JOURNAL OF BACTERIOLOGY, Oct. 1970, p. 581-584
Vol. 104, No. 1

Printed in U.S.A.

NOTES

Cytochemical Localization of Catalase Activity in Yeast Peroxisomes

H.-P. HOFFMANN, A. SZABO, AND C. J. AVERS

Department of Biological Sciences, Douglass Campus, Rutgers University, New Brunswick, New Jersey 08903

Received for publication 4 May 1970

Diaminobenzidine oxidation product occurred in peroxisomes and in the intracristate spaces of mitochondria. The reaction was inhibited only in peroxisomes when 3-amino-1,2,4-triazole was present, but cyanide and azide inhibited deposition in both kinds of organelles.

Peroxisomes have been identified in *Saccharomyces cerevisiae* (2, 16) by the criteria of ultrastructure and enzyme activities (5). Because of problems in obtaining, by centrifugation, peroxisome and mitochondrial fractions which are free of cross-contamination, we were unable to provide clear evidence showing the unique location of catalase in peroxisomes of bakers' yeast (16). In the present cytochemical study, however, we found that catalase peroxidatic activity (6) was restricted to peroxisomes, whereas enzymes other than catalase probably are responsible for reduction of H_2O_2 in mitochondria of these same cells. We used modifications of the Graham-Karnovsky (8) diaminobenzidine (DAB) method, which has proved successful for both plant (7) and animal (4, 6, 14) materials.

Wild-type strain iso-N of *S. cerevisiae* (3) was grown at 25 C in a liquid semisynthetic medium (12) supplemented with 1 or 10% glucose. Cells were harvested in the late exponential phase (3) and were fixed immediately in a cacodylate-buffered glutaraldehyde-paraformaldehyde mixture (9) at 0 to 4 C for 4 hr (or as long as 16 hr for some materials). After three washes in water and two washes in the same buffer solution as was to be used in the staining medium, about 0.5 g (wet weight) of cells was incubated in stoppered test tubes at 37 C for 1 to 2 hr in a freshly prepared incubation medium containing 10 mg of 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, Mo.) dissolved in 5 ml of an alkaline buffer [50 mM Veronal acetate, *p*H 9.0; 50 mM 2-amino-2-methyl-1-propanol, *p*H 9.1; or 100 mM tris(hydroxymethyl)aminomethane-chloride, *p*H 8.6 to 8.9], and 0.007% H_2O_2. The tubes were put in ice to stop the reaction, after which the cells were rinsed once with buffer and then either were postfixed for 1 hr in 5% $NaMnO_4$ in 0.1 M cacodylate buffer (*p*H 7.2) or first were converted to spheroplasts (1, 13) and then were postfixed for 12 hr at 4 C in 2% OsO_4 in Millonig's buffer (11). The fixed cells were rinsed in water, embedded in 2% warmed agar, and stained for 1 hr at room temperature in a mixture of equal parts of saturated aqueous uranyl acetate and 100% acetone; they were then dehydrated rapidly in a graded acetone series and embedded in Epon 812 epoxy resin (10). Sections of 40- to 90-nm thickness were cut with diamond knives and sometimes stained with lead citrate (15) before scanning and photography, for which we used an RCA 3G electron microscope at an accelerating voltage of 50 kv.

The DAB oxidation product occurred in peroxisomes and in the intracristate spaces of mitochondria (Fig. 1). No crystalloid or tubular inclusions were present in peroxisomes fixed in either $NaMnO_4$ or OsO_4, but there was a less electron-dense region in the matrix (Fig. 2). The organelles usually were round or dumbbell-shaped and often were in clusters near the cell periphery. Sometimes peroxisomes occurred in very close association, separated only by a thin electron-transparent line (Fig. 2).

