voltage of 50 V for 2 hr. The Buffer solution contained 0.3 M boric acid and 0.075 M NaOH at pH 8.2. The strip was stained either with 0.5% Alcian Blue 8GS in 3% acetic acid (pH 3) or with 1% Ponceau 3R in 6% trichloroacetic acid.

\textbf{DEAE-cellulose column chromatography.} The sonicated soluble ovomucin (100 mg) was applied to a DEAE-cellulose column (2 \times 40 cm) previously equilibrated with 0.01 M Tris-HCl buffer, pH 8.0. Elution was carried out with 1000 ml of a 0~0.5 M linear gradient of NaCl in 0.01 M Tris-HCl buffer, pH 8.0, and the extinction at 490 nm was determined.

\textbf{Carbohydrate analysis.} Total hexose content was determined by the orcinol method in which an equimolar mixture of galactose and mannose was used as the standard.\textsuperscript{24} Galactose and mannose were determined by gas-liquid chromatography.\textsuperscript{25} Samples (5~20 mg) were hydrolysed with 1 N H\textsubscript{2}SO\textsubscript{4} (2 ml) at
Dissociation of Ovomucin by Sonication

100°C in sealed ampules for 3 hr. After removal of SO$_4^{2-}$ by passage of the hydrolysate through a Dowex 1 (acetate form) column, the hydrolysate was lyophilized, and then reacted for 15 min at 80°C with pyridine-hexamethyl disilazane-trimethylsilyl chloride (5:2:1). Sample (2 µl) was injected onto a glass column (2 m) packed with 1.5% Silicone OV-1 on Shimalite W (201p) 80/100. The column was maintained at 170°C until all of the hexose peaks had emerged.

Total hexosamine content was determined by Elson-Morgan's method, as modified by Neuhaus and Letzring, in which glucosamine was used as a standard. Galactosamine and glucosamine were determined by using a JLC-5AH automatic amino acid analyser (Japan Electron Optics Laboratory). Samples (4 mg) were hydrolysed with 3 N HCl (1 ml) at 100°C for 4 hr in sealed ampules. The hydrolysates were evaporated to dryness and then dissolved in 0.01 N HCl.

Sialic acid content was determined by the thio-barbituric acid method after hydrolysis of samples (4~10 mg) with 0.1 N H$_2$SO$_4$ (2 ml) at 80°C for 1 hr in sealed ampules.

Ester sulfate was determined after hydrolysis with 6 N HCl at 100°C for 9 hr in sealed ampules by the method of Dodgson and Spencer.

**Amino acid analysis.** Dry samples (4 mg) were hydrolysed at 110°C for 24 hr with 6 N HCl (2 ml) in sealed evacuated ampules. The hydrolysates were dried and dissolved in 0.01 N HCl. Amino acid analyses were carried out with a JLC-5AH automatic amino acid analyser. Tryptophan was determined in unhydrolysed samples by the method of Goodwin and Morton. Cysteine was determined by the method of Ellman.

**RESULTS**

As the soluble ovomucin was eluted at the void volume of Sepharose 4B column, it was successful to isolate the soluble ovomucin free from lysozyme (Fig. 1).

Figure 2 shows influences of the sonication on molecular weight (8.3 × 10$^6$) and reduced viscosity (580 ml/g) of the soluble ovomucin. Both decreased markedly with time of sonication: namely, they decreased to about a half at 10 sec and about one sixth at 60 sec. When the soluble ovomucin was sonicated for longer than 60 sec, no further remarkable decrease was shown in molecular weight and reduced viscosity.

After 10 min of the sonication, low molecular weight materials (MW < 10,000) which may be degradation products of the soluble ovomucin were found. They contained only a little nitrogen, hexose and sialic acid accounting for less than 1% of total nitrogen and hexose, and 4% of total sialic acid of the soluble ovomucin.

Figure 3 shows the concentration dependence of the reduced viscosity of ovomucins. Although the concentration dependence of the soluble ovomucin was large, such a dependence was not found with the sonicated soluble ovomucin and the sonicated and reduced solu-
FIG. 3. Concentration Dependence of Reduced Viscosity of Ovomucins.

-○-, the soluble ovomucin; ●●-, sonicated soluble ovomucin; △-△, sonicated & reduced soluble ovomucin.

A reduced viscosity of the soluble ovomucin was very high compared with that of the sonicated soluble ovomucin.

