Interconversion between Monomer and Dimer of the 7S Globulin of Soybean Seed

Chiharu IIBUCHI and Kazutomo IMAHORI*

Department of Agricultural Chemistry, Faculty of Agriculture,
*Department of Biochemistry, Faculty of Medicine, The University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

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The 7S globulin of soybean seed was purified on concanavalin A-Sepharose 4B. It exists as monomers at a high ionic strength or at a low pH, whereas it associates to form dimers under the opposite conditions. The sedimentation coefficient of the monomer is 5.6S, and that of the dimer 10S, at a protein concentration of 0.16%. Since the interconversion between the monomer and the dimer proceeds fairly rapidly, the ultracentrifugal pattern of the mixture shows a single peak. The apparent sedimentation coefficient of the mixture successively changes between 5.6S and 10S, corresponding to the ratio of the amounts of the monomer and the dimer. The monomer reversibly dissociates into subunits with 5S at urea or 0.01 N HCl.

The storage protein of soybean seed consists of four components, having sedimentation coefficients of 2, 7, 11 and 15S, respectively, in phosphate buffer, pH 7.6, ionic strength of 0.5.\(^{1,2}\) The main components are the 7S and 11S globulins. Naismith\(^1\) reported also that the sedimentation coefficient of the 7S globulin became about 9S (the so-called 9S form\(^3\)) when the ionic strength of the solution was increased to 0.1 at pH 7.6. The interconversion of 7S\(\rightarrow\)9S with ionic strength at higher pH than the isoelectric point of the 7S globulin\(^4\) has been considered as its specific and characteristic feature. Naismith\(^1\) considered that the 9S form was a dimer of the 7S globulin. The hypothesis was supported by Roberts and Briggs\(^3\) and Koshiyama\(^5,6\) through determination of the molecular weights of the 7S and 9S forms.

Roberts and Briggs\(^3\) reported that at an intermediate ionic strength it was possible to observe varying amounts of the two sedimenting species. Koshiyama\(^5\) showed an ultracentrifugal pattern having two peaks of 7S and 9S at an ionic strength of 0.35. He also reported that the 7S globulin dissociated into the 5S and 2S forms in 0.01 N HCl.

The authors will present a new opinion about the sedimentation coefficient and the interconversion between the monomer and the dimer of the 7S globulin. The so-called 7S globulin at 0.5 ionic strength is not a monomer, but a mixture of the monomer and the dimer. The sedimentation coefficient of the monomer is 5.6S, and that of the dimer is 10S, at a protein concentration of 0.16%. The monomer associates to form the dimer with an increase of ionic strength or pH. At an intermediate ionic strength or pH, the protein exists as a mixture of the monomer and the dimer. The ultracentrifugal pattern of the mixture shows a single peak, unlike the result of Koshiyama,\(^5\) and the apparent sedimentation coefficient changes successively between 5.6S and 10S. The value of 7S, therefore, is only an apparent sedimentation coefficient of a mixture. The results can be explained on the basis of the theory of Gilbert.\(^7\)

This paper also describes the dissociation of the protein into subunits. Physico-chemical properties of the monomer, dimer and subunits will be reported in the next paper.

MATERIALS AND METHODS

Soybean meal. The strain of soybean used was
Norin Nigo, 1973 crop. Soybean was mealed with a Waring blender and defatted with ethyl ether for more than 24 hr in a Soxhlet extractor.

Buffer solutions
Phosphate buffer. The standard solution consisted of 0.0026 M KH$_2$PO$_4$ and 0.0325 M K$_2$HPO$_4$, pH being adjusted to 7.6. Since the ionic strength of this solution is 0.1, this is referred as the 0.1 µ standard solution. In some cases, 0.4 M NaCl was added to the above solution to raise the ionic strength to 0.5. This solution is referred as the 0.5 µ standard buffer.2)

Citrate buffer. The solution contained 15 mM citric acid and pH was adjusted to 3, 3.6, 4.0, 4.5 or 5.8 with Na$_2$HPO$_4$.

Glycine buffer. The solution contained 20 mM glycine and pH was adjusted to 10 with NaOH.

Procedure of purification
Soybean protein was extracted from defatted soybean meal with 50 mm 2-mercaptoethanol, and then the extract was centrifuged. The pH of the supernatant was adjusted to the isoelectric point of the 7S globulin, namely 5, with HCl to precipitate a large portion of the protein in the supernatant. The precipitate was collected and suspended in water. The suspension was adjusted to pH 7 with NaOH to dissolve the protein. After dialysis of the solution against the 0.5 µ standard buffer containing 50 mm 2-mercaptoethanol, it was applied to a column of Bio-Gel A-0.5 M (3×200 cm) and eluted with the same buffer at 4°C to remove aggregated protein and the 2S globulin. The fractions of the 7S and 11S globulin were eluted together as a single main peak. The same elution pattern was reported for Sephadex G-2008) or Sepharose 4B9) column chromatography. The main fraction eluted was subjected to affinity chromatography on concanavalin A-Sepharose. The 7S globulin adsorbed to the column was eluted by α-methyl-D-mannoside.10) The eluate gave a single peak on the ultracentrifugal analysis and was identified as the 7S globulin from its sedimentation velocity.

