Purification of the 7S Component of Soybean Proteins

Sir:

Ultracentrifugal studies^{1,2)} on the water-extractable soybean proteins have revealed four fractions with sedimentation constants of about 2, 7, 11 and 15S at pH 7.6, 0.5 ionic strength. Among them, the 11S component has been extensively studied^{3~8)} and purified ultracentrifugally from the cold-insoluble fraction by gel filtration.⁹⁾

However, the 7S component has not been studied so much. Recently, Roberts and Briggs¹⁰⁾ obtained a 7S protein preparation of about 90 per cent ultracentrifugal purity by ammonium sulfate fractionation and gel filtration and discussed its characteristic properties.

The present communication is concerned with purification of the 7S component of soy-

bean proteins by cooling, addition of calcium chloride and gel filtration.

A standard buffer (a phosphate-sodium chloride buffer, pH 7.60, 0.5 ionic strength containing 0.01M mercaptoethanol) was used as eluant in gel filtration and as solvent in ultracentrifugation. The 2S contaminant was eliminated from the crude 7S protein, which was prepared by the method shown in Fig. 1, by gel filtration with Sephadex G-100 (3.0×90cm column) as shown in Fig. 3B. Subsequently, a single peak was obtained by repeating gel filtration with Sephadex G-200 (2.0×200cm column) three times as shown in Fig. 2C. The effluent was concentrated in a cellophane tubing under a pressure with Sephadex G-25. As shown in Fig. 3D, the purified 7S protein by gel filtration exhibits

FIG. 1. Schematic Outline of the Preparation of the Crude 7S Protein.

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FIG. 2. Purification of the 7S Protein by Gel Filtration with Sephadex G-200.

A: First step gel filtration of the partially purified 7S protein with Sephadex G-100.
B: Rechromatography I.
C: Rechromatography II.
Each 5 ml effluent was collected.

FIG. 3. Ultracentrifugal Patterns of Each Purification Step.

A: The crude 7S protein.
B: The partially purified 7S protein with Sephadex G-100.
C: The partially purified 7S protein with Sephadex G-200 shown in Fig. 2A.
D: The purified 7S protein shown in Fig. 2C.
Photographs were taken after 45 min. of centrifugation at 55430 r.p.m. Direction of sedimentation is from right to left.

a single peak ultracentrifugally and has $S_{20,w}$ value of 7.61S. Fig. 4 shows electrophoretic patterns of the purified 7S protein at pH 7.50 (a phosphate-sodium chloride buffer, 0.1 ionic

FIG. 4. Electrophoretic Patterns of the Purified 7S Protein.

A: Taken at 2% protein concentration after 91 min. in pH 7.50 buffer at 6 mA, 4°C.
B: Taken at 1.2% protein concentration after 100 min. in pH 9.50 buffer at 6 mA, 4°C.

strength) and at pH 9.50 (a glycine-sodium hydroxide-sodium chloride buffer, 0.1 ionic strength).

Considering the above-mentioned results, the purified 7S protein seems to be homogeneous ultracentrifugally and electrophoretically.

Naismith and Roberts et al. tried to purify the 7S component of soybean proteins by various methods. However, they could not obtain the pure preparation, and discussed isomerization of the 7S protein using an impure sample.

To this purifying procedure of the 7S component of soybean proteins is applied the fractionating method³⁾ of the 11S protein from the aqueous extract of soybean meal. The 11S contaminant, which could not be eliminated by Roberts, was removed by gel filtration with Sephadex G-200.

Hasegawa et al.¹²⁾ isolated four 7S proteins, which had sedimentation constants calculated for 7.8, 7.5, 7.2 and 6.0S respectively. The purified 7S protein of this study may be one of them, but this assumption is not proved yet.

The author wishes to express his sincerest thanks to Prof. Y. Sakurai, Assist. Prof. M. Fujimaki, Prof. K. Arima and Prof. Y. Ikeda of the University of Tokyo for their kind guidance

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and valuable suggestions. Thanks are due to Department of Agricultural Chemistry, the University of Tokyo, for ultracentrifugal analysis. The author also wishes to thank Dr. M. Mogi, Dr. N. Iguchi and Mr. E. Ichishima for their kind advices and encouragements

and Miss. T. Ichikawa for her kind assistance.

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Received July 8, 1965