Observations on the Dissociation of β-Conglycinin into Subunits by Heat Treatment

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A heat-induced transparent solution consisting of dissociates can be prepared under appropriate conditions: β-conglycinin preparations, pH, and salt affected the thermal dissociation. Experimental conditions studying quantitative dissociation into subunits and analyzing the dissociation process using gel filtration without artifact are examined. β-Conglycinin dissolved in distilled water (0.5% w/v, pH 7.5) can be heated without turbidity even at 100 °C for 30 min. The elution buffer, 3.2 mM potassium phosphate (pH 7.6, $I = 0.01$), did not affect the gel filtration profile of heat-induced products, whereas buffers of ionic strength above 0.02 caused aggregation of dissociate and a decrease in a portion of the dissociation peak. It was found that upon heating, β-conglycinin dissociated into its subunits and they can exist in dissociated form unless salt is added to the system. The main factor governing these dissociation characteristics of β-conglycinin is considered to be that the mutual repulsion forces arising from hydrophilic domains of the subunits are superior to hydrophobic association.

INTRODUCTION

Proteins are generally aggregated as a result of heat denaturation (Privalov, 1979). The heat denaturation of globular protein is proposed as follows: the conversion of a native molecule to a denatured state involving dissociation into subunits and rupture of hydrophobic bonds followed by irreversible denaturation and aggregation through the exposed hydrophobic regions. A third phase is the formation of larger protein aggregates. However, the initial protein unfolding steps have not been sufficiently investigated, since aggregation causes a great problem for all physical methods. Recently, it has been reported that under the appropriate conditions hen egg ovalbumin (Hegg et al., 1979; Hatta et al., 1986) and proteins with lower contents of hydrophobic groups (Shimada and Matsushita, 1980) did not show turbidity upon heating and formed transparent solutions and gels.

β-Conglycinin is a complex protein that exhibits polymorphism (B_0–B_6) in its subunit composition consisting of different combinations of three subunits, α, α′, and β (Thanh and Shibasaki, 1978; Yamauchi et al., 1981; Sykes and Gayler, 1981). β-Conglycinin has a characteristic of 7S ⇌ 9S (dimer of 7S) interconversion with a change of ionic strength; it possesses a protomer form (7S; trimeric structure) at high ionic strength above 0.5 and a dimer form (9–10S; hexameric structure) at low ionic strength below 0.2. At very low ionic strength ($I < 0.01$), the α subunit dissociated from the protomer (Thanh and Shibasaki, 1979). In the case of β-conglycinin, the dissociation step is also unclear. Thermal denaturation of soybean globulins has been extensively investigated with respect to the process of aggregation of denatured molecules (Damodaran and Kinsella, 1982; Mori et al., 1982; Iwabuchi and Shibasaki, 1981; Yamagishi et al., 1987) and following gel network formation (Nakamura et al., 1984; Hermansson, 1986). However, there are few reports concerning thermal denaturation of β-conglycinin (Hashizume et al., 1975; Hashizume and Watanabe, 1979; Iwabuchi and Shibasaki, 1981; Nakamura et al., 1986) compared with what is known about glycinin. Among these studies on thermal aggregation with turbidity, only our previous works have proposed that under limited salt-free conditions a transparent solution of β-conglycinin has been prepared by heating at 100 °C for 5 min, and the protein composition of this heated solution was dissociated subunits (Iwabuchi and Shibasaki, 1982; Iwabuchi and Yamauchi, 1984).

Turbidity arising from protein aggregation disturbs the progress of thermal denaturation research using optical measurements. The aim of the present study, therefore, is to establish the experimental conditions that will reproducibly yield a clear solution after heating and to obtain a better understanding of the thermal dissociation of β-conglycinin from the evaluation of the effects of heating temperature, pH, and ionic strength, using gel filtration and polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Materials. Soybeans (*Glycine max* var. Raiden) were finely ground and defatted with hexane at room temperature. Defatted soybean meals were extracted with 0.03 M Tris-HCl (pH 8.0) buffer in the presence of 10 mM 2-mercaptoethanol (2-ME) at a solvent-to-meal ratio of 20:1 at room temperature. After removal of the glycinin fraction by isoelectric precipitation at pH 6.4, the β-conglycinin fraction was precipitated at pH 4.8 according to the method of Thanh and Shibasaki (1976a). This crude protein fraction was further fractionated with ammonium sulfate, and purified β-conglycinin was finally obtained by gel filtration on a Sepharose CL-6B column according to a previously reported method (Iwabuchi and Yamauchi, 1987). Sepharose CL-6B was purchased from Pharmacia Co., and all other reagent grade chemicals were obtained from Nakarai Chemicals Co.

Separation of β-conglycinin molecular species (B_1–B_6) into its components, group A ($B_1 + B_2$), group B ($B_3 + B_4$), and group C ($B_5 + B_6$), was carried out according to the method of Thanh and Shibasaki (1976b, 1978).

