Chromatographic and Sedimentation Behavior of a Purified 7S Protein in Soybean Globulins[1]

I. KOSHIYAMA, Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan

ABSTRACT

A 7S protein of soybean globulins, isolated by cooling an aqueous meal extract, adding $CaCl_2$ and performing gel filtration with Sephadex G-100 and G-200, was homogeneous ultracentrifugally, electrophoretically, and chromatographically on hydroxylapatite and Sephadex G-200. This preparation corresponded to fraction D of the chromatogram of soybean globulins on hydroxylapatite by Wolf and Sly (Arch. Biochem. Biophys. 110; 47; 1965) and peak 3 in gel filtration of water-extractable soybean proteins by Hasegawa *et al.* (Agr. Biol. Chem. 27: 878; 1963). This protein underwent quantitatively a reversible 7S⇄9S isomerization with change of ionic strength from $\mu = 0.1$ to $\mu = 0.5$. In 0.01*N* HCl, this protein separated into two small components, but in a small amount of NaCl they associated into only one peak having a sedimentation coefficient of approximately 7S quantitatively. The isoelectric point was at pH 4.90.

Soybean globulins consist of two major proteins, which have sedimentation coefficients of approximately 7S and 11S (1,2,3). It is very important and necessary for the utilization of soybean proteins to investigate their protein molecules, with the use of purified samples.

One of the major problems on soybean proteins has been the difficult isolation and purification of individual protein components revealed in ultracentrifugal analysis. The 11S component was obtained ultracentrifugally pure from the cold-insoluble fraction by Mitsuda *et al.* (4). The 7S component, however, was observed to be much more heterogeneous than the 11S, by Hasegawa *et al.* (5), Wolf and Sly (6), and Vaintraub (7). This is probably one reason why less work has been done on this component.

Recently, a 7S protein which showed the characteristic 7S ⇄ 9S dimerization was isolated approximately 90% pure by Roberts and Briggs (8).

In the previous paper (9), isolation and purification of a 7S protein of soybean globulins was reported. The physical homogeneity and chromatographic and sedimentation characteristics of the protein are described here.

MATERIALS AND METHODS

Materials

Samples. Water-extractable proteins, soybean globulins, and purified 7S protein were prepared as described earlier (9,10).

Buffer. Potassium phosphate-sodium chloride buffer (0.035*M* potassium phosphate buffer, 0.4*M* sodium chloride containing 0.01*M* 2-mercaptoethanol, pH 7.60, 0.5 ionic strength (2)), was used as the standard buffer.

Sedimentation Analysis

Ultracentrifugal analysis was performed with a Hitachi UCA-1 centrifuge at 55,430 r.p.m. in the buffer at pH 7.60 and at 51,200 r.p.m. in the buffer at pH 2.50, and in 0.01*N* hydrochloric acid. Routine assay runs were made at 20°C.

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Electrophoresis

Electrophoresis was carried out with a Hitachi HTD-1 Tiselius-type apparatus. The specific conductance of the 7S protein solution was measured with a Yokokawa-type K-1 Kohlraush bridge. The electrophoretic migration was carried out in glycine-hydrochloric acid buffer over a pH range of 2.04 to 3.05, in sodium acetate-acetic acid buffer of pH 4.17 and 5.10, in sodium phosphate buffer of pH 6.08 and 7.11, in sodium 5,5-diethylbarbiturate-hydrochloric acid buffer of pH 7.90, and glycine-sodium hydroxide buffer of pH 9.58. Each buffer was adjusted to 0.2 ionic strength with sodium chloride.

Ultraviolet Absorption

Ultraviolet absorption spectra were measured with a Hitachi recording spectrophotometer.

Chromatography

Chromatography on Sephadex G-200 was performed with a column (2×200 cm.) equilibrated with the standard buffer according to the procedures of a previous paper (9) and Hasegawa *et al.* (5).