Elution patterns in density gradient electrophoresis of the soluble ovomucin and the sonicated soluble ovomucin are shown in Fig. 4. The soluble ovomucin was detected as one broad asymmetrical peak, whereas the sonicated soluble ovomucin showed two symmetrical peaks.

Figure 5 shows cellulose acetate electrophoretic patterns of the soluble ovomucin and the sonicated soluble ovomucin. The electrophoretic pattern of the soluble ovomucin stained either by Ponceau or by Alcian Blue showed a broad band near the origin. On the other hand, that of the sonicated soluble ovomucin showed two bands; one was a fast moving component which was not stained with Ponceau but was stained with Alcian Blue, while the other was a slow moving component which was not stained with Alcian Blue but was stained with Ponceau.

DEAE-cellulose column chromatogram of the sonicated soluble ovomucin is shown in Fig. 6. It was shown that the sonicated soluble
ovomucin consisted of two components, peak I and peak II. Peak I was eluted by NaCl of around 0.15 M and coincided with the slow moving component on the cellulose acetate electrophoresis. Peak II was eluted by NaCl of around 0.3 M and coincided with the fast moving component on the cellulose acetate electrophoresis. As determined by triangulation of the peaks revised by the hexose content of peak I (6.3%) and peak II (19.8%), peak I accounted for approx. 87% of the sonicated soluble ovomucin and peak II accounted for approx. 13%.

Carbohydrate composition and amino acid composition of peak I and peak II components are given in Tables I and II. Results of Kato and Sato (reduced S, F-component) and Robinson and Monsey (reduced α, β-ovomucin) are quoted in order to be compared with the present results. Carbohydrate content of

### Table I. Carbohydrate Composition of Peak I and Peak II

<table>
<thead>
<tr>
<th>Component</th>
<th>Peak I</th>
<th>S-(^a)</th>
<th>α-(^b)</th>
<th>Peak II</th>
<th>F-(^c)</th>
<th>β-(^d)</th>
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<tr>
<td>Hexose</td>
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<td>6.4</td>
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<td>0.4</td>
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<td>N-Acetylated-hexosamine</td>
<td>7.5</td>
<td>8.2</td>
<td>7.3</td>
<td>19.1</td>
<td>22.5</td>
<td>19.7</td>
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<tr>
<td>N-Acetylated-galactosamine</td>
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<td>0</td>
<td>0.6</td>
<td>8.0</td>
<td>9.7</td>
<td>8.7</td>
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<tr>
<td>N-Acetylated-glucosamine</td>
<td>7.5</td>
<td>8.2</td>
<td>6.7</td>
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<td>Sialic acid</td>
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<td>0.8</td>
<td>1.0</td>
<td>12.6</td>
<td>11.4</td>
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<td>Sulfate</td>
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<td>0.06</td>
<td>0.7</td>
<td>3.1</td>
<td>1.2</td>
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\(^a\) Reduced S-component; Kato and Sato (1971).  
\(^b\) Reduced α-ovomucin; Robinson and Monsey (1971).  
\(^c\) Reduced F-component; Kato and Sato (1971).  
\(^d\) Reduced β-ovomucin; Robinson and Monsey (1971).

