bean proteins. However, in the case of E-CBP, the alkalinity alone did not decrease the nutritive values (Table VI).

Feeding tests with rats showed the absence of toxicity in the isolates, proving the efficiency of the heat treatment. On the other hand, the rats that received crude extract protein in the diet showed 100% mortality within the first 5 days of the experiment. The average intake of food by these animals was very low (1.5 g/rat). Examination of the organs after death did not show any visible lesions, probably due to the potency of the toxin, which killed the animals at very low intake.

Protein intake, body weight gain, and NPU are shown in Table VI for the different treatments. One can see that the consumption of diets which contained the isolates and casein was similar, indicating good acceptability of the castor bean protein. Satisfactory food intake and healthy appearance of the animals at the end of the experiment testified to the complete detoxification of ricin.

The average of all NPU values of the isolates was 36% (similar to wheat gluten). The NPU of EI (pH 11) was 37% and that of EI (NaOH 0.5%) was 46%. The addition of 3% L-lysine and 1.54% DL-methionine elevated the NPU value of EI (pH 11) to 49 or 73% that of casein.

In relation to the presence of allergens no objective measurements were carried out on the different materials. However, during the work with CBP the senior author became sensitized by the allergens manifesting sneezing, respiration difficulties, and general discomfort. These manifestations were completely absent when working with the isolates, suggesting that the allergens must have been eliminated to a great extent, if not completely, by the isolation procedure. This indirect observation is confirmed by Coulson et al. (1960) who could not detect any allergenic or allergic reaction due to castor bean proteins precipitated by heat coagulation.

The results of this investigation suggest that castor bean pomace could be considered as a source for large scale production of protein isolates. Further studies should be carried out on the technological, economical, toxicological, and nutritional aspects of these products both as animal and possibly as human foods. One should also look for some functional properties and applications of these proteins other than as food ingredients.

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Studies on Factors of Solubilization of Insoluble Ovomucin during Thick White Thinning

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Insoluble ovomucin prepared in this experiment was successfully incubated under aseptic conditions for 15 days at 30 °C in various solutions. Half-amounts of insoluble ovomucin were gradually solubilized when they were incubated in a buffer solution of pH ~9.5 for 15 days. When the ionic strength of the buffer solution was 0.1, the solubilized parts consisted of a lot of the carbohydrate poor component and only a little of the carbohydrate rich component, but much carbohydrate rich component was solubilized when insoluble ovomucin was incubated in a 5% ovalbumin solution with an ionic strength of 0.1 at pH 9.6 or in a buffer solution with ionic strengths of 0.0001 and 0.001 at pH 10 without other components. From the presumption that a high concentration of ovalbumin may reduce ionic activity in solution, it was suggested that an increase of pH during storage, lowered ionic strength by egg white proteins, and raised storage temperature may be the main causal factors in the solubilization of the carbohydrate rich component from insoluble ovomucin.

Many workers have hitherto been concerned with clarifying the mechanism of thick white thinning and it has been accepted that the main factors causing the thinning are not microbiological but are inherent in the chemical properties of the egg white itself (Feeney et al., 1951; Baliga et al., 1964). A number of workers (Hawthorne, 1950; Cotterill and Winter, 1955; Brooks and Hale, 1959, 1961; Robinson, 1972) have proposed that interaction

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between ovomucin and lysozyme was responsible for the rigidity of thick white. Hawthorne (1950) suggested that egg white thinning might result from the slow insolubilization of ovomucin caused by its combination with lysozyme. Cotterill and Winter (1955) found maximum interaction between ovomucin and lysozyme at a pH of 7 and, as the egg white became more alkaline, less complex was formed. Brooks and Hale (1959, 1961) suggested that a network of ovomucin chains associated with or cross-linked by lysozyme molecules be postulated to account for changes during thinning of thick white.

