MICROBODIES OF SOYBEAN COTYLEDON MESOPHYLL

KUO-CHUN LIU, A. J. PAPPELIS, AND H. M. KAPLAN
Department of Botany, and Department of Physiology, Southern Illinois University, Carbondale, Illinois 62901

ABSTRACT.—Microbodies were found in the upper mesophyll cells of soybean [*Glycine max* (L.) Merr., var. Wayne] cotyledon from the germinating stage (24 hr after planting) to cotyledon abscission (12 hr day, 12 hr night, 25°C, and 700 ft-c). These organelles were associated with endoplasmic reticulum, mitochondria, and chloroplasts. The shape of these organelles changed from oval, circular, or elongate in the earlier stage of seedling development to circular forms when cotyledons became yellow. The microbodies in senescent cells often lacked a continuous bounding membrane.

The occurrence, structure and the enzymatic function of plant microbodies is a subject of increasing interest to many investigators. Some of the characteristics of plant microbodies can be summarized as follows: they are bounded by a single membrane; they have a diameter from 0.5 to 1.5 μ; a dense granular stroma; and they are often associated with endoplasmic reticulum. Some are reported to contain crystals. These characteristics apply equally to two types of cell particulates isolated from homogenates. One of these, obtained from leaves, is involved in photo-respiration and is referred to as a peroxisome. The second, obtained from endosperm, is involved in the formation of succinate from fatty acids and is referred to as a glyoxysome. In cotyledons, although glyoxysomes appear early and are replaced by peroxisomes, the cytological events associated with these changes are unclear. Much of the literature describing plant microbodies and their associated enzymes has recently been reviewed (Beevers, 1969; Breidenbach, 1969; Gruber, *et al.*, 1970; Tolbert and Yamazaki, 1969; and Vigil, 1970).

No description of soybean microbodies has yet been published. In our recent study of cellular senescence in soybean cotyledons, we found organelles similar to published electronmicrographs and descriptions of glyoxysomes and peroxisomes. This paper presents some of our observations.

MATERIALS AND METHODS

Soybean [*Glycine max* (L.) Merr., var. Wayne] seeds were planted at a depth of 5 cm in sand and peat mixture (1:1 by volume) and grown in a growth chamber (25° C both for 12 hr of 700-ft-c of light and 12 hr of dark period). Sampling was at 24 hr intervals after planting for six days (at which time the hypocotyls were about 5 cm above the soil surface). On the sixth day, seedlings were standardized for uniform height and for uninjured cotyledons. All other seedlings were re-

moved. After the sixth day, sampling was obtained at three-day intervals until cotyledon abscission.

For each sample, tissue blocks of 1 x 1 x 4 mm were cut from the upper central area of the cotyledon. The blocks were fixed with 3% glutaraldehyde in 0.066 M phosphate buffer at pH 7.4 for 4 hours. After rinsing three times with the same buffer, the tissues were post-fixed in a 1:1 mixture of 2% osmium tetroxide and the above buffer for two hours. Both fixations were at room temperature. The tissues were dehydrated through an ethanol series, treated with propylene oxide and embedded in Epon 812 (Luft, 1961).

Sections were obtained with a diamond knife on a Reichert Om-U2 ultramicrotome, mounted on 200 mesh copper grids, stained in uranyl acetate (Watson, 1958), and counter-stained with lead citrate (Reynolds, 1963), and examined with a Hitachi HU-11A electron microscope at 50 KV.

Results

We did not find microbodies in cotyledons sampled 24 hrs after planting. Single membrane bounded electron dense, granulate organelles (Figs. 1-3) were observed in samples obtained at 48 hr after planting and in all other subsequent samples. Endoplasmic reticulum was associated with or in the vicinity of those organelles. The diameters of the dense organelles ranged from 0.5 to 1.3 μ, which is in the range of plant microbodies. We concluded that these were microbodies.

The shapes of the microbodies were spherical, elongated, irregular, or dumbbell (Figs. 2-6). Elongated microbodies had diameters of 0.3 μ at the narrowest region to 1.3 μ at the widest region. The stroma appeared as electron dense granules with transparent areas scattered within it. A single membrane was clearly identified as surrounding the microbodies of the earlier samples (Figs. 1-4). The bounding membrane of the microbodies from the sample of yellow cotyledon (21 days after planting) could not be seen clearly, but the endoplasmic reticulum associated with the organelles persisted (Figs. 5-6). The diameters of the microbodies in senescent cells were about 0.5 to 1.0 μ.

In addition to the close association between the microbodies and endoplasmic reticulum, in most of the cells the microbodies and chloroplasts were appressed (Figs. 3-4), changing the shape of the microbody at the place where the close spatial association occurred. Similar close associations between microbodies and mitochondria, and between mitochondria and chloroplasts were also observed.

