Immunoelectron Microscopic Observation of the Behaviors of Peroxisomal Enzymes Inducibly Synthesized in an n-Alkane-Utilizable Yeast Cell, Candida tropicalis

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ABSTRACT. We reported that immunoelectron microscopy was an excellent tool for determining the subcellular localization of thiolase isozymes, acetoacetyl-CoA thiolase (T-I) and 3-ketoacyl-CoA thiolase (T-III) in n-alkane-grown Candida tropicalis cells (KAMASAWA, N. et al., 92. Cell Struct. Fund., 17: 203-207). Current investigation on the visualization of other peroxisomal enzymes, acyl-CoA oxidase (ACO), catalase (KAT), carnitine acetyltransferase (CAT), isocitrate lyase (ICL) and malate synthase (MS), showed that ACO localized in peroxisomes, KAT in peroxisomes and cytoplasm, and CAT in peroxisomes, mitochondria and cytoplasm. Most of ICL and MS were found in peroxisomes. These results agreed with previous biochemical studies and supported the presumed roles of these enzymes. The same technique was applied to study the process of synthesis and localization of these enzymes early in the cultivation period in n-alkane medium when peroxisomes began to proliferate. ACO and T-III were rapidly induced after transfer of cells from glucose- to n-alkane-media. There was a drastic change of their location from cytoplasm to peroxisomes between 1 h and 2 h after the transfer, while T-I, KAT and CAT were moderately induced in cytoplasm and their location was gradually changed to each organelle. ICL and MS, the key enzymes in the glyoxylate cycle, were already localized in peroxisomes in the glucose-grown cells and respective inducible enzymes also were gradually localized there. This visual analysis is useful for the vivid elucidation of the process of peroxisome proliferation and enzyme transport within a cell.

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Abbreviations: ACO, acyl-CoA oxidase; KAT, catalase; T-I, acetoacetyl-CoA thiolase; T-III, 3-ketoacyl-CoA thiolase; CAT, carnitine acetyltransferase; ICL, isocitrate lyase; MS, malate synthase.

When Candida tropicalis cells were grown in n-alkane as a sole carbon source, numerous peroxisomes (microbodies) proliferated in the cells. From our investigation on the structures and functions of peroxisomes in alkane metabolism, we found the peroxisomal enzymes to play an indispensable role in fatty acid metabolism (7, 8, 12). It is now generally accepted that peroxisomes proliferate by development and division of pre-existing organelles, and acquire supplies of proteins and lipids from the outside. Peroxisomal enzymes are encoded by nuclear genes, synthesized on free cytosolic polysomes and post-translationally imported into the organelles (1, 5 for reviews). Import of a part of the peroxisomal proteins requires specific signals: peroxisome targeting signals (PTSs), the two known being PTS1 and PTS2, and some of the receptor protein machinery on the surface of the peroxisomes (2, 10, 11). From the genetic approaches, peroxisome assembly protein (PAS) genes are isolated from Saccharomyces cerevisiae as essential genes of peroxisome biogenesis (6 for review). However, very little is known about when and how the proteins move from cytoplasm into the organelles.

The technique of immunoelectron microscopy is able to vividly visualize the behaviors of the inducibly synthesized protein in the cells, and is useful in clearly elucidating simultaneous phenomena of the proliferation of subcellular organelles and the transport of enzymes within them.

We describe here findings by immunoelectron microscopy of the behaviors of the peroxisomal enzymes synthesized, imported and localized into the organelles linked with peroxisome proliferation.
MATERIALS AND METHODS

Strain and Growth. Candida tropicalis pK233 (ATCC 20336) was grown in a medium containing an n-alkane mixture (C10-C14) as described (14).

Conventional Electron Microscopy. Yeast cells were fixed with 2.5% glutaraldehyde, postfixed with 1.5% KMnO4 and embedded in Quetol 653 resin as described (8).

Immunoelectron Microscopy. Immunoelectron microscopy of yeast cells was performed generally according to the method described (3), except that it was modified by dehydration of specimens through a graded ethanol series (50 to 95%, v/v). LR white resin (The London Resin Co. Ltd.) was used to embed the specimens followed by polymerization for 24 h at 50°C.

Immunostaining of Ultrathin Sections. Immunostaining was done as described (3). For the primary antibody, we used antisera against each enzyme diluted 1:2,000 and non-immune rabbit serum for the negative control.

Antisera. The antiserum against each purified enzyme, acetyl-CoA oxidase (ACO), catalase (KAT), acetoacetyl-CoA thiolase (T-III), isocitrate lyase (ICL) and malate synthase (MS) was prepared as reported (3). For the primary antibody, we used antisera against each enzyme diluted 1:2,000 and non-immune rabbit serum for the negative control.

RESULTS AND DISCUSSION

Location of enzymes at logarithmic phase. Candida tropicalis pK233 cells were cultured in n-alkane medium to the mid-logarithmic phase, and localization of the enzymes, ACO, KAT, CAT, ICL and MS in the cells was observed by immunoelectron microscopy. Figure 1a shows a cell image reacted with non-immuno rabbit serum as a control experiment. Nucleus (N), vacuole (V), mitochondria (M) and peroxisomes (P) are clearly visible, and no specific gold particles are observed. The gold particles reacting with the antiserum against ACO existed only in peroxisomes (Fig. 1b). KAT was seen in peroxisomes and cyttoplasm but not in mitochondria (Fig. 1c). We reported that KAT was localized in the matrix of peroxisomes by cytochemical reaction with 3,3’-diaminobenzidine (9), but immuno-gold labelings showed that KAT was at the periphery in the present study. The gold particles for CAT were visible not only in peroxisomes and cytoplasm but also in mitochondria (Fig. 1d). Most gold particles for ICL and MS existed in peroxisomes (Figs. 1e and f). Isozymes of thiolases, T-I and T-III were also observed in peroxisomes and a part of T-I was seen in cytoplasm (3). The same result was obtained on the cells embedded in LR white resin. The ultrastructure of the cell was clear compared with that seen by the Lowicryl embedding method, although there were fewer gold particles in the cells (Figs. 1g and h). These results agreed with biochemical studies previously reported (reviewed in ref. 4), and supported the presumed role of each enzyme in the cell.