Preparation of Protein Solutions. During the course of attempts to isolate β-conglycinin, we noticed that dialysis greatly affects the quality of protein samples. Therefore, the effect of time of dialysis was investigated. The gel-filtrated β-conglycinin solution, 32.5 mM potassium phosphate buffer containing 0.4 M NaCl, pH 7.6, was dialyzed against 20–50 volumes of distilled water with six or seven changes for 3 days in cellulose tubing (Union Carbide Co.).

To avoid the formation of extensive precipitation during dialysis, we stopped dialyzing at pH 6.4–6.6, where a slight amount

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of precipitate appeared. The dialyzed protein solution was then divided into four parts and back-titrated with 0.1 N NaOH to pH 7.0, 7.3, 7.5, and 8.0, respectively. These four aliquots, obtained finally as a clear solution at each pH, were freeze-dried. In contrast, if the β-conglycinin solution was exhaustively dialyzed below pH 6.0, large amounts of β-conglycinin were precipitated and remained partly insoluble even after back-titration to pH 8.0.

Heat Treatment. Freeze-dried materials were dissolved in distilled water or in appropriate buffers to give a 0.5% solution and centrifuged. Four-milliliter aliquots were then transferred to test tubes (16 × 125 mm) with a screw cap. Sample solutions were heated to the desired temperature and held for the desired period of time by immersion in a temperature-controlled water bath (±0.01 °C). Heated samples were removed from the bath after different intervals and cooled in cold water.

Buffers. Nonbuffered solutions of various ionic strengths were prepared by adding NaCl to distilled water. Buffered solutions of potassium phosphate, pH 7.6, with various ionic strengths were prepared according to previous methods (Iwabuchi and Yamauchi, 1984). All solutions used for heat treatment did not contain 2-ME.

Sepharose CL-6B Gel Chromatography. Heat-treated protein solution was applied to a 1.0 × 110 cm column of Sepharose CL-6B equilibrated with a eluting buffer at room temperature. Previously we reported that thermally induced dissociates of β-conglycinin have been readily renatured to the original structure by the addition of salt (Iwabuchi and Shibasaki, 1982). Therefore, it is necessary to study the effect of ionic strength contained in an elution buffer on the thermally dissociated protein sample during gel filtration. Experimental details for elution buffer are given below (see Results).

The void volume (V_0) was taken as the peak of the elution profile of Blue Dextran 2000 (Pharmacia Co.). Twenty milligrams of protein dissolved in 4 mL of water was applied to the column. Fractions (1 mL) were collected, and the elution profiles were determined by the absorbance at 280 nm.

Turbidity Measurements. The turbidity of protein solutions formed by heating was recorded at 420 nm.

Ultracentrifugation. Ultracentrifugal analysis of the heat-induced dissociates was performed with a Hitachi UCA-1 ultracentrifuge at 55 430 rpm using a 0.69% protein concentration. The sedimentation coefficient was represented as $s_{20,w}$.

Polyacrylamide Gel Electrophoresis (PAGE). The Ornstein–Davis (native) system gel electrophoresis and urea–sodium dodecyl sulfate (SDS)–PAGE were carried out according to previous methods (Iwabuchi and Shibasaki, 1982).

Two-Dimensional PAGE. First, the protein components of unheated and heat-denatured β-conglycinins were separated by a native system PAGE using glass tubes (2.5 × 80 mm) of 6.5% polyacrylamide gel. Second, the subunits composed of these separated components were further fractionated by a Laemmli system SDS–PAGE (Laemmli, 1970) using a slab gel (130 × 130 mm) of 10% polyacrylamide gel.

RESULTS

Factors Affecting Electrostatic States of β-Conglycinin Molecules. Influence of Careful Adjustment of pH. The effect of different preparations of β-conglycinin on the thermal dissociation was investigated. Table I shows the conditions for preparation of freeze-dried materials, the pH values of the β-conglycinin solution shown in the 0.5% solution, and turbidity at 420 nm measured after heating. The solutions of protein samples I and II, of which the pH values were close to 7.5 and 7.2, respectively, had no turbidity even after heating at 100 °C for 5 min, while the solutions of samples III and IV, of which the pH values were 6.9, formed a slight amount of thermal aggregates as indicated by the increase in OD_{420nm}. This turbidity was narrowly caught with the naked eye. Such a slight amount of turbidity, however, makes it impossible to study the conformational changes by using optical measurements. Thus, turbidity development was largely dependent on the period of dialysis and careful adjustment of the back-titration pH, which seems to have an influence on the electrostatic characteristics of the β-conglycinin molecule.

Table I. Effect of Dialyzing Period and pH on the Quality of Prepared β-Conglycinin

sample	conditions for preparation	pH dissolved in water[a]	turbidity at 420 nm[b]
I	dialyzed to pH 6.4–6.6 and back-titrated to pH 8.0	7.5	0.007
II	dialyzed to pH 6.4–6.6 and back-titrated to pH 7.5	7.2	0.007
III	dialyzed to pH 6.4–6.6 and back-titrated to pH 7.3	6.9	0.037
IV	dialyzed to pH 6.4–6.6 and back-titrated to pH 7.0	6.9	0.037

[a] pH values of 0.5% solutions of β-conglycinin dissolved in distilled water were measured. [b] Protein solutions of 0.5% concentration dissolved in distilled water were heated at 100 °C for 5 min, and then turbidity was measured.

