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ARTICLE

A New Method of Separating Ovomucin from Egg White

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Ovomucin, a key component in maintaining the viscous nature of egg white, is a glycoprotein contributing to 2−4% of the total egg albumin protein. Preparation of pure ovomucin remains a challenge due to the presence of coprecipitated proteins, mainly ovalbumin and lysozyme. The objectives of the study were to determine the effect of different salt concentrations on the extractability of ovomucin and to develop a simple method to purify ovomucin that could be adapted for further scale-up production. The protein compositions of ovomucin extracts were significantly affected by salt concentrations. The concentration of ovalbumin was increased, whereas that of lysozyme was decreased in the ovomucin extracts at increasing salt concentrations up to 500 mM; lysozyme was the major contaminant at low salt concentrations (<100 mM), whereas ovalbumin was the major contaminant at high concentrations (≥200 mM). A 2-step method was developed for the first time to prepare ovomucin with a purity of greater than 90%. Egg white was first extracted in the presence of 100 mM NaCl at pH 6.0 to produce a precipitate containing moderate coprecipitated ovalbumin (14.6%) and lysozyme (15.9%); the contaminated proteins in the precipitate were further removed by using 500 mM NaCl. The yield of ovomucin was determined to be 400.2 mg/100 g of egg white. This 2-step method is simple, environmentally friendly, and easy for scale-up preparation.

KEYWORDS: Egg white; ovomucin; lysozyme; ovalbumin; isoelectric precipitation; sodium chloride

INTRODUCTION

Ovomucin, a key component in maintaining the viscous nature of egg white, is a glycoprotein contributing to 2.0−4.0% of the total egg albumin protein (*1*). It is composed of a carbohydrate-poor component (α-ovomucin, which constitutes α1 and α2 subunits with a difference in molecular weight) and a carbohydrate-rich component (*β*-ovomucin) with a reported isoelectric pH between 4.5 and 5.0 and a molecular weight of 5.5−8.3 × 10^3 kDa (*2*, *3*). The molecular weight of α-ovomucin was estimated to be 210 kDa by Robinson and Monsey (*3*). However, some researchers estimated that the molecular weights of α1 and α2 ovomucin are 150 kDa and 220 kDa, respectively, and that of *β*-ovomucin is 400 kDa (*4*).

Ovomucin was proposed to play an important role in egg white thinning during storage of shell eggs; it was hypothesized that egg white thinning is associated with the degradation of ovomucin by reduction of disulfide bonds or dissociation of the lysozyme−ovomucin complex (*5*, *6*). In addition to its excellent foaming and emulsifying properties (*7*), ovomucin was found to display hypocholesterolemic property (*8*). Moreover, ovomucin-derived peptides have been demonstrated to have antiviral and antitumor activities (*9*, *10*). Its excellent functional and biological properties indicate its wide potential applications in the functional food and neutraceutical industries.

Ovomucin can be extracted from egg whites by isoelectric precipitation (IP) including dilution of egg albumin with water followed by acidification, but the preparation of high purity ovomucin was hampered due to the coprecipitation of ovalbumin and lysozyme (*3*, *11*, *12*). Ovalbumin, with its pI of 4.5 and molecular weight of 45 kDa, is a phosphoglycoprotein constituting about 60% (w/w) of the egg white protein. Lysozyme, with its pI of 10.7 and molecular weight of 14.3 kDa, accounts for ∼3.5% (w/w) of the egg white proteins. The other coprecipitated proteins are ovomucoid and ovotransferrin, whose pIs are 4.1 and 6.1, respectively (*13*). The gelatinous ovomucin precipitate thus obtained is, however, contaminated with other egg albumin proteins, which cannot be removed by repeated water and 2% KCl washing procedures (*12*). Ovomucin prepared from egg white by the conventional method without 2% KCl washing was reported to have a purity of 64% (*14*). Further, KCl washing was reported to cause a loss of ovomucin (*15*, *16*). In the literature, gel filtration chromatography was the only method used to purify ovomucin for research purposes (*17*); but this is not suitable for large-scale production. Therefore, the challenge remains to prepare a large quantity of highly pure ovomucin. The objectives of the study were to determine the effect of different salt concentrations on the extractability of ovomucin and to develop a simple method to purify ovomucin that could be adapted for further scale-up production.

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MATERIALS AND METHODS

Materials. Fresh eggs laid within 24 h from White Leghorn were collected in the morning from the Poultry Research Centre farm of the University of Alberta (Edmonton, Canada) and used within the same day for ovomucin preparation. Standard proteins (ovalbumin, ovotransferrin, ovomucoid, and lysozyme) were obtained from Neova Technologies Inc., Abbotsford BC, Canada. Sodium dihydrogen phosphate monohydrate, disodium orthophosphate heptahydrate, and hydrochloric acid were purchased from Fisher Scientific (Ottawa, ON, Canada). 2-Mercaptoethanol was obtained from MP Biomedicals, LLC (Solon, OH, USA), and sodium chloride was purchased from Acros Organics (Morris Plains, NJ, USA).

