Dissociation into Subunits of a 7S Protein in Soybean Globulins with Urea and Sodium Dodecyl Sulfate

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A 7S protein isolated from soybean globulins was dissociated into a similar slow-sedimenting material (subunit) by the treatment of urea and sodium dodecyl sulfate (SDS).

Sedimentation coefficients of the subunits obtained by treating with 8 M urea and 0.25% SDS were 1.35 S and 2.00 S, and molecular weights were 22,500 and 34,000, respectively. These subunits by the both treatments were apparently different in conformation from the results of optical rotatory dispersion, *i.e.*, urea treatment caused the almost complete unfolding of the subunit structure. On the contrary, SDS treatment contributed new partial formation of α -helical conformation for the subunits.

These dissociations were extremely disturbed by the presence of sodium chloride.

There was a possibility that a 7S protein in soybean globulins¹⁾ had a multi-subunit structure from N-terminal analysis.²⁾ On the other hand, recently, the 11S protein which was another major component of soybean globulins was clarified to have twelve polypeptide chains (subunits), eight of which end in glycine, two in phenylalanine and two in either leucine or isoleucine.³⁾

In general, urea and an anionic detergent, sodium dodecyl sulfate (SDS), was often used to dissociate the proteins into the subunits. Though the final objective of this study is to isolate the individual subunits of which the 7S protein is made up and to make the subunit structure clear, this paper deals with the dissociation of the protein by two treatments with urea and the detergent, and subsequently, those subunits obtained by the both treatments

are physico-chemically compared using sedimentation and optical rotatory dispersion.

MATERIALS AND METHODS

Protein sample. According to the method described in a previous paper,¹⁾ the 7S protein was prepared. This 7S protein is called γ -conglycinin by Catsimpoolas.^{4~6)}

Ultracentrifugation. Sedimentation analysis was performed with a Hitachi UCA-1 centrifuge at 55,430 rpm in the 0.5 μ standard buffer (0.0325 M K_2HPO_4 , 0.0026 M KH_2PO_4 , 0.4 M NaCl, pH 7.60⁷⁾) and 0.01 M tris-hydrochloric acid buffer, pH 8.20, and 51,200 rpm in 0.01 N hydrochloric acid. Routine assay runs were made at 20°C and protein concentration was 0.70%.

Molecular weight. Molecular weights were determined by the short column method of Yphantis⁸⁾

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using a eight channel cell.

Optical rotatory dispersion measurements. Optical rotatory dispersion was measured with a Japan Spectroscopic model ORD/UV-5 recording spectropolarimeter (double prism, double monochromator) employing a xenon arc lamp as the source of radiation. Measurements were made at a room temperature near 20°C. For the measurements, in the visible and near-ultraviolet region (300~600 mμ), 100 mm cylindrical cell was used with the protein concentration of 0.35% to 0.45%.

The resulting data were interpreted by the Moffit-Young equation,^{9,10)}

$$[m']_{\lambda} = 3/(n^2 + 2) \cdot M_0/100 \cdot [\alpha]_{\lambda} \\ = \lambda_0^2/(\lambda^2 - \lambda_0^2) \cdot a_0 + \lambda_0^4/(\lambda^2 - \lambda_0^2)^2 \cdot b_0 \quad (1)$$

and the one-term Drude equation,^{9,10)}

$$[\alpha]_{\lambda} = A/(\lambda^2 - \lambda_c^2) \quad (2)$$

In the equation (1), $[m']_{\lambda}$ is the reduced mean residue rotation of the polypeptide at the wave-length of λ , n is the refractive index of solvent, λ_0 is the absorption wave-length associated with the rotation assumed to be 212 mμ, and M_0 is the mean residue weight.

The refractive index value (n) was measured with an Abbe refractometer, and the dispersion of refractive index was neglected. As the mean molecular

weight of residues (M_0), the value of 107.4 was employed based on the data previously reported.²⁾

The parameters, a_0 and b_0 , were evaluated from the intercept and slope, respectively, of a plot of $[m']_{\lambda} \cdot (\lambda^2 - \lambda_0^2)$ versus $1/(\lambda^2 - \lambda_0^2)$. The dispersion constant λ_c in the equation (2) was similarly obtained from the slope of a plot of $[\alpha]_{\lambda} \cdot \lambda^2$ versus $[\alpha]_{\lambda}$.

For the measurement in the ultraviolet region (200~250 mμ), 1.0 mm cell was employed. The protein concentration was 0.15% to 0.25%.

RESULTS

Sedimentation behavior of the 7S protein in the presence of SDS

When the 7S protein was overnight treated with 0.25% SDS (8.68×10^{-3} M) in 0.01 M tris-hydrochloric acid buffer of pH 8.20, it was converted to a slow-sedimenting component having a sedimentation constant of 1.91 S (Fig. 1, A). Treating with a lower concentration of the detergent (0.025%) gave two peaks (10.29 S and 2.02 s) in the sedimentation pattern as shown in Fig. 1, B, but these two peaks changed to a single slow-sedimenting form (1.83 S) by the long time treatment (Fig. 1, C).