### Table II. Amino Acid Composition of Peak I and Peak II

<table>
<thead>
<tr>
<th></th>
<th>Peak I</th>
<th>S-(^a)</th>
<th>α-(^b)</th>
<th>Peak II</th>
<th>F-(^c)</th>
<th>β-(^d)</th>
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<tr>
<td>Lysine</td>
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<td>6.6</td>
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<td>5.8</td>
<td>5.1</td>
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<tr>
<td>Histidine</td>
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<td>1.6</td>
<td>2.1</td>
<td>1.9</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.7</td>
<td>2.2</td>
<td>2.7</td>
<td>2.4</td>
<td>2.4</td>
<td>2.6</td>
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<tr>
<td>Aspartic acid</td>
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<td>13.9</td>
<td>9.9</td>
<td>4.8</td>
<td>8.7</td>
<td>7.2</td>
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<tr>
<td>Threonine</td>
<td>6.6</td>
<td>8.0</td>
<td>7.5</td>
<td>13.5</td>
<td>14.7</td>
<td>13.3</td>
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<td>Serine</td>
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<td>8.4</td>
<td>8.1</td>
<td>15.2</td>
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<td>14.4</td>
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<td>Glutamic acid</td>
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<td>11.4</td>
<td>6.5</td>
<td>8.1</td>
<td>8.6</td>
</tr>
<tr>
<td>Proline</td>
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<td>3.2</td>
<td>5.4</td>
<td>7.2</td>
<td>4.9</td>
<td>7.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>7.4</td>
<td>8.2</td>
<td>6.8</td>
<td>5.3</td>
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<td>4.6</td>
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<td>Alanine</td>
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<td>4.9</td>
<td>5.2</td>
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<td>6.4</td>
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<tr>
<td>Cystine (half)</td>
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<td>8.7</td>
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<td>2.3</td>
<td>5.4</td>
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<tr>
<td>Valine</td>
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<td>4.2</td>
<td>5.5</td>
<td>4.9</td>
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<tr>
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<td>2.3</td>
<td>0.8</td>
<td>1.1</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Isoleucine</td>
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<td>4.7</td>
<td>4.6</td>
<td>8.6</td>
<td>4.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Leucine</td>
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<td>6.2</td>
<td>6.4</td>
<td>7.4</td>
<td>7.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.6</td>
<td>3.7</td>
<td>3.4</td>
<td>5.1</td>
<td>2.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.9</td>
<td>4.5</td>
<td>4.4</td>
<td>2.5</td>
<td>3.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.3</td>
<td>—</td>
<td>1.3</td>
<td>2.0</td>
<td>—</td>
<td>0.8</td>
</tr>
</tbody>
</table>

\(^a\) Reduced S-component.  
\(^b\) Reduced α-ovomucin.  
\(^c\) Reduced F-component; Kato and Sato (1971).  
\(^d\) Reduced β-ovomucin; Robinson and Monsey (1971).
peak I and peak II were 15% and 55% per dry matter, respectively. Carbohydrate composition of peak I was almost the same as the reduced S-component or the reduced α-ovomucin, and likewise carbohydrate composition of peak II was almost the same as the reduced F-component or the reduced β-ovomucin (Table I). Prominent amino acids of peak I were aspartic acid and glutamic acid, and those of peak II were threonine and serine. Amino acid composition of peak I was similar to that of the reduced S-component or the reduced α-ovomucin except for isoleucine and half cystine. Amino acid composition of peak II was also similar to that of the reduced F-component or the reduced β-ovomucin except for acidic amino acid, isoleucine, tyrosine and half cystine (Table II).

Molecular weight and intrinsic viscosity of the soluble ovomucin, the sonicated soluble ovomucin and the sonicated and reduced soluble ovomucin are given in Table III. The

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>Intrinsic viscosity</th>
</tr>
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<tbody>
<tr>
<td>(×10,000)</td>
<td>(ml/g)</td>
</tr>
<tr>
<td>The soluble ovomucin</td>
<td>830 (±40)</td>
</tr>
<tr>
<td>Sonicated soluble ovomucin</td>
<td>110 (±15)</td>
</tr>
<tr>
<td>Sonicated and reduced soluble ovomucin</td>
<td>23 (±3)</td>
</tr>
</tbody>
</table>

* The values were means of three trials.

values of molecular weight were means obtained from three trials of light scattering measurements. Molecular weight and intrinsic viscosity of the sonicated soluble ovomucin were intermediate value of those of the soluble ovomucin and the sonicated and reduced soluble ovomucin.

DISCUSSION

Young and Gardner(27) prepared ovomucin as follows. A 10 ml portion of egg white diluted five-fold in 0.85 % saline was applied to a Sepharose 4B column and eluted with 0.85 % saline. On the other hand, in our experiment, ovomucin was dissolved after removal of water soluble proteins, applied to a Sepharose 4B column and eluted with 0.1 M Menzel buffer containing 0.4 M NaCl. The amount of the soluble ovomucin preparation obtained by our method was ten fold as much as that obtained by the method of Young and Gardner.(27)

The soluble ovomucin had a large molecular weight of 8.3 × 10^6 and high viscosity (Table III). The mol. wt. determined by sedimentation of the ovomucin prepared by Lanni et al.(11) was 7.6 × 10^6, being consistent with our result. However, mol. wt. of ovomucin from thick white prepared by Tomimatsu and Donovan was larger (27-56 × 10^6 in 1.1 M KCl, pH 7.9; by light scattering).(13) Intrinsic viscosity of the soluble ovomucin was 365 ml/g (Fig. 3) and comparable to that of the ovomucin from thick white prepared by Kato et al. (260 ml/g)(18) and Donovan and White (280 ml/g; i=0.11, pH 9.7). (32)

The intact soluble ovomucin could not be separated into two components, carbohydrate rich and poor component, by density gradient electrophoresis (Fig. 4), cellulose acetate electrophoresis (Fig. 5) and electrofocusing, whereas the ovomucin reduced with mercaptoethanol could be separated into two components by density gradient electrophoresis(33) and density gradient ultracentrifugation. (2)

From these results, it was considered that the soluble ovomucin occurred as aggregated and polymerized molecules which were formed by interaction between carbohydrate rich and poor component.

