Plant Physiol. (1978) 61, 1040–1042

Short Communication

Evidence That the Castor Bean Allergens Are the Albumin Storage Proteins in the Protein Bodies of Castor Bean[1]

Received for publication October 11, 1977 and in revised form January 16, 1978

RICHARD J. YOULE[2] AND ANTHONY H. C. HUANG[3]
Department of Biology, University of South Carolina, Columbia, South Carolina 29208

ABSTRACT

The well characterized castor bean (*Ricinus communis L.*) allergens were identified as the low molecular weight albumin storage proteins in the matrix of the protein bodies in the endosperm. The methods of identification involved molecular weight estimation, amino acid composition, stability at 100° C, solubility in various solvents, gel electrophoresis, and immunological techniques. The finding explains the wide distribution of allergens in various seeds.

Potent allergens of protein nature in the castor bean have been studied decades ago by Spies and coworkers (7–11; see reviews 1, 8) and more recently by other investigators (2, 4, 5). These castor bean allergens, studied intensively by Spies' group, are a group of microheterogeneous proteins of low mol wt with closely similar chemical properties. Although they have been well characterized, the cellular and physiological aspects of these proteins in the castor bean such as the *in vivo* function, subcellular localization, and per cent of total seed protein are completely unknown.

Recently, we reported the presence of albumin storage proteins in the castor bean (15). The albumin storage proteins occupy 40% of the total seed proteins and are present in the matrix of the protein bodies (12, 14). A careful literature survey of the known properties of the castor bean allergens and of the albumin storage proteins revealed some similarities, such as the amino acid composition, the low mol wt, and the solubility in water. In this communication, we present evidence that the castor bean allergens are indeed the albumin storage proteins in the protein bodies of castor bean.

MATERIALS AND METHODS

Preparation of Protein Fractions. The matrix proteins, the lectins (4–6S albumins), the 11S globulins, the 2S[4] albumins, and the total proteins of castor bean (*Ricinus communis* var. Hale) were purified as described (14, 15). CB-1A allergens were generously supplied by J. R. Spies.

SDS-Polyacrylamide Gel Electrophoresis. Slab gel electrophoresis was performed in 15% polyacrylamide gels with SDS and a discontinuous buffer system (13). Protein samples were incubated at room temperature for 30 min, or at 100 C for 2 min in 0.01 M Tris-HCl (pH 6.8) and 1% SDS. Mol wt standards were used as described (14).

Immunological Techniques. Each 2.27-kg New Zealand White rabbit was injected in the footpads and the back subcutaneously with 2 mg of CB-1A mixed in complete Freund's adjuvant. After 1 week, they were injected in the back subcutaneously with 2 mg of CB-1A mixed in incomplete Freund's adjuvant. They were bled 10 days after the second injection and the γ-globulins were purified by the procedure of Kendall (3). The γ-globulins were dialyzed against 0.01 M Na-phosphate buffer (pH 7.5), 0.05 M NaCl, and after centrifugation at 10,000*g* for 30 min, the supernatant fraction was retained. γ-Globulins were also prepared by an identical procedure from untreated rabbits as controls.

Double diffusion tests were performed according to Ouchterlony (6). Equal amounts of protein samples to be tested were applied to each well except that twice the amount of the organelle matrix proteins and the total castor bean proteins were used.

In the electrophoretic analysis of the antigenicity of the 2S albumins, 0.08 mg of the 2S albumins were mixed with 200 μl of 10 mM Na-phosphate buffer (pH 7.5) and 15 mM NaCl, and 200 μl of either anti-CB-1A γ-globulins or unsensitized γ-globulins. The solution was incubated at 23 C for 1 hr and then at 4 C for 16 hr. After centrifugation at 10,000*g* for 30 min, the supernatant fraction was lyophilized to dryness. The lyophilized powder was dissolved in 100 μl of 10 mM Na-phosphate buffer (pH 7.5) and 15 mM NaCl and 100 μl of similar γ-globulins. After similar incubation and centrifugation, the supernatant fractions were analyzed by SDS-polyacrylamide gel electrophoresis.

RESULTS

The properties of the well characterized castor bean allergens, the CB-1A fraction, and our purified 2S albumin storage proteins were compared.

