MICROBODIES IN METHANOL-ASSIMILATING YEASTS

VEENHUIS, M; KREGERVANRIJ, NJW; HARDER, W; Kreger-van Rij, N.J.W.

Published in:
Archives of Microbiology

DOI:
10.1007/BF00428343

Arch. Microbiol. 102, 41–44 (1975)

Microbodies in Methanol-Assimilating Yeasts

J. P. VAN DIJKEN, M. VEENHUIS, N. J. W. KREGER-VAN RIJ, and W. HARDER
Departments of Microbiology and Electron Microscopy, University of Groningen, Haren (Gr.)

Received August 19, 1974

Abstract. Cells of 3 yeast species capable of assimilating methanol have been examined by electron microscopy. When grown on methanol as the sole source of carbon and energy they contained many microbodies. Cells grown on glucose or ethanol either did not contain such bodies at all, or only to a limited extent.

Key words: Yeasts — Methanol — Microbodies.

Since the recent discovery of a number of yeasts which can utilize methanol as the sole source of carbon and energy, much progress has been made with respect to the enzymology of methanol metabolism in these organisms. It has been shown that the dissimilation of methanol is mediated by a methanol oxidase, catalase, and NAD dependent formaldehyde—and formate dehydrogenases (Fujii and Tonomura, 1972). The same authors (1973) obtained indications that the ribulose phosphate pathway of formaldehyde fixation is involved in the assimilation of methanol in yeasts. However, as yet, nothing is known about the localization of the assimilatory and dissimilatory enzyme systems involved in methanol metabolism in the yeast cell. Recent work in our laboratories has revealed a special intracellular organization of a number of yeasts during growth on methanol. This communication describes observations made on the ultrastructure of these C_1 utilizers.

Microorganisms and Cultivation. Strains of *Hansenula polymorpha* de Morais et Maia, CBS 4732, *Pichia pinus* (Holst) Phaff, CBS 5098 and *Candida boidinii* Ramirez, CBS 2429 were grown in a mineral medium supplemented with vitamins and either methanol, ethanol or glucose (0.3% w/v) as the sole source of carbon and energy (van Dijken and Harder, 1974). *H. polymorpha* was grown in batch and in chemostat cultures, whereas the other two species were batch-cultivated. Subcellular fractions were prepared via protoplast formation with snail gut enzyme (Helicase) followed by mechanical breakage in a Potter Elvehjem homogenizer and differential centrifugation.

Electron Microscopy. For the preparation of specimens for sectioning, the yeast cells were harvested and washed with water. Two methods of fixation were employed: (1) with 1.5% $KMnO_4$ for 20 min at room temperature, and (2) with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 for 3 h at 0°C, followed by washing in the same buffer and postfixation with 1% OsO_4 in cacodylate buffer for 16 h at 4°C. During dehydration in an ethanol series, the cells were poststained with a saturated solution of uranyl acetate in 100% ethanol. They were embedded in Epon 812, and part of the sections were stained with lead citrate. Freeze-etch replicas were made in a Balzer's unit as described by Moor (1964).

The cells of the three yeast species grown on methanol contained microbodies. The bodies had a homogeneous matrix and were surrounded by a unit membrane (Figs. 1 and 2) which showed smooth fracture faces (Figs. 3 and 4). Under certain conditions the contents of the microbodies were found to be crystalline, namely in freeze-etched cells of *H. polymorpha* grown in a chemostat culture (Fig. 5) and in a glutaraldehyde-OsO_4 fixed subcellular fraction of such a culture (Fig. 6). When *H. polymorpha* was grown in batch culture with methanol and cells were fixed with $KMnO_4$ or glutaraldehyde-OsO_4, no crystalloids were observed in the microbodies. In batch cultures up to 6 per section were found. Cells from the chemostat culture contained the largest number of profiles of microbodies per section, namely up to 12. They often filled a large part of the cell. Generally, the microbodies clustered, and, when closely packed, had flat surfaces between them (Figs. 1 and 2). The free surfaces were convex or flat. The greatest diameter of the profiles was 1.5 μ. Microbodies also occurred in young buds (Fig. 7). Occasionally, a strand of endoplasmic reticulum surrounded a microbody partially. Apart from the microbodies, the cells contained the usual organelles such as nucleus, mitochondria, vacuole(s) and endoplasmic reticulum.

