# Albumin Storage Proteins in the Protein Bodies of Castor Bean[1]

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## ABSTRACT

Of the total protein in the protein bodies of castor bean (*Ricinus communis* L.), approximately 40% is represented by a group of closely related albumins localized in the matrix of the organelle. This group of albumins has a sedimentation value of 2S and is resolved into several proteins of molecular weight around 12,000 daltons by sodium dodecyl sulfate-acrylamide gel electrophoresis. It has a high content of glutamate/glutamine and undergoes rapid degradation during the early stage of germination. In view of the abundance and ubiquitous occurrence of albumins in various seeds, we suggest that albumins, in addition to globulins, glutelins, and prolamines, are important storage proteins in seeds.

Since the classic studies of seed proteins by Osborne (19) and Danielsson (6), the storage proteins of legumes and oil seeds have been considered to be globulins that are insoluble in water but soluble in salt solution. Water-soluble proteins, albumins, also exist in these seeds. Albumins have not been shown to be storage proteins, but are generally thought to be enzymes and other metabolic proteins (1, 2, 4, 7, 17). However, such consideration cannot explain the high content of albumins in many seeds (3, 5, 10).

In this paper, we report the characterization of a major group of albumins isolated from the protein bodies of castor bean. We provide ample evidence to demonstrate its physiological role as a storage protein. In view of the abundance and ubiquitous occurrence of albumins in various seeds, we suggest that albumins are important storage proteins in seeds. We also establish the occurrence of two major but diverse classes of storage proteins having distinct suborganelle localization in the protein bodies of a seed.

## MATERIALS AND METHODS

**Preparation of Protein Fractions.** Three g of dry deshelled castor bean (*Ricinus communis* L. var. Hale) were ground in 100 ml of 1 M NaCl, 0.035 M Na-phosphate buffer (pH 7.5) for 20 min in a VirTis 45 homogenizer. The crude extract was centrifuged at 10,000g for 30 min and the supernatant fraction beneath the fat layer was used as the total protein extract.

Protein bodies from dry castor bean were isolated and purified by the nonaqueous technique reported previously (25). The purified protein bodies were lysed and subfractionated on a sucrose gradient to yield the matrix fraction and the crystalloid fraction (25).

The 2S albumin proteins were purified from the matrix fraction of protein bodies isolated by the following method

(23). Dry castor beans were ground in glycerol and the protein bodies were pelleted by centrifugation. The protein bodies were lysed by the addition of water and the matrix fraction was separated from the other organelle components by centrifugation on a sucrose gradient (25). The matrix proteins were applied to a Sephadex G-50 column (2.6 × 90 cm) equilibrated with 0.05 M imidazole-HCl buffer (pH 7) and eluted with the same buffer. Fractions were collected and assayed for protein composition by 15% gel electrophoresis. The 4–6S proteins were eluted in the void volume and the 2S proteins were eluted afterward. The 2S protein fractions were slightly contaminated by some of the 4–6S proteins (the lectins) which were presumably retained by sugar-binding interaction with dextran gel (18, 23). All fractions containing the 2S proteins were pooled, concentrated by lyophilization, and rechromatographed on the same column of Sephadex G-50. All of the eluted fractions with the 2S proteins were again pooled and the combined eluate showed no contamination on 15% gel electrophoresis.

**Determination of Sedimentation Values by Ultracentrifugation.** Protein fractions were prepared for ultracentrifugation by dissolving in or dialyzing against 35 mM Na-phosphate buffer (pH 7.5) and 1 M NaCl. Purified protein bodies in hexane and $CCl_4$ were mixed with the buffer, and all of the organic solvents were allowed to evaporate under $N_2$. After this treatment which solubilized the crystalloid and matrix proteins, the globoids and membrane were removed by centrifugation. Since catalase dissociated into its subunits in 1 M NaCl, the enzyme was dissolved in the buffer without NaCl.

Sedimentation analyses were performed by sucrose gradient centrifugation according to Martin and Ames (15) with modification (10). Each 1-ml protein sample was applied on top of a linear density gradient composed of 34 ml of 5 to 30% (w/w) sucrose. The gradient solution also contained 35 mM Na-phosphate buffer (pH 7.5) and 1 M NaCl. In the centrifugation of catalase, NaCl was omitted in the gradient solution. The gradient was centrifuged at 25,000 rpm for 24 hr in a Beckman L2-65B ultracentrifuge using a SW 27 rotor. The gradient was fractionated into 1-ml fractions and the protein was assayed according to Lowry et al. (13) with BSA as the standard. Myoglobin (type II, Sigma) and bovine liver catalase (type C-10, Sigma) were used as markers for sedimentation values. Catalase was assayed as described (14) and myoglobin was assayed by the Lowry method.

