The Fine Structure of Microbodies in the Yeast *Pichia pastoris*

Microbodies or peroxisomes have been detected in the cells of animals[1] and plants[2,3] and in microorganisms[4]. These cytoplasmic organelles are surrounded by a unit membrane, and possess a fine granular inner structure as was revealed by electronmicroscopy. Their function in cell metabolism has only partly been elucidated, but according to our present knowledge, all microbodies are characterized by the presence of catalase and a number of oxidases. In rat liver microbodies catalase, urate oxidase, D-amino acid oxidase, L-α-hydroxyacid oxidase and isocitrate dehydrogenase have been demonstrated[5]. In microbodies of rat liver or plant leaves, tubular or crystalline regions have been observed[5,6]. HOFMANN et al.[7] showed uneven distribution of peroxidase activity of catalase in microbodies of *Saccharomyces cerevisiae* after cytochemical staining with diaminobenzidine (DAB). Similar results were obtained with alkane-assimilating yeasts by OSUMI et al.[8].

In our studies, the occurrence of microbodies and their internal structure has been investigated in a methanol-assimilating yeast.

Materials and methods. The methanol-assimilating yeast investigated was *Pichia pastoris* CBS 704. The test organism was grown in a 10 l Marubishi laboratory fermentor on mineral salts medium supplemented with

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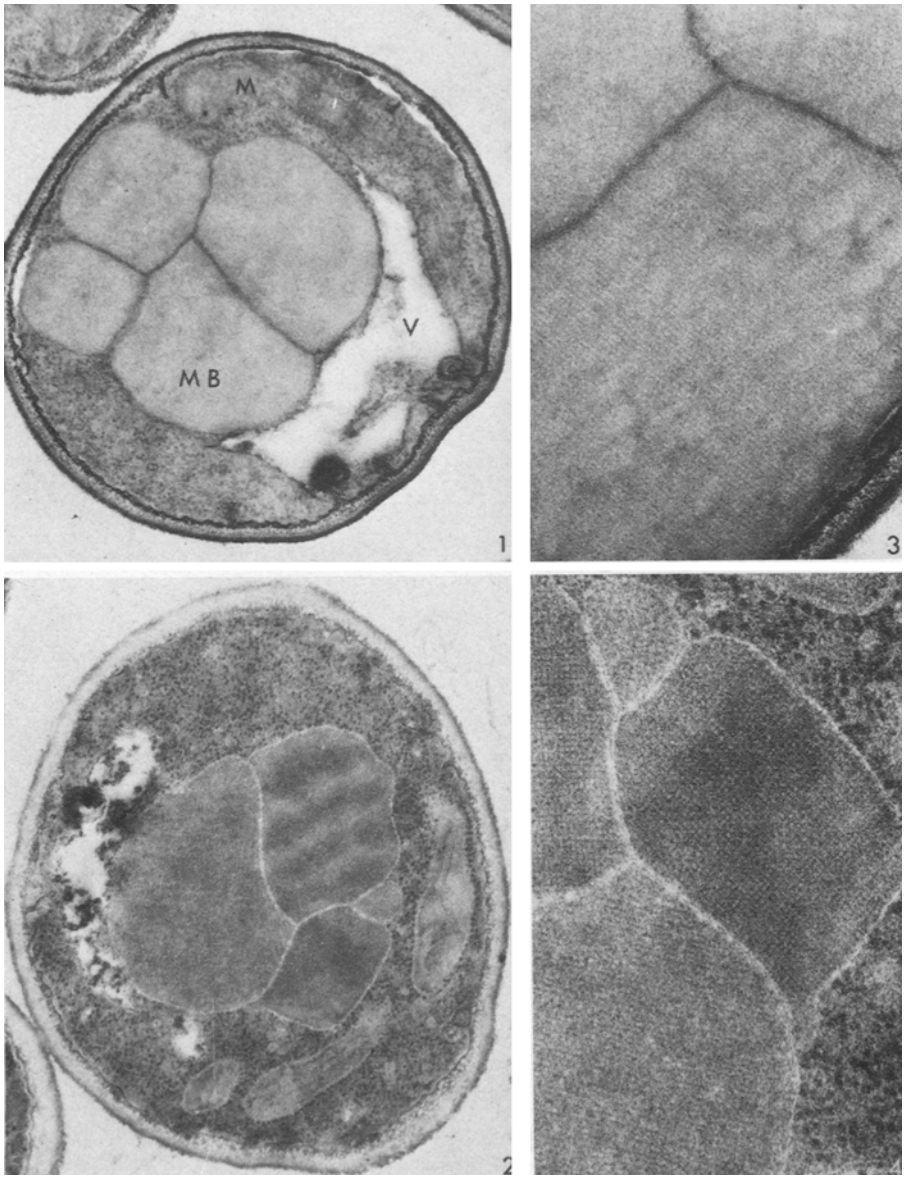

Fig. 1 and 2. *Pichia pastoris*, grown in batch culture on methanol. Section through a cell showing a number of microbodies (MB) with crystalline internal structure, mitochondria (M) and vacuoles (V) after fixation with paraformaldehyde/glutaraldehyde. ×34,000 and × 39,000 respectively.

Fig. 3 and 4. Details of crystalline matrix in microbodies with striated structures (Figure 3) and spacing of protein molecules (Figure 4). ×76,000 and ×96,000 respectively.

vitamins at 29 °C[9]. The aeration rate was 0.57 l air/l/min. Methanol was added as a vapor to the aeration air. Cells were harvested at various intervals in the course of 62 h.

