Short Communication

Immunocytochemical Analysis Shows that Glyoxysomes Are Directly Transformed to Leaf Peroxisomes during Greening of Pumpkin Cotyledons

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ABSTRACT

The functional transition of glyoxysomes to leaf peroxisomes occurs during greening of germinating pumpkin cotyledons (Cucurbita sp. Amakuri Nankin). The immunocytochemical analysis using A-gold method was employed in the analysis of the transition using glyoxysomal specific citrate synthase immunoglobulin G and leaf peroxisomal specific glycolate oxidase immunoglobulin G. The labeling density of citrate synthase was decreased in the microbodies during the greening, whereas that of glycolate oxidase was dramatically increased. Double labeling experiments using different sizes of protein A-gold particles show that both the glyoxysomal and the leaf peroxisomal enzymes coexist in the microbody of the transitional stage indicating that glyoxysomes are directly transformed to leaf peroxisomes during greening.

There exist two functionally different types of microbody in plant tissues. Glyoxysomes are unique organelles engaged in the degradation of reserve oil via β-oxidation and glyoxylate cycle in germinated fatty seeds such as pumpkin (Cucurbita sp.) (1, 5). Upon greening, the cotyledons become photosynthetic organs; functional leaf peroxisomes, with a crucial role in photosynthesis, appear together with chloroplasts. The important question is raised of whether the functional transition of microbodies proceeds in a linear and continuous fashion or whether a discontinuous, two-step sequence operates therein. Indeed, two drastically opposing hypotheses, i.e. 'one-population' and 'two-population' models, have been proposed (1). According to the one-population hypothesis, glyoxysomes are directly transformed to leaf peroxisomes during greening of cotyledons, accompanying the insertion of newly synthesized leaf peroxisome-specific enzymes and the concomitant breakdown of glyoxysome-specific enzymes (2, 12). On the contrary, the two-population hypothesis postulates that glyoxysomes are broken down and leaf peroxisomes are newly synthesized de novo (6, 7). As schematically illustrated in Figure 1, according to the one-population hypoth-

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Two-population hypothesis

[Diagram of two-population hypothesis]

Fig. 1. The two hypothetical mechanisms proposed for the transition of glyoxysomes to leaf peroxisomes in fatty seedlings. (●), Glyoxysome-specific enzyme; (○), leaf peroxisome-specific enzyme; (▲), enzymes known to exist in both glyoxysomes and leaf peroxisomes, e.g. catalase and malate dehydrogenase.
different microbodies, never coexisting in the same organelle during the step of glyoxysome-leaf peroxisome transition.

On the basis of cytochemical investigation, Burk and Trelease (3) have shown that 94 to 97% of the microbodies isolated from cucumber cotyledons at the transition stage are stainable for malate synthase (glyoxysomal enzyme) and glycolate oxidase (leaf peroxisomal enzyme). However, the method employed by these workers does not provide the direct demonstration showing that both microbody enzymes co-exist in the same microbodies and the criticism has been propounded (2).

The immunocytochemical protein A-gold technique using different sizes of gold particles provides us with an opportunity to clarify the localization of two different antigens in living cells (11). Accordingly, to resolve the two hypothetical mechanisms described above we have used this technique employing the two antibodies raised against the glyoxysome-specific citrate synthase and the leaf peroxisome-specific glycolate oxidase as probe.

MATERIALS AND METHODS

Plant growth condition was described previously (9). Pumpkin cotyledons grown under the three different conditions described in “Results and Discussion” were harvested and sliced (1 mm thick) with a razor blade. The slices were treated for 2 h with fixative containing 4% paraformaldehyde, 1% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4). Postosmication was omitted. The sections were dehydrated in graded ethanol at -20°C and embedded in Lowicryl-K4M (3). Thin sections were cut by a glass knife fitted to an LKB Ultratome and mounted on nickel grids coated with a Formvar membrane.

Preparation for antibodies were described previously against glycolate oxidase (8) and citrate synthase (10). The specific immunocytochemical cross-reactivities of the antisera used are most crucial in this study, and we have found that both antiglycolate oxidase IgG and anti-citrate synthase IgG react only with the purified preparation of antigenic enzyme molecules employing the immunoblotting techniques. Immunocytochemical protein A-gold labeling procedures were exactly the same as described by Yokota et al. (15). The sections were examined under a Hitachi H 600 electron microscope at 100 kV.