**Gel Electrophoresis.** Disc and slab gel electrophoresis was performed in 7.5% or 15% polyacrylamide gel with SDS and a discontinuous buffer system (24, 25). Protein samples were incubated at room temperature for 30 min or boiled for 2 min in 0.01 M tris-HCl buffer (pH 6.8), 1% SDS, and as appropriate, 1% β-mercaptoethanol. Molecular wt standards were used as described previously (25).

**Protein Hydrolysis and Amino Acid Analysis.** After dialysis against water and lyophilization, the protein samples were hydrolyzed to amino acids with 4 N methanesulfonic acid *in vacuo*. The procedure that preserved tryptophan was followed (20). The protein samples were reduced with DTT for half-

cystine analysis. Amino acid analyses were performed on a Beckman 120-C instrument.

**Germination.** Castor beans were soaked overnight in running tap water and germinated in moist vermiculite in 30 C in darkness. The endosperm at various stages was ground with a mortar and pestle in grinding medium (11). All of the extracts were made to the same volume (2.66 ml/endosperm pair) with grinding medium. The extracts were centrifuged at 5,000g for 10 min and the supernatant fractions beneath the fat layer were used for gel electrophoresis.

## RESULTS

**Sedimentation Values of Proteins.** As reported previously (23, 25), most of the protein in the castor bean endosperm was restricted to the protein bodies. When the proteins of the protein bodies were extracted and centrifuged on a sucrose gradient, three protein fractions were obtained (Fig. 1). The two major protein fractions had approximate sedimentation values of 2S and 11S, and a minor protein fraction possessed a sedimentation value of 4-6S. A similar protein profile was obtained from a total protein extract of the seeds (data not

shown). When the protein bodies were subfractionated as described before (25), the albumins of the organelle matrix yielded the 2S and 4-6S fractions whereas the globulin of the crystalloids comprised the 11S fraction. The 2S and 4-6S fractions in the organelle matrix were purified by gel filtration chromatography and affinity chromatography (18), respectively, for identification and characterization (see next section). The 2S fraction represented proteins of low mol wt (around 12,000 daltons), and the 4-6S fraction was identified as mostly the castor bean lectins. The identification of these protein fractions was further checked by their protein patterns in 7.5% SDS-acrylamide gel after electrophoresis and the data (gel patterns in Fig. 1) were in agreement with those reported earlier (25). As estimated from the protein profile in the sucrose gradient of the extracted proteins of the protein bodies (Fig. 1), approximately 40% of the protein was represented by the 2S proteins, 10% by the 4-6S proteins, and 50% by the 11S proteins.

**Protein Composition of 2S Proteins.** In order to obtain good resolution of proteins of low mol wt by gel electrophoresis, we used polyacrylamide gels of high density (15% gel). Such a gel system gave good resolution of proteins of low mol wt, but was not suitable for the identification of proteins of high mol wt. Using this gel system, the organelle matrix was resolved into two groups of proteins (Fig. 2). The protein bands at the upper portion of the gel represented the 4-6S fraction containing mostly the castor bean lectins, which had higher mol wt (18, 25). The three protein bands at the lower portion of the gel were identified as the 2S proteins by using the isolated 2S fraction. Sucrose gradient centrifugation of the isolated 2S proteins in the absence of 1 M NaCl showed that they still migrated with a sedimentation value of 2S, which was approxi-
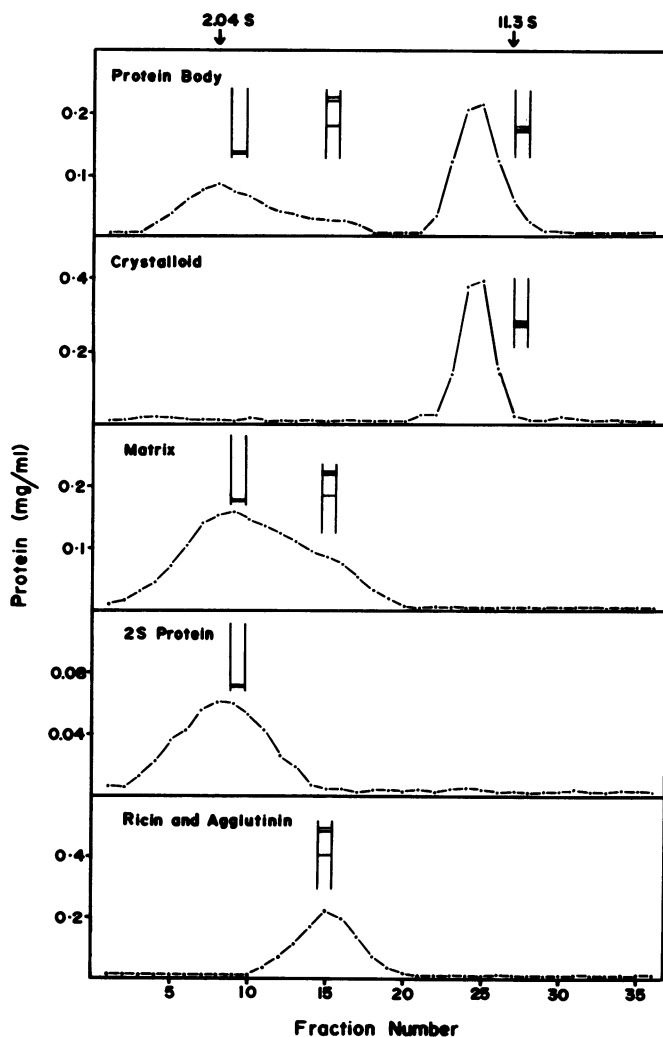