SDS-Polyacrylamide Gel Electrophoresis and Mol Wt. After electrophoresis in SDS-polyacrylamide gels of high density (15%), the proteins of the isolated protein bodies of castor bean were resolved into two major groups (Fig. 1a). A group of protein bands of high mol wt occurred at the upper portion of the gel, representing the 11S globulins and the 4–6S albumins and their dissociated subunits (12, 14, 15). Another group of two major protein bands was present at the lower portion of the gel, representing the 2S albumins. The 2S albumins appeared as several protein bands with mol wt around 11,000 daltons. Their low mol wt (Fig. 1a) and their estimated sedimentation value (15) are similar to those of the castor bean allergens reported earlier (9, 10). The isolated 2S albumins and the CB-1A had similar electrophoretic patterns although the amount of the lower band was reduced in the CB-1A (Fig. 1a).

[1] This work was supported by National Science Foundation Grant BMS 75-02320.

[2] Present address: Laboratory of Neurochemistry, National Institute of Mental Health, Bethesda, Md. 20014.

[3] To whom reprint requests should be sent.

[4] Abbreviations: 2S albumins: 2S albumin storage proteins; CB-1A: CB-1A fraction.

Immunological Studies. Rabbit γ-globulins raised against the CB-1A were used to test the antigenic relationship between the CB-1A and the 2S albumins. In the Ouchterlony diffusion test (Fig. 2), the γ-globulins formed two precipitin lines with the total seed proteins, the matrix proteins of protein bodies, and the isolated 2S albumins. The two precipitin lines in each sample were continuous with corresponding precipitin lines of other samples. No reaction was observed with the 11S globulins of the crystalloids or the 4–6S albumins of the organelle matrix. No proteins in the total seed proteins other than the 2S albumins had an immunological relationship with the CB-1A. The two corresponding precipitin lines of the CB-1A and of the 2S albumins did not form a spur at the junction but were continuous and exhibited a crossover, suggesting that the two protein fractions contained indistinguishable antigens which occurred in different proportions. Such identity and unequal proportionality of the two protein fractions are in good agreement with their protein patterns in polyacrylamide gels (Fig. 1a).

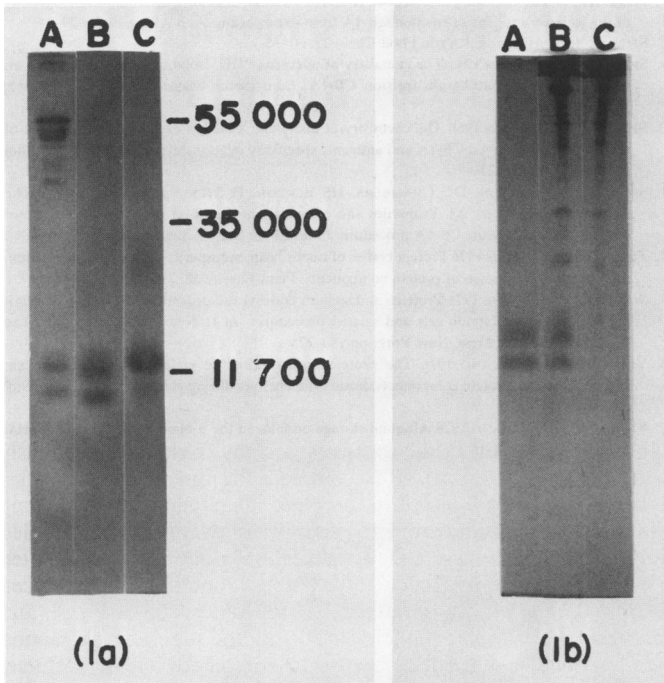

FIG. 1. a: Photograph of SDS-acrylamide gels after electrophoresis of total protein bodies (A), isolated 2S albumins (B), and CB-1A allergens (C) of castor bean. Numbers on the right are estimated mol wt in daltons. b: Immunological analysis of the antigenicity of the 2S albumins toward anti-CB-1A γ-globulins. An equal amount of 2S albumins was untreated (A), or treated with either unsensitized γ-globulins (B), or anti-CB-1A γ-globulins (C). After centrifugation to remove the antigen-antibody complexes, an equal amount of the supernatant fraction was analyzed by SDS-polyacrylamide gel electrophoresis.