Effects of Salt Concentration on the Extraction of Ovomucin. Ovomucin was prepared as a function of sodium chloride (NaCl) concentrations at 0, 2.5, 5, 10, 50, 100, 200, and 500 mM. Egg white was manually separated from the egg yolk and then homogenized using a stirrer (IKA, RCT B S1, IKA Works Inc., Wilmington, NC, USA) for 30 min at ambient (22 °C) temperature. Stirring speed was set at 350 RPM to avoid foaming. To 180 mL of salt solution at various concentrations, 60 mL of homogenized egg white solution was added in centrifuge tubes. The pH of the solution was then adjusted to 6.0 using 1 N or 0.1 N HCl, while stirring gently at ambient temperature (22 °C) for 30 min using Forma Orbital Shaker (Model 416, Thermo Electron Corporation, Ohio, USA). The centrifuge tubes were kept at 4 °C overnight, and the precipitates (crude ovomucin) were then separated by centrifugation at 15,300*g* for 10 min by using an Avanti J-E refrigerated centrifuge (Beckman Coulter, Inc., Fullerton, CA, USA), freeze-dried, and stored at −20 °C until analysis. All of the experiments were carried out in duplicate.

Preparation of Ovomucin Using a 2-Step Method. The newly proposed protocol for the preparation of ovomucin is given in **Figure 1**. Ovomucin was first prepared using isoelectric precipitation in the presence of 100 mM NaCl solution. The dispersion was kept overnight at 4 °C and separated by centrifugation at 15,300*g* for 10 min at 4 °C. The precipitate was further suspended in 500 mM NaCl solution while stirring for 4 h followed by overnight settling at 4 °C. After centrifugation at 15,300*g* for 10 min at 4 °C, the precipitate was freeze-dried and stored at −20 °C until analysis. Here onward, this method will be termed as the 2-step extraction method.

Yield Determination. In the literature, the yield of ovomucin was normally expressed as weight of ovomucin (mg) out of 100 g of egg albumen. The yield was calculated by taking into account the protein concentration and purity percentage using the formula

$$\text{yield} = \frac{W_1 \times \text{protein\%} \times \text{purity\%}}{100 \text{ g of egg white}} \quad (1)$$

where, W_1 = weight of freeze-dried ovomucin powder obtained based on 100 g of egg white; protein % = protein content of freeze-dried ovomucin in percentage; and purity % = ovomucin purity in percentage.

Percentage yield was also calculated as a ratio of ovomucin weight between ovomucin obtained and ovomucin in raw egg white by using the following formula:

$$\text{yield} = \frac{W_1 \times \text{protein\%(ovomucin powder)} \times \text{purity\%(ovomucin powder)}}{W_2 \times \text{protein\%(egg white)} \times \text{ovomucin purity\%egg white}} \times 100 \quad (2)$$

where W_1 = weight of ovomucin powder prepared from W_2 of egg white and W_2 = weight of egg white required to prepare W_1.

Gel Filtration Chromatography (GFC). The purity of the prepared ovomucin was determined by using a High-load

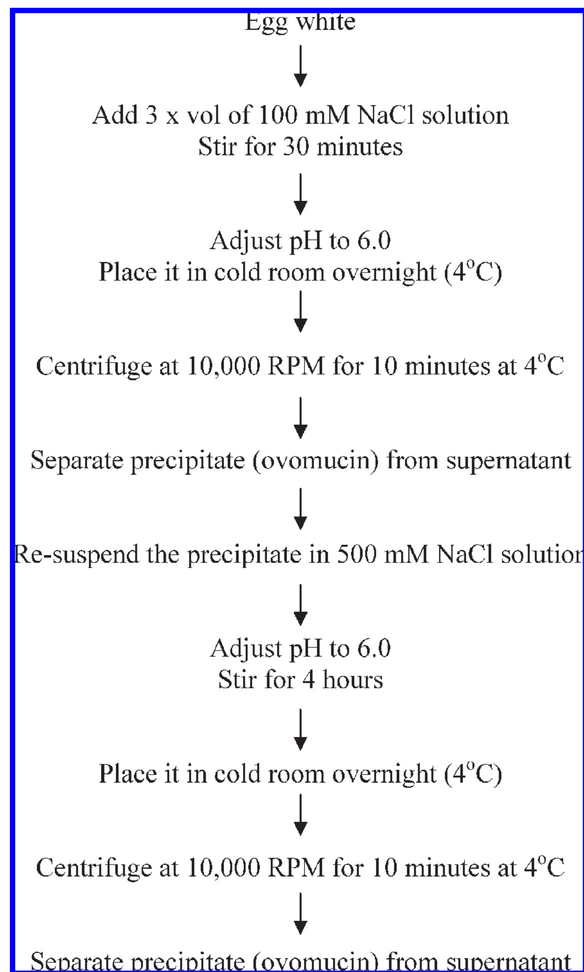

Figure 1. Protocol for ovomucin preparation by precipitation using 100 mM NaCl followed by 500 mM NaCl solution.

16/60 column (Superdex 200 preparatory grade, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) coupled with Fast-Performance Liquid Chromatography (FPLC) as described (*14*). Samples, at a concentration of 5 mg/mL, were prepared by dissolving ovomucin in 100 mM sodium phosphate buffer (pH 7.0) containing sodium dodecyl sulfate (50 mg/mL) and β-mercaptoethanol (10 μL/mL) by overnight stirring (200 RPM) at ambient (22 °C) temperature using an IKA, RCT B S1 stirrer (IKA Works Inc., Wilmington, NC, USA). The samples were filtered through mixed esters of a cellulose (MCE) syringe filter (0.45 μm, Fisher Scientific, Canada). The injection volume was 3 mL, and the column was eluted with 100 mM phosphate buffer (pH 7.0) containing SDS (5 mg/mL) and β-mercaptoethanol (1 μL/mL) at a flow rate of 1 mL/min monitoring at 280 nm.