In the previous paper (Kato and Sato, 1971; Kato et al., 1970, 1971) we showed that ovomucin, which was contained as insoluble matter in fresh thick white, consisted of a carbohydrate poor component (noted as the S-component) and a carbohydrate rich component (noted as the F-component); the latter component was gradually dissociated and solubilized into the liquid part of thick white during the natural thinning of samples of the thick egg white. Robinson and Monsey (1972a) also obtained similar results on the behavior of ovomucin during the natural thinning by analytical ultracentrifugation and they designated the carbohydrate poor component as α-ovomucin and the carbohydrate rich component as β-ovomucin (Robinson and Monsey, 1971).

Recently, Dam (1971) suggested that changes in ovomucin might be sufficient to explain thinning of the gel structure in egg white during its storage. Donovan et al. (1972) suggested that alkaline hydrolysis of the disulfide bonds of ovomucin was responsible for the thinning. Tomimatsu and Donovan (1972) reported the pH, ionic strength, temperature, and time dependence of the weighted average molecular weight of ovomucin. Their investigations put stress on the result from incubation of prepared ovomucin, instead of analyzing the changes of materials in thick white during its storage.

The present paper deals with the dependence of dissociation and the solubilization of the two components from prepared ovomucin during its incubation on pH, ionic strength, and coexisting materials.

MATERIALS AND METHODS

Ovomucin. Ovomucin was prepared by washing the gel-like precipitate with 2% KCl and distilled water, which was ultracentrifugally separated from fresh thick white, according to the method of Kato et al. (1970). In this report it is denoted as fresh ins-ovomucin (ins means insoluble, corresponding to ovomucin gel B or OMG_0 in previous reports; Kato et al., 1970; Kato and Sato, 1972). Fresh ins-ovomucin prepared in this experiment showed an area ratio of 5:5 in S-component to F-component by elution patterns from density gradient electrophoresis of samples reduced by mercaptoethanol, as shown in Figure 2B. Sialic acid content of the sample was 9.3 ± 0.5%. These data almost coincide with those in our previous report (Kato et al., 1971, 1972).

Other Materials. Ovalbumin, ovomucoid, and lysozyme were prepared by the methods of Sorensen and Hoyrup (1918), Fredericq and Deutch (1949), and Alderton and Fevold (1946), respectively. The purities of the prepared proteins were confirmed by the acrylamide gel electrophoretic patterns. From their ash contents of less than 1.0%, it was conceived that the influences of their ash content on ionic strength were less than 0.008 in 5% solutions of the proteins. Cysteine hydrochloride was purchased from Tokyo Kasei Kogyo Co. Ltd., Tokyo.

Incubation of Fresh Ins-Ovomucin and Analysis. Fresh ins-ovomucin was weighed into sterile test tubes. Sterile buffer solutions with various pH values and ionic strengths were added to each test tube and these test tubes were incubated for 15 days at 30 °C after shaking them until the fresh ins-ovomucin was fully swollen. In the cases of incubation with ovalbumin, ovomucoid, lysozyme, or cysteine, their solutions filtered with microfilter under aseptic conditions were added to the samples in the test tubes. The concentration of fresh ins-ovomucin was 0.2% throughout this experiment and small amounts of merthiolate were added to these tubes for antisepsis. A viable count of bacterial cells was determined after incubation and subsequently the contents of those tubes containing no bacterial cells were centrifuged at 13 000g for 15 min. The insoluble parts were washed with 2% KCl until the washing was free from protein, suspended in distilled water, dialyzed against distilled water, and then lyophilized. These lyophilizates were weighed and denoted as remained ins-ovomucin. The percentage of remained ins-ovomucin to fresh ins-ovomucin was denoted as ROM. The sialic acid content of each remained ins-ovomucin was determined by the method described later and density gradient electrophoresis for remained ins-ovomucin was carried out after its solubilization with mercaptoethanol in Menzel buffer (pH 9.6, ionic strength 0.24) for 1 h at 30 °C. The sialic acid contents in fresh and remained ins-ovomucin were determined by the thiobarbituric acid method of Warren (1959) after hydrolyzing them with 0.1 N H_2SO_4 for 1 h at 80 °C in sealed ampules. Standard sialic acid was purchased from Seikagaku Kogyo Co. Ltd., Tokyo. The sialic acid content in remained ins-ovomucin was expressed as the ratio of the percentage of sialic acid in remained ins-ovomucin to that in fresh ins-ovomucin and will be referred to below as the relative content.