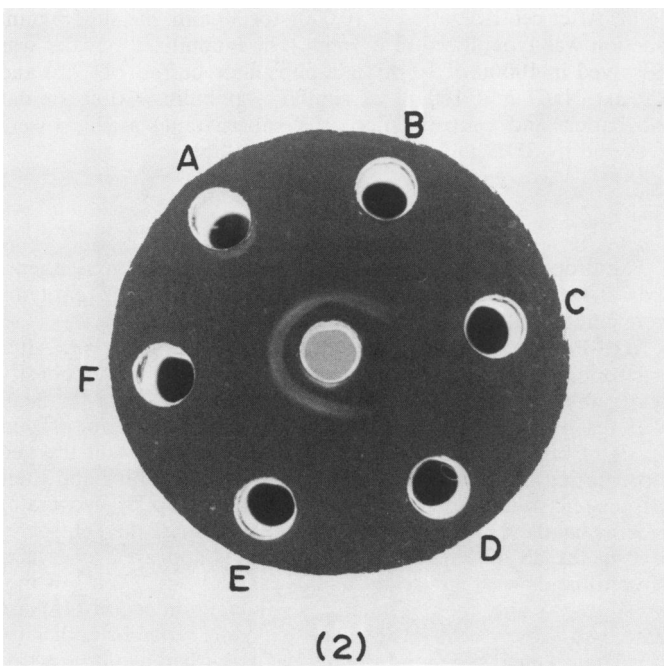

FIG. 2. Photograph of Ouchterlony double diffusion tests of rabbit γ-globulins prepared against CB-1A allergens. Central well: rabbit γ-globulins; A: 2S albumins; B: CB-1A; C: 11S globulins; D: 4–6S albumin; E: matrix proteins of protein bodies; F: total seed proteins.

Another approach was performed to study the immunological relationship between the 2S albumins and the CB-1A. The 2S albumins were incubated with γ-globulins prepared from rabbits previously sensitized with CB-1A or with control γ-globulins obtained from unsensitized rabbits. After incubation, the precipitate was removed by centrifugation and the supernatant fraction was analyzed by SDS-acrylamide gel electrophoresis. As shown in Figure 1b, the 2S albumins were completely precipitated by the γ-globulins prepared against CB-1A whereas they were still present after treatment with control γ-globulins. The finding indicates that all of the 2S albumins are antigenically related to the CB-1A.

Amino Acid Composition. An examination of the published data on the amino acid composition of the CB-1A (11) and of the 2S albumins (15) reveals great similarity. The 2S albumins are storage proteins with unique amino acid composition, notably the high amount of arginine (9.4%) and glutamate/glutamine (30%), and the low content of aspartate/asparagine (4.4%). These figures are in good agreement with the amount of arginine (11.2%), glutamate/glutamine (29.2%), and aspartate/asparagine (5.7%) in CB-1A. Although the amino acid composition of the CB-1A was analyzed some 30 years ago using microbiological assays, the data match well with our amino acid data on the 2S albumins obtained with an amino acid analyzer.

Solubility and Heat Stability. Spies and Coulson (9) prepared the CB-1A from the total extract of castor bean by retaining those proteins that were heat-stable and that went through a series of steps of differential solubility precipitation in various solvents. To compare with the properties of the CB-1A, the 2S albumins were tested for their heat stability and solubility in similar solvents. The 2S albumins were water-soluble and remained in solution after treatment at 100 C for 10 min. When they were treated with 75% ethanol, 70% precipitated; 50% of their precipitate was soluble in a solution of 10% lead acetate and 25% ethanol. Thus, a large portion (35%) of the 2S albumins were retained by Spies' procedure for the preparation of the CB-1A.

