MICROBIAL ASSIMILATION OF METHANOL INDUCTION AND FUNCTION OF CATALASE IN *CANDIDA BOIDINII*

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1. Introduction

As described in a previous report an inducibly formed FAD dependent alcohol oxidase has been isolated from the yeast *Candida boidinii* growing on methanol as sole source of carbon and energy [1]. This enzyme was characterized to catalyze the following reaction: $CH_3OH + O_2 \rightarrow HCHO + H_2O_2$. In view of the H_2O_2 generation of the methanol oxidizing enzyme we investigated the regulation and the peroxidase activity of catalase, since methanol oxidation by catalase has been shown in rat liver [2] and also in methanol utilizing bacteria [3] and yeasts [4].

In this paper we report that in *Candida boidinii* growing on methanol catalase is inducibly formed and that formaldehyde production in vitro is also accomplished by peroxidation of methanol and H_2O_2, which is generated by the alcohol oxidase reaction.

2. Materials and methods

Actidion (cycloheximide) was purchased from Serva, Heidelberg. 3-Amino-1, 2, 4-triazole was obtained from Ega-Chemie, Steinheim, purified beef liver catalase and NAD from Boehringer, Mannheim.

Candida boidinii was grown at 30°C in 500 ml Erlenmeyer flasks containing 100 ml basal medium as previously described [5]. Cells were harvested at the end of logarithmic growth phase and cell-free extracts were prepared using an X-press [1]. Alcohol oxidase was purified from cell-free extracts as described earlier [1].

Catalase was assayed by the decrease in absorbance at 240 nm as H_2O_2 was decomposed [6]. The reaction mixture contained in 3.0 ml 100 μmoles potassium phosphate buffer pH 7.0, 40 μmoles H_2O_2 and cell-free extract (0.001–0.02 mg protein). The extinction coefficient was 4.36×10^4 cm^2/mole. Alcohol oxidase activity was measured by the determination of formaldehyde described by Tani et al. [7] except 100 μmoles methanol were used. Formaldehyde dehydrogenase was assayed by the increase in absorbance at 340 nm caused by NAD reduction [8]. Specific activity is expressed as 1 μmole of converted substrate per minute per mg protein. Protein was determined by the method of Lowry et al. [9].

O_2-consumption was measured in a Warburg apparatus (Gilson respirometer) at 30°C. Each vessel contained 300 μmoles potassium phosphate buffer pH 7.5, 100 μmoles methanol and cell-free extract (1 mg protein) or alcohol oxidase (0.1 mg protein) at a total volume of 3.0 ml. The centre well contained 0.2 ml of 20% (w/v) KOH for CO_2 absorption. The reaction was stopped by addition of 0.5 ml of 5% (v/v) acetic acid after 30 min. Formaldehyde was determined by the method of Frisell and Mackansie [10].

3. Results

3.1. *Induction of catalase*

As shown in table 1 there are significant differences in catalase activity of cell-free extracts from *Candida boidinii* grown on different carbon sources. The activity in methanol grown cells is increased about five-fold in comparison with cells cultivated on glucose or ethanol.

Table 1
Catalase activity of *Candida boidinii* grown on different carbon sources

Carbon source (1% w/v)	Specific activity (U/mg protein)
Methanol	1450
Glucose	310
Ethanol	320

After transferring of glucose grown cells to a methanol medium catalase activity increases rapidly (fig. 1). The full activity is reached after an incubation time of about 5 hr, whereas the alcohol oxidase takes about 30 hr [8].

As synthesis of catalase during aeration of anaerobically grown cells of *Saccharomyces cerevisiae* is regulated by catabolite repression [11] the inducible formation of catalase in *Candida boidinii* is also repressed by glucose. If actidion is added to the growing culture (approx. 2×10^{10} cells/ml) at a concentration of 8 mg/ml increase of catalase activity was not observed. These facts suggest that catalase is synthesized de novo.

