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IDENTIFICATION OF THE 7S GLOBULIN WITH β-CONGLYCININ IN SOYBEAN SEEDS

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Abstract—The 7S globulin, a major ultracentrifugal component with the 11S globulin, was identical with β-conglycinin one of four antigenic components in the reserve proteins of soybean seeds (*Glycine max*). Double gel immunodiffusion and immunoelectrophoresis in agar gel were used for their identification. In addition, some characteristic properties on ultracentrifugation and in carbohydrate content agreed well between the proteins. Their MWs were *ca* 180000.

INTRODUCTION

Four components with sedimentation values of *ca* 2, 7, 11 and 15S have been accepted as the protein components of soybean globulins at 0·5 ionic strength, pH 7·6 [1–4]. Among them, the major components are the 7 and 11S fractions. A portion of the 7S fraction dimerizes at 0·1 ionic strength, pH 7·6 to form a 9S peak [5–7].

More recently, Catsimpoolas *et al.* [8,9] isolated and characterized 4 proteins, namely α, β, γ-conglycinin and glycinin [10] as the major antigenically different components of the reserve soybean proteins. They reported that glycinin was identical with the 11S globulin and γ-conglycinin with the 7S globulin isolated by one of us [11,12]. α-Conglycinin was a 2S soybean globulin component and β-conglycinin the major component of a crude 7S soybean protein prepared by Roberts and Briggs [5]. However, the relation between the ultracentrifugal and the antigenic compositions, particularly in the 7S globulin, β- and γ-conglycinin is still not well understood.

This paper describes some characteristics of β-conglycinin and the identification of the protein with the 7S globulin isolated by one of us is discussed.

RESULTS

A single peak was obtained as a typical elution pattern of the crude 7S protein [5] subjected to DEAE-Sephadex A-50 chromatography. This crude preparation was free of the 11S fraction not only ultracentrifugally, but also immunologically. The single peak was separated from Fraction 1 to 6 in order of elution. Although Fraction 1 to 4 contained quantitatively only one antigenic protein which gave an arc band to the monospecific anti-7S b-antigen serum, another protein which gave an immunoprecipitin band to the monospecific anti-7S a-antigen serum was contained in Fraction 5 and 6 as a contaminant. Therefore, Fractions 1 to 4 were prepared as a β-conglycinin fraction. Studies on 7S a-antigen will be reported separately. In this paper, the 7S globulin [11,12] was temporarily named as 7S b-antigen.

Both β-conglycinin and 7S b-antigen (the 7S globulin) gave one precipitin band which fused completely at the end of the two bands against the antisera prepared separately for both the proteins. Although these results indicate that the 2 proteins are antigenically identical, the possibility exists that two or more precipitin bands may coincide in double gel immunodiffusion. To eliminate this possibility, they were analysed by immunoelectrophoresis in agarose. Both β-conglycinin and 7S b-antigen gave the same migrating precipitin lines to the anode which fused at the original sample well against anti-7S b-antigen and anti-β-conglycinin serum. Furthermore, the migrating position of β-conglycinin revealed in the precipitin band coincided well that of Catsimpoolas [9]. Thus, β-conglycinin was considered to be immunologically identical with the 7S b-antigen (the 7S globulin).

The crude 7S protein [5] showed a dimerization reaction with change of ionic strength from 0·5–0·1 at pH 7·6. The reaction was also a characteristic property of the 7S globulin (7S b-antigen) [6]. β-Conglycinin showed the typical dimerization reaction with the change of ionic strength 0·5–0·1, i.e. $s_{20,w}$ values in 0·5 and 0·1 ionic strength were 7·3S and 10·1S, respectively at the protein concentration of 0·43%.

The dependence of MW on protein concentration for β-conglycinin was measured by Yphantis' method [13]. About 90 min were required to attain equilibrium at 10400 rpm and 21·4°. Extrapolation to zero concentration of the protein gave the MW of 181000. This value agreed with that of 180000 in the 7S globulin [14].

The 7S globulin is a glycoprotein [15,16] in addition to an agglutinin [17,18] in soybean seeds. The carbohydrate and hexosamine contents of β-conglycinin were 4·04 and 1·25%, respectively. These values corresponded

well with 3·75 and 1·19% of the 7S globulin. The carbohydrate/hexosamine ratio was also identical in them, i.e. 3·22 for the former and 3·15 for the latter.

The broad areas over the nearer part to the condensing gel in addition to the fastest moving major band were similarly stained in the patterns of disc electrophoresis of both the proteins. These slower moving broad band areas increased gradually with storage, particularly when the proteins were stored in lower ionic strength than 0·1. However, the disc electrophoretic patterns of both the preparations purified further by preparative-scale polyacrylamide gel electrophoresis gave a single band and their mobilities were identical.