Identification of F- or S-components in the fraction separated through density gradient electrophoresis was carried out by cellulose acetate electrophoresis. The ratio of the F-component to the sum of the F- and S-components in ins-ovomucin was calculated from the area of separated peaks in the elution patterns of density gradient electrophoresis and is denoted as the F-ratio.

The supernatant in the centrifugation after incubation of fresh ins-ovomucin was applied on the column (2.5 × 50 cm) of a Sepharose 4B with Menzel buffer (pH 9.6, ionic strength 0.24). Fractions eluted at the void volume of the column were collected and concentrated. These concentrates contain solubilized ovomucin. Cellulose acetate electrophoresis of each concentrate was performed in order to make sure that it did not contain any protein except solubilized ovomucin, and then density gradient electrophoresis was carried out without reduction by mercaptoethanol. The F-ratio in solubilized ovomucin was also measured by the same procedure as in remained ins-ovomucin.

Density Gradient Electrophoresis. Density gradient electrophoresis was carried out using an LKB 7900 Uniphor column (2.5 × 50 cm) according to the method of Kato and Sato (1971). A column linear density gradient was formed with decreasing concentration, from bottom to top, of 40% (w/w) sucrose in 0.3 M borate buffer at pH 8.2. A sample was added to the top of the column. All runs were performed for 24 h at a constant voltage of 300 V at 4 °C. After electrophoresis, the effluent was fractionated in tubes by 6 ml and the elution pattern was drawn by measuring the extinction at 280 nm of the fractional effluents.

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 current of 0.2 mA/cm for 1 h.

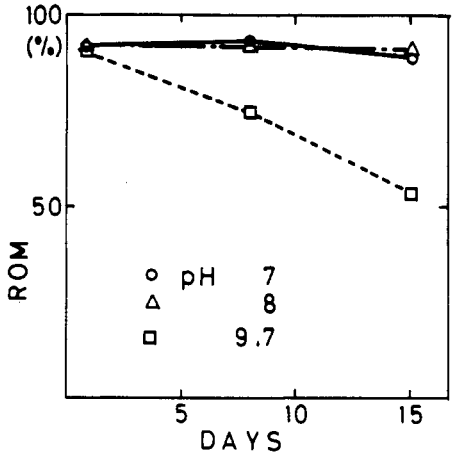

Figure 1. Incubating time dependence of solubilization of fresh ins-ovomucin: ROM, percentage of remained ins-ovomucin to fresh ins-ovomucin; concentration of fresh ins-ovomucin, 0.2%; ionic strength, 0.1 (0.1 M $NaHCO_3$ adjusted to pH 7, 8, or 9.7 with 0.1 M HCl or $^1/_{30}$ M Na_2CO_3); incubating temperature, 30 °C.

The buffer solution used contained 0.3 M boric acid and 0.075 M NaOH at pH 8.2. The strip was stained with either 0.5% Alcian Blue 8GS in 3% acetic acid (pH 3) or 1% Amido Black 10 B in 7% acetic acid, followed by washing with 3% acetic acid. The F-component was stained by Alcian Blue and Amido Black 10 B, and the S-component was stained only by Amido Black 10 B.

RESULTS

Influence of pH on Solubilization of Fresh Ins-Ovomucin. The ionic strength of egg white was estimated to be about 0.1 being calculated from the concentration of salts determined by Sato et al. (1960). It is generally accepted that the pH of newly laid egg white is 7.5–8.0 and that it increases to about 9.5 after a week of storage at 30 °C. On the basis of these facts found in eggs stored in their shells, the experimental conditions on solubilization of fresh ins-ovomucin were set up.

Figure 1 shows the change of ROM over time during incubation of fresh ins-ovomucin at 30 °C and ionic strength 0.1 under the conditions of pH 7, 8, and 9.7. Fresh ins-ovomucin was solubilized gradually at pH 9.7 with elapsed time, but was not solubilized at pH 7 and 8. Finally, about half of the fresh ins-ovomucin was solubilized in 15 days at pH 9.7 and 30 °C.