RESULTS AND DISCUSSION

From the developmental analysis of greening pumpkin cotyledons, glyoxysomal enzyme activities show a maximum of 4 to 5 d after germination in the dark; upon illumination of the etiolated cotyledons, the microbody transition was found to be completed in the next 4 to 5 d (9, 13). Therefore, we employed three stages of cotyledons for the immunocytochemical analysis: (a) 5-d-old etiolated cotyledons (glyoxysomal stage), (b) 7-d-old greening cotyledons grown for 5 d in the dark and for 2 d in the light (transitional stage), and (c) 10-d-old green cotyledons grown for 5 d in the dark and for 5 d in the light (leaf peroxisomal stage). Results of the serial examination of these specimens by protein A-gold labeling EM are given in Figure 2, in which the gold particles represent the antigen sites for citrate synthase and glycolate oxidase. As presented, both are exclusively present in microbodies (mb) but barely detectable in plastids (ch), mitochondria (mt), or other cellular components such as vacuole (va) and lipid bodies (lb). However, the labeling density for glycolate oxidase was extremely low in the etiolated cotyledons (Fig. 2A), whereas it was dramatically increased in the cotyledons grown in the light (Fig. 2B and C). The profile of the labeling density for citrate synthase was completely the reverse, and there were few gold particles after greening (Fig. 2F).

The most crucial experiment shows the results of double labeling using different sizes of protein A-gold particles. It is clear that glycolate oxidase (small gold particle, small arrowhead) and citrate synthase (large gold particle, large arrowhead) coexist in the microbody during greening (Fig. 3A). However, in the section of the cotyledons treated with IgG fraction from nonimmunized rabbit, followed by protein A-gold conjugation, no specific labeling was discernible (Fig. 3B). It should be mentioned that all microbodies seen in the same electron micrograph section at this transitional stage are labeled by both particles. Overall experimental results show that the microbodies of the transition stage contain both glyoxysomal and leaf peroxisomal enzymes and that glyoxysomes are directly transformed to leaf peroxisomes.

![Fig. 2. Sections of Lowicryl K4M-embedded pumpkin cotyledons incubated with antiglycolate oxidase (A, B, C) or anticitrate synthase (D, E, F) IgG, followed by protein A-gold complex conjugation. (A, B, C), immunocytochemical labeling of glycolate oxidase; (D, E, F), that of citrate synthase. A and D, 5-d-old etiolated cotyledon; B and E, 7-d-old greening cotyledon grown for 5 d in the dark and for 2 d in the light; C and F, 10-d-old green cotyledon grown for 5 d in the dark and for 5 d in the light. Original magnification: A, × 47,000; B, × 35,000; C, × 45,000; D, × 40,000; E, × 52,000; F, × 34,000. ch, chloroplasts; lb, lipid bodies; mt, mitochondria; mb, microbodies (glyoxysomes and leaf peroxisomes); va, vacuole. Experimental details are described in the text. Bars are 1 μm. Since the materials were not postosmicated, membrane structures in all figures are negative images.](https://www.plantphysiol.org/content/8/6/314.full)
during greening.

Several intriguing questions arise: how are glyoxysomal enzymes destroyed selectively during greening and how are enzymes present in both microbodies, such as catalase and malate dehydrogenase, destroyed partially? In relation to these questions, we have found that the enzymically inactive catalase precursor is present in microbodies (14) and accumulates during the step of glyoxysome-leaf peroxisome transition (13). During the continuous one-population transition of the microbodies, as was demonstrated to occur in the present study, characteristic regulatory mechanisms may control the synthesis and degradation of the individual enzyme molecules. Therefore, studies of the synthesis and degradation of each microbody enzyme, like that of the catalase molecule, will undoubtedly aid us in answering these intriguing questions. It should be pointed out that no one has ever established whether there is a change in the number of microbodies per cell during the transition stage; we are currently engaged in analyzing this problem using cytochemical methods. Further investigations, employing various experimental approaches, will be necessary to elucidate all aspects of the microbody transition.

Note Added in Proof. After submission of this manuscript, we have noted that a paper by D. E. Titus and W. M. Becker was just published: Investigation of the glyoxysomes-peroxisomes transition in germinating cucumber cotyledons using double-label immunoelectron microscopy (1985 J Cell Biol 101: 1288-1299), in which the authors independently reached the same conclusion to ours for microbody transition in cucumber cotyledons using the protein A gold technique, although they have used different enzymes.

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LITERATURE CITED

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