FIG. 1. Sucrose gradient centrifugation of castor bean proteins. Each linear gradient was composed of 34 ml of 5% (w/w) to 30% sucrose. Centrifugation was carried out in a Beckman SW 27 rotor for 24 hr at 25,000 rpm. Myoglobin (2.04S) and catalase (11.3S) were used as markers of sedimentation values. The protein pattern in 7.5% SDS-acrylamide gel was obtained as described earlier (25).
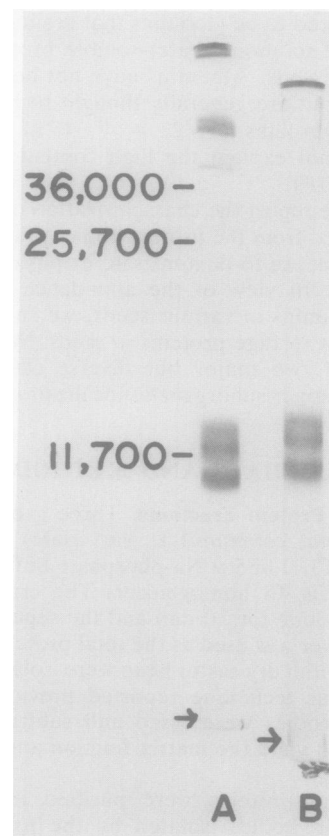


FIG. 2. Photograph of 15% SDS-acrylamide gels after electrophoresis of isolated organelle matrix of castor bean protein bodies (A) and purified 2S proteins (B). Arrows indicate positions of tracking dye. Numbers on left are estimated mol wt in daltons.

mately of a mol wt of 12,000 daltons (15). They were probably equivalent to the two protein bands of low mol wt observed previously (23). The crystalloids (22, 23, 25) and the 11S proteins (12) of the castor bean had been described earlier. In the present study, the 11S proteins were reduced to numerous protein bands of lower mol wt in SDS-acrylamide gel electrophoresis after exhaustive dissociation with SDS and reduction with β-mercaptoethanol (data not shown). All of these protein bands were of higher mol wt than those of the 2S proteins (Fig. 2B), indicating that the 2S proteins were not subunits of the 11S protein.

**Amino Acid Composition.** The total seed protein, the purified 2S proteins, and the 11S protein were subjected to amino acid analysis (Table I). The 11S protein showed an amino acid profile characteristic of storage proteins (4, 9), and distinct from metabolic and structural proteins (4, 8). The values of glutamine/glutamic acid, asparagine/aspartic acid, arginine and tryptophan were analogous to those in other 11S seed proteins (9). The 2S proteins also exhibited an amino acid composition with characteristics similar to those of other seed storage proteins; yet differences existed. The values of glutamine/glutamic acid and arginine were high, a characteristic of storage protein. The contents of leucine and asparagine/aspartic acid were comparatively low, whereas the values of cysteine and serine were high. Ricin and agglutinin (the lectins) were the two major 4–6S proteins, representing some 10% of the protein in the protein bodies. Their amino acid compositions as reported (18) were distinctly different from those of the 2S and 11S proteins (Table I). The amino acid composition of the total seed protein matched well with the combined values of the 2S proteins, 11S protein, and the lectins, when their relative amounts in the seed were taken into account.

