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Scanning Electron Microscopy of Soybean Protein Bodies

Abstract

Protein bodies prepared from defatted soybean flour contained numerous spherical particles 1–3 μ in diameter, plus amorphous material, when examined in a scanning electron microscope. Full-fat and defatted soybean flours contained particles 1–10 μ in diameter. The larger protein bodies apparently disrupted during isolation. The scanning technique is a simple and rapid method for observing the effects of various treatments on subcellular seed particles of this size.

Many seed proteins are located in discrete cellular organelles called aleurone grains or protein bodies which have been isolated from several seeds. Characterization of protein bodies usually involves light and transmission electron microscopy. The scanning electron microscope has the advantage of a large depth of focus and direct viewing of samples without staining or sectioning. I have examined protein bodies isolated from mature soybeans and found, for the first time, that these materials are readily observed with the scanning electron microscope which should be useful in studies of the effects of grinding, defatting, and drying conditions on integrity of protein bodies.

Kanrich variety soybeans were cracked, dehulled and ground in an Alpine 160Z Kolleplex pin mill. The resulting meal was defatted at room temperature with pentane-hexane, air-dried, and material passing through a 325-mesh sieve was used to isolate the protein bodies by sucrose density gradient centrifugation at 25 C as described by Tombs (1). The sucrose solution containing the protein body fraction was diluted with distilled water, centrifuged, the protein bodies washed two to three times with water to remove sucrose, and finally dried in the centrifuge tube in a vacuum desiccator or freeze-dried. The protein body samples contained 82–83% protein (nitrogen X 5.8) in good agreement with values reported by Tombs (1) for his unfractionated preparations.

Ultracentrifugal analysis of the proteins in the protein body preparations confirmed that these are the storage sites of the major soybean proteins. Analyses in standard buffer (2) revealed 2S, 7S, 11S, and 15S components similar to those observed in the water-

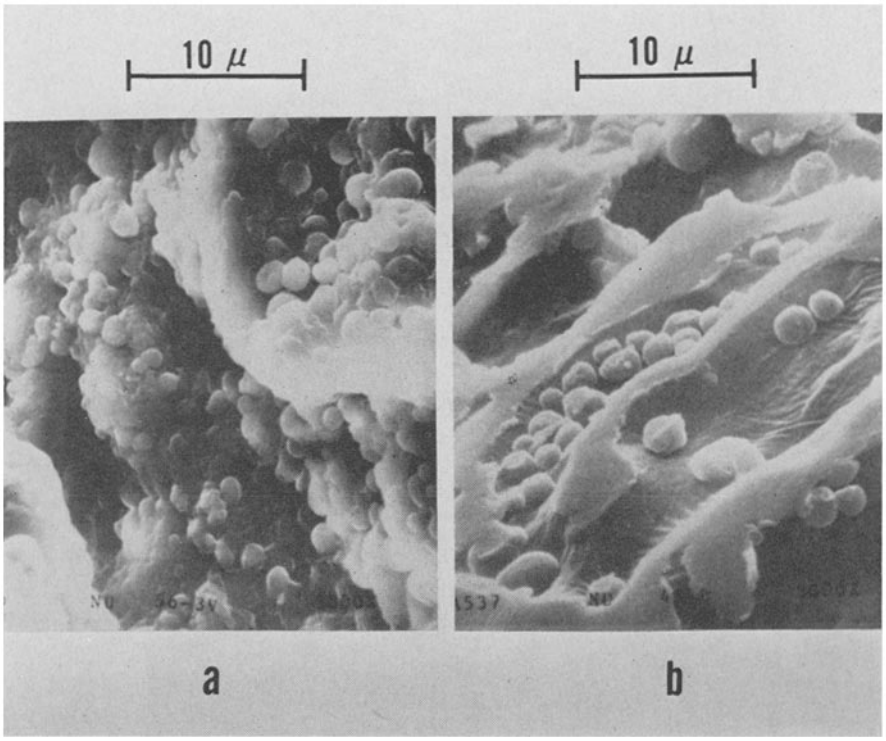

FIG. 1. Scanning electron micrograph of (a) soybean protein bodies and (b) pellet fraction obtained by sucrose density gradient centrifugation (× 3000). The samples were sprinkled onto a specimen holder covered with double-coated cellulose adhesive tape, coated with gold and then examined in a Stereoscan Mark II scanning electron microscope (Cambridge Instrument Co., Ltd., London, England).