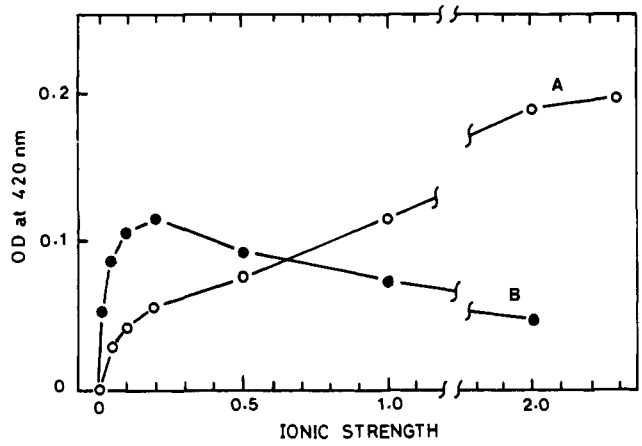

Figure 1. Turbidity developed by heating the β-conglycinin solutions in the presence of various ionic strengths using potassium phosphate, pH 7.6 (A), and NaCl (B). Protein (0.5% w/v) was dissolved in various ionic strength buffers and heated at 100 °C for 5 min. Turbidity was measured at 420 nm.

Shielding of repulsion forces among denatured β-conglycinin resulted in a profound increase in turbidity upon heating (Figure 1). These readily aggregating characteristics are in good agreement with previous reports (Iwabuchi and Yamauchi, 1984; Hashizume et al., 1975; Hashizume and Watanabe, 1979).

Results obtained by turbidity measurements described above (Table I; Figure 1) demonstrated the fact that under the limited salt-free conditions and pH range above 7.2 a transparent solution of β-conglycinin was obtained upon heating at 100 °C for 5 min. These conditions for preparing a transparent solution of β-conglycinin are similar to those for ovalbumin: that is, a transparent gel of ovalbumin is prepared as neutral pH (7.5) and low ionic strength (0.2 M NaCl) (Hatta et al., 1986).

Ultracentrifugal Analysis of the Heat-Induced Dissociates. The heat-induced products of β-conglycinin (unless otherwise stated, sample I shown in Table I was used as the protein sample for the following thermal denaturation study) formed upon heating (100 °C for 5 min) in distilled water were analyzed by ultracentrifugation (Figure 2). The results show that the heat-denatured β-conglycinin is composed mainly of 3.45S, which refers to dissociated monomers, although a small peak of 9.7S was recognized. This value of 3.45S is close to the $s_{20,w}$ of the monomer (3.1S) dissociated at low ionic strength of 0.01 (Thanh and Shibasaki, 1979).

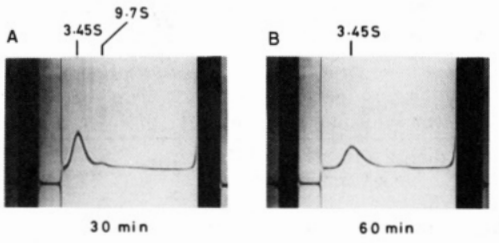

Figure 2. Ultracentrifugal patterns of heat-dissociated β-conglycinin. β-Conglycinin (0.69% w/v) dissolved in distilled water was heated at 100 °C for 5 min and cooled. Photographs were taken 30 (A) and 60 min (B) after 55 430 rpm was reached. The sedimentation coefficient is shown as $s_{20,w}$ in Svedberg units.

Influence of Ionic Strength in the Eluting Buffer for Gel Filtration. Gel filtration is available for obtaining data on thermal denaturation which causes changes in the quaternary structure, i.e., dissociation into subunits, association of these subunits, and aggregation into large molecules. However, as indicated by the results mentioned above, the charged groups of β-conglycinin molecules exposed by heating should be influenced by the salts included in the elution buffer.

The protein solutions of heat-denatured β-conglycinin, which is established to be completely dissociated into monomers as shown in Figure 2, were analyzed on a Sepharose CL-6B column. When the phosphate buffer of low ionic strength ($I = 0.01$) was used as the eluting buffer, the elution profile gave a symmetrical single peak ($V_e/V_0 = 1.48$) and native system PAGE showed the presence of dissociated monomers at relative mobility (R_m) of 0.53 (Figure 3) (see the unheated control in Figure 4b). The increase in ionic strength of the phosphate buffer from 0.01 to 0.05 resulted in a significant appearance of a shoulder peak at $V_e/V_0 = 1.22$ following a decrease in the portion of the dissociate peak at $V_e/V_0 = 1.48$ (Figure 3b–d). When the unbuffered 0.01 M NaCl solution was used as the eluting buffer, extensively aggregated components at $V_e/V_0 = 1.0$ (Figure 3e) were newly appeared. By use of the native system PAGE, it was clarified that the components at $V_e/V_0 = 1.35$ and 1.0 referred to the renatured β-conglycinin and aggregates of higher molecules, respectively, as shown by the inserts in Figure 3. The renaturation of β-conglycinin during gel filtration was dependent upon the salt concentration and salt species; this phenomenon is in good agreement with a previous study (Iwabuchi and Shibasaki, 1982). Thus, it is remarkable that the ionic strength of the phosphate buffer above 0.02 had great influence on the elution profiles of the thermally dissociated products. These results indicate that in the reaction system of phosphate buffer the protein association reaction occurs reversibly at the subunit level. On the other hand, in the reaction system containing NaCl, irreversible aggregation occurs more randomly and forms higher molecule complexes.