**Developmental Changes of 2S Proteins During Germination.** The present gel system, designed to yield good resolution of proteins of low mol wt, was used to study the developmental change of the 2S proteins during germination. As shown in

Figure 3, the three 2S protein bands disappeared rapidly after the 1st day of germination. The disappearance of the 2S proteins coincided with the degradation of the 11S protein reported previously (25). In our previous report (25), we used a gel system that gave good resolution only of proteins of high mol wt. There, the 2S proteins migrated as one protein band of low mol wt along with the tracking dye and possibly other compounds of low mol wt, and thus the disappearance of the 2S proteins during germination escaped our attention.

## DISCUSSION

The 2S albumins of castor bean are shown to be localized within the protein bodies, to constitute 40% of the total seed protein, to degrade rapidly during germination, and to have an amino acid composition characteristic of storage proteins. Based upon these findings, we conclude that the 2S albumins are storage proteins. Our findings provide the first experimental evidence that albumins in ungerminated seeds are storage proteins. Our data also show that in castor bean, there are two major classes of storage proteins with distinct chemical properties and suborganelle compartmentation in the protein bodies: the 11S globulin in the crystalloid and the 2S albumins in the organelle matrix. The 2S and 11S storage proteins contain high amounts, 30% and 16% respectively, of glutamate/glutamine. Such an amino acid composition presumably reflects the storage role of the proteins, since during germination, 40% of the amino acid transported from the storage endosperm to the growing embryonic axis is glutamine (21). Albumin storage

Table I. Amino Acid Composition of Castor Bean Proteins.

|      | Total Protein | 2S Proteins | 11S Proteins | Ricin[1] | Agglutinin[1] |
|------|---------------|-------------|--------------|----------|----------------|
| Try  | 0.6           | trace       | 1.0          | ND[3]    | ND[3]          |
| Lys  | 2.6           | 3.0         | 2.0          | 1.7      | 2.3            |
| His  | 1.5           | 0.8         | 1.9          | 1.0      | 1.1            |
| Arg  | 8.8           | 9.4         | 9.2          | 6.3      | 5.3            |
| Cys[2] | 3.5         | 8.5         | 2.9          | 9.8      | 9.8            |
| Asx  | 9.8           | 4.4         | 11.4         | 12.1     | 11.9           |
| Thr  | 4.4           | 1.4         | 4.2          | 7.0      | 7.4            |
| Ser  | 7.5           | 11.8        | 9.1          | 6.8      | 6.9            |
| Glx  | 18.6          | 30.0        | 16.0         | 9.8      | 10.0           |
| Pro  | 4.2           | 2.5         | 3.5          | 4.9      | 5.3            |
| Gly  | 7.2           | 8.4         | 8.4          | 7.0      | 7.0            |
| Ala  | 6.2           | 3.7         | 6.3          | 7.4      | 6.4            |
| Val  | 6.2           | 4.6         | 6.2          | 5.6      | 6.1            |
| Met  | 1.7           | 1.6         | 2.0          | 0.8      | 0.7            |
| Ile  | 5.1           | 3.0         | 4.1          | 6.3      | 6.7            |
| Leu  | 6.5           | 4.2         | 6.1          | 8.5      | 8.2            |
| Tyr  | 2.5           | 1.8         | 2.6          | 2.7      | 2.3            |
| Phe  | 3.1           | 0.9         | 3.3          | 3.3      | 3.5            |

[1] Data obtained from Nicolson et al (18). Because of the difference in terminology, we assume RCAI and RCAII (18) to be agglutinin and ricin (25), respectively.

[2] Total Protein, 2S proteins, and 11S proteins had cysteine determined as S-sulfocysteine. The cysteine in ricin and agglutinin was determined as half cystine.

[3] N.D., not determined.



FIG. 3. Photograph of 15% SDS-acrylamide gels after electrophoresis of total extracts of castor bean endosperm at various days of germination. D and S denote dry and soaked beans, respectively. The gel was designed to give good resolution of proteins of low mol wt and thus the proteins of high mol wt were not well resolved.

proteins may be quite common among seeds in view of the substantial amount of albumin in many seeds (3, 5, 10). Whether or not in other seeds they have characteristics similar to those of the 2S proteins in castor bean remains to be seen.

Of nutritional interest, the castor bean contains a substantial amount of 2S albumins that possess high cysteine content. Such a system offers a novel example of possible genetic manipulation of seed protein for nutritive value. The amino acid contents of nutritionally important seeds have been genetically manipulated by changing the relative amounts of the respective classes of proteins (16). Albumins in both corn and pea have amino acid contents nutritionally superior to the bulk of the seed proteins, and valuable varieties of corn with high albumin content have been bred (4, 16). A question arises as to how much the relative proportion of the various classes of the seed protein can be altered (16, 17). Castor bean is a clear example that seeds with a high amount of storage albumin do exist.

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