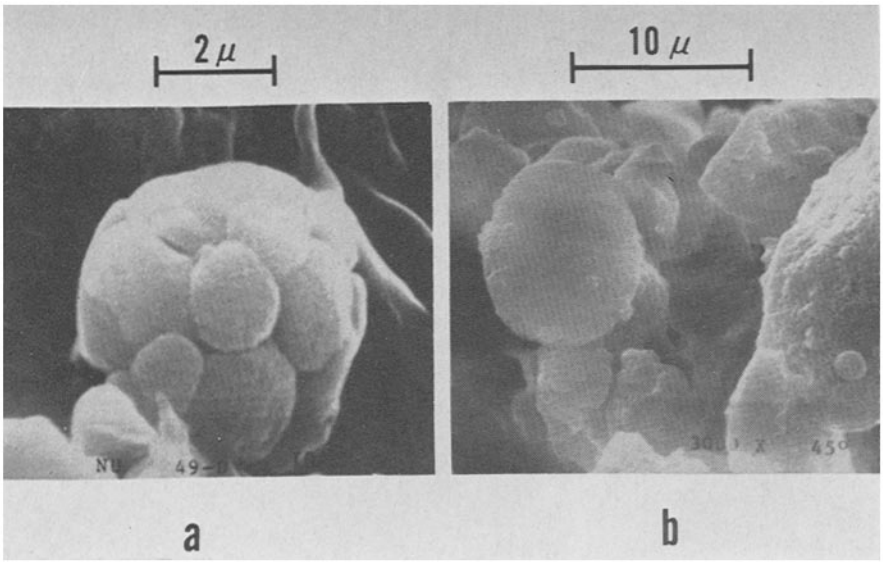

FIG. 2. Scanning electron micrograph of (a) aggregate of protein bodies in pellet fraction (× 10,000) and (b) protein bodies in undefatted ground soybeans (× 3000).

extractable protein mixture of defatted soybean meal, except that the 2S fraction was lower in concentration than in the water-extractable proteins. Tombs concluded on the basis of polyacrylamide gel electrophoresis that the protein bodies contained only the 11S ultracentrifugal component. Immunoelectrophoresis has since shown that the body proteins do not differ greatly from the proteins isolated from soybean cotyledons (3), in agreement with my ultracentrifugal analyses.

Examination of the vacuum-dried protein bodies in the scanning electron microscope revealed numerous spherical or nearly spherical particles 1–3 μ in diameter, plus some amorphous material (Fig. 1a). Freeze-dried preparations contained more amorphous material and a greater number of small particles (1 μ or less in diameter) than the vacuum-dried sample; the freeze-dried particles also appeared to be coated with the amorphous fraction. Protein bodies in soybean cotyledons are reported to be 2–20 μ in diameter (1) but none of the particles in my preparations were larger than about 3 μ in diameter.

The density gradient separation also yielded a pellet fraction, which consists primarily of the water-insoluble polysaccharides of soybeans. The pellet fraction (Fig. 1b) consisted of a filmy material interspersed with particles similar to those seen in Figure 1a. The largest individual particle in the pellet fraction was about 4 μ in diameter. A particle 5 μ in diameter was observed in the pellet fraction, but it was obviously an aggregate of particles 1–2 μ in diameter (Fig. 2a). The soybean flour (before and after defatting) used for isolation of the protein bodies, however, contained particles 1–10 μ in diameter (Fig. 2b) which is the size expected for protein bodies on the basis of work by others (1,4–6).

Since the flour contained particles considerably larger than those in either the isolated protein body preparation or the pellet fraction, the large protein bodies apparently did not survive the isolation process. The amorphous material in the protein body preparation (Fig. 1a) may thus be protein released by rupture of the larger protein bodies. Breakdown of protein bodies appeared to be more extensive in freeze-dried preparations than in the samples dried in a vacuum desiccator. Perhaps the large protein bodies are less stable to drying than the small protein bodies. Soybean protein bodies, 2–10 μ in diameter, are obtained when a nonaqueous density gradient is used (5); stability of the larger protein bodies may be greater in nonaqueous systems than in the aqueous system used here. Damage to protein bodies isolated by sucrose density gradient centrifugation has been observed (1), but no particle size selectivity in stability was reported.

It seems unlikely that the large particles observed in Figure 2b are starch granules because (a) mature soybeans contain little or no starch, although the large particles are quite numerous in the ground soybean sample; (b) because wheat starch granules are stabilized in the presence of sucrose (7), similar granules in soybeans would be expected to remain intact during the isolation process and to appear in the pellet fraction because of their higher density as compared to the density of the protein bodies (1). The large particles, however, were not observed in the pellet layer (Fig. 1b).

The aggregate of particles shown in Figure 2a probably is a clump of protein bodies, which survived initial grinding of the soybeans, as well as homogenization during isolation. Alternatively, the aggregate may have formed during washing and centrifugation. Such well-defined clusters of particles were not observed, however, in the protein body preparation (Fig. 1a) which contained a much higher concentration of protein bodies than the pellet fraction.

W. J. WOLF
Northern Regional Research Laboratory
No. Utiliz. Res. Dev. Div.
ARS, USDA
Peoria, Illinois 61604

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