When the sample solution was eluted with distilled water or phosphate buffer of low ionic strength ($I = 0.01$), there were no changes between these elution profiles. Therefore, among the elution profiles in Figure 3, only the profile of Figure 3a represents the real pattern of the denatured products of β-conglycinin. Generally, the eluting buffer for Sepharose CL-6B required at least 0.02 ionic strength to suppress the ionic interaction between the proteins and the gel matrix (Pharmacia Technical Bulletin, "Gel Filtration"). However, our results show that 3.2 mM potassium phosphate ($I = 0.01$, pH 7.6) can be used successfully as an eluting buffer for Sepharose CL-6B. From the results of elution profiles using a Sephadex G-200

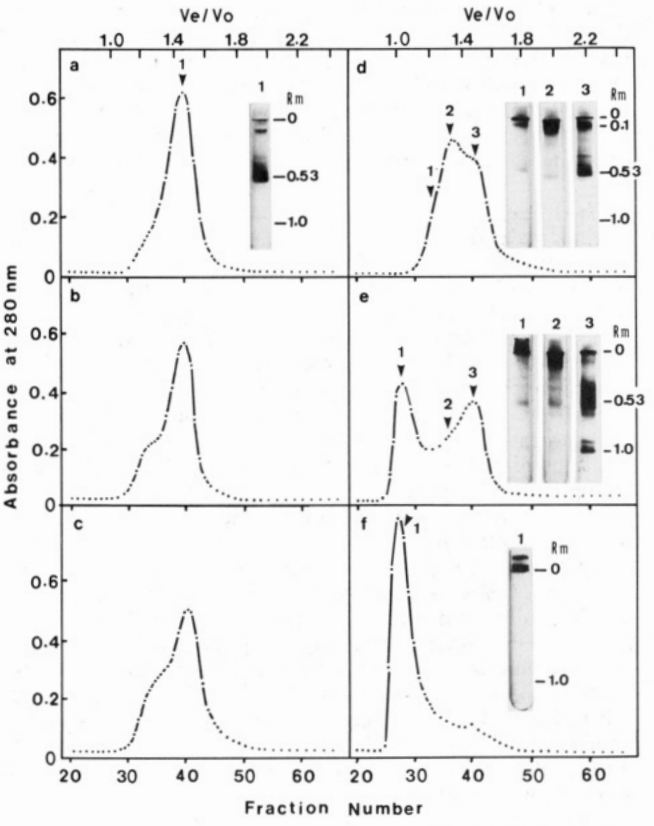

Figure 3. Effect of ionic strength contained in the eluting buffer on the elution profiles of heat-induced dissociates of β-conglycinin. β-Conglycinin solution (20 mg/4 mL of distilled water) was heat-treated at 100 °C for 5 min and then injected onto the Sepharose CL-6B column (1 × 110 cm). The elution profiles were obtained by eluting the column using 3.2 mM phosphate buffer ($I = 0.01$, pH 7.6) (a), 6.3 mM phosphate buffer ($I = 0.02$, pH 7.6) (b), 12.6 mM phosphate buffer ($I = 0.04$, pH 7.6) (c), 15.8 mM phosphate buffer ($I = 0.05$, pH 7.6) (d), and unbuffered solution of 10 mM NaCl ($I = 0.01$) (e). Extremely aggregated β-conglycinin prepared by heating in the presence of 0.1 M NaCl is shown (f). Inserts show the Ornstein–Davis (native) system PAGE of the protein solutions indicated by the arrow. The scale of the relative mobility values (R_m) was assigned by taking the separating line of the stacking and separating gels as the 0.00 points and the position of the fastest moving dye as 1.00.

column under the same conditions used for Sepharose CL-6B, it was found that Sephadex G-200 is not adequate for monitoring the thermal dissociation of β-conglycinin since a large peak of higher molecular weight except for the dissociate peak was newly formed during elution (data not shown).

Thermal denaturation of β-conglycinin using each preparation of samples I–IV shown in Table I was investigated by gel filtration under the eluting conditions described above. The elution profiles of native β-conglycinin that is dissolved in the 32.5 mM phosphate buffer, pH 7.6, containing 0.4 M NaCl and 0.02% sodium azide showed a symmetrical peak at $V_e/V_0 = 1.33$ (Figure 4a). The quaternary structure of β-conglycinin was stabilized under the higher ionic strength as supported by the insert PAGE pattern. The quaternary structure, however, is labile in distilled water or in very low ionic strength ($I \sim 0.01$) buffer and is partly broken down, following the formation of the dissociated subunit peak at $V_e/V_0 = 1.48$ composed of α and α′ subunits (Figures 4b and 8a). When the preparations of samples I and II were used as β-conglycinin for studying thermal denaturation, the elution profiles gave a major peak of dissociated subunits at $V_e/V_0 = 1.48$, although a slight peak at $V_e/V_0 = 1.30$ appeared