Molecular weight and reduced viscosity of the soluble ovomucin decreased with the time of sonication (Fig. 2) and the sonicated soluble ovomucin could be separated into two components by density gradient electrophoresis, cellulose acetate electrophoresis and DEAE-cellulose column chromatography (Figs. 4, 5, 6). As the sonicated soluble ovomucin contained no cysteine, it was considered that the soluble ovomucin can dissociate into smaller molecules through disruption of interaction between carbohydrate rich and poor component by sonication without reduction of disulfide bonds.
However, it is not clear which bonds were disrupt by sonication, hydrogen bond, electrostatic bond, ester bond or any others. It was considered that the soluble ovomucin was degraded only at the joining sites of aggregated molecules considering the facts that i) the amounts of nitrogen and hexose of small molecular materials obtained by sonication of the soluble ovomucin were less than 1% of those of the soluble ovomucin and the amount of the sialic acid liberated was less than 4% of that of the soluble ovomucin, accounting for 0.08% of dry weight of the soluble ovomucin, as the soluble ovomucin contains only about 2% sialic acid; ii) carbohydrate and amino acid compositions of peak I component (sonicated α-ovomucin) were almost the same as those of the reduced S-component or the reduced α-ovomucin and those of peak II component (sonicated β-ovomucin) were almost the same as those of the reduced F-component or reduced β-ovomucin; iii) it was reported that 2% of ovomucin was disintegrated into low molecular materials when ovomucin was solubilized by reduction of disulfide bonds with thioglycolate and mercaptoethanol.

As carbohydrate and amino acid compositions of peak I were almost the same as those of the reduced S-component or the reduced α-ovomucin (Tables I, II), peak I may well be designated as sonicated α-ovomucin. On the other hand, carbohydrate composition of peak II was almost the same as that of the reduced F-component or the reduced β-ovomucin (Table I), and amino acid composition of peak II was similar to that of the reduced F-component or the reduced β-ovomucin except for several amino acids (aspartic acid, glutamic acid, half cystine, isoleucine and tyrosine) (Table II). The reduced β-ovomucin contains only about 30% of protein moieties of reduced α-ovomucin, so that a small contamination of the reduced α-ovomucin may considerably affects amino acid composition. Therefore, assuming that the composition of peak II is the same as that of the reduced F-component or the reduced β-ovomucin, peak II may be designated as sonicated β-ovomucin.

Intrinsic viscosity of the sonicated soluble ovomucin was larger than that of the sonicated and reduced soluble ovomucin and molecular weight of the sonicated soluble ovomucin was five times greater than those of the sonicated and reduced soluble ovomucin (Table III). Robinson and Monsey reported that molecular weight of the reduced α-ovomucin (ovomucin monomer) was $2.1 \times 10^6$. Tomimatsu and Donovan also determined molecular weight of ovomucin by light scattering to be $40 \times 10^6$ in 1.1 M KCl of pH 7.9, $23 \times 10^6$ in 6.5 M guanidine-HCl and $2.2 \times 10^6$ in 0.1 N NaOH where disulfide bonds of ovomucin were split by hydroxide ion. As molecular weight obtained by us with the sonicated and reduced soluble ovomucin was consistent with their results, the minimum unit of ovomucin with mol. wt. of $2.1 \sim 2.3 \times 10^6$ is considered to be monomeric ovomucin. The above results suggests that the sonicated soluble ovomucin is an intermediate of the monomeric ovomucin and the aggregated, polymerized ovomucin. It is also suggested that the sonicated α-ovomucin molecule or the sonicated β-ovomucin molecule is composed of monomeric α-ovomucin or monomeric β-ovomucin cross-linked by disulfide bonds, respectively, and the soluble ovomucin is composed of the sonicated α- and β-ovomucin molecules cross-linked by some specific bonds. Insoluble ovomucin, reported earlier by authors, is perhaps comprehended as one of the aggregated and polymerized ovomucins.

REFERENCES