The concentrations of coprecipitated proteins were calculated from standard protein curves, and the concentration of ovomucin was calculated by subtracting the amount of coprecipitated proteins according to Hiidenhovi et al. (*14*). All of the determinations were carried out in duplicate.

Sodium Dodecyl Sulfate−Polyacrylmide Gel Electrophoresis (SDS−PAGE). SDS−PAGE was carried out (*18*) using 10−20% ready gels (Bio-Rad Laboratories, Inc., Hercules, CA) at a constant voltage mode in a Mini-PROTEAN tetra cell (Bio-Rad Laboratories, Inc., Hercules, CA) attached to a PowerPac Basic electrophoresis apparatus (Bio-Rad Laboratories Inc., 1000 Alfred Nobel Drive, Hercules, California, USA). The loaded amount of proteins was

50 μg for all of the samples. Protein markers of high range molecular weight obtained from Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA) were loaded into a separate well for comparison of molecular weight. The gels were scanned using an Alpha Innotech gel scanner (Alpha Innotech Corp., San Leandro, CA) with FluorChem SP software.

Proximate Composition. The moisture and ash contents were determined by the AOAC method (*19*). Fat content was estimated using the Goldfish extraction method (*19*) and expressed as g/100 g (dry weight basis) sample. Protein content was determined using a TruSpec CN carbon/nitrogen determinator (Leco Corp., St. Joseph, MI) and multiplying nitrogen content by a factor of 6.25.

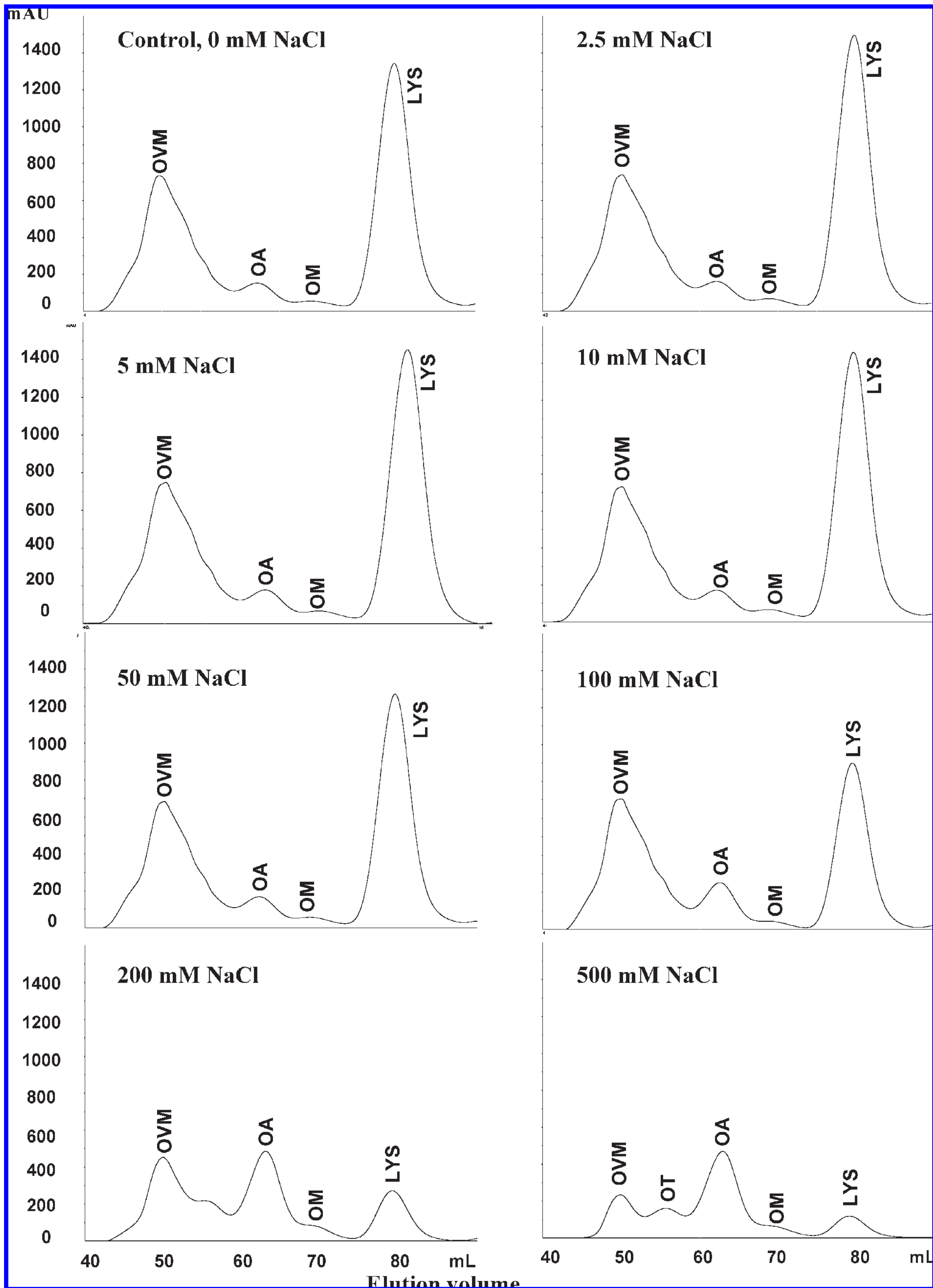

Figure 2. Gel filtration chromatogram of ovomucin prepared as a function of NaCl concentrations. OVM, ovomucin; OT, ovotransferrin; OA, ovalbumin; OM, ovomucoid; LYS, lysozyme.