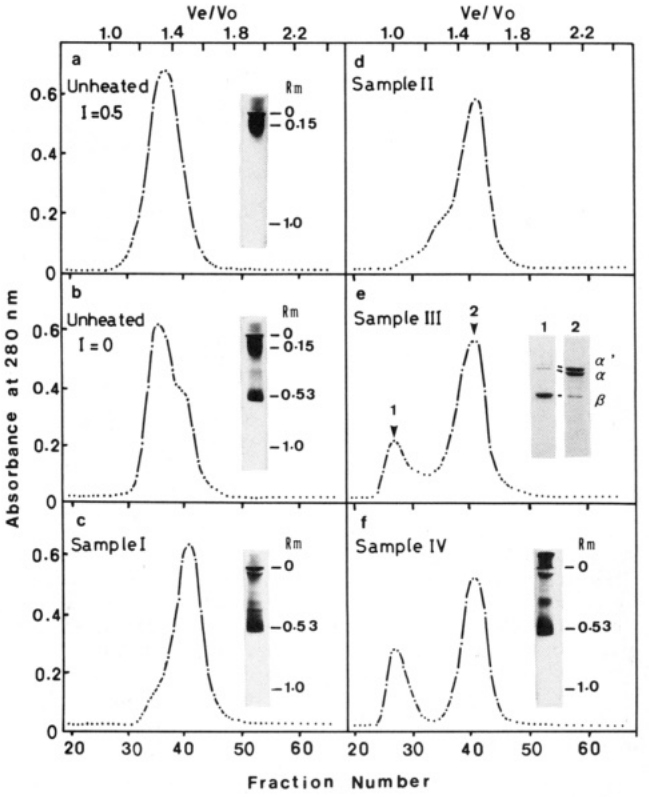

Figure 4. Gel filtration analysis of the effect of preparations on the thermal dissociation of β-conglycinin into subunits. Protein samples I–IV refer to Table I. Each preparation (20 mg) was dissolved in 4 mL of distilled water and heated at 100 °C for 5 min. Illustrated are the elution profiles obtained with the Sepharose CL-6B column (1 × 110 cm). Elution profiles of b–e were obtained with the elution buffer of 3.2 mM potassium phosphate (pH 7.6). Profiles a and b are shown for unheated β-conglycinin solutions dissolved in 32.5 mM potassium phosphate containing 0.4 M NaCl (I = 0.5, pH 7.6) and dissolved in distilled water, respectively, and eluted with 32.5 mM phosphate buffer containing 0.4 M NaCl (I = 0.5, pH 7.6) and 3.2 mM phosphate buffer (I = 0.01, pH 7.6), respectively. Inserts show the native system PAGE of unfractionated whole protein solutions unheated (a and b) and heated (c and f). Insert in (e) shows the SDS–PAGE of the fractions indicated by the arrow.

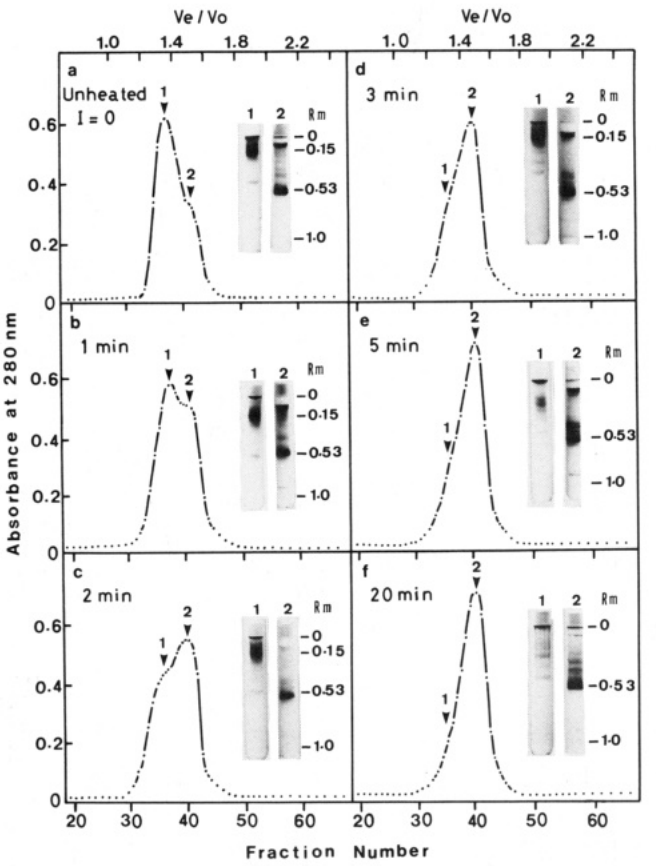

Figure 5. Chromatography of β-conglycinins heat-treated at 65 °C for various times on a column of Sepharose CL-6B (1 × 110 cm) eluted with 3.2 mM phosphate buffer (I = 0.01, pH 7.6). Elution profiles are shown for unheated β-conglycinin dissolved in distilled water (a) and denatured β-conglycinin heated at 65 °C for 1 (b), 2 (c), 3 (d), 5 (e), and 20 min (f). Inserts show the native system PAGE of the protein solutions indicated by the arrow.