There was no DAB oxidation product in either organelle type when the incubation media lacked H_2O_2 or contained 10 to 50 mM KCN or 0.1 M sodium azide in a complete reaction mixture (Fig. 3). On the other hand, 20 mM 3-amino-1,2,4-triazole (Aldrich Chemical Co., Milwaukee, Wis.) completely inhibited the

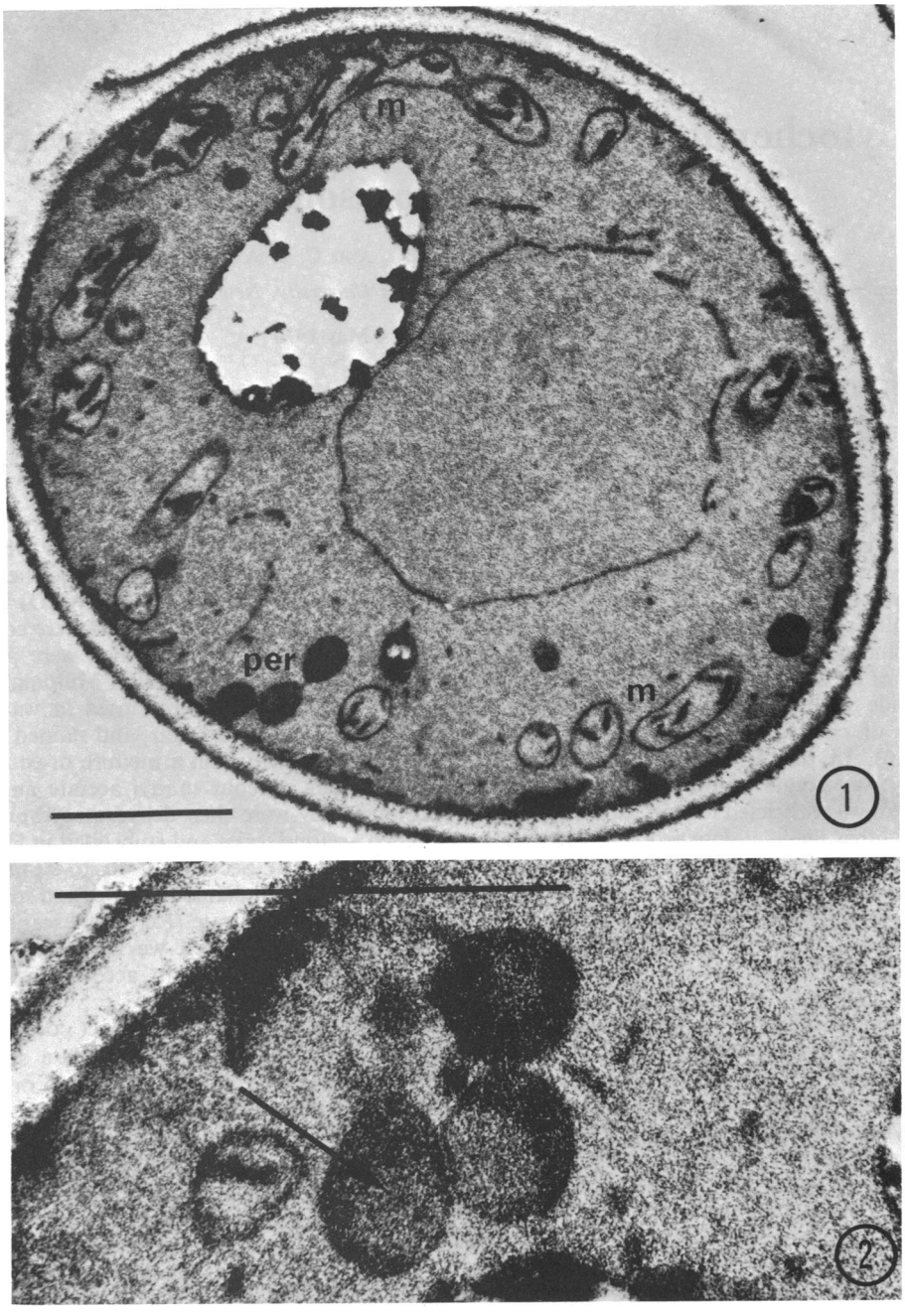

FIG. 1. *DAB reaction product localization in peroxisomes (per) and intracristate spaces of mitochondria (m) in a thin section of $NaMnO_4$-fixed yeast. The bar represents 1 μm in all photographs.*

FIG. 2. *Peroxisomes containing DAB reaction product, but showing an area of lower electron density (at arrows) within each organelle.*

cytochemical reaction in peroxisomes but was ineffective in mitochondria (Fig. 4).