Dissociation to subunits of the 7S protein

FIG. 1. Ultracentrifugal Patterns of the 7S Protein in the Presence of SDS.

A: 0.25% SDS.

B: 0.025% SDS.

C: 0.025% SDS. Prolonged treatment during 11 days.

Photographs were taken after 100 min of sedimentation for A, 41 min for B and 60 min for C.

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FIG. 2. Effect of Ionic Strength (0.5μ) in the Presence of SDS on Stability of Ultracentrifugal Patterns of the 7S Protein.

A: 0.025% SDS (upper). Dialysed against the 0.5μ standard buffer for 3 days (bottom).

B: 0.25% SDS (upper). Dialysed against the 0.5μ standard buffer for 3 days (bottom).

Photographs were taken after 40 min of sedimentation.

FIG. 3. Ultracentrifugal Patterns of the 7S Protein in 0.01 N HCl Containing 0.025% SDS (A), and 0.025% SDS and 0.1 M NaCl.

Photographs were taken after 40 min of sedimentation at 51,200 rpm.

with SDS was extremely disturbed in the presence of sodium chloride. As shown in the upper row of Fig. 2, B, conversion of the protein to the slow-sedimenting form was not observed even by the overnight treatment with 0.25% solution of the detergent containing 0.5 M sodium chloride. Rather, non-specific association was observed in the sedimentation pattern. The protein treated with SDS in the presence of sodium chloride

easily restored a sedimentation peak of 7S by dialysis against the 0.5μ standard buffer overnight as shown in the lower row of Fig. 2. But, the protein treated with the detergent without sodium chloride precipitated in the 0.5μ standard buffer.

In 0.01 N hydrochloric acid, the 7S protein precipitated by the treatment with 0.25% SDS, and associated non-specifically in 0.025% solution of the detergent as shown in Fig. 3, A. Similar effect of sodium chloride in the treatment with SDS on sedimentation pattern was also observed in the acidic solution of 0.01 N hydrochloric acid as well as in the alkaline solution of pH 8.20. Figure 3, B shows the results of the presence of 0.1 M sodium chloride.

Dissociation to subunits of the 7S protein with urea

Results of urea treatment to the 7S protein were shown in Fig. 4, *i.e.*, the sedimentation coefficients ($s_{20,w}$) of the protein diminished following the curve A in Fig. 4 with the increase of urea concentration. However, by the presence of 0.5 M sodium chloride, dissociation of the protein with urea, especially in lower concentration of urea, was extremely interfered as shown in Fig. 4, B₁.

In this experiment, to dissociate the protein into subunits having sedimentation coefficients lower than 2S, at least 5 M urea needed in the absence of sodium chloride and more than 6 M urea in the presence of it.

Most part of the 7S protein treated with various concentrations of urea in the presence of 0.5 M sodium chloride easily restored a sedimentation pattern of 7S as shown in Fig. 4, B₂ by dialysis against the 0.5μ standard buffer, though non-specific aggregation somewhat occurred in the small part of the protein with the increase of urea concentration. But, the phenomena were not observed in the protein treated with urea in the absence of sodium chloride.

On the other hand, it was easily observed that the protein dissociated into subunits

FIG. 4. Correlation of Sedimentation Constant of the 7S Protein and Urea Concentration.

A: Treated in 0.01 M Tris-HCl buffer, pH 8.20.

B₁: Treated in the buffer containing 0.5 M NaCl.

B₂: Dialysed B₁ against the 0.5 μ standard buffer for 2 days.

C: Treated in 0.01 N HCl.

smaller than 2 S in 0.01 N hydrochloric acid, even in 2 M urea.

From the results obtained above, it can be said that both urea and SDS have an ability to dissociate the 7S protein into subunits having a similar sedimentation coefficient.

Comparison of the subunits obtained by the treatments of SDS and urea

From the results of this experiment, the subunits obtained by treating with SDS was similar to those with urea, ultracentrifugally in appearance. However, it is a question whether these subunits are completely the same or not. Accordingly, it is necessary to compare these subunits physico-chemically.

Ultracentrifugal properties

1. *Sedimentation coefficient.* Figure 5 shows the relationship between sedimentation coefficients and protein concentration for each series of dilutions, *i.e.*, the values of $s_{20,w}^0$ with

FIG. 5. Sedimentation Rate of the 7S Protein as a Function of Concentration in 8 M Urea and 0.25% SDS.

●—●: in 8 M urea
○—○: in 0.25% SDS

FIG. 6. Molecular Weight of the 7S Protein by the Yphantis' Method as a Function in 8 M Urea and 0.25% SDS.