Discussion

In fatty cotyledons, stored lipids are converted to carbohydrates in the early post-germinative stages. The microbodies observed in the soybean cotyledons at this stage were assumed to be glyoxysomes. As the cotyledons expand and emerge from the soil, they become photosynthetic organs. The microbodies observed in soybean cotyledons associated with the chloroplasts at this stage of growth are assumed to be peroxisomes. A time must exist when both

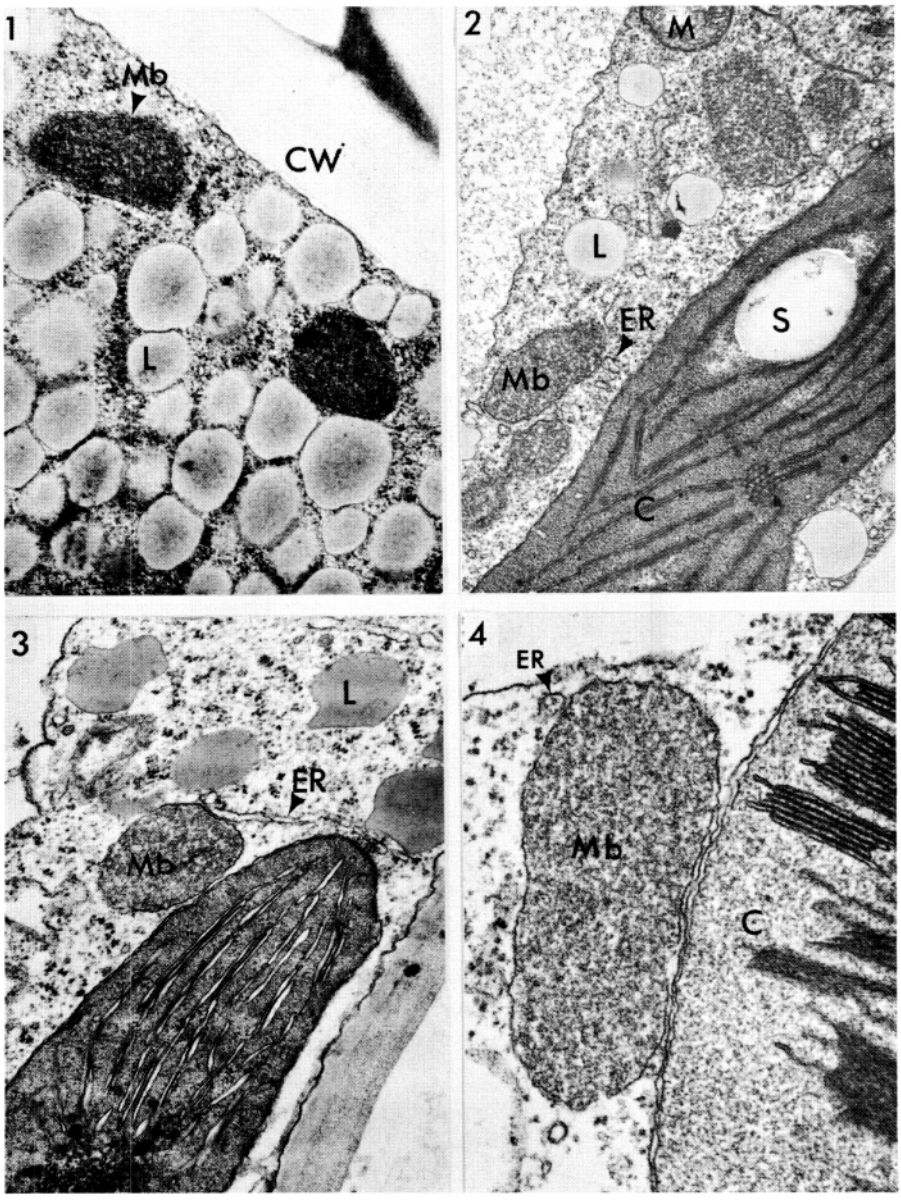

PLATE I

FIGURE 1. Two microbodies (Mb) among lipid bodies (L) in a cotyledon from three day old seedling; Cw, cell wall, x26,000.

FIGURE 2. Microbody (Mb) with endoplasmic reticulum (ER) in a cotyledon from four day old seedling. Mitochondrion (M), chloroplast (C), and starch grain (S) also can be identified, x26,000.

FIGURE 3. Microbody (Mb) associated with endoplasmic reticulum (ER) and developing chloroplast (C) in a cotyledon from five day old seedling, x33,000.

FIGURE 4. Microbody (Mb) with endoplasmic reticulum (ER), and chloroplast (C) in a cotyledon from a ten day old seedling, x52,000.