The cytochemical data indicate that peroxidatic activities of different enzymes occur in different parts of the cell. Catalase appears to be confined to peroxisomes, whereas peroxidases are mitochondrial in location (14, 17). An earlier study showed that cytochrome *c* peroxidase activity

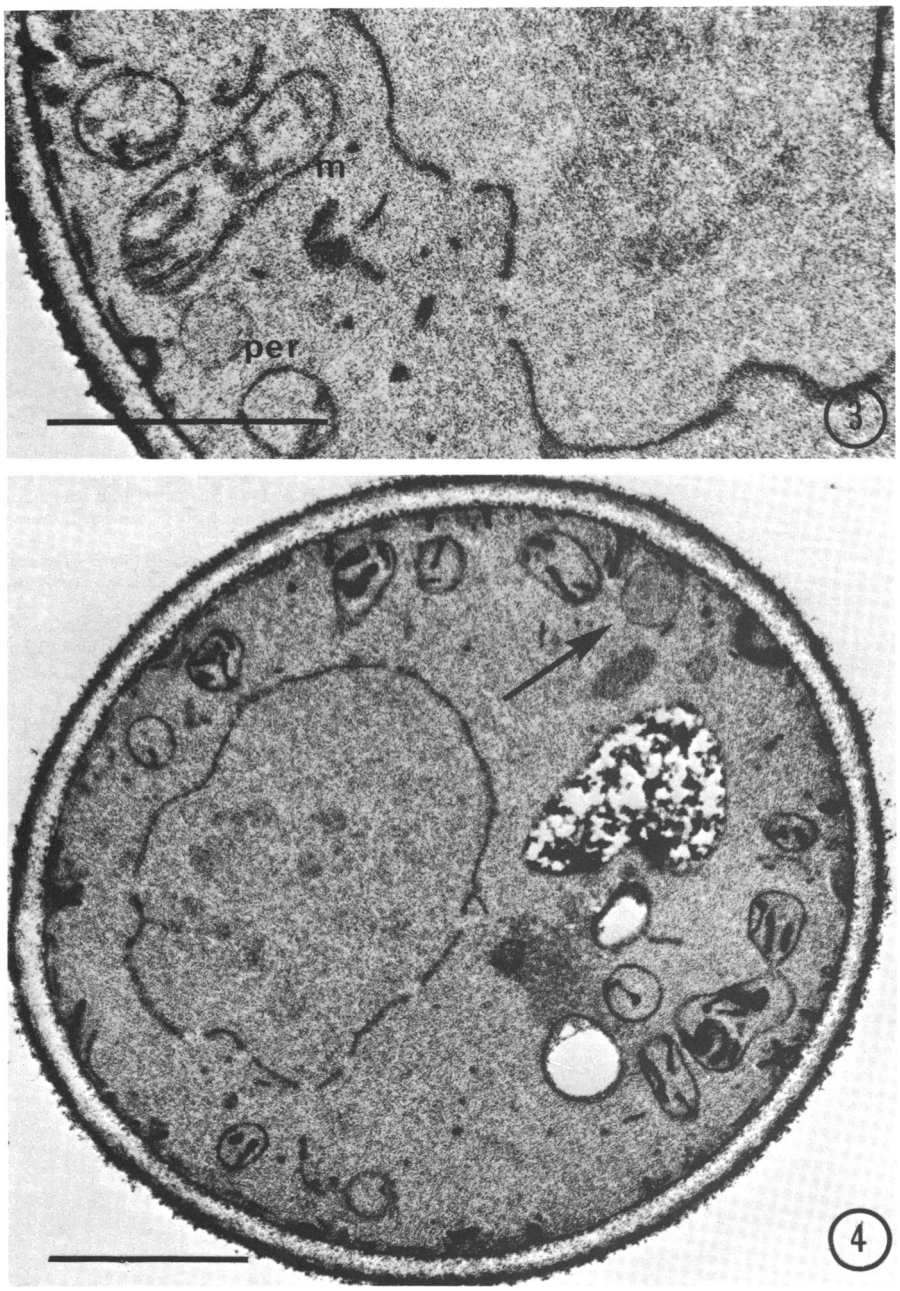

FIG. 3. *KCN control showing absence of DAB reaction product from peroxisomes (per) and mitochondria (m).*