Chromatography on hydroxylapatite, prepared by the method of Anacker and Stoy (11), was conducted with a column (3×6.5 cm.) equilibrated with 0.03*M* potassium phosphate buffer containing 0.01*M* 2-mercaptoethanol, pH 7.60, at room temperature, according to the procedure of Wolf and Sly (6). Elution was carried out with a linear gradient of the same buffer from 0.03 to 0.60*M;* a flow rate of 5–6 ml. per hour was used, as recommended by Vaintraub (7).

Protein Concentration

Protein concentration was routinely determined by light absorption at 280 mμ with a Hitachi P-1 spectrophotometer, and turbidity at 420 mμ with a Klett-Summerson photoelectric colorimeter (12). Phosphorus concentration was done by the method of Nakamura (13).

RESULTS

Physical Homogeneity of 7S Protein

A previous study (9) indicated that a 7S protein in soybean globulins could be purified with comparative ease from a meal extract by cooling, adding calcium chloride, and performing gel filtration with Sephadex G-100 and G-200, successively. To ascertain the physical homogeneity of this protein, ultracentrifugal analysis and electrophoresis were carried out.

Ultracentrifugal Analysis. The sedimentation patterns are illustrated in Fig. 1. The photographs, taken at intervals of 21 min., indicate homogeneity. A sedimentation constant $S_{20,w}$ was calculated to be 7.50S at a concentration of 0.50%.

Electrophoresis. Schlieren diagrams of the ascending and descending electrophoretic boundaries over a pH range 2.04–9.58 at 4°C. are shown in Fig. 2. The photographs were taken 120 min. after the start of electrophoresis with a current of 6 ma. As shown, this 7S protein is also electrophoretically homogeneous.

From these results, this 7S protein is considered to be physically homogeneous.

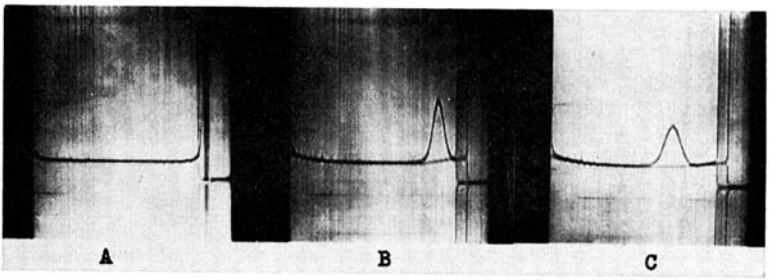

Fig. 1. Ultracentrifugal patterns of the 7S protein. The concentration of the protein was 0.50% in the standard buffer. Bar angle was 70° for A, B, and C.

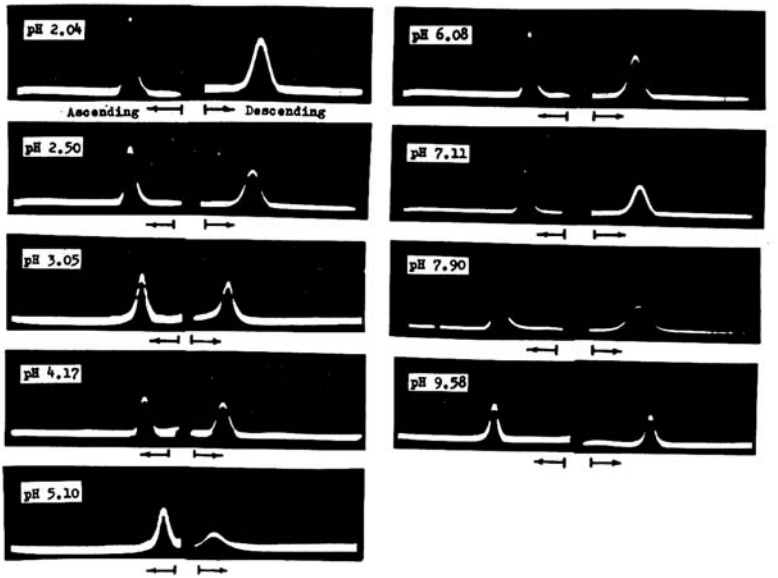

Fig. 2. Electrophoretic patterns of the 7S protein. Bar angle was 60°–80°.