Cells of the 3 yeast species grown in media with glucose or ethanol did not show this accumulation of microbodies (Fig. 8), although in glucose- and ethanol-grown cells of *P. pinus* a few microbodies of smaller dimensions (0.2—0.5 μ) were observed. From experiments in which cells of *H. polymorpha* grown on glucose were transferred to mineral medium with methanol, it

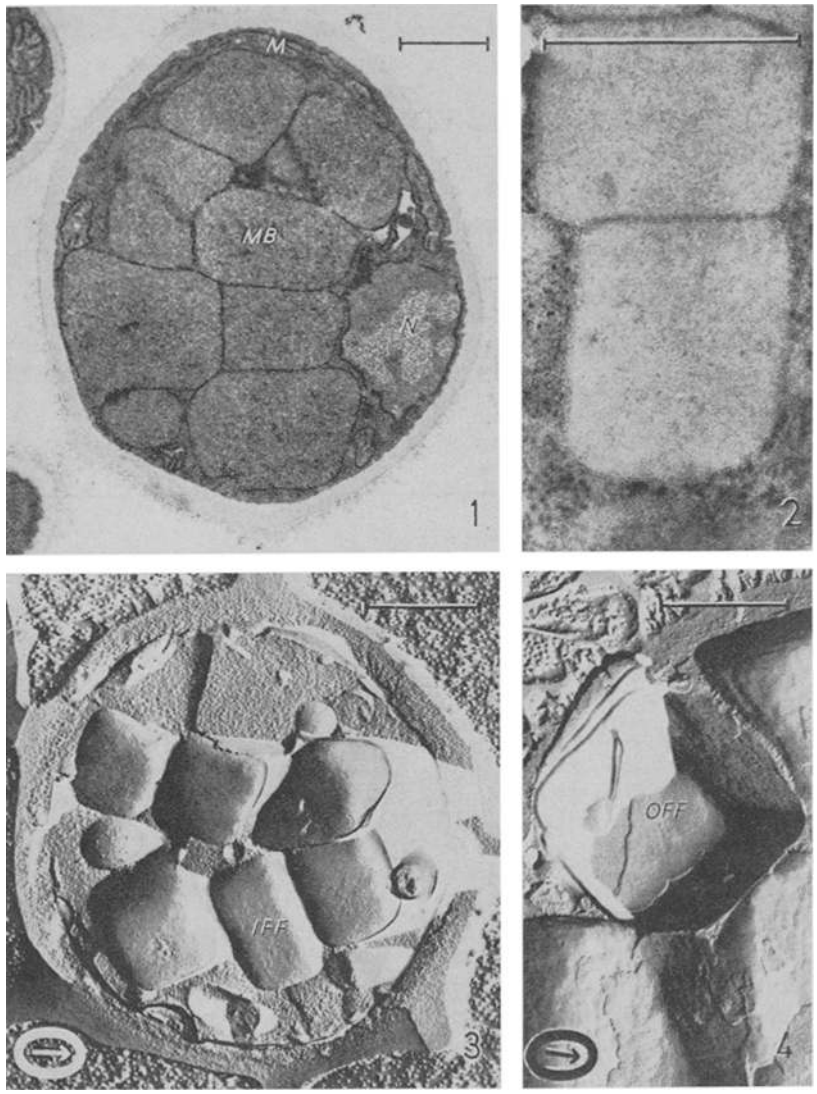

Fig. 1. *H. polymorpha*. Section through a cell from a chemostat culture ($D = 0.10\ h^{-1}$; C source: methanol) with many microbodies (*MB*). Nucleus (*N*) and mitochondria (*M*) are visible. ($KMnO_4$, lead citrate). In all figures the marker represents 0.5 μ