(Figure 4c,d). When the preparations of samples III and IV were used as protein samples, the gel filtration profiles gave a new peak of aggregate at V_e/V_0 = 1.0 consisting mainly of β subunits (Figure 4e,f). The component of this peak was equal with the extensively aggregated compounds with turbidity which are formed by heating in the presence of 0.1 M NaCl (Figure 3f). The appearance of this component caused turbidity of β-conglycinin solutions in the case of samples III and IV (see Table I).

From the results of turbidity measurements and elution profiles, the conditions for obtaining a transparent solution of β-conglycinin upon heating and monitoring the thermal dissociation without aggregate formation have been established as follows. (1) Sample preparations: dialysis against distilled water must be stopped at the point pH 6.4–6.6 and then the protein solution is back-titrated to pH 8.0 before freeze-drying. (2) Gel filtration for monitoring thermal dissociation: to avoid the formation of secondary products during gel filtration analysis, it is adequate to use Sepharose CL-6B and an eluting buffer of low ionic strength buffer (3.2 mM potassium phosphate, pH 7.6, I = 0.01, not containing NaCl).

Thermal Dissociation Study of β-Conglycinin. The time course of the dissociation into subunits by heating was followed with gel filtration according to the conditions mentioned above. The thermal dissociation of β-conglycinin was well-documented at 65 °C (Figure 5). After heating for 20 min, the elution profile showed a symmetrical single peak composed of dissociated subunits which is also shown by the insert PAGE patterns. These results indicated that thermal dissociation of β-conglycinin was nearly finished at 65 °C after 20 min.

The effect of temperature (60–100 °C) on the dissociation of β-conglycinin is also shown in Figure 6. The elution profiles and native system PAGE indicated that the thermal energy at 60 °C is insufficient to bring about the complete dissociation of β-conglycinin into subunits since the relatively large size of the shoulder remained even after 20 min (Figure 6b). This shoulder (V_e/V_0 = 1.20) was composed of undissociated β-conglycinin of R_m 0.15 as shown by native PAGE. Heating at 70 °C for 5 min and at 100 °C for 1 min resulted in complete dissociation into subunits. Continuous heating at 100 °C for 30 min resulted in no aggregated peaks of large molecules at V_e/V_0 = 1.0, although the shoulder peak at V_e/V_0 = 1.20 appeared slightly (Figure 6h). It is necessary to confirm the protein component of these peaks by SDS–PAGE and native system PAGE.

SDS–PAGE Analysis of Thermal Dissociation of β-Conglycinin. β-Conglycinin molecules have a heterogeneity of seven isomers. Its subunit structure is determined to be B_1, $\alpha'\beta\beta$; B_2, $\alpha\beta\beta$; B_3, $\alpha'\alpha\beta$; B_4, $\alpha\alpha\beta$; B_5, $\alpha'\alpha\alpha$; B_6, $\alpha\alpha\alpha$ (Thanh and Shibasaki, 1978), and B_0, $\beta\beta\beta$ (Yamauchi et al., 1981; Sykes and Gayler, 1981). One of the reasons for shouldering of the elution peaks in Figures