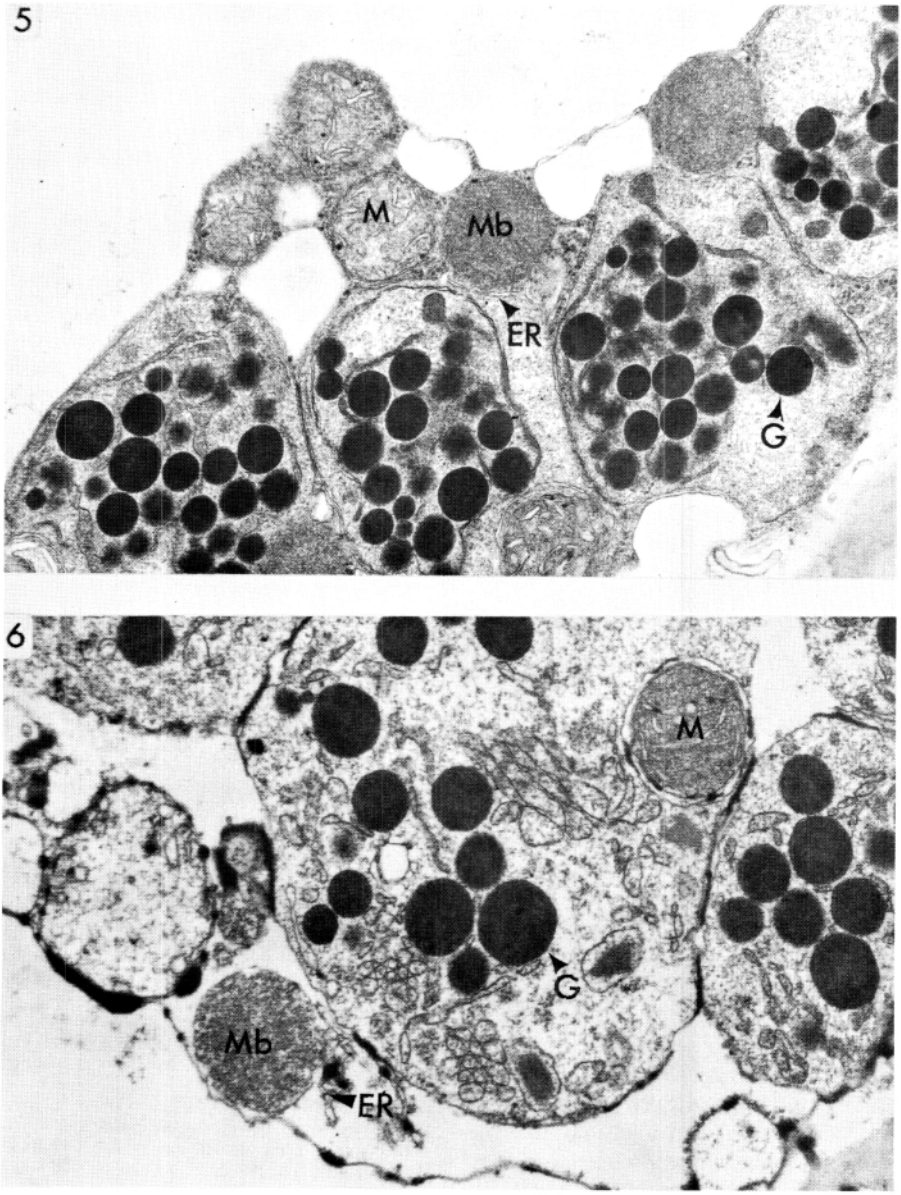

PLATE II

FIGURE 5. Microbodies (Mb) associate with endoplasmic reticulum (ER) from yellow cotyledon of 21 day old seedling. Degenerated mitochondrion (M) and degenerated chloroplasts with electron dense globules (G) also can be seen, x35,000.

FIGURE 6. As Figure 5, x44,000.

types of microbodies exist within the same cell of a cotyledon. Gruber *et al.* (1970) showed that microbodies are present at three distinct stages of cotyledon development of sunflower, cucumber, and tomato. The microbodies progress from catalase-containing particles located among lipid and protein bodies, to glyoxysomes closely associated with lipid bodies, to peroxisomes frequently appressed to chloroplasts. Although a transitional period occurred involving decline of glyoxysomes and a rapid rise of peroxisomes, the origin of the particles and their mode of destruction were not described. They did not determine whether any of the particle types were derived from preexisting microbodies or whether each arise as a separate population.

Vigil (1970) demonstrated that castor bean cotyledon microbodies showed membrane continuity with rough endoplasmic reticulum, suggesting a mode of formation similar to that in animal cells. As cotyledon development progresses, some microbodies disappear *in toto* by sequestration into autophagic vacuoles. The loss of enzymes did not appear to occur prior to digestion of the sequestered microbodies.

The rapid loss of lipids during germination can be seen in Figs. 1-4, the latter being sampled five days after planting. Starch was observed in developing proplastids at the stage of germination when seedlings were still underground. The glucose for this starch synthesis was believed to be derived from the conversion of lipids to hexose in a process involving glyoxysomes. The close association of microbodies and chloroplasts at a later stage is interpreted to imply that peroxisomal photorespiration occurs in soybean cotyledons.

The change in shape of plant microbodies from oval, elongate, and irregular in young tissue to almost perfectly circular (Figs. 5-6) in old tissue, and the inability to visualize a distinct bounding membrane, are the only noticeable changes during senescence. The chloroplast changes during aging (osmiophilic bodies, disruption and loss of grana stacks, and the appearance of circular fragments of lamellae) are similar to those reported by Barr and Arntzen (1969).

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