Amino Acid Analysis. Amino acid analysis was performed on a Beckman System 6300 High Performance Analyzer using postcolumn ninhydrin methodology with Pickering Laboratories 15 cm sodium column and Pickering's sodium eluent buffers. Data was collected and analyzed using Beckman System Gold software. Samples were hydrolyzed under vacuum in 6 N HCl and 0.1% phenol for 1 h at 160 °C.

Statistical Analysis. All data were subjected to Analysis of Variance using the general linear models procedure of SAS (SAS version 9.0, SAS Institute, Cary, NC, USA, 2006), and significant differences were determined using Duncan's multiple range test at the level of $p < 0.05$ (*20*).

RESULTS AND DISCUSSION

Effect of Salt Concentrations on Protein Composition of Ovomucin. Although ovomucin can be easily prepared using isoelectric point precipitation, the purity of ovomucin was low due to the coprecipitation of other egg white proteins, mainly ovalbumin and lysozyme (*11*, *12*). Salt is known to play an important role in the solubility characteristics of proteins via influencing protein−protein and protein−solvent interactions (*21*). In the study, the effect of salt concentrations on the extractability of ovomucin was studied. The protein composition of the ovomucin precipitates prepared using isoelectric precipitation at different salt concentrations was analyzed by gel filtration chromatography (**Figure 2**). Gel filtration chromatographs of standard proteins viz. ovotransferrin, ovalbumin, ovomucoid, and lysozyme revealed that peak elution volumes were at 57.6, 64.4, 71.2, and 81.3 mL, respectively. The peak elution volume of ovomucin in the present study was 50.0 mL, compared to the previously reported value of 56 mL by Adachi et al. (*22*). Our results showed that the major contaminated proteins in the ovomucin precipitates are ovalbumin and lysozyme; at increasing salt concentrations, the peak corresponding to the ovalbumin fraction increased, whereas the peak corresponding to lysozyme decreased.

Our study showed that adding salt could significantly affect the protein composition of ovomucin (**Table 1**). Compared with the control (precipitation at pH 6 without salt addition and without 2% KCl washing), the lysozyme concentration in the precipitate was not affected at low salt concentrations but was significantly reduced at concentrations greater than 50 mM; the greatest reduction of lysozyme concentration in the precipitates was observed at salt concentrations of 200 and 500 mM. However, the content of ovalbumin was increased at increasing salt concentrations up to 200 mM. Ovomucoid was not significantly affected by salts to a concentration up to 200 mM. Ovotransferrin was found to be below the detection level at lower concentrations and was measurable only at 500 mM salt concentration by the GFC method but was detected by SDS−PAGE in all of the treatments (**Figure 3**); it is known that SDS−PAGE is more sensitive than the GFC method. Ovomucin extracts as

Table 1. Protein Composition of Ovomucin Samples Prepared As a Function of Salt Concentrations[a]

treatments	ovotransferrin (%)	ovalbumin (%)	ovomucoid (%)	lysozyme (%)	ovomucin (%)
control, 0 mM	—	7.6 c (0.4)	3.2 a (0.8)	25.5 b,c (0.5)	63.8 a,b (0.2)
2.5 mM	—	8.9 c (0.1)	5.4 a (2.4)	27.0 a,b (1.2)	58.8 b,c (1.2)
5 mM	—	9.6 c (2.2)	4.9 a (2.7)	26.0 a,b,c (0.9)	59.6 b (5.7)
10 mM	—	9.4 c (0.5)	4.3 a (0.3)	27.3 a (0.3)	59.0 b,c (0.0)
50 mM	—	11.0 c (1.3)	5.5 a (2.1)	24.9 c (0.5)	58.7 b,c (4.0)
100 mM	—	14.6 b (0.4)	2.2 a (0.4)	15.9 d (0.5)	67.4 a (0.4)
200 mM	—	41.7 a (2.9)	—	5.8 e (0.8)	52.6 c,d (2.1)
500 mM	7.2 (0.3)	41.9 a (1.6)	—	3.0 f (0.5)	48.0 d (1.3)

[a] Given values are mean values of duplicate trials. Values in parentheses represent standard deviation. Dissimilar letters in the same column denote significant difference ($P < 0.05$); — denotes below detection level. All samples were at the same pH of 6.0 under various salt concentrations.