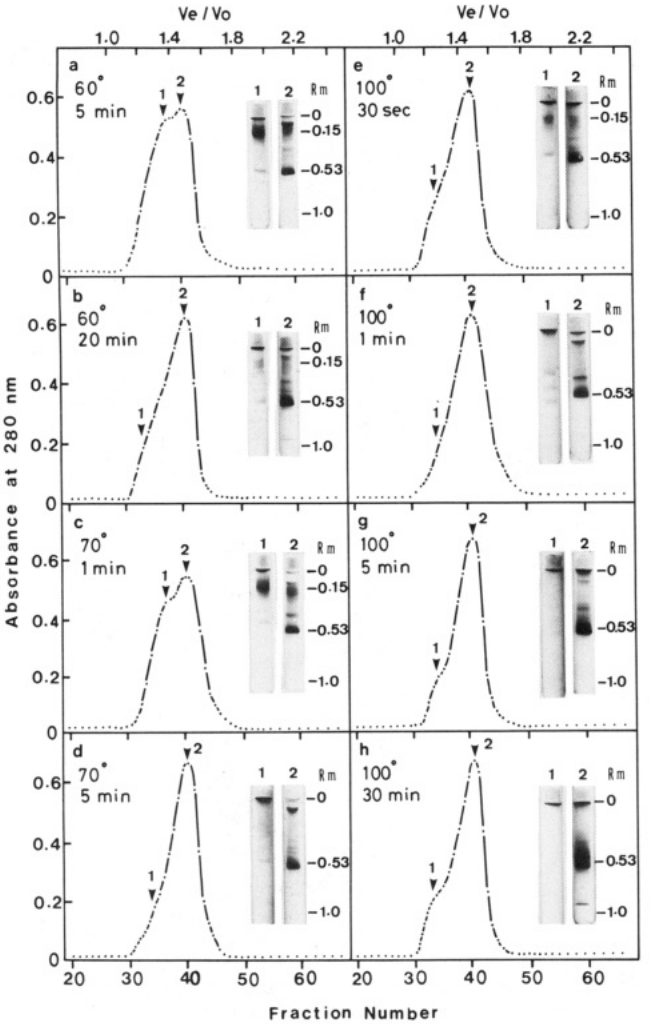

Figure 6. Thermal dissociation of β-conglycinin at various temperatures. Elution profiles are shown for heat-treated β-conglycinin at 60 °C for 5 min (a), 60 °C for 20 min (b), 70 °C for 1 min (c), 70 °C for 5 min (d), 100 °C for 30 s (e), 100 °C for 1 min (f), 100 °C for 5 min (g), and 100 °C for 30 min (h), eluted with 3.2 mM phosphate buffer (I = 0.01, pH 7.6). Inserts show the native system PAGE of the protein solutions indicated by the arrow.

5 and 6 is considered to be due to the heterogeneity of β-conglycinin. Therefore, by use of the fractionated β-conglycinins of molecular species of group A ($B_1 + B_2$), group B ($B_3 + B_4$), and group C ($B_5 + B_6$) prepared according to the method of Thanh and Shibasaki (1976b), changes in proteins accompanying heating were monitored by two-dimensional electrophoresis (native system PAGE × SDS–PAGE) (Figure 7). Two-dimensional electrophoretic analysis of unheated whole β-conglycinin revealed that under the salt-free conditions a part of the α and α′ subunits was dissociated from B_5 and/or B_6 but no β subunits were detectable (Figure 7a). Heat treatment at 70 °C for 5 min induced complete dissociation of the quaternary structure of groups B and C (Figure 7c,d); however, a part of the quaternary structure of β subunit predominant molecular species, group A, remained undissociated (Figure 7b). There is a possibility that dissociated β subunits might reassociate.

The subunit component of the elution profiles of β-conglycinin in Figure 5 was analyzed by single-dimensional SDS–PAGE (Figure 8). Compared with the α′, α, and β subunit bands of unheated conglycinin (see Figure 8a, whole), heating disrupted the balance of the α′/α/β ratio; heating at 65 °C for 1 min induced a great reduction of

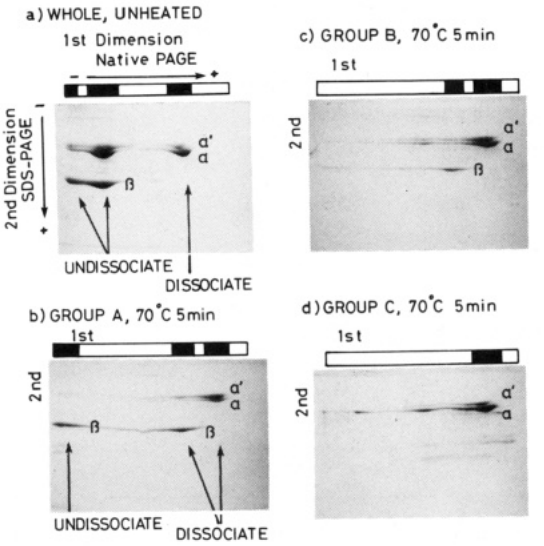

Figure 7. Two-dimensional electrophoretic analyses resolved by Ornstein–Davis system electrophoresis (native PAGE) × Laemmli system electrophoresis (SDS–PAGE) of (a) whole β-conglycinin (unheated, dissolved in water), (b) group A ($\alpha'\beta\beta$ and $\alpha\beta\beta$) heated at 70 °C for 5 min, (c) group B ($\alpha'\alpha\beta$ and $\alpha\alpha\beta$) heated at 70 °C for 5 min, and (d) group C ($\alpha'\alpha\alpha$ and $\alpha\alpha\alpha$) heated at 70 °C for 5 min. The first-dimensional tube gel of native PAGE was laid on the second-dimensional slab of SDS–PAGE.

α subunit from the undissociated peak at fractions 33–38, and the α subunit band of the dissociated peak at fractions 40–45 was increased (Figure 8b). With increasing heating time, dissociation of α′ subunit followed. Although β subunits have a tendency to resist dissociation, prolonged heating (65 °C for 20 min) brought about the dissociation of β subunits since the α′/α/β ratio (Figure 8f) recovered close to that of unheated ratio in Figure 8a. Further heating (100 °C for 5 min) seems to lead to a reassociation of the dissociated β subunits (Figure 8g).