From the experimental results described above, it may be concluded that β-conglycinin is identical with the 7S globulin (7S b-antigen).

DISCUSSION

Catsimpoolas *et al.* [8–10] reported that γ-conglycinin was the 7S globulin isolated by one of the authors [11,12]. Their results however, differ from the conclusion obtained from our experiments. When the elution pattern of the reserve soybean proteins by gel filtration with Sephadex G-150 was minutely investigated, γ-conglycinin of Catsimpoolas eluted mainly after the main peak, whereas β-conglycinin eluted in the main peak with the 11S globulin. Moreover, judging from the area in which γ-conglycinin eluted, the protein might be a minor component. However, the 7S globulin eluted in the main peak of gel filtration using Sephadex G-200 with the 11S globulin. If the elution pattern of Sephadex G-200 is not substantially different from that of Sephadex G-150 and γ-conglycinin is the 7S globulin as mentioned by Catsimpoolas, γ-conglycinin must also elute in the main peak with the 11S globulin in the gel filtration of the reserve soybean proteins with Sephadex G-150. In order to prove the identity of β-conglycinin and the 7S globulin, their dissimilar subunits present in both dissociated proteins which have been found by Catsimpoolas [19] must be compared. The discrepancy between the results of our experiments and those of Catsimpoolas probably derive from the difference of the preparative procedure of the 7S globulin.

Roberts and Briggs [5] determined the MW of the 7S protein which represented mostly β-conglycinin as 330000. On the other hand, the 7S globulin (7S b-antigen) had the value of *ca* 180000 [14]. The possibilities were discussed for the discrepancy in MW of the 2 proteins previously [14]. In addition to the possibilities, the partial association of the 7S globulin preparation in storage should be also considered. The bands moving slower than a single band of the 7S globulin arose apparently when the protein was stored in 5 mM KPi buffer containing 10 mM 2-mercaptoethanol, pH 7·6 for a week. The similar pattern was also obtained from β-conglycinin by Catsimpoolas [9]. He reported that the multiple components of β-conglycinin were attributed to the characteristic association properties of the protein since they formed only one fused immunoprecipitin band on disc immunoelectrophoresis. Thus, the slower moving bands from the 7S globulin were also likely to be the associated products. The artifact formation in disc electrophoresis on polyacrylamide gel was likely to arise after storage at low ionic strength. These associated bands could not dissociate to an original single band even if they were dialysed against the 0·5 μ standard buffer.

EXPERIMENTAL

Protein samples. The purified 7S globulin (7S b-antigen) was prepared as described previously [11,12]. β-Conglycinin was purified by chromatography with DEAE-Sephadex A-50 from a crude 7S protein prepared by Roberts and Briggs [5] according to the method of ref. [9]. Both proteins were further purified by preparative-scale polyacrylamide gel electrophoresis.

Preparation of antisera. Young adult white rabbits were immunized by 2 intramuscular injections into each foot pad at 1 month intervals of a 1% antigen soln in the 0·5 μ standard buffer [4] (32·5 mM K_2HPO_4, 2·6 mM KH_2PO_4, 0·4 M NaCl, 0·01 M 2-mercaptoethanol, pH 7·6) without 2-mercaptoethanol mixed and homogenized with an equal vol of Freund's adjuvant. The injection vol of each antigen was 0·4 ml. After a month, the rabbits were given a 1 ml booster injection of the 0·1% antigen soln into the marginal ear vein and bled after 10 days from the carotid artery by cardiac puncture. The sera was stored at 4° overnight to remove the clot and stored at −80° after centrifugation at 800 *g* for 15 min.

Immunochemical methods. Immunoelectrophoresis in agar gel was performed by the general procedure described in ref. [20]. The gel medium consisted of 1% agarose in Tris–Veronal–HCl buffer, pH 8·6, 0·025 ionic strength. Electrophoresis was carried out using a 50 μg of protein sample with a current of 5 mA per microscope slide glass (2·6 × 7·5 cm) for 90 min at room temp. The precipitin arcs were stained with amido black 10B. Double gel immunodiffusion was performed on the glass slide according to the method of ref [21]. The gel medium consisted of a 1% Bacto-agar soln in the 0·5 μ standard buffer. The reaction was allowed to diffuse at 37° for 18 hr. A single diffusion technique was done for immunological quantitative determination of antigenic proteins by using a glass tube (1 × 50 mm) according to the procedure of ref. [22].

Sedimentation analysis. Ultracentrifugation was carried out at 20° and 512000 rpm in an analytical ultracentrifuge.

MW was determined by the sedimentation equilibrium method of ref. [13] using an eight-channel short column cell.