Table I shows the influences of the various pH values on solubilization of fresh ins-ovomucin and the relative content of sialic acid in remained ins-ovomucin after incubation of fresh ins-ovomucin for 15 days under the conditions of ionic strength 0.1 and 30 °C. ROM was about 90% between pH 7 and 8, and gradually decreased with the increase of pH and was 45% at pH 10. If the solubilized part consists mainly of the carbohydrate rich component as shown in the previous report on the experimental storage of shell eggs (in the experiment, increased amounts of only the F-component of soluble ovomucin in the liquid part of stored thick white were found; Kato et al., 1970), the relative content of sialic acid in remained ins-ovomucin would be expected to decrease accompanied by the release of the carbohydrate rich component. However, against our expectation, the relative content of sialic acid in the remained ins-ovomucin gradually increased with the increase of pH. This seems to show that considerable amounts of F-component were contained in the remained ins-ovomucin. This was verified by the elution pattern of density gradient electrophoresis of remained ins-ovomucin which was obtained after 15 days incubation of fresh ins-ovomucin at pH 9.6, ionic strength 0.1 and 30 °C as shown in Figure 2.

Table I. Influence of pH on ROM[a] and Relative Content of Sialic Acid[b] in Remained Ins-Ovomucin[c]

pH[d]	ROM, %	Rel content of sialic acid
7.0	89	1.06
8.0	90	1.05
8.7	80	1.06
9.0	64	1.09
9.7	54	1.27
10.0	45	1.30

[a] Percentage of remained ins-ovomucin to fresh ins-ovomucin. The values represent the mean of two replications. Experimental error is within ±5%. [b] Ratio of sialic acid percentage of remained ins-ovomucin to sialic acid percentage of fresh ins-ovomucin. The values represent the mean of two replications. Experimental error is within ±0.05. [c] Incubating time, 15 days; other incubating conditions are the same as described in Figure 1. [d] 0.1 M $NaHCO_3$ adjusted to given pH with $^1/_{30}$ M Na_2CO_3 above pH 8.7 and with 0.1 M HCl below pH 8.0.

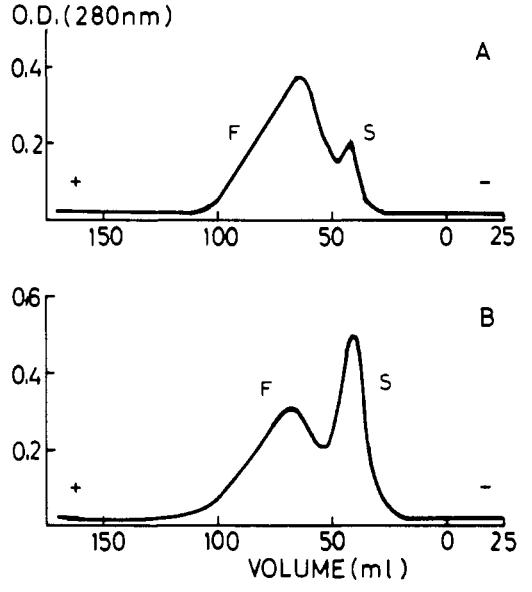

Figure 2. Elution patterns in density gradient electrophoresis of remained ins-ovomucin: (A) remained ins-ovomucin in incubating fresh ins-ovomucin at pH 9.6 and ionic strength 0.1 for 15 days (other conditions are all the same as described in Figure 1); (B) fresh ins-ovomucin.

It was, therefore, necessary to try to find other conditions to solubilize more F-component from fresh ins-ovomucin. Furthermore, influences of various ionic strengths and the coexistence of other proteins on solubilization of fresh ins-ovomucin were investigated.

Influence of Various Ionic Strengths on Solubilization of Fresh Ins-Ovomucin. Table II shows the influences of various ionic strengths on solubilization of ins-ovomucin and the relative content of sialic acid in remained ins-ovomucin at pH 10.