To examine the chromatographic and sedimentation characteristics of this 7S preparation, the following examinations were performed.

Chromatographic Characteristics of 7S Protein

Column Chromatography with Sephadex G-200. As shown in Fig. 3, A, an elution pattern very similar to that reported by Hasegawa *et al.* (5) was obtained for the water-extractable soybean proteins. Figure 3, C, shows that the present 7S preparation eluted in the same position as that of peak 3 in A. It is obvious, however, that a slight difference exists in the eluting position between the 7S and the 11S protein as shown in B.

Column Chromatography on Hydroxylapatite. Figure 4 shows elution diagrams of the 7S and soybean globulin preparations. The 7S protein eluted in the position of fraction D in the elution pattern of soybean globulins. Frac-

tion B separated only two peaks instead of four as reported by Vaintraub (7), even though a slow flow rate was used.

Sedimentation Characteristics of 7S Protein

Effect of Ionic Strength. The 7S protein isolated by Roberts and Briggs (8) was shown to undergo a reversible 7S ⇄ 9S isomerization, depending on

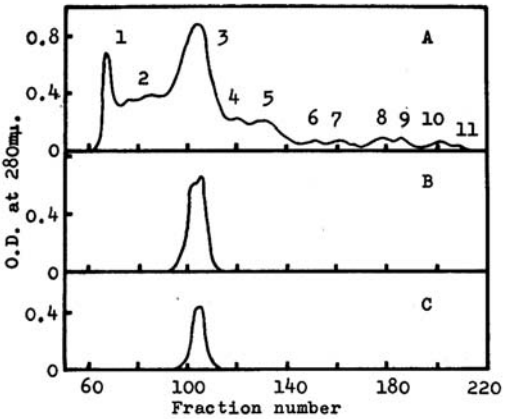

Fig. 3. Column chromatography of various soybean proteins on Sephadex G-200. Eluant: the standard buffer. Flow rate: 10 ml./hr. Fraction volume: 3.94 ml. A, 150 mg. of water-extractable protein. B, 50 mg. of 11S protein and 57 mg. of 7S protein. C, 30 mg. of 7S protein.

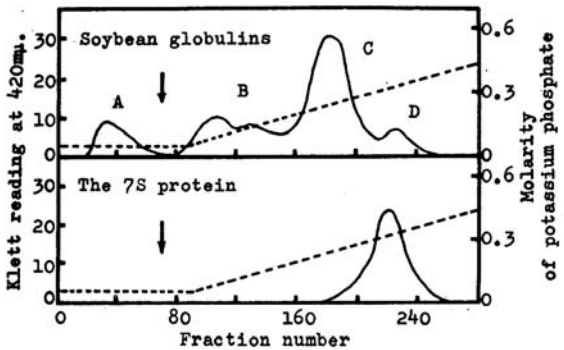

Fig. 4. Gradient chromatography of soybean globulins and 7S protein on hydroxylapatite. Fraction volume: 2.70 ml. Sample: 164.85 mg. of soybean globulins and 29.20 mg. of 7S protein in 5 ml. of starting buffer. Vertical arrow indicates point of start of gradient elution; dashed curve is gradient of potassium phosphate. Protein concentration was measured by turbidity at 420 mμ with a Klett-Summerson photoelectric colorimeter.

change of ionic strength. As shown in Fig. 5, the reversible reaction of association and dissociation was observed also in this purified 7S protein with a change of ionic strength from 0.1 to 0.5.

The $S_{20,w}$ for the associated form of the 7S protein was calculated to be 11.49S at a concentration of 0.25%.