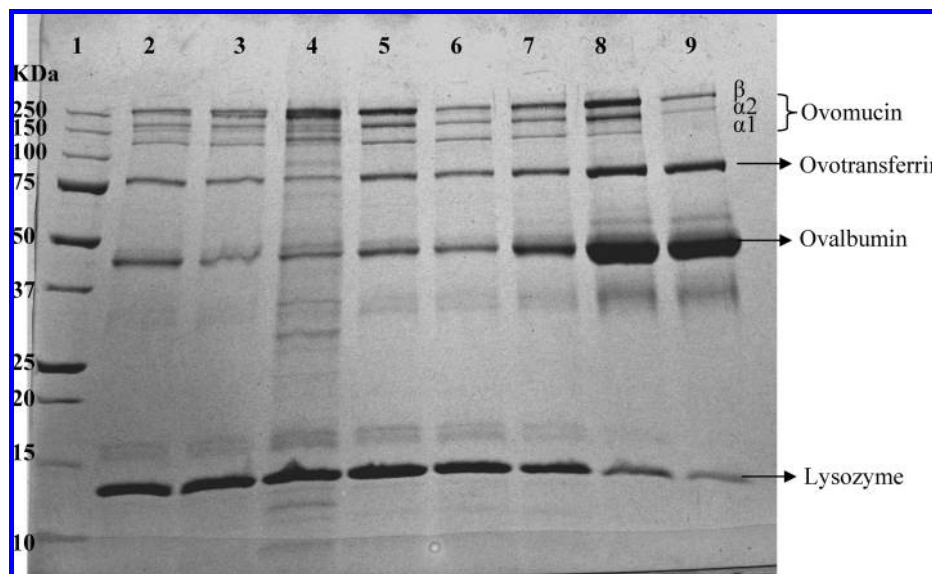

Figure 3. SDS−PAGE analysis of ovomucin extracts at different salt concentrations (lane 1, standard molecular weight markers; lane 2, 0 mM; lane 3, 2.5 mM; lane 4, 5 mM; lane 5, 10 mM; lane 6, 50 mM; lane 7, 100 mM; lane 8, 200 mM; lane 9, 500 mM).

a function of salt concentrations were also analyzed by using SDS−PAGE (**Figure 3**). Ovomucin was thought to be composed of a carbohydrate-poor component (α-ovomucin) and a carbohydrate-rich component (*β*-ovomucin) (*2*, *3*). Our results showed that ovomucin is composed of 3 components as reported (*4*) and that the molecular weights of *β*, α2, and α1 were 400,000, 220,000, and 150,000 Da, respectively. The intensity of ovalbumin was increased, whereas that of lysozyme was decreased with increasing salt concentrations, which was in good alignment with the results from the GFC analysis (**Table 1**). Ovotransferrin showed a trend similar to that of ovalbumin.

The control ovomucin sample (without salt addition and KCl washing) showed a purity of 63.8% (**Table 1**); this value was comparable to the previously reported value of 64% (*14*). Compared with the control, there was no significant ($P > 0.05$) change in the purity of ovomucin up to a NaCl concentration of 50 mM because of an increasing trend in ovalbumin content and a decreasing trend in lysozyme content (**Table 1**). The purity of prepared ovomucin attained a maximum value of 67.4% at a salt concentration of 100 mM due to the moderate coprecipitated levels of the 2 major contaminated proteins. Ovomucin precipitate at a concentration of 200 mM had the lowest amount of lysozyme but the highest amount of ovalbumin. Above these salt concentrations (≥200 mM), the purity of ovomucin decreased significantly with a significant increase in the content of ovalbumin and a significant decrease in lysozyme content ($P < 0.05$).

Ovomucin has been shown to be present in egg white as a complex with lysozyme (*23*) and other egg white proteins (*24*). It was estimated that 1.97 g of lysozyme could bind 2 g of ovomucin at pH 7.4 (*6*). The interactions between lysozyme and ovomucin are mainly electrostatic interactions and may involve two basic groups of the lysozyme molecule and one sialic acid residue of the ovomucin complex (*6*). Other secondary stabilizing forces (such as hydrophobic and hydrogen interactions) may aid in the stabilization of the complex. It is known that an ionic environment weakens the electrostatic interaction between the protein molecules by electrostatic shielding. A decrease in electrostatic interaction at high salt concentration is responsible for the observed decrease in the content of lysozyme at high salt concentrations. Magnesium ions and sodium chloride were also reported to decrease the interaction between ovomucin and lysozyme (*6*). However, at higher salt concentrations, ovalbumin precipitated more along with ovomucin. At increasing salt concentration, more of the water becomes associated with the ions and thus reduces the solvation layer and increases protein−protein interactions (*25*). Consequently, less water is available to partake in the solvation layer around the protein, which exposes hydrophobic patches on the protein surface and causes proteins to exhibit hydrophobic interactions, aggregate, and precipitate from solution.

Table 2. Yield of Ovomucin Prepared in the Presence of Various Salt Concentrations[a]

treatments	ovomucin content (mg/100 g of egg albumen)	ovomucin expressed as percentage of ovomucin to egg white (%)
control, 0 mM	281.5 b,c (0.9)	64.8 b,c (0.2)
2.5 mM	330.0 a (6.7)	75.9 a (1.5)
5 mM	265.0 c (15.5)	61.0 c (5.8)
10 mM	271.9 b,c (1.2)	62.6 b,c (0.2)
50 mM	264.5 c (17.8)	60.9 c (4.1)
100 mM	309.5 a,b (2.1)	71.2 a,b (0.5)
200 mM	244.6 c,d (9.9)	56.3 c,d (2.2)
500 mM	217.7 d (8.1)	50.1 d (1.8)

[a] Given values are mean values of duplicate trials; values in parentheses represent standard deviation; dissimilar letters in the same column denote significant difference ($P < 0.05$).