In Table II ROM decreased with ionic strengths of more than 0.1, and the relative content of sialic acid was always more than 1.0. It was revealed that less carbohydrate rich component was solubilized out into solution in the range of ionic strength 0.1–0.2. In the region of less than 0.1 of ionic strength, ROM was 45–69% and increased little by little with the decrease of ionic strength, and the relative content of sialic acid of remained ins-ovomucin gradually decreased with the decrease of ionic strength. The observation of the relative content of sialic acid was confirmed by the elution patterns from the density gradient

Table II. Influence of Ionic Strength at pH 10[a]

Ionic strength[b]	ROM, %	Rel content of sialic acid	F-ratio[c] in solubilized ovomucin
0.0001	69	0.54	0.9
0.001	58	0.67	0.7
0.01	47	1.10	0.5
0.1	45	1.30	0.1
0.12	53	1.30	nd[d]
0.16	41	1.30	nd
0.24	32	1.31	nd

[a] Incubating temperature, 30 °C; incubating time, 15 days. The values represent the mean of two replications. Experimental error is within ±5% in ROM and within ±0.05 in the relative content of sialic acid and the F-ratio. [b] Na_2CO_3 solution with given ionic strength was adjusted to pH 10 with the corresponding ionic strength $NaHCO_3$ except for ionic strength 0.0001, in which 0.0001 M NaOH was used. [c] Area ratio of F-component in solubilized ovomucin which was measured by elution pattern of density gradient electrophoresis. [d] Not determined.

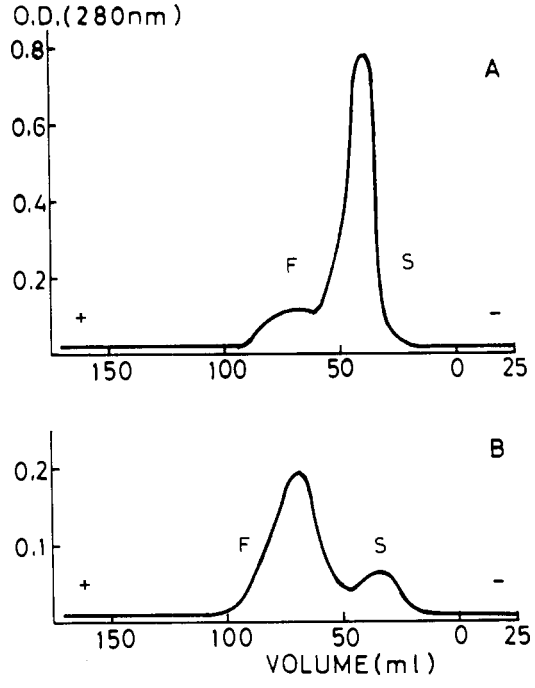

Figure 3. Elution patterns in density gradient electrophoresis of solubilized ovomucin recovered from incubating solution with ionic strength 0.1 (A) or 0.0001 (B) at pH 10. Incubating conditions are all the same as in Table II except for ionic strength.

electrophoresis of solubilized parts in 15 days incubation of fresh ins-ovomucin at pH 10 and 30 °C under ionic strengths 0.1 and 0.0001. As shown in Figure 3 the area ratio of solubilized F-component to S-component in incubation at ionic strength 0.0001 was larger than that in incubation at ionic strength 0.1.

Influence of Other Proteins on Solubilization of Fresh Ins-Ovomucin. Taking coexistence of other proteins in natural thinning into consideration, the influences of ovalbumin, ovomucoid, and lysozyme on solubilization of the F-component from fresh ins-ovomucin were tested, respectively, under the conditions of ionic strength 0.1 at 30 °C and pH 9.5. The results are shown in Table III.

When 5% ovalbumin, 1.5% ovomucoid, or 0.6% lysozyme was added to 0.2% ins-ovomucin suspension at pH 9.5 and ionic strength 0.1 and each mixture was incubated for 15 days at 30 °C, the amounts of remained ins-ovomucin were 34, 21, and 110%, respectively.