Sedimentation analysis.
Sedimentation velocity analysis was performed at 55,430 rpm at 20°C with a Hitachi-UCA-1A analytical centrifuge. Sedimentation coefficients were calculated from the Schlieren pattern.

Reagent
All reagents were of analytical grade. Bio-Gel A-0.5 M (100~200 mesh) was purchased from BIO-RAD Laboratories (U. S. Std.) and concanavalin A-Sepharose from Pharmacia Fine Chemicals, Sweden.

RESULTS
1. Purity of the 7S globulin.
Sedimentation analysis of the purified 7S globulin gave a single peak in the 0.5 µ standard buffer, pH 7.6, as shown in the upper pattern of Fig. 1. The sedimentation coefficient under these conditions was 7.3S. In the 0.1 µ standard buffer, the protein showed again a single peak, as shown in the lower pattern of Fig. 1. However, the sedimentation coefficient in this case was 10.0S. When the ionic strength of this solution was increased to 0.5, the sedimentation coefficient of the peak was shifted to 7.3S. As will be described below this reflects a shift of the equilibrium between the monomer and the dimer.

Fig. 1. Sedimentation Pattern of the 7S Globulin. Upper pattern, in the 0.5 µ standard buffer; lower pattern, in the 0.1 µ standard buffer. Photograph was taken at 20 min after reaching to a speed of 55,430 rpm. Protein concentration was 0.4%. M, Meniscus; B, Bottom.

2. Influence of ionic strength on ultracentrifugal pattern and sedimentation coefficient of the 7S globulin
An adequate amount of NaCl or water was added to the 0.1 µ standard buffer, pH 7.6, to prepare twelve solutions of various ionic strengths from 0.025 to 1.2 as indicated in Fig. 2. A solution of the 7S globulin was dialyzed overnight against each of these twelve buffer solutions and sedimentation coefficients were determined for each sample. All of the sedimentation patterns, including those at the intermediate ionic strengths, showed each a single peak, as shown in Fig. 3, in contradiction with the results of Koshiyama6) and Roberts and
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3. Influence of Ionic Strength

As shown in Fig. 2, the sedimentation coefficient of the 7S globulin was 10S at ionic strengths of less than 0.2, and it was 5.6S at ionic strengths above 0.8. At the intermediate ionic strengths, it varied between 10 and 5.6S. These results showed that the ratio of the amounts of the monomer and the dimer changed successively with the ionic strength, and that the equilibrium between these two states was established very rapidly. These results also showed that the sedimentation coefficient of the monomer of the 7S globulin is not 7S but 5.6S, and that of the dimer is 10S. This conclusion was confirmed by the measurement of the molecular weights of the monomer and dimer, which will be shown in the next paper.

3. Influence of pH

The 7S globulin was dialyzed overnight against solutions of pH 2 (0.01 N HCl), 3, 3.6, 4.0, 4.5, 5.8 (citrate buffer), 7.6 (the 0.1 μ standard buffer), or 10 (glycine buffer). The sedimentation coefficient of each sample was determined as shown in Fig. 4. All of the sedimentation patterns (except at pH 2) showed a single peak. The sedimentation coefficients varied from 5.5 to 10, in accordance with the variation of pH. These results showed that the protein existed as monomers at pH about 3, and that they associated to form dimers at or above pH 6. At the intermediate pH the protein existed as a mixture of monomers and dimers, as in the case of the intermediate ionic strength. At pH 4.5, the protein was precipitated, because this pH was close to its isoelectric point.

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At pH 2, the sedimentation pattern showed two peaks, representing the fractions of 5S and 2S. The fact showed that a part of the monomers was dissociated into sub-com-
ponents in very acidic conditions. Such a sedimentation pattern at pH 2 has been reported by Koshiyama$^5$ and Roberts and Briggs,$^3$ although their interpretations for the fact were different from that of the present paper.

At alkaline pH values (7.6, 8.5 and 10) where the protein existed as dimers, a part of the dimers was dissociated into monomers if the ionic strength of the solution was raised to 0.5 by adding 0.4 M NaCl, and consequently the sedimentation coefficient of the protein became about 7S, which could be expected from Fig. 2.

4. Influence of organic solvent

Dioxan, N, N$'\text{-dimethyl formamide, acetoni-}$

tril and dimethyl sulfoxide, below 20% in
the 0.1 μ standard buffer, gave no effect on the interconversion between the 7S form and the 9S form. When the concentration of the solvents was more than 30%, the protein was aggregated.