Fig. 2. *H. polymorpha*. Section of a cell from a batch culture (C source: methanol) showing two profiles of microbodies surrounded by a unit membrane. (Glutaraldehyde — OsO_4, lead citrate)

Figs. 3—5. Freeze-etched preparations of cells of *H. polymorpha* from a chemostat culture ($D = 0.02\ h^{-1}$; C source: methanol). The direction of shadowing is indicated by arrows. In Fig. 3 several microbodies of a typical rectangular shape with rounded edges are present. The smooth inner fracture face (*IFF*) of the surrounding membrane is visible. In Fig. 4 the smooth outer fracture face (*OFF*) can be seen. Fig. 5 shows a cross fractured microbody with crystalline contents (for Fig. 5 see p. 43)

appeared that the formation of microbodies was inducible. They became visible 1—3 h after the transfer, although few cells showed only one or two profiles at that time. Oxidation of methanol by whole cells and growth of the organism became apparent approximately 20 h after the transfer.

Avers and Federman (1968) observed microbodies in cells of the yeast *Saccharomyces cerevisiae*. These bodies measured 0.2—0.4 μ and the largest number of profiles per section in a diploid strain was seven, in a haploid one three. In contrast with *S. cerevisiae*, microbodies in *H. polymorpha*, *P. pinus* and *C. boidinii* grown on methanol, are more numerous and larger. Their absence or rare occurrence in cells of these yeasts grown on other carbon compounds suggests that these microbodies are involved in methanol metabolism. Since moreover, catalase and methanoloxidase, a H_2O_2 producing flavoprotein oxidase, are involved in the dis-