Induced synthesis of catalase in anaerobically grown cultures of *Rhodopseudomonas spheroides* was shown to be achieved by aeration or addition of H_2O_2 [12]. In order to find out if the H_2O_2 generated by alcohol oxidase reaction or an intermediate of methanol metabolism in *Candida boidinii* is the inducer we used a

Table 2
Induction of catalase and formaldehyde dehydrogenase in a methanol negative mutant of *Candida boidinii* (strain 4_5) after transferring of the glucose grown cells to a methanol medium

Time (hr)	Catalase (U/mg protein)	Formaldehyde dehydrogenase (U/mg protein)
0	330	0.04
5	680	0.3
23	1420	0.41

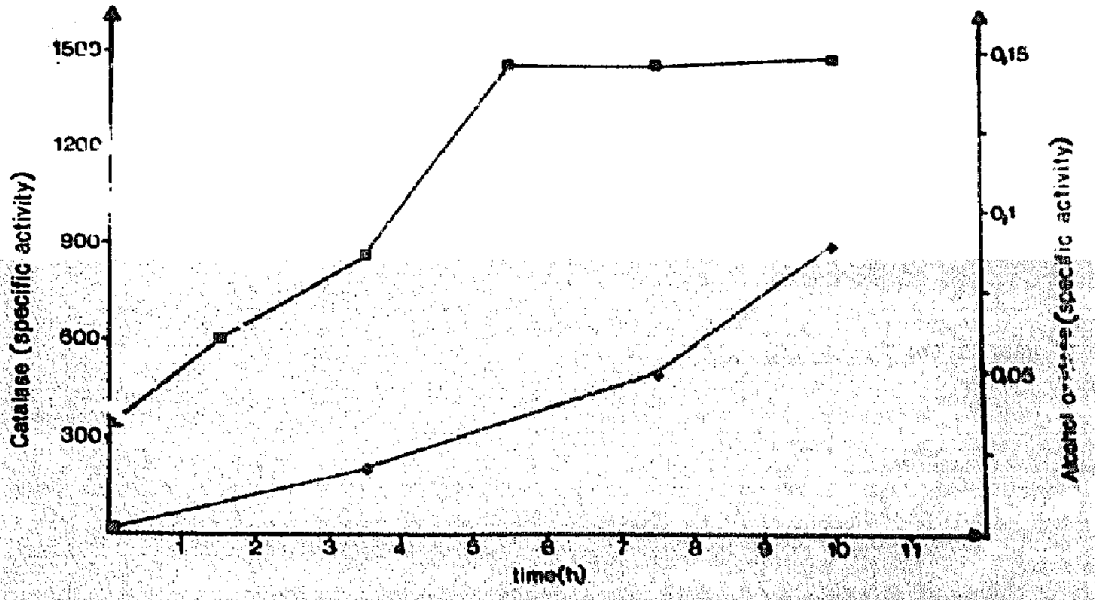


Fig. 1. Induction of catalase (▪——▪) and alcohol oxidase (•——•) of glucose grown cells in a methanol medium.

Table 3
Stoichiometry of methanol oxidation by crude extract and alcohol oxidase of *Candida boidinii*

Enzyme	Protein (mg)	Additions	O_2 consumption (μmole)	Formaldehyde production (μmole)
Crude extract	1	—	5.6	10.4
Crude extract	1	Triazole (0.15 M)	5.7	5.4
Alcohol oxidase	0.1	—	5.2	5.9
Alcohol oxidase	0.1	Triazole (0.15 M)	5.3	5.6
Alcohol oxidase	0.1	Catalase (1000 units)	5.3	10.8

O_2-Consumption and formaldehyde production were assayed as described in Materials and methods.