After centrifugation the cells were washed with 0.067 *M* phosphate buffer pH 5.8, with addition of 0.09 *M* NaCl. The pellet was resuspended in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde. After 2 h at 20 °C, the cells were washed twice, and incubated in 1% OsO_4 for 16 h at 4 °C, then washed twice and embedded in 2% agar. All the treatments mentioned above were carried out in phosphate buffer/NaCl. The cells were dehydrated in an alcohol series, stained with 1.5% uranyl acetate for 1.5 h at the 50% alcohol step, and finally embedded in Epon. Thin sections were cut with an LKB microtome, poststained with 2% uranyl acetate for 2 min, with Reynolds lead citrate for 5 min, washed, and finally examined with a Philips E.M. 201.

Results and discussion. We have confirmed the observation of OSUMI et al.[8] that the occurrence of microbodies in yeast depends on the age of the culture and on the substrate for growth. In our studies they have not been found in glucose-grown cells but, in methanol-grown cells of *Pichia pastoris*, varying numbers up to 8 were observed (Figures 1 and 2), with highest frequences in exponentially growing cells. These microbodies are separated from each other and from the cytoplasm by a single unit membrane, and are often more or less rectangular. OSUMI et al.[8] showed these organelles to be present in various yeasts grown on hydrocarbons and in *Kloeckera* sp. grown on methanol, and VAN DIJKEN et al.[10] detected microbodies in the methanol-assimilating yeasts *Hansenula polymorpha*, *Pichia pinus* and *Candida boidinii*.

Striated or crystalline areas were observed occasionally depending on the plane of section (Figures 3 and 4). Contrary to results obtained with microbodies from animal or plant cells[1,6], the crystalline structure was evenly distributed over the entire volume of microbodies in methanol – assimilating yeasts, and may be responsible for their rectangular shape. These observations confirm the results of VAN DIJKEN et al.[10] with freeze-etched preparations of *Hansenula polymorpha* from a chemostat culture. Crystalline structures could, as reported in this paper, only be obtained by fixation of the cells with paraformaldehyde/glutaraldehyde. $KMnO_4$ fixation demonstrated the outlines of microbodies, but did not reveal their internal structure, as was also found by OSUMI et al.[8].

The periodicity calculated from striated areas (Figure 3) was about 125 Å, and varied between 95 and 105 Å in crystalline regions. The nature of the enzymes present in yeast microbodies, apart from catalase which was demonstrated by cytochemical staining, is still unknown, owing to difficulties in obtaining isolated and intact preparations of these organelles. However, there is a striking correlation between the occurrence of microbodies and the catalase content of yeast cells. Methanol, as a substrate for growth, increased the catalase content of the cells 5-fold[11], and *n*-alkane 10 to 50-fold[12] as compared with glucose-grown cells. Therefore, one might ponder on the possibility that the crystalline nature of yeast microbodies may be provoked primarily by the high content of catalase.

In methanol-grown yeasts, this enzyme was demonstrated to degrade H_2O_2 both by catalase and by peroxidase activity with methanol as a substrate[11]. Studies on liver peroxisomes revealed that formaldehyde and formiate were also peroxidatively metabolized[5]. It is not yet clear whether these reactions play a role in yeast metabolism.

Though the significance of microbodies in yeast has still to be elucidated, both alkane- and methanol-assimilating yeasts offer opportunities for studying this intriguing phenomenon.

Zusammenfassung. In auf Methanol gewachsenen Zellen von *Pichia pastoris* wurden in «Microbodies» oder Peroxisomen nach Fixierung mit Paraformaldehyd/Glutaraldehyd kristalline Strukturen beobachtet.

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Activité antimicrobienne de *Candida hordei* DE MIRANDA et DIEM

Antimicrobial Activity of *Candida hordei* DE MIRANDA and DIEM

L'aptitude à produire des antibiotiques est rarement observée chez les levures. Au cours de nos recherches sur les microorganismes de la phyllosphère de l'Orge, nous avons néanmoins constaté que les cultures de *Candida hordei*, une levure récemment décrite par DE MIRANDA et DIEM[1], étaient capables d'inhiber la croissance de divers bactéries et champignons. Cette note rapporte les premiers résultats de nos travaux sur ce phénomène.

Matériel et méthodes. L'activité antimicrobienne de *C. hordei* est d'abord mise en évidence par la méthode classique des stries. A l'aide d'une suspension de spores de *Colletotrichum graminicola* (Ces.) Wilson et de divers autres microorganismes unicellulaires (Figure 1), on trace des stries perpendiculairement à des cultures de *C. hordei* âgées de 3, 5 et 8 jours et obtenues sur le milieu ci-dessous: glucose, 20 g; asparagine, 1,5 g; KH_2PO_4, 0,5 g; K_2HPO_4, 0,5 g; $MgSO_4$, 7 H_2O, 0,5 g; KCl, 0,5 g; extrait de levure, 1 g; gélose, 15 g; eau, 1 litre; pH ajusté à 6. Après 12 h d'incubation, le nombre de spores germées de *C. graminicola* est évalué directement au microscope tandis que, pour les microorganismes unicellulaires, l'inhibition de leur croissance est enregistrée après 3 jours d'incuba-

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