Table III. Effect of Ovalbumin, Ovomucoid, and Lysozyme[a]

Coexisting protein	ROM, %	Rel content of sialic acid	F-ratio[b] Solubilized ovomucin	F-ratio[b] Remained ins-ovomucin
None	47	1.35	0.1	0.9
5% ovalbumin	34	0.53	0.7	0.3
1.5% ovomucoid	21	1.07	0.4	<0.8[c]
0.6% lysozyme	110	0.73	nd[d]	nd
0.3% lysozyme	nd	nd	nd	0.95

[a] Incubation was carried out under the conditions of ionic strength 0.1 at 30 °C and pH 9.5, except in the case where no coexisting proteins were added and ionic strength 0.1 at 30 °C and pH 10 were used. The values represent the mean of two replications. Experimental error is within ±5% in ROM and within ±0.05 in relative content of sialic acid and F-ratio. [b] Area ratio of F-component in solubilized ovomucin or remained ins-ovomucin which was measured by elution pattern of density gradient electrophoresis. [c] Calculated value from ROM and F-ratio of solubilized ovomucin. [d] Not determined.

The excess percentage of remained ins-ovomucin in storage with 0.6% lysozyme seems to be due to amounts of unremoved lysozyme in the determination of remained ins-ovomucin. The relative content of sialic acid was calculated to be 0.73 because of the incorrect value of remained ins-ovomucin contaminated with lysozyme. In such a case it was better to measure the F-ratio in remained ins-ovomucin. In another experiment carried out with 0.3% lysozyme, the F-ratio of remained ins-ovomucin calculated from the elution pattern of density gradient electrophoresis after its reductive solubilization was 0.95, reflecting that only a little of the F-component was contained in the solubilized part.

Because the relative content of sialic acid of remained ins-ovomucin decreased to 0.53 and the relative area ratio of the F-component in the solubilized part increased to 0.7 in the presence of ovalbumin, it is reasonable to think that the presence of 5% ovalbumin makes solubilization of the F-component easier than either the presence of 1.5% ovomucoid or the absence of any proteins other than fresh ins-ovomucin, under the conditions of pH 9.6–10 and ionic strength 0.1.

One of the most probable and inherent functions of these proteins for the selective solubilization of the F-component seems to be the reducing power for the disulfide bond by the SH groups buried in the ovalbumin structure which are exposed through its alkaline denaturation. A scientific approach to the function of the exposed SH groups was carried out at pH 9.0–9.4 by adding cysteine to the fresh ins-ovomucin suspension showing ionic strength 0.1.

Table IV shows that the F-ratio in the solubilized part from fresh ins-ovomucin increased to 0.4 by adding 2.5 mg/ml cysteine. This concentration of cysteine corresponds to 2.5 times the amount of SH groups contained in 6% ovalbumin in egg white, presuming that 4 mol of exposed SH groups is contained in 1 mol of denatured ovalbumin. It seems likely that the characteristic function of ovalbumin for the F-component solubilization in ionic strength 0.1 is not due to exposure of the SH groups in ovalbumin.

Another experiment was carried out with reference to the effect of *N*-ethylmaleimide (MalNEt), a blocking agent of the sulfhydryl group, on thick white thinning. When homogenized thick white which contained 1.75 mg of

Table IV. Influence of Cysteine on Solubilization of Fresh Ins-Ovomucin[a]

Cysteine, mg/ml	F-ratio in solubilized ovomucin[b]
0	0.15
1.24	0.3
2.48	0.4
4.96	0.4
12.4	0.45

[a] Incubating conditions are all the same as in Figure 3A. pH was controlled including cysteine hydrochloride to 9.0–9.4. [b] Area ratio of F-component in solubilized ovomucin which was measured by elution patterns of density gradient electrophoresis. The values represent the mean of two replications. Experimental error is within ±0.05.

MalNEt per ml of thick white was stored for 10 days at 30 °C under aseptic conditions, the solubilized ovomucin which was recovered from the liquefied part of thick white stored with MalNEt contained as much F-component as that in solubilized ovomucin obtained from thick white stored without MalNEt. This fact suggests that natural thinning was not affected by MalNEt.

Considering that conditions, such as ionic strength 0.001–0.0001 and pH 9.5–10 or the presence of ovalbumin at ionic strength 0.1 and pH 9.5–10, are useful for solubilization of the F-component, it is estimated that higher contents of soluble proteins, for example, 5% ovalbumin in this experiment, may reduce ionic strength through its own ion-binding function. If so, OH ions in lower ionic strength would be essential for releasing the F-component from fresh ins-ovomucin.

