Microbodies in Methanol-grown *Candida boidinii*

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SUMMARY

Intracellular structures were observed in *Candida boidinii* grown in a medium containing methanol as the sole source of carbon and energy; these structures were absent in the same organism grown in the presence of glucose or ethanol. These substrate-specific structures are ultrastructurally similar to microbodies. Studies with sphaeroplast lysates and a mutant lacking alcohol-oxidase activity indicate that the alcohol oxidase may be located in these microbodies.

INTRODUCTION

As described in previous reports (Sahm & Wagner, 1973*a*; Roggenkamp, Sahm & Wagner, 1974), an FAD-dependent alcohol oxidase and a catalase are inducibly formed in *Candida boidinii* growing on methanol. In plant and animal cells flavin oxidase and catalase have been found to be at least partly located in microbodies (De Duve & Baudhuin, 1966; Tolbert, 1971). Furthermore, Kormendy & Wayman (1974) observed the presence of substrate-specific structures in *Candida utilis* when this yeast was grown on *n*-butane or *n*-butanol. We describe microbodies in *C. boidinii* grown on methanol, and give some evidence that the alcohol oxidase is located within these particles.

METHODS

Preparation of extracts. *Candida boidinii* was cultivated in a basal medium as previously described (Sahm & Wagner, 1972). Cells were harvested in the late exponential growth phase, and extracts were prepared either by using an X-Press (Sahm & Wagner, 1973*a*) or by the osmotic lysis of sphaeroplasts. Cells were converted to sphaeroplasts by treating with digestive enzymes from *Helix pomatia* as described by Kováč, Bednářová & Greksák (1968). About 90 % of the cells were converted to osmotically labile sphaeroplasts within 60 min at 30 °C. The sphaeroplasts were lysed by lowering the mannitol concentration to 0.44 M and by agitation with a Potter-Elvehjem homogenizer. Intact cells were removed from the crude sphaeroplast lysate and X-Press extract by low-speed centrifugation at 500 *g* for 10 min. These extracts were subjected to further centrifugation at 5000 *g* for 30 min to sediment the particulate matter.

Enzyme assays. All spectrophotometric assays were performed on a Zeiss PMQII recording spectrophotometer at 30 °C. The following enzymes were assayed by published pro-

cedures: alcohol oxidase (Sahm & Wagner, 1973*a*); catalase, EC. 1.11.1.6 (Roggenkamp *et al.* 1974); formaldehyde dehydrogenase, EC. 1.2.1.1 and formate dehydrogenase, EC. 1.2.1.2 (Sahm & Wagner, 1973*b*).

Protein determination. Protein was assayed by the Folin–Ciocalteu method as described by Lowry *et al.* (1951). Standards were prepared with crystalline bovine serum albumin.

Electron microscopy. Cells were fixed in 3 % (w/v) glutaraldehyde in 50 mM-phosphate buffer pH 6.9, for 2 or 16 h. Following a thorough rinsing in the same buffer, cells were postfixed either in 2 % (w/v) OsO₄ buffered with 50 mM-phosphate pH 6.9 for 2 h, or in unbuffered 2 % (w/v) KMnO₄ for 20 min, and then washed and embedded in 2.5 % (w/v) agar. These agar blocks were dehydrated in a graded ethanol series, treated with propylene oxide, and embedded in Epon. Sections were cut with glass knives on an LKB Ultratome, stained with lead citrate (Reynolds, 1963), and examined in a Philips EM 300 electron microscope.

RESULTS AND DISCUSSION

The electron micrographs in Fig. 1 show the typical structure of *C. boidinii* grown on methanol. Each cell contained a cluster of bodies varying in size from 0.4 to 1 µm in diameter; these bodies were surrounded by a single membrane and they may therefore be described as microbodies (Tolbert, 1971). Crystalloid inclusions were observed in these microbodies after postfixation in OsO₄ (Fig. 1*b*). The age of the yeast did not influence the presence of these microbodies. However, no sign of their presence was found in yeast grown on glucose or ethanol (Fig. 2) indicating that these structures may be associated with methanol metabolism.

This hypothesis is supported by the fact that about 80 % of the alcohol-oxidase activity was found in particles which were collected from the sphaeroplast lysates by centrifugation for 30 min at 5000 *g* (Table 1). A parallel study on cells broken by the X-Press showed that under these conditions only 20 % of alcohol-oxidase activity was found in the sediment. These results indicate that the alcohol oxidase is particulate. While the last two enzymes of the dissimilatory methanol pathway, the formaldehyde dehydrogenase and the formate dehydrogenase (Sahm & Wagner, 1973*b*), are soluble enzymes, part of the catalase activity is also particulate (Table 1).

Further evidence that the alcohol oxidase may be located in these microbodies is given by the mutant 4₈ of *C. boidinii*, which is unable to grow on methanol because it lacks the alcohol oxidase (Sahm & Wagner, 1973*a*; Roggenkamp *et al.* 1974). After growth on glucose, cells of this mutant incubated for 24 h in a methanol medium contained a cluster of microbodies, but in contrast to those in wild type, they lacked any crystalloid inclusions after postfixation in OsO₄ (Fig. 3). This finding suggests that the inclusions in the microbodies represented crystals of the alcohol oxidase. The size of these crystals indicates that this enzyme must constitute a considerable proportion of the total protein in the microbodies, an observation consistent with biochemical results on the alcohol-oxidase content of *C. boidinii* (Sahm & Wagner, 1973*a*).

The crystalloid inclusions described in this report resemble the crystalloid inclusions which have been observed in microbodies of *Nicotiana tabacum* (Frederick & Newcomb, 1969) and in rat-liver cells (Baudhuin, Beaufay & De Duve, 1965). However, the latter types were associated with catalase and urate oxidase, respectively. Since in *C. boidinii* the methanol seems to be oxidized by the alcohol oxidase as well as by the peroxidative action of catalase using the H₂O₂ which is generated by the alcohol-oxidase reaction (Roggenkamp *et al.* 1974), it may be that besides the alcohol oxidase, part of the catalase activity is located in

Fig. 1. *Candida boidinii* grown on methanol (a) postfixed in KMnO_4 , (b) postfixed in OsO_4 . M, microbody; N, nucleus; V, vacuole. Inset: Higher magnification of a crystalloid core.

Fig. 2. *Candida boidinii* grown on ethanol, postfixed in KMnO_4 .

Fig. 3. Mutant 48 of *C. boidinii* incubated for 24 h in a methanol medium, postfixed in OsO_4 . The microbodies (M) are without crystalloid cores.

Table 1. *Distribution of alcohol oxidase, catalase, formaldehyde dehydrogenase and formate dehydrogenase between the supernatants and sediments of lysates and extracts after centrifugation at 5000 g for 30 min*

Enzyme	Total enzyme units ($\mu\text{mol}/\text{min}/5\text{ ml}$)					
	Sphaeroplast lysate			X-Press extract		
	Lysate	Supernatant	Sediment	Extract	Supernatant	Sediment
Alcohol oxidase	4.2	0.7	3.1	4.1	2.8	0.8
Catalase	32 540	21 240	10 050	35 580	33 210	1390
Formaldehyde dehydrogenase	14.2	12.6	1.3	15.1	13.6	1.2
Formate dehydrogenase	4.8	4.4	0.2	4.9	4.6	0.2
Protein content (mg/5 ml)	20.5	11.5	8.3	22.1	16.3	6.0

these microbodies. If this were so, the microbodies could be designated as peroxisomes (De Duve & Baudhuin, 1966).

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