DISCUSSION

In this paper, it was clarified that thermal denaturation of β-conglycinin is markedly sensitive to changes in pH and ionic strength, which do influence the electrostatic state of protein molecule. This specific denaturation behavior probably originated from the structural feature of β-conglycinin. Recently, Doyle et al. (1986) reported complete amino acid sequence data of the α′ subunit. Their data indicate that the α′ subunit (the α subunit has a sequence highly similar to that of the α′ subunit) has very unusual amino acid sequences: the 150 amino acid N-terminal segment is largely polar and is probably located at the exterior of the molecules, whereas the β subunit consisting of a highly hydrophobic residues did not contain this hydrophilic insert. The quaternary structure of β-conglycinin is stabilized at high ionic strength ($I \geq 0.5$) owing to an enhancement of hydrophobic interactions but becomes unstable at low ionic strengths following the dissociation of α and/or α′ subunits. The β subunits, however, were not dissociated by the removal of salt (see Figure 7a). At higher temperature the disruption of the quaternary structure is accelerated by thermal energy and the dissociated form is stable at higher temperature or after cooling unless salt is added in the protein dispersions. It seems that aggregation is not essential in thermal denaturation of β-conglycinin, since the formation of aggregated products depends only slightly on heating but largely on the electrostatic atmosphere surrounding the protein molecules.

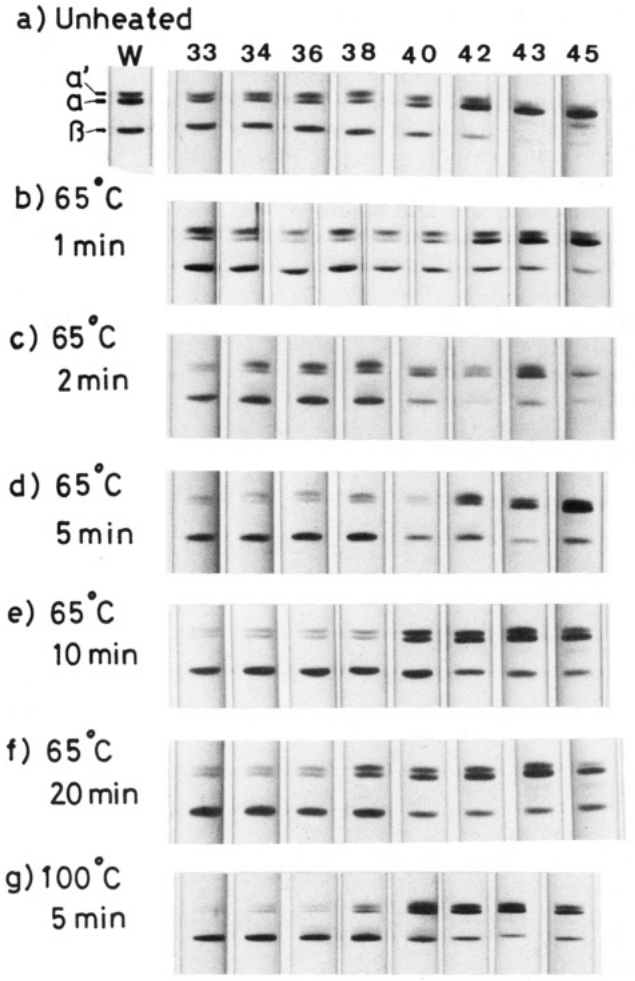

Figure 8. Urea–SDS–PAGE analyses of Sepharose CL-6B eluted fractions of unheated β-conglycinins (a) and β-conglycinins heat-treated at 65 °C for 1 (b), 2 (c), 5 (d), 10 (e), and 20 min (f) and at 100 °C for 5 min (g). Fraction numbers (33–45) refer to the numbers of Figures 5–7.

The direct action of solvent components on protein molecules can be classified in terms of electrostatic and hydrophobic interactions. We discuss the electrostatic circumstances of the dissociated form of β-conglycinin. A change in the electrostatic environment can be achieved in two ways: by the addition of neutral electrolyte or by a change in pH. A slight change in pH of β-conglycinin preparations shown in Table I should lead to small changes in the equilibria of acidic and basic residues, especially the N-terminal domain of α′ and α subunits, but results in a significant effect on the thermal denaturation behavior: that is, thermal denaturation of β-conglycinin varied distinctly from dissociation to aggregation within the narrow pH range from 7.5 to 6.9 (Figure 4). It is known that a salt concentration as low as 0.1 M will suffice to reduce electrostatic effects on polyelectrolytes (Tanford, 1968). Therefore, the aggregation phenomena of β-conglycinin suggest that the reduction of the net surface charge density of heat-induced dissociate promotes hydrophobic aggregation and development of turbidity in the system.

The internal bondings, both hydrophobic and electrostatic, are disrupted during heating due to intramolecular events. Because of the instability of these exposed hydrophobic regions and the tendency to associate soon with each other, thermal denaturation is usually followed by irreversible aggregation and formation of large molecule aggregates. It is interesting to compare the thermally denatured products of ovalbumin with those of β-conglycinin. Both proteins yield transparent solutions upon heating. However, in the case of ovalbumin, the thermal products are composed of soluble aggregates which will lead to the fundamental components of gel structure (Hatta et al., 1986). On the other hand, under the carefully controlled conditions described above, even the exposure of hydrophobic sites upon denaturation did not cause the aggregation of dissociated subunits (Figure 3a). This is because the mutual repulsion forces probably arising from the N-terminal hydrophilic domains of subunits which are generated at pH 7.5 are superior to the hydrophobic association. This structural feature alone is a factor governing the characteristics of thermal denaturation of β-conglycinin.

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