The yield of ovomucin precipitates prepared as a function of NaCl concentrations ranged from 217.7 to 330.0 mg/100 g of egg albumen or from 50.1% to 75.9% expressed in ovomucin percentages (**Table 2**). A wide range of ovomucin yield was reported in literature. The yield of the control sample was 281.5 mg/100 g of egg albumen in the study, compared to reported values of 90−117 mg/100 g of albumen after an exhaustive washing procedure by Brooks and Hale (*26*), or 530 mg/100 g of whole egg white (*27*). A yield of 120 mg of ovomucin in 100 g of egg white was reported, but the KCl washings contained 360 mg of ovomucin (*16*). The maximum yield was observed at a salt concentration of 2.5 mM, followed by 100 mM NaCl concentration ($P > 0.05$).

Ovomucin Prepared by a 2-Step Method. The above results suggested that maximum ovomucin purity can be achieved by precipitating using 100 mM NaCl concentration, wherein the concentrations of the major coprecipitating proteins were at the medium level (the concentrations of ovalbumin and lysozyme were 14.6% and 15.9%, respectively). It was also noticed that the lowest lysozyme concentration was found at 500 mM NaCl, whereas the highest ovalbumin concentration was at salt concentrations greater than 200 mM. The reason for the difference in the interaction of ovalbumin and lysozyme with salt may be due to the high amount of arginine (12.7%) in lysozyme and glutamic acid (16.5%) in ovalbumin (*28*). On the basis of the above two findings, a new method was proposed to precipitate ovomucin at 100 mM NaCl solution to achieve low ovalbumin contamination and further to remove lysozyme in the second step by using 500 mM NaCl concentration as shown in **Figure 1**. A gel filtration chromatogram of ovomucin prepared using the 2-step extraction method is given in **Figure 4B**. The peaks from the gel filtration curve showed the presence of a major peak corresponding to ovomucin and 2 minor peaks corresponding to ovalbumin and lysozyme, which denote an increased purity of ovomucin. The purity of the ovomucin was 94.6% (**Table 3**); the percentages of coprecipitated proteins, ovalbumin and lysozyme, were 3.7% and 1.8%, respectively. The gel filtration chromatogram of ovomucin prepared using the conventional method (dilution with water, precipitation at pH 6, followed by washing the precipitate using 2% KCl until the supernatant becomes protein free) is given (**Figure 4A**) for comparison. Contamination of ovomucin prepared by the conventional method is evident from peaks corresponding to ovalbumin, ovomucoid, and lysozyme. The purity of the ovomucin prepared by the conventional method was 66.8% due to a high concentration of ovalbumin (14.8%), ovomucoid (9.0%), and lysozyme (9.5%). To test weather the overnight settling procedure is necessary, we reduced the settling time from overnight to 2 h. As shown in **Figure 4C**, a purity of 93.0% was obtained (**Table 3**), which is comparable to the value obtained from overnight settling. Our results showed that highly pure ovomucin can be prepared by using the 2-step method of salt extraction and that the purity was not affected by reducing the settling time from overnight settling to 2 h of settling. It is clear that the 2-step method can

considerably reduce the coprecipitated proteins during ovomucin extraction. Ovomucin with a purity of 80% was reported by using gel permeation chromatography (*29*). The yield of crude ovomucin prepared using the conventional method was 185.5 mg/100 g of egg albumen, compared to 400.2 mg/100 g of egg white prepared by using the 2-step extraction method (**Table 4**). A similar yield of 391.3 mg/100 g of egg white was obtained at the reduced settling time in the study. The difference in the yield of ovomucin prepared by different researchers (*8*, *11*, *25*) may be attributed to the difference in the methods of preparations.