DISCUSSION

Various methods of ovomucin preparation have been devised by many workers (Hawthorne, 1950; MacDonnel et al., 1951; Brooks and Hale, 1961; Robinson and Monsey, 1971; Donovan et al., 1972). The method adopted in our laboratory at present consists of ultracentrifuging blended thick white and washing the precipitate with 2% KCl solution, distilled water, and deionized water in order. The ovomucin obtained was considered to be insoluble type ovomucin, perhaps native type, although soluble type ovomucin was contained in the supernatant in the ultracentrifuge. Almost the same amounts of F- and S-components were found in a reduced solution of the insoluble ovomucin prepared from the gel-like precipitate in ultracentrifugation of fresh thick white and almost all of the ovomucin prepared from the supernatant (liquid part in thick white) consisted of only the S-component (Kato et al., 1970). Although the ratio of α- to β-ovomucin in the "ovomucin complex" prepared by Robinson and Monsey (1972a) is 71.3:28.6 in the peak areas of the ultracentrifugal pattern, the measured ratio of S- to F-component in fresh ins-ovomucin prepared in this experiment is 5:5 in the peak areas of the elution pattern by density gradient electrophoresis (Figure 2). The F-ratio in fresh ins-ovomucin calculated from measured values of ROM, the relative content of sialic acid, and the F-ratio of solubilized ovomucin or remained ins-ovomucin after the incubation experiment were in the range of 0.45–0.59. It would be, therefore, in accordance with the measured value, with allowance for experimental error. As the amino acids and the carbohydrate compositions of α- and β-ovomucin prepared by Robinson and Monsey (1971) are in fair agreement with those of S- and F-components prepared by us (Kato and Sato, 1971, 1972), it seems likely that α- and β-ovomucin correspond to the S- and F-components, respectively. Therefore, the difference between the two ratios measured by them and by us may be, perhaps, due to the fact that our samples are free from the component contained in the supernatant separated from thick white by the ultracentrifuge.

In this experiment, fresh ins-ovomucin was successfully incubated under aseptic conditions, and was partially solubilized, showing about 100% recovery of ovomucin, calculated from the total amount of solubilized and remained insoluble ovomucin (with allowance for about 5% loss in experimental procedure), under all conditions of this experiment. Therefore, microbiological factors should not be considered in the solubilization of the fresh ins-ovomucin in this experiment.

Kato et al. (1971), Kato and Sato (1972), and Robinson and Monsey (1972a) suggested that thick white thinning was accompanied by release of the carbohydrate rich component from native ovomucin. In 15 days incubation at 30 °C under the conditions of pH 10 and ionic strength 0.0001 and also in 15 days incubation with 5% ovalbumin under the conditions of pH 9.6 and ionic strength 0.1, ROM's were 69 and 34%, respectively, and F-ratios of their remained ins-ovomucin were 0.3 and 0.3, respectively. In other incubating conditions of this experiment, F-ratios in remained ins-ovomucin were about 0.8 or more, although ROM's were sometimes 21 or 47. These results obtained under the former conditions of this experiment are near the results in the storage experiment of thick white (Kato et al., 1971), in which recovery of insoluble ovomucin from stored thick white and its F-ratio become about 50% and 0.3, respectively, in 10 days of storage at 30 °C (Kato et al., 1970, 1971).

In our previous reports (Kato et al., 1970; Kato and Sato, 1972) no shortage of recovery of ovomucin was found by weight determination of solubilized and insoluble ovomucin and no conversion of F-component to S-component or vice versa was found by carbohydrate and amino acid analyses of both components dissociated from the original insoluble ovomucin. On the basis of these results the relative content of sialic acid in remained ins-ovomucin would be a convenient index for solubilization of the F-component. The percentages of sialic acid in fresh ins-ovomucin and the S- and the F-components were found to be 9.3 ± 0.5, 0.5–0.8, and 11.4–14.5, respectively. In the complete release of the S-component, the relative content of sialic acid in remained ins-ovomucin would be 1.2–1.5 (= 11.4–14.5/9.3), and, on the contrary, in the complete release of the F-component, the relative content of sialic acid in remained ins-ovomucin would be 0.05–0.08 (= 0.5–0.8/9.3). When the relative content of sialic acid is less than 1.0 (its value in the native state), it may be estimated that the F-component is to be more solubilized from fresh ins-ovomucin. When the relative content of sialic acid is more than 1.0, it may be estimated that the S-component is to be more solubilized from fresh ins-ovomucin.