In yeast, the β-oxidation system responsible for fatty acid degradation is exclusively peroxisomal and not mitochondrial. The complete degradation of fatty acids to acetyl-CoA is carried out in peroxisomes, from which acetyl-CoA required for citrate synthesis must be transported to mitochondria. CAT catalyzes the reversible replacement of CoA and carnitine to the acetyl group, and seems to constitute an acetylcarnitine shuttle system between peroxisomes and mitochondria (13). Immunoelectron microscopic observations visually supported this hypothesis in the yeast cells.

Synthesis and localization of the enzymes early in cultivation. To observe the transport of synthesized peroxisomal enzymes, we further studied the behaviors of these enzymes after the transfer of cells from glucose- to alkane- media, especially after 1 h or 2 h. This period seems to be important, because thereafter peroxisomes begin to proliferate and the metabolism of carbon sources drastically changes (8). Despite such a significant event, the subcellular fractionation of peroxisomes is very difficult because of the low level of cell growth and the low efficiency of cell wall lysis. Figures 2a–2h show cell images fixed with KMnO4, in which nucleus (N), vacuole (V) and mitochondria (M) can be discriminated. 0 h cultured cells (cells before transfer from glucose- to alkane- media and 22 h cultured cells in glucose medium) had profuse electron-dense granules, and about 0–3 peroxisomes (P) per sectioned cell (Fig. 2a). The electron-dense granules are thought to be glycogen in nature. Cells cultured in alkane for 1 h also had electron-dense granules, and 1–3 peroxisomes per sectioned cell (Fig. 2a2). In 2 h cultured cells, electron-dense granules were scattered in the cytoplasm and peroxisomes, and CAT is also in mitochondria in 1 h cells. ICL and MS exist in peroxisomes in 0 h cells. a) ×12,000, b)–h) ×30,000. Bar = 500 nm.

Fig. 1. Immunoelectron micrographs of Candida tropicalis cells grown on n-alkane medium to mid-log phase showing the location of peroxisomal proteins. a) Control experiment with non-immuno serum showing no specific gold particles. b) Immunogold labeling with antiserum against ACO, c) KAT, d) CAT, e) ICL, f) MS, g) T-III, h) T-I. Gold particles for ACO and T-III localized in peroxisomes, KAT and T-I in peroxisomes and cytoplasm, CAT also in mitochondria. Most gold particles for ICL and MS were in peroxisomes and a few in cytoplasm. CW: cell wall, N: nucleus, V: vacuole, M: mitochondrion, P: peroxisome × 30,000. Bar = 500 nm.

Fig. 2. Electron and immunoelectron micrographs of Candida tropicalis cells. Behavior of the peroxisomal enzymes after the transfer of cells from glucose- to alkane- media. a) Cell image fixed with KMnO4. b) Immunogold labeling with antiserum against ACO, c) T-III, d) T-I, e) KAT, f) CAT, g) ICL, h)MS. In each figure, they are shown as 1:0 h cultured cells (before transfer to alkane-medium), 2:1 h cultured in alkane-medium, 3:2 h cultured. Gold particles of ACO and T-III are scattered in cytoplasm at 1 h, and there location then drastically changes to the peroxisomes. T-I and KAT are observed earlier in both cytoplasm and peroxisomes, and CAT is also in mitochondria in 1 h cells. ICL and MS exist in peroxisomes in 0 h cells. a) ×12,000, b)–h) ×30,000. Bar = 500 nm.
Fig. 1.
Fig. 2. a–d.
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Fig. 2, e–h.

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ules had disappeared and the cytoplasm was uniform; peroxisomes had proliferated and numbered 2–8 per sectioned cell (Fig. 2a).

These cells were reacted with antisera against seven peroxisomal enzymes, respectively, and the results are shown in Figs. 2b–2h. ACO and T-III were rapidly induced by changing carbon sources; there were few gold particles for these enzymes in 0 h cells; they were scattered in cytoplasm after 1 h; and then their location drastically changed from cytoplasm to peroxisomes at 2 h (Figs. 2b and 2c). Some of these particles seemed to exist outside the peroxisomal membrane (→), which may indicate the pathway of the transport process. This pattern of localization did not change until the log-phase after 2 h. T-I, KAT and CAT were more moderately induced and synthesized in cytoplasm than ACO and T-III, then gradually located in each organelle (Figs. 2d, 2e, 2f). KAT and T-I were observed earlier in both cytoplasm and peroxisomes, and CAT was also seen in mitochondria in 1 h cells. ICL and MS, key enzymes in the glyoxylate cycle, were found in peroxisomes in glucose-grown cells and their pattern of location did not change (Figs. 2g, 2h). Candida cells have only a few peroxisomes when grown on glucose (7), and these contain relatively high levels of ICL and MS compared with enzymes composed of the β-oxidation system (16). This study also demonstrated by visualization that these enzymes pre-existed in peroxisomes in glucose-grown cells.

These immunoelectron microscopic studies made it possible to clearly demonstrate the synthesis of the peroxisomal enzymes and the process of their transport into the organelles. The fact was disclosed that all enzymes were not simultaneously transported into proliferating peroxisomes after synthesis. Further investigation should be done on what function determines the transport-order of transported proteins and regulates the machinery involved in the gene expression of these proteins.

REFERENCES


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