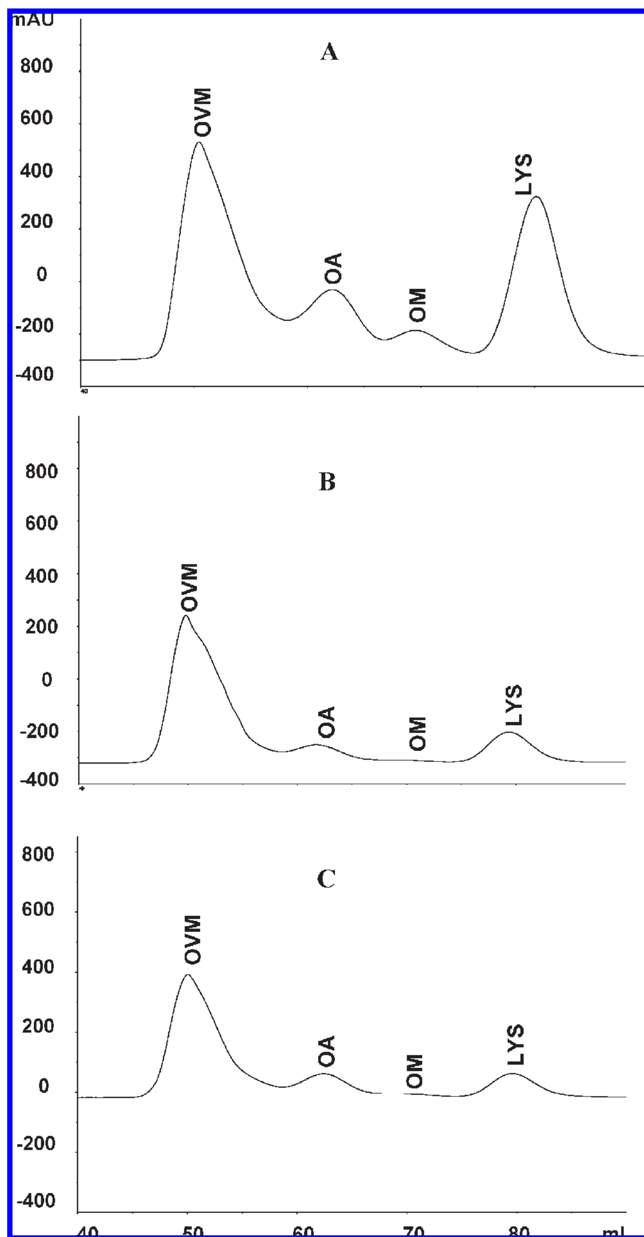

Figure 4. Gel filtration chromatogram of ovomucin prepared (**A**) by the conventional method, (**B**) by precipitation using 100 mM followed by 500 mM NaCl solution, and (**C**) by precipitation using 100 mM followed by 500 mM NaCl solution at reduced time.

The prepared ovomucin was further analyzed by SDS−PAGE. SDS−PAGE revealed strong bands above 250 kDa and one band between 250 kDa and 150 kDa, which corresponds to ovomucin subunits (**Figure 5**). Only faint bands were seen for ovalbumin (45 kDa) and lysozyme (14.5 kDa), which further confirms the gel filtration results. Ovomucin is the only protein in egg white protein having a molecular weight as high as this. The molecular weight of α-ovomucin was estimated to be 210 kDa (*3*). SDS−PAGE of ovomucin showed bands at 150, 220, and 400 kDa corresponding to α1, α2, and β ovomucin, respectively (*4*). In the present study, the purified ovomucin showed bands corresponding to β- and α2 subunits of ovomucin as suggested by Itoh et al. (*4*). The band corresponding to α1 was absent likely because the α1 subunit was washed off at the second step of 500 mM NaCl extraction; the SDS−PAGE pattern of ovomucin prepared at different salt concentrations also revealed the absence of a band corresponding to α1 ovomucin at this concentration (500 mM) (**Figure 3**). There was no difference between the bands of ovomucin prepared at different settling times. The SDS−PAGE pattern of ovomucin prepared using the conventional method showed less intense bands for ovomucin and more intense bands corresponding to ovalbumin, lysozyme, and ovotransferrin. SDS−PAGE of freeze-dried egg white is given for comparison.

Proximate composition of ovomucin prepared by the 2-step method (after dialysis) revealed a protein content of 69.7% (**Table 5**). The protein content of ovomucin isolated from thick white and thin white was found to be 66.1% and 71.5%, respectively (*22*). The protein content of the control ovomucin (without salt addition and without KCl washing) and freeze-dried egg white was found to be high due to the low contents of moisture and ash. Fat content of all the samples were found to be minimum. Information on the proximate composition of ovomucin is scarce except for its protein content. Donovan et al. (*11*) showed that the

Table 4. Yield of Ovomucin Prepared Using Various Methods[a]

treatments	ovomucin content (mg/100 g of egg albumen)	ovomucin expressed as the percentage of ovomucin in egg white (%)
conventional ovomucin	185.5 b (1.8)	61.8 b (0.6)
ovomucin prepared by 2-step method	400.2 a (4.8)	92.1 a (1.1)
ovomucin prepared at reduced time	391.3 a (2.4)	90.0 a (0.5)

[a] Given values are means of duplicate trials; values in parentheses represent standard deviation; dissimilar letters in the same column denote significant difference ($P < 0.05$).

Table 3. Protein Composition of Ovomucin Samples Prepared Using Various Methods[a]

treatments	ovotransferrin (%)	ovalbumin (%)	ovomucoid (%)	lysozyme (%)	ovomucin (%)
conventional ovomucin	0.0	14.8 a (0.0)	8.9 a (0.6)	9.5 a (0.0)	66.8 b (0.6)
ovomucin prepared by 2-step method	0.0	3.7 b (0.1)	1.1 b (0.8)	1.8 b (0.1)	94.6 a (1.1)
ovomucin prepared at reduced time	0.0	5.2 b (0.1)	0.4 b (0.1)	1.4 c (0.1)	93.0 a (0.6)

[a] Given values are mean values of duplicate trials; values in parentheses represent standard deviation; dissimilar letters in the same column denote significant difference ($P < 0.05$).

