Globulin and Albumin-2 Associated with Protein Bodies in *Amaranthus cruentus* Seeds

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To examine whether albumin-2, a specific protein found only in amaranth seeds so far, is associated with protein bodies, we isolated protein bodies from *Amaranthus cruentus* seed embryos by rate-zonal centrifugation with a sucrose gradient. Most protein bodies in the final preparation were intact when observed by electron microscopy. Profiles by SDS-PAGE showed that the isolated protein bodies contained globulin and albumin-2.

Key words: *Amaranthus cruentus* L.; protein body; globulin; albumin-2

Amaranth (genus *Amaranthus*) seeds contain 14–16% crude protein with a well-balanced amino acid composition.† Abrasion of seeds has shown more of the protein to be in the seed embryo than in the starchy perisperm.‡ We have used electron microscopy to find protein bodies 2–5 μm in diameter that contain phytin globoids in embryos (cotyledonous and radicle cells) of *A. cruentus* seeds.§ The protein bodies are similar morphologically to legumin vacuolar protein bodies in their lack of attached polysomes.

The major protein of amaranth seeds is an oligomeric globulin with high molecular weight.¶ We have extracted two albumin fractions, albumin-1 and -2, from defatted flour of seeds of *A. hypochondriacus*, *A. cruentus*,¶ A. caudatus, and *A. hybridus* (Konishi et al., unpublished data). Albumin-1 is extractable with 0.5 M NaCl or water and albumin-2 can be extracted only with water after exhaustive extraction of globulin and albumin-1. As far as we know, albumin-2 is found only in amaranth seeds; because no protein corresponding to albumin-2 has been extracted from the related species quinoa (*Chenopodium quinoa* Willd.) or buckwheat (*Fagopyrum esculentum* Moench).¶ In addition, albumin-2, but not albumin-1, is protected from proteolysis when flour of amaranth seeds is treated with protease for 30 min at 20°C.¶ These results suggest that albumin-2, together with globulin, is associated with protein bodies.

In this study, we therefore isolated protein bodies from *A. cruentus* seed embryos by rate-zonal centrifugation with a discontinuous sucrose gradient and examined proteins in the bodies by SDS-PAGE.

*A. cruentus* seeds were the generous gift of the Rodale Research Center (Emmaus, PA, USA). For isolation of protein bodies from seeds, aqueous and nonaqueous methods are generally used, but both approaches were unsuccessful when whole seeds of amaranth were used. One reason might be contamination by perisperm starch granules during centrifugal fractionation, because the granules are smaller (about 1 μm in diameter) than protein bodies.

We therefore used embryonic tissues as the starting material for isolation of protein bodies in this study. In amaranth seeds, the embryo surrounds the perisperm, so embryonic tissue was obtained by mechanical force of the seeds themselves when they were soaked in water overnight at 4°C. The embryonic tissue (1 g) was homogenized with 0.5 M sucrose with a Teflon Potter-Elvehjem homogenizer. The homogenate was passed through a 145-mesh screen for removal of unbroken tissues and the cell wall fraction and was then centrifuged (100 × *g*, 1 min). The resulting supernatant was collected and centrifuged (3,500 × *g*, 10 min) for precipitation of protein bodies. The precipitate was suspended in 0.1 ml of 0.5 M sucrose and put on a 40% (w/v) sucrose solution (1.4 ml), followed by centrifugation (10,000 rpm, 60 min) with a Beckman Microfuge E centrifuge. The precipitated protein bodies were examined for integrity by electron microscopy and then analyzed by SDS-PAGE. The buoyant density of the isolated protein bodies was measured by Nycodenz (Nycoderm, Oslo, Norway) isopycnic centrifugation.

Figure 1A shows an electron micrograph of the embryos of dry seeds, in which protein bodies are densely electron-stained. These bodies included phytin globoids that are seen as voids, probably because globoid crystals had fallen out during thin-sectioning, as occurred previously.¶ Figure 1B shows the ultrastructure of unbroken tissues retained on the 145-mesh screen after homogenization of the water-soaked seed embryo, in which subcellular organelles were more loosely packed within the cells and were now swollen. The lipid bodies, in particular, surrounding protein bodies, were more spherical than those in the dry seeds. The structure of protein bodies, however, was maintained, with a membrane, and were densely electron-stained. The final preparation of protein bodies was contaminated with starch granules (Fig. 1C). A few of the protein bodies were unevenly

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stained, suggesting that some proteins had leaked out of the bodies (Fig. 1D), but most of the protein bodies were densely stained (Fig. 1E).

Figure 2 shows profiles by SDS-PAGE of proteins extracted with 0.1% Triton X-100 from the protein body fraction, together with those of globulin, albumin-1, and albumin-2 extracted from defatted A. cruentus seed flour as described previously. The globulin fraction contained five polypeptides with molecular masses of 41, 17, 16, 15.5, and <14 kDa, in agreement with the results of Gorinstein et al. The albumin-2 fraction also had five components, with molecular masses of 56, 35, 31, 25, and 22 kDa, as we reported previously. The isolated protein bodies contained all of these components of globulin and albumin-2. The buoyant density of the isolated protein bodies was 1.27–1.28 g/ml on Nycodenz gradient centrifugation (Fig. 3).

In general, seed storage proteins are water-insoluble: globulin, prolamin, and glutenin. However, albumin is a storage protein in legumes such as pea, mung bean, and castor bean, even though globulins are more abundant storage proteins in these legumes.

This study showed that globulin and albumin-2 are components of protein bodies in amaranth seeds, in which they are storage proteins. These proteins were broken down rapidly during germination (Konishi et al., manuscript in preparation).

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Fig. 2. SDS-PAGE of Proteins Extracted from Isolated Protein Bodies, and of Albumin-1, Albumin-2, and Globulin.

Proteins were extracted with 0.1% Triton X-100 from isolated protein bodies. Albumin-1, albumin-2, and globulin fractions were extracted from the defatted flour of A. cruentus seeds as described previously. SDS-PAGE was done by the method of Laemmli (10) with a 15% separating gel. PB, protein body; A2, albumin-2; G, globulin; A1, albumin-1; M, molecular mass standards (Bio-Rad Laboratories): phosphorylase (97.4 kDa), bovine serum albumin (66.2 kDa), egg white ovalbumin (42.7 kDa), bovine carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa), and egg white lysozyme (14.4 kDa).

Fig. 3. Nycodenz Isopycnic Centrifugation of Protein Bodies Isolated from A. cruentus Seeds.

Isolated protein bodies were suspended in 5 ml of 30.3% Nycodenz solution containing 5 mM Tris-HCl (pH 7.5), 3 mM KCl, 0.3 mM EDTA, and 7.45% sucrose, and the suspension put on a discontinuous gradient of 45.5% and 60.6% Nycodenz containing the same buffer (5 ml each) in a tube. Tubes were centrifuged at 40,000×g for 16 h in a Hitachi 55P-2 ultracentrifuge with an RP30-2 angle rotor. After centrifugation, 2.2-ml fractions were collected. Densities (g/ml) of the Nycodenz of the fractions were measured spectrophotometrically as recommended by the manufacturer (Nycodex Pharma, Oslo, Norway). Protein was measured by the method of Bradford (17).