Effect of pH. According to the present experiments, the 7S protein in 0.03*M* borax-HCl buffer, pH 2.50, showed only one peak having a sedimentation coefficient of 7.30S at concentration of 0.70% (Fig. 6).

However (Fig. 6), in 0.01*N* hydrochloric acid (pH 2) this preparation was divided into two components having sedimentation coefficients of 1.92S and 5.47S as observed in a preparation of Roberts and Briggs. These two components were converted into only one peak having a sedimentation coefficient of 7.19S at a concentration of 0.70% by overnight dialysis against 0.01*N* HCl containing 0.1*M* sodium chloride (Fig. 6).

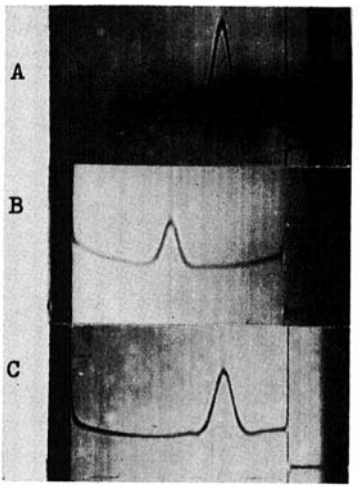

Fig. 5 (left). Ultracentrifugal patterns of the 7S protein in buffers of various ionic strength. A, $\mu = 0.5$, pH 7.60; B, $\mu = 0.1$, pH 7.60; C, $\mu = 0.1 \rightarrow \mu = 0.5$. Photographs were taken after 40 min. of centrifugation at 55,430 r.p.m.

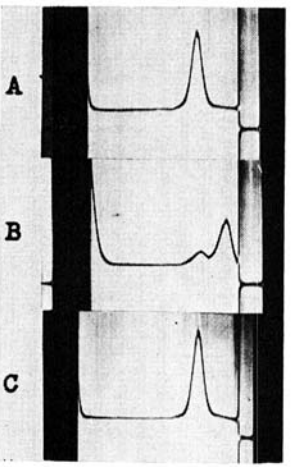

Fig. 6 (right). Ultracentrifugal patterns of 7S protein in acidic conditions. A, in 0.03*M* borax-HCl buffer, pH 2.50; B, in 0.01*N* HCl; C, in 0.01*N* HCl containing 0.1*M* NaCl. Photos A and C taken after 40 min.; B, 56 min.

The buffer prepared by adding HCl to borax was naturally considered to contain a small amount of NaCl. Therefore, from the result of Fig. 6, a single peak observed in borax-HCl buffer seemed to arise as an effect of the presence of NaCl.

Isoelectric Point

Mobility as a function of pH is plotted in Fig. 7. The mobilities of the protein in the buffer of 0.2 ionic strength were calculated from the descending boundaries. The curve indicates that the isoelectric point lies at pH 4.90.

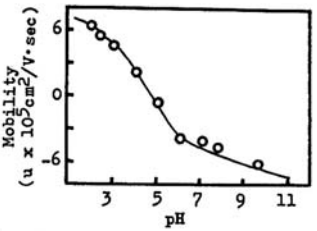

Fig. 7 (left). Effect of pH on electrophoretic mobility of 7S protein.

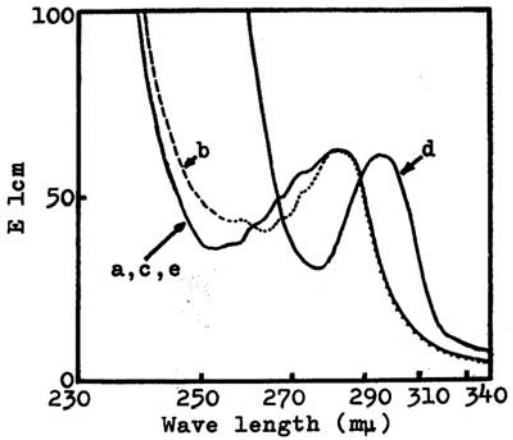

Fig. 8 (right). Ultraviolet absorption spectra of 7S protein: a, in 0.03*M* borax-HCl buffer, pH 2.50; b, in 0.01*M* borax-NaOH buffer, pH 10.0; c, in 0.01*M* sodium phosphate buffer, pH 7.93; d, in 0.1*N* NaOH; e, in the standard buffer.