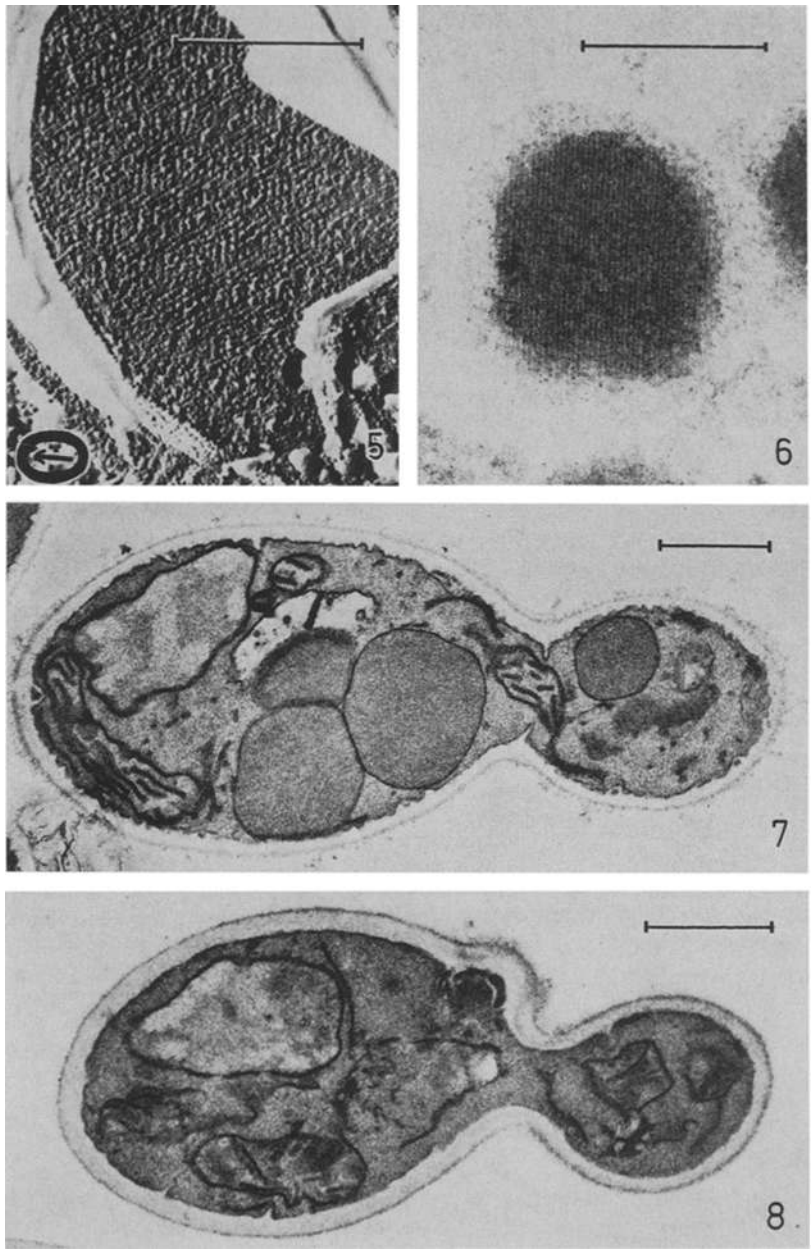

Fig. 6. Section through a microbody in a subcellular fraction prepared from *H. polymorpha* cells grown in a chemostat culture ($D = 0.10\,h^{-1}$; C source: methanol). A lattice structure is visible in the isolated microbody (Glutaraldehyde — OsO_4, lead citrate)

Fig. 7. Section of budding cell of *Pichia pinus* from a batch culture (C source: methanol) with microbodies in mother cell and bud. $KMnO_4$

Fig. 8. Section through a budding cell of *H. polymorpha* grown in batch culture with glucose as the carbon source. Microbodies were absent

similation of methanol in yeasts (Fujii and Tonomura, 1972), the microbodies may be peroxisomes. Peroxisomes have been found in yeasts (Avers, 1971), plants (Vigil, 1973) and animal tissues (De Duve and Baudhuin, 1966). In plant and animal tissue crystalline inclusions have been observed in microbodies, and their appearance has been ascribed to conditions of growth (Frederick *et al.*, 1968). In addition methods of fixation may be decisive. Our results with methanol-assimilating yeasts seem to be in agreement with this observation.

Apart from methanol oxidase and catalase, enzymes for the assimilation of methanol might be present in the microbodies.

Acknowledgements. J. P. van Dijken was supported by a grant from Shell Research Ltd., Sittingbourne, Kent, U.K. The work of N.J.W. Kreger-van Rij was supported by the Netherlands Organization for the advancement of Pure Research (Z.W.O.). The authors wish to thank Drs. J.B.M. Meiberg for his interest and valuable discussions.

Dr. W. Harder, Department of Microbiology, Biological Centre, Kerklaan 30, Haren (Gr.), The Netherlands

References

Avers, C.J.: Peroxisomes of yeast and other fungi. Sub-Cell. Biochem. **1**, 25–37 (1971)

Avers, C.J., Federman, M.: The occurrence in yeast of cytoplasmic granules which resemble microbodies. J. Cell Biol. **37**, 555–559 (1968)

van Dijken, J.P., Harder, W.: Optimal conditions for the enrichment of methanol-assimilating yeasts. J. gen. Microbiol. **84**, 409–411 (1974)

De Duve, C., Baudhuin, P.: Peroxisomes (microbodies and related particles). Physiol. Rev. **46**, 323–357 (1966)

Frederick, S.E., Newcomb, E.H., Vigil, E.L., Wergin, W.P.: Fine-structural characterization of plant microbodies. Planta (Berl.) **81**, 229–252 (1968)

Fujii, T., Tonomura, K.: Oxidation of methanol, formaldehyde and formate by a *Candida* species. Agr. Biol. Chem. **36**, 2297–2306 (1972)

Fujii, T., Tonomura, K.: Hexose-phosphate as an intermediate in the assimilation of methanol by *Candida* sp. Agr. Biol. Chem. **37**, 447–449 (1973)

Moor, H.: Die Gefrier-Fixation lebender Zellen und ihre Anwendung in der Elektronenmikroskopie. Z. Zellforsch. **62**, 546–580 (1964)

Vigil, E.L.: Structure and function of plant microbodies. Sub-Cell. Biochem. **2**, 237–285 (1973)