FIG. 4. *Thin section of cell after DAB reaction in the presence of aminotriazole. Peroxisome lacks deposit (at arrow), whereas mitochondria contain electron-dense product within the cristae.*

yielded a grossly particulate reaction product along cytoplasmic and mitochondrial membranes (1). The intracristate pattern of DAB reaction product may be due to the activity of some peroxidase which is different from cytochrome *c* peroxidase (1, 17), or to the difference between DAB and the diphenylamines used in the separate cytochemical test systems (1, 6). At the least,

the use of selective inhibitors in cytochemical reaction mixtures has revealed the compartmentation of peroxidatic enzyme activities in peroxisomes and mitochondria in yeast, and probably in other cell types, too (14).

This study was supported by a grant from the National Science Foundation (GB-7439). One of us (A.S.) was a Public Health Service predoctoral fellow (1 FO1-GM-41713-02).

We thank Gracia Ulrickson for excellent technical assistance.

LITERATURE CITED

1. Avers, C. J. 1967. Distribution of cytochrome *c* peroxidase activity in wild-type and petite cells of bakers' yeast grown aerobically and anaerobically. J. Bacteriol. **94**:1225–1235.
2. Avers, C. J., and M. Federman. 1968. The occurrence in yeast of cytoplasmic granules which resemble microbodies. J. Cell Biol. **37**:555–559.
3. Avers, C. J., C. R. Pfeffer, and M. W. Rancourt. 1965. Acriflavine induction of different kinds of "petite" mitochondrial populations in *Saccharomyces cerevisiae*. J. Bacteriol. **90**: 481–494.
4. Beard, M. E., and A. B. Novikoff. 1969. Distribution of peroxisomes (microbodies) in the nephron of the rat. J. Cell Biol. **42**:501–518.
5. De Duve, C., and P. Baudhuin. 1966. Peroxisomes (microbodies and related particles). Physiol. Rev. **46**:323–357.
6. Fahimi, H. D. 1969. Cytochemical localization of peroxidatic activity of catalase in rat hepatic microbodies (peroxisomes). J. Cell Biol. **43**:275–288.
7. Frederick, S. E., and E. H. Newcomb. 1969. Cytochemical localization of catalase in leaf microbodies (peroxisomes). J. Cell Biol. **43**:343–353.
8. Graham, R. C., and M. J. Karnovsky. 1965. The histochemical demonstration of uricase activity. J. Histochem. Cytochem. **13**:448–453.
9. Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. **27**:137A–138A.
10. Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**:409–414.
11. Millonig, G. 1962. Further observations on a phosphate buffer for osmium solutions in fixation. Proc. 5th Intern. Conf. Electron Microsc. **2**:8. Academic Press Inc., New York.
12. Nagai, S. 1959. Induction of respiration-deficient mutation in yeast by various synthetic dyes. Science **130**:1188–1189.
13. Neal, W. K., II, H.-P. Hoffmann, C. J. Avers, and C. A. Price. 1970. Derepression of mitochondria in yeast spheroplasts. Biochem. Biophys. Res. Commun. **38**:414–422.
14. Novikoff, A. B., and S. Goldfischer. 1969. Visualization of peroxisomes (microbodies) and mitochondria with diaminobenzidine. J. Histochem. Cytochem. **17**:675–680.
15. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17**:208–212.
16. Szabo, A., and C. J. Avers. 1969. Some aspects of regulation of peroxisomes and mitochondria in yeast. Ann. N.Y. Acad. Sci. **168**:302–312.
17. Yonetani, T., and T. Ohnishi. 1966. Cytochrome *c* peroxidase, a mitochondrial enzyme in yeast. J. Biol. Chem. **241**:2983–2984.