If this calculation could be applied to the results of this experiment, being supported by the elution pattern of density gradient electrophoresis, it might be concluded that the conditions suitable for solubilization of the F-component would be ionic strength 0.001–0.0001 at pH 10 and ionic strength 0.1 at pH 9.6 in the presence of 5% ovalbumin. It would be, therefore, rather reasonable to estimate that an alkaline condition with a lower ionic strength or with a higher one in the presence of 5% ovalbumin is necessary for solubilizing only or primarily the F-component from fresh ins-ovomucin.

The function of ovalbumin in higher ionic strength would not be considered to be mainly the reaction of

exposed sulfhydryl groups from denatured ovalbumin, on the basis of the results shown in Table IV, accompanied by S-component solubilization by cysteine, and on the basis of the experimental fact that blocking sulfhydryl groups of ovalbumin with MalNEt does not affect natural thinning. In the experiment shown in Table IV, the ionic strength of cysteine itself might be considered. However, cysteine is a weak electrolyte and, if dissociated considerably, total ionic strength in the test solution would be in the range between 0.1 and 0.2. No influences of the ionic strengths in such a range on the selective solubilization of the F-component were recognized from the relative content of sialic acid shown in Table II. The invariability of the F-ratio in solubilized ovomucin on addition of cysteine seems to be due to the simultaneous solubilization of the F- and S-components of ins-ovomucin, just as in the case when mercaptoethanol or dithiothreitol solubilizes ins-ovomucin. Therefore, it seems likely that the main function of ovalbumin may well be estimated to be the reduction of ionic strength in thick white.

Donovan et al. (1972) reported that when ovomucin solution with 0.1 M KCl was exposed to alkaline, above pH 11, its intrinsic viscosity fell down close to that of ovomucin whose disulfide bonds were split by an excess of reducing agent, and they suggested that this alkaline degradation of the disulfide bond by the hydroxide ion was responsible for the thinning of egg white during storage. From our results, it is a certainty that solubilization of fresh ins-ovomucin can take place at pH 9.5 and ionic strength 0.1, but the solubilized part under these conditions consisted of a lot of the S-component and a little of the F-component, while it does not in natural conditions.

Robinson and Monsey (1972b) reported that either 0.01 M magnesium salt or 0.03 M sodium chloride also reduced the losses of the β-ovomucin which normally occur during liquefaction of thick egg white. However, amounts of "ovomucin complex" obtained after storage with 0.03 M sodium chloride were much smaller (89 mg/100 ml of thick white) than those obtained from newly laid thick white (142 mg/100 ml of thick white). On the basis of our experimental results, it was considered that 0.03 M sodium chloride might promote the losses of the S-component (Robinson's α-ovomucin) perhaps by increasing the number of free ions.

If it is true that thick white thinning is caused by release of the carbohydrate rich component during storage, it may be suggested from our results that both alkaline pH and low ionic strength are responsible for the release of the F-component from insoluble ovomucin which occurs in natural thinning of thick egg white.

Although Hawthorne (1950), Cotterill and Winter (1955), and, in recent years, Robinson (1972) have postulated that ovomucin–lysozyme interaction influences the gel structure of thick white, it is recognized that insoluble ovomucin holds gel properties in itself and is solubilized as pH increases when it is free from lysozyme. Donovan et al. (1972) showed that a change in the kinetic viscosity of a 0.2% solution of ovomucin did not depend upon the presence or absence of an equal weight concentration of lysozyme. Our present viewpoint resembles Donovan's opinion (Donovan et al., 1972).

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