Ultraviolet Absorption Spectra

As shown in Fig. 8, ultraviolet absorption spectra of 7S protein were measured at various pH values. No change was found in the absorption spectrum of the protein in the standard buffer ($\mu = 0.5$), the borax-HCl buffer, pH 2.50 ($\mu = 0.03$), and sodium phosphate buffer, pH 7.93 ($\mu = 0.01$).

In borax-NaOH buffer, pH 10.0, however, there existed a slight shift of the minimum point in the direction of long wave length, but no change of the maximum was observed. On the other hand, a striking change of the maximum and minimum points of the spectrum occurred in 0.1*N* NaOH solution, indicating dissociation of tyrosyl-OH groups.

The $E^{1\%}_{280\ m\mu}$ value for this 7S protein was 5.47 in this experiment. The ratio of 280 to 260 mμ in the standard buffer was estimated to be 1.36.

DISCUSSION

It has been observed that soybean globulins contain several 7S components which are similar ultracentrifugally but which differ chromatographically.

Hasegawa *et al.* (5) showed that water-extractable soybean proteins consisted of nine protein components by gel filtration with Sephadex G-200 and that four components of them possessed the sedimentation coefficient of approximately 7S. One of these 7S components was eluted with the 11S protein in peak 3 and was the major 7S component of water-extractable soybean proteins. This was also ascertained in the present experiments, by complete coincidence with Hasegawa's data. It is clear that the 7S fraction purified in this paper is the major 7S protein, since it was eluted in peak 3.

Wolf and Sly (6) recognized at least two components of the 7S protein in soybean globulins by column chromatography on hydroxylapatite. Further, it was found that one of these 7S components had the 7S ⇄ 9S dimerization characteristics. They found that it is eluted in fraction D (Fig. 4). Its property agreed with that of the 7S protein isolated by Roberts and Briggs (8).

Recently, Vaintraub (7) examined the fractionation of soybean globulins by column chromatography on hydroxylapatite according to the procedure of Wolf and Sly. When the elution rate was lowered to 16 ml./cm.^{2}/hr., fraction B was divided into four peaks: one is a 2S component and the other three are the 7S components which have different amino terminal groups.

The purified 7S protein used in the present experiments corresponded to the 7S protein of peak 3 by Hasegawa *et al.* and fraction D by Wolf and Sly (Figs. 3 and 4). This protein also exhibited the association-dissociation reaction reported by Roberts and Briggs.

From these results, this protein is considered to be the major 7S component in soybean globulins, and the 7S protein isolated by Roberts and Briggs is assumed to be the same protein as this purified 7S protein. This is examined and ascertained in more detail in another paper (14), by N-terminal analysis of this protein.

There were four shoulders between 250 and 280 mμ on ultraviolet absorption spectra of this 7S protein in the usual buffers. The tryptophan content in this 7S protein is so low that its effect on the spectrum is considered to be negligible (14). Therefore, these shoulders should be ascribed to the absorption of phenylalanine (15). The tyrosyl-OH groups dissociate in the alkaline solution at pH 9.97, and the resulting phenoxide ion causes the absorption spectrum to shift toward the long wave length. However, the red shift of the absorption spectrum of the 7S protein did not occur at pH 10.0. This indicates that tyrosyl-OH groups are binding through the hydrogen bonds with the side-chain carboxyl groups or are buried in the hydrophobic region of the molecule. This was also observed through the difference spectrum of the 7S protein by Fukushima (16).

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