●—●: in 8 M urea
○—○: in 0.25% SDS

0.25% SDS and 8 M urea were 2.00 S and 1.35 S, respectively.

2. *Molecular weight.* The weight average molecular weights of the subunits obtained by two treatments were plotted in Fig. 6 as a function of protein concentration, assuming the partial specific volume of 0.725 ml/g. The molecular weights obtained by extrapolation to infinite dilution were 34,000 for SDS treatment and 22,500 for urea treatment, respectively.

Therefore, it was considered that the dissociation products with two reagents were apparently different in molecular size each other.

Optical rotatory dispersion properties

An each optical rotatory dispersion of the 7S protein under three conditions, *i.e.*, in the 0.5μ standard buffer, in 0.01 M tris-hydrochloric acid buffer of pH 8.20 containing 8 M urea and in the buffer containing 0.25% SDS, was measured in the visible and near-ultraviolet regions ($300\sim 600\text{ m}\mu$). The results were shown in Table I.

According to Table I, a more larger change in the specific rotation, $[\alpha]_D$, and a_0 value to the negative direction occurred upon the treatment of urea than that of SDS. On the other hand, the b_0 value, which can be used as measure of α helix content of protein, changed from -38 to -74 with 0.25% SDS and to zero with 8 M urea, respectively. These results seemed to indicate that the treatment with 8 M urea caused the almost complete unfolding of the protein as a whole. On the contrary, the treatment with 0.25% SDS may be said from a_0 and b_0 values not only to cause the incomplete unfolding of the protein, but also to contribute new partial formation of a helical conformation.

The changes in the α -helix content from $-b_0$ value by both treatments with 8 M urea and 0.25% SDS can be confirmed by changes

FIG. 7. Mean Residue Rotation of the 7S Protein as a Function of Wave Length.

- a: dissolved in the 0.5μ standard buffer.
- b: treated with 0.25% SDS.
- c: treated with 8 M urea.

in the magnitude of the trough at the critical wave length of $233\text{ m}\mu$. As shown in Fig. 7 and the last column of Table I ($[m']_{233}$), in 8 M urea solution the trough at $233\text{ m}\mu$ completely disappeared. However, the amplitude of negative Cotton effect at $233\text{ m}\mu$ in 0.25% SDS solution increased about two times as large as that of the negative protein.

From the results of sedimentation and optical rotatory dispersion, it might be concluded that the subunits obtained by the both treatments were surely different in molecular size and conformation each other. However, there was a full possibility that the subunits obtained by treating with SDS made the complexes with the detergent, and consequently the molecular size was larger than that of them obtained with urea. Therefore, it could not be concluded within the limits of this experiment whether the subunits were essentially different or not.

DISCUSSION

It had been observed that there were new formation of a partial α -helical conformation by treating with SDS in many proteins, *i.e.*,

TABLE I. OPTICAL ROTATORY DISPERSION DATA OF THE 7S PROTEIN

Parameters	Native ^{a)}	8 M urea ^{b)} treatment	0.25% SDS ^{b)} treatment
$[\alpha]_D$	-44.1	-109.3	-71.9
a_0	-246	-600	-371
b_0	-38	0	-74
$\lambda_c (\text{m}\mu)$	226	216	227
$[M']_{233}$	-1890	—	-3740

^{a)} Dissolved in the 0.5μ standard buffer.

^{b)} Treated with 0.01 M tris-hydrochloric acid buffer of pH 8.20 containing urea or SDS.

chymotrypsinogen A, trypsin, soybean trypsin inhibitor, histones¹¹⁾ and aspergillopeptidase A¹²⁾ as well as in this protein. Moreover, recently, it was reported that the apparent micellar molecular weights of the protein-SDS complexes, such as thyroglobulin, ovalbumin, ovomucoid, lysozyme, bovine γ -globulin and Tamm & Horsfall urinary glycoprotein, approximately the same (34,000~41,000) irrespective of the molecular weight of the protein to which they were attached. Therefore, it may be said that the results from this experiment give a new example for the proteins mentioned above.

Fukushima¹⁴⁾ supposed that α -helix content of the native 7S protein was very low, because the $-b_0$ value of it was 5° . In this experiment, the $-b_0$ value of the native protein was 38° as shown in Table I. From the content of α -helix was estimated to be about 6% (for 100% right-handed α -helix, $b_0 = -630$).

In any case, the native 7S protein is considered to have a very low α -helix content.

The molecular weight obtained by treating with 8M urea was about one ninth of the parent molecule. The results agreed almost to the numbers of subunits obtained by N-terminal amino acid analysis.²⁾

The action of stabilization to the gross structure of the 7S protein by addition of sodium chloride in the treatments of urea and SDS was also recognized in that of acid.¹⁵⁾ The reason cannot be discussed within the limits of this experiment, but it is a very interesting subject for a future study.

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