Disc electrophoresis. The details were carried out as described in refs [23,24] with 6·8% polyacrylamide gel concn as a separating gel. Proteins were detected by staining with 0·5% amido black 10B dye in 7% HOAc soln. For preparative gel electrophoresis the column was prepared by casting a separating gel (6·8%, 2 cm ht) and a stacking gel (3·5%, 1 cm ht). The 0·5% sample was applied directly on the top of the stacking gel with a micro tube pump. Electrophoresis was performed for 12 hr at 50 mA eluting with 0·1 M Tris–HCl buffer, pH 8·1 at 0°. The bottom of the separating gel was washed with the buffer during the electrophoresis using a micro tube pump at 55 ml per hr. Fractions (5·5 ml) were collected and the UV absorbance was measured.

DEAE–Sephadex chromatography. The same column size (4 × 40 cm) for DEAE-Sephadex A-50 chromatography as that described in [9] was used. About 240 mg of the crude 7S protein prepared according to ref. [5] dissolved in 10 ml of the starting buffer (32·5 mM K_2HPO_4, 2·6 mM KH_2PO_4, 0·1 M NaCl, 0·01 M 2-mercaptoethanol, pH 7·6) was put on the column. Elution was performed with the same buffer containing NaCl in gradient concn 0·1–1 M. The mixing chamber contained 350 ml of the starting buffer and the reservoir chamber an equal vol of M NaCl adjusted to pH 7·6. The NaCl concn was determined by the procedure of ref. [25].

Others. Phenol–H_2SO_4 method of Dubois *et al.* [26] was used for determination of total carbohydrate. Hexosamine content was determined by the method of ref. [27]. Mannose and glucosamine were used to prepare the standard curves. Protein concn was measured by turbidity at 420 nm [28].

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REFERENCES

1. Danielson, C. E. (1949) *Biochem. J.* **44,** 387.
2. Naismith, W. E. F. (1955) *Biochim. Biophys. Acta* **16,** 203.
3. Wolf, W. J. and Briggs, D. R. (1956) *Arch. Biochem. Biophys.* **63,** 40.
4. Wolf, W. J. and Briggs, D. R. (1959) *Arch. Biochem. Biophys* **85,** 186.
5. Roberts, R. C. and Briggs, D. R. (1965) *Cereal Chem.* **42,** 71.
6. Koshiyama, I. (1968) *Cereal Chem.* **45,** 405.
7. Koshiyama, I. (1968) *Agr. Biol. Chem. Tokyo* **32,** 879.
8. Catsimpoolas, N., Leuthner, E. and Meyer, E. W. (1968) *Arch. Biochem. Biophys.* **127,** 338.
9. Catsimpoolas, N. and Ekenstam, C. (1969) *Arch. Biochem. Biophys.* **129,** 490.
10. Catsimpoolas, N. (1969) *Cereal Chem.* **46,** 369.
11. Koshiyama, I. (1965) *Agr. Biol. Chem. Tokyo* **29,** 885.
12. Koshiyama, I. (1972) *Agr. Biol. Chem. Tokyo* **36,** 2257.
13. Yphantis, D. A. (1960) *Ann. N.Y. Acad. Sci.* **88,** 586.
14. Koshiyama, I. (1968) *Cereal Chem.* **45,** 394.
15. Koshiyama, I. (1966) *Agr. Biol. Chem. Tokyo* **30,** 646.
16. Koshiyama, I. (1969) *Arch. Biochem. Biophys.* **130,** 370.
17. Lis, H., Sharon, N. and Katchalski, E. (1966) *J. Biol. Chem.* **241,** 684.
18. Lis, H., Sharon, N. and Katchalski, E. (1969) *Biochim. Biophys. Acta* **192,** 364.
19. Catsimpoolas, N. (1970) *Cereal Chem.* **47,** 70.
20. Graber, P. and Williams, C. A. (1953) *Biochim. Biophys. Acta* **10,** 193.
21. Ouchterlony, O. (1967) in *Handbook of Experimental Immunology* (Weir, D. M. ed.), p. 655, Blackwell, Oxford.
22. Catsimpoolas, N. and Meyer, E. W. (1968) *Arch. Biochem. Biophys.* **125,** 742.
23. Ornstein, L. (1964) *Ann. N.Y. Acad. Sci.* **121,** Art 2, 321.
24. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121,** Art 2, 404.
25. Vogel, A. I. (1961) in *Quantitative Inorganic Analysis*, p. 258, Longmans, London.
26. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) *Anal. Chem.* **28,** 350.
27. Elson, L. A. and Morgan, W. T. (1933) *Biochem. J.* **27,** 1824.
28. Luck, J. M., Rasmussen, P. S., Satake, K. and Tsvetikov, A. N. (1958) *J. Biol. Chem.* **233,** 1407.