5. Behavior in 0.01 N HCl

The behavior of the 7S globulin in 0.01 N HCl was almost the same as the results reported by Koshiyama$^5$ and Roberts and Briggs.$^3$

The ultracentrifugal pattern showed two peaks having sedimentation coefficients of 5.5S (that of monomer) and about 2S. The 2S fraction increased with storage time, while the 5.5S monomer fraction correspondingly decreased, as shown in Fig. 5. The results showed that the monomer protein was dissociated into smaller molecules under these conditions. A mixture of the 5.5S and 2S forms completely reconstructed the 7S form after dialysis against the 0.5 μ standard buffer, unless the protein was kept at pH 2 for more than one day. After standing it for seven days in 0.01 N HCl, the recovery of the 7S form was about 40% in the same dialyzing conditions. The extent of the reconstruction was calculated from the area of the peak of the sedimentation pattern.

6. Influence of urea and guanidine HCl

As shown in Fig. 6, the upper pattern, the

7S globulin was dissociated into smaller components having 1.5S by 5 M urea in the 0.1 μ standard buffer containing 50 mM 2-mercapto-

ethanol. Most of the smaller components recombined to form the 7S globulin after removing urea by dialysis against 0.5 μ standard buffer, as shown in Fig. 7. As shown in Fig. 6, the middle and lower patterns, the 7S globulin was also dissociated into sub-components having a sedimentation coefficient of 1.6S by 5 M guanidine HCl dissolved in the 0.1 μ standard buffer or by 5 M urea dissolved in the 15 mM citrate buffer, pH 4.0, containing 50 mM 2-mercaptoethanol. The sub-components were considered to be the subunits of the 7S globulin. By adding 0.4 M
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FIG. 6. Influence of Urea or Guanidine HCl on Sedimentation Pattern of the 7S Globulin.
Upper, in the 0.1 \( \mu \) standard buffer containing 5 M urea; middle, in the 0.1 \( \mu \) standard buffer containing 5M guanidine HCl; lower, in citrate buffer containing 5 M urea, pH 4.0. Photographs were taken at 10, 100 and 10 min, respectively, after reaching to a speed of 60,000 rpm with a synthetic boundary cell. Protein concentrations were 0.15, 0.54 and 0.25%, respectively.

FIG. 7. Reconstruction by the Removal of Urea.
Upper, the 7S globulin; lower, reconstructed 7S globulin. Photograph was taken after 30 min of centrifugation at 55,430 rpm. Protein concentrations of these samples were both 0.4%.

NaCl to the solution of urea or guanidine HCl, the dissociation of the protein into subunits was considerably prevented, as reported by Koshiyama.5)

DISCUSSION

It has been believed that the 7S globulin, one of the soybean storage proteins, existed as monomers having a sedimentation coefficient of 7S in a solution of an ionic strength of 0.5, pH 7.6, and that they were dimerized into the 9S form by changing the ionic strength to 0.1.1) At an intermediate ionic strength, the ultracentrifugal pattern showed two peaks of monomers and dimers.3,5) However, our results were contradictory to a part of this dimerization model and revealed a new fact concerning dimerization.

Our results clearly indicated that in the neutral pH region the protein existed as monomers having a sedimentation coefficient of 5.6S if the ionic strength was above 0.8. On the other hand, when the ionic strength was below 0.2 the protein molecules were associated into dimers having a sedimentation coefficient of 10S. When the ionic strength was intermediate between 0.2 and 0.8 the protein gave also a single peak on sedimentation analysis. The sedimentation coefficient continuously changed from 10 to 5.6 as the ionic strength increased from 0.2 to 0.8. These results can be explained by the theory of Gilbert.7) This theory proposed that a mixture of monomers and dimers should give a single peak on sedimentation analysis if they are in a rapid dynamic equilibrium and the sedimentation coefficient increases as the equilibrium shifts to a dimer form. Accordingly, in the solution of an intermediate ionic strength the protein should exist as a mixture of monomers and dimers although it gave a single peak on sedimentation analysis.

It is worthy to note that a sedimentation coefficient of 7S, which has been used to characterize the molecular size of this protein, was obtained at an ionic strength of 0.5 and pH 7.6, where the protein is a mixture of monomers and dimers. In this sense it may be appropriate to use the 5.6S globulin rather than the 7S globulin. However, as will be described in the following paper the so-called 7S globulin fraction is a mixture of several molecular species. On the other hand, the name of the 7S globulin is widespread and has been used for a long time. Thus, the use of this name would be allowable although it is trivial and conventional.

Our results, especially those at the inter-
mediate ionic strength region, are contradictory with those previously reported. Roberts and Briggs\textsuperscript{3} and Koshiyama\textsuperscript{5} reported that two peaks corresponding to monomers and dimers appeared on sedimentation analysis while we observed only one peak. At present we can not explain this discrepancy.

The interconversion between monomers and dimers could be achieved by changing the pH of the solution. This result in combination with the previous one\textsuperscript{3} suggests that electrostatic force is responsible for dimerization. Since the plot of Fig. 4 is similar to the titration curve of carboxyl group,\textsuperscript{11} it is deduced that a carboxylate ion plays a major part of the electrostatic interaction.

Although we defined the fraction having a sedimentation coefficient of 5.6S as monomers, it consists of subunits. This was shown by ultracentrifugation of this protein in an extremely acidic condition, which gave two peaks corresponding to monomers and subunits. The subunit structure of the molecule will be discussed in the following paper.

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REFERENCES
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