DISCUSSION

By various criteria, we establish that the 2S albumins are the well characterized CB-1A allergens of the castor bean. When Spies and co-workers prepared their CB-1A allergens, they followed, without modification, a procedure which they established in the preparation of allergens from cotton seeds. The fractionation procedure employed heat stability and differential solubility in various solvents of the proteins. The yield and proportion of individual components of a protein mixture in such a fractionation procedure could be variable depending on many factors, such as concentration and the occurrence of other proteins and compounds. Since the recovery of the CB-1A was not checked, its protein components might not be in the same proportion as those of all of the castor bean allergens *in vivo*. On the other hand, we utilize mainly the solubility in water, rate sucrose gradient centrifugation, and gel filtration to recover the 2S albumins quantitatively. The two diverse procedures of the preparation should

explain the unequal proportion of the various protein components (Fig. 1a) and antigens (Fig. 2) in the two fractions.

The present identification provides a direct bridge in our knowledge between the chemistry of the castor bean allergens and the cellular and physiological aspects of the albumin storage proteins. The wealth of knowledge on the castor bean allergens such as the detailed antigenic properties of the microheterogeneous components (7, 10) greatly improves our understanding of storage proteins. Our recent finding (in preparation) that the low mol wt albumin storage proteins are widely distributed among seeds relates to the occurrence of similar allergens in many oilseeds as reported by Spies *et al.* (11).

Acknowledgments—We sincerely thank J. R. Spies for several inspiring discussions and a generous supply of the invaluable CB-1A fraction. We also thank M. Felder for his assistance in antibody preparation.

LITERATURE CITED

1. BERRENS L 1971 The chemistry of atopic allergens. Monogr Allergy 7: 74–103
2. DAUSSANT J, RL ORY, LL LAYTON 1976 Characterization of proteins and allergens in germinating castor seeds by immunochemical techniques. J Agric Food Chem 24: 103–107
3. KENDALL FE 1938 The use of immunological method for the identification and determination of human serum protein. Cold Spring Harbor Symp Quant Biol 6: 376–384
4. LAYTON LL, BT DANTE, LK MOSS, NH DYE, F DEEDS 1961 Electrophoretic fractionation of soluble antigenic proteins from the seed of *Ricinus communis* (castor bean). J Am Oil Chem Soc 38: 405–410
5. LAYTON LL, E YAMANAKA, S LEE, TW GREEN 1962 Multiple allergies to the pollen and seed antigens of *Ricinus communis* (castor bean). J Allergy 33: 232–235
6. OUCHTERLONY O 1968 Handbook of Immunodiffusion and Immunoelectrophoretic Techniques. Ann Arbor-Humphrey Science Publishers, Ann Arbor, Mich
7. SPIES JR 1967 The chemistry of allergens. XIX. On the number of antigens and the homogeneity of the isolated antigens of fraction CB-1A from castor beans. Ann Allergens 25: 29–34
8. SPIES JR 1974 Allergens. J Agric Food Chem 22: 30–36
9. SPIES JR, EJ COULSON 1943 The chemistry of allergens. VIII. Isolation and properties of an active protein-polysaccharide fraction CB-1A, from castor beans. J Am Chem Soc 65: 1720–1725
10. SPIES JR, EJ COULSON 1964 The chemistry of allergens. XVI. Ion exchange fractionation of the castor bean allergen CB-1A and antigenic specificity relationships of the fractions. J Biol Chem 239: 1818–1827
11. SPIES JR, EJ COULSON, DC CHAMBERS, HS BERTON, H STEVENS, JH SHIMP 1951 The chemistry of allergens. XI. Properties and composition of natural proteases isolated from oilseeds and nuts by the CS-1A procedure. J Am Chem Soc 73: 3995–4001
12. TULLY RE, H BEEVERS 1976 Protein bodies of castor bean endosperm: isolation, fractionation, and the characterization of protein components. Plant Physiol 58: 710–716
13. WEBER K, M OSBORNE 1975 Proteins and sodium dodecyl sulfate: molecular weight determination on polyacrylamide gels and related procedures. *In* H Neurath, RL Hill, eds, The Proteins, Academic Press, New York, pp 180–225
14. YOULE RJ, AHC HUANG 1976 The protein bodies from the endosperm of castor bean: subfractionation, protein components, lectins and changes during germination. Plant Physiol 58: 703–709
15. YOULE RJ, AHC HUANG 1978 Albumin storage proteins in the protein bodies of castor bean. Plant Physiol 61: 13–16