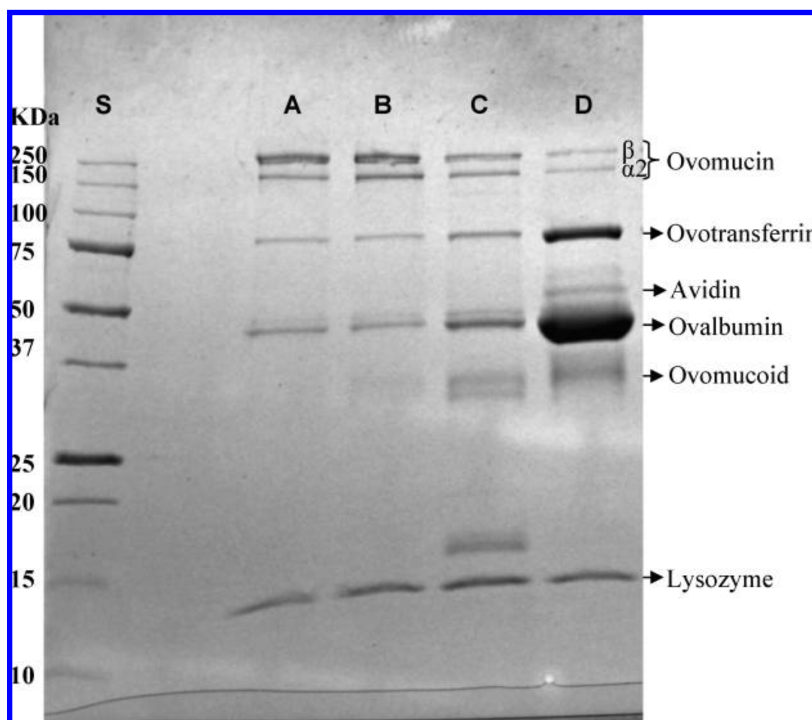

Figure 5. SDS−PAGE pattern of ovomucin prepared under various conditions; egg white is given for comparison. (S) Standard marker; (A) precipitation with 100 mM NaCl followed by 500 mM NaCl, with overnight settling; (B) precipitation with 100 mM NaCl followed by 500 mM NaCl, with 2 h of settling; (C) conventional ovomucin; (D) egg white protein.

Table 5. Proximate Composition of Ovomucin Prepared by the 2-Step Precipitation Method[a]

samples	moisture (g/100 g)	protein (g/100 g)	fat (g/100 g)	ash (g/100 g)
ovomucin prepared by 2 step extraction	4.1 b (0.2)	69.7 c (0.4)	0.2 b (0.0)	9.5 b (0.4)
ovomucin prepared by conventional method	2.6 c (0.1)	60.1 d (1.4)	0.6 a (0.1)	33.5 a (0.5)
control ovomucin (without salt addition and without KCl washing)	2.5 c (0.1)	88.2 a (0.1)	0.7 a (0.1)	4.7 c (0.1)
freeze-dried egg white	5.5 a (0.3)	84.5 b (0.2)	0.3 b (0.0)	5.8 c (0.1)

[a] Given values are means of duplicate trials; values in parentheses represent standard deviation; dissimilar letters in the same column denote significant difference ($P < 0.05$).

Table 6. Amino Acid[a] Composition of Ovomucin Prepared by the 2-Step Precipitation Method: Comparison with Previously Published Data of Ovomucin Prepared by Different Methods

amino acids	this study	Donovan et al. (*11*)[b]	Robinson and Monsey (*3*)[c]	Young and Gardner (*17*)[d]
lysine	7.4	5.5	6.3	5.3
histidine	2.5	2.0	2.3	1.9
arginine	4.6	3.43	3.2	3.1
aspartic acid	12.0	9.6	9.2	8.7
threonine	7.4	6.4	8.5	9.0
serine	6.8	8.8	9.4	9. 6
glutamic acid	12.0	11.2	10.3	10.0
glycine	7.7	6.1	6.0	6.9
alanine	6.4	6.5	4.8	6.0
cysteine	4.7	4.3	7.3	8.1
valine	7.8	7.6	6.2	6.4
methionine	2.0	2.7	2.1	0.6
isoleucine	5.5	5.2	4.4	4.5
leucine	8.1	7.8	6.7	7.1
tyrosine	3.4	3.2	3.0	2.7
phenylanlanine	3. 9	4.5	4.0	3.5
cysteic acid	0.33	n.d.	n.d.	n.d.
tryptophan	n.d.[e]	1.5	0.8	n.d.

[a] Expressed as mol/100 moles. [b] Modified MacDonnell et al. (1951) method. [c] Brooks and Hale (1961) method. [d] Gel filtration method. [e] n.d., not determined.

proximate composition of air equilibrated freeze-dried ovomucin was 12.6% nitrogen, 9.24% moisture, and 2.11% ash. The higher ash content in ovomucin prepared by the conventional method is due to KCl washing.

In general, amino acid composition of ovomucin prepared by the 2-step method (**Table 6**) is comparable with that of previous reports (*11*, *12*). However, serine content was found to be slightly lower than that in previous works, whereas lysine, arginine, aspartic acid, glutamic acid, and glycine were found to be in higher proportion. Because the prepared ovomucin contained both β- and α2 subunits of ovomucin and the β- ovomucin contained more carbohydrate and less protein than that of α-ovomucin, amino acid composition of the prepared ovomucin reflects predominantly that of α-ovomucin.

A 2-step method of ovomucin extraction was developed for the first time to purify ovomucin at a purity of greater than 90%, compared with the purity of less than 70% in the literature by the conventional method. Our method is simple, environmentally friendly, and easy for scale-up preparations. Ovomucin has been reported to have vast potential to be developed as functional foods and neutraceutical ingredients; the development of a method to purify ovomucin will facility our further work, which is under way to explore this great potential.

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