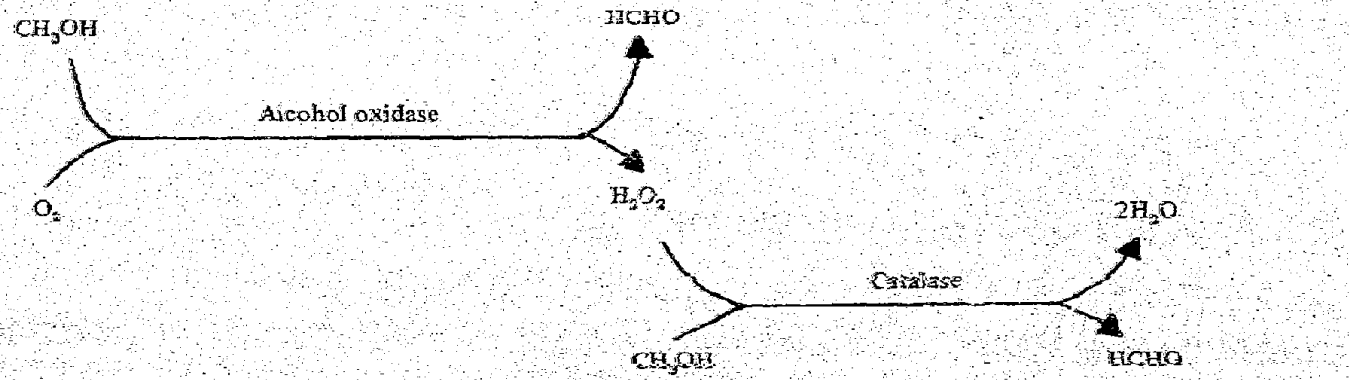
methanol negative strain of the yeast. This mutant (strain 4_8) is unable to grow on methanol because of lacking the methanol oxidizing enzyme [1]. Induction of catalase and formaldehyde dehydrogenase in a methanol medium (table 2) indicates that the catalase may be induced by methanol itself and not by H_2O_2.

3.2. *Methanol oxidation by catalase*

Measurements of O_2 consumption during methanol oxidation by purified alcohol oxidase (without catalase activity) showed that 1 mole O_2 was consumed as 1 mole formaldehyde and 1 mole H_2O_2 were produced [1]. In case of the cell-free extract 1 mole O_2 was consumed as 2 moles formaldehyde were produced (table 3). If the extract was incubated with 3-amino-1, 2, 4-triazole, a specific inhibitor of catalase [13], stoichiometry of methanol oxidation was the same as if purified alcohol oxidase was used, because the formaldehyde production was reduced to 50%. Since alcohol oxidase is not inhibited by 3-amino-1, 2, 4-triazole, the second mole formaldehyde per mole O_2 using crude extract is due to peroxidative activity of catalase. This result is confirmed by the fact that addition of beef liver catalase to purified alcohol oxidase causes twice as much formaldehyde production (table 3). As shown by the stoichiometry all H_2O_2 generated by alcohol oxidase reaction is used for formaldehyde formation and not for peroxidation of formaldehyde or formate under these conditions [2].

4. Discussion

Cell-free extract of *Candida boidinii* can oxidize methanol to formaldehyde by two reactions. The first is via an alcohol oxidase which requires oxygen and produces hydrogen peroxide besides formaldehyde. The second is the peroxidatic action of catalase using the H_2O_2 which is generated by alcohol oxidase.



The catalase may be induced by methanol and not by H_2O_2, since the induction of this enzyme occurs also in a mutant lacking the alcohol oxidase, after transferring of glucose grown cells to a methanol medium. In plant cells and yeasts most of the catalase has been found in peroxisomes, which all contain H_2O_2 producing oxidases, too [14,15]. Recently Fukui could show that increase in catalase activity paralleled the appearance of microbodies (peroxisomes) in *Candida* strains growing on *n*-alkanes [16]. Therefore we further investigated if inducibly formed catalase and alcohol oxidase in *Candida boidinii* are also located in microbodies.

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References

[1] Sahm, H. and Wagner, F. (1973) Eur. J. Biochem. 36, 250–256.
[2] Aebi, H., Koblet, H. and Von Wartburg, J. P. (1957) Helv. Physiol. Acta 15, 384–399.
[3] Harrington, A. A. and Kallio, R. E. (1960) Can. J. Microbiol. 6, 1–7.
[4] Fujii, T. and Tonomura, K. (1972) Agr. Biol. Chem. 36, 2297–2306.
[5] Sahm, H. and Wagner, F. (1972) Arch. Mikrobiol. 84, 29–42.
[6] Bergmeyer, H.-U., Gawehn, K. and Graßl, H. (1970) in: Methoden der enzymatischen Analyse (Bergmeyer, H.-U., ed.) Bd. 1 pp. 388–488, Verlag Chemie, Weinheim.
[7] Tani, Y., Miya, T. and Ogata, K. (1972) Agr. Biol. Chem. 36, 76–83.
[8] Sahm, H. and Wagner, F. (1973) Arch. Mikrobiol. 90, 263–268.
[9] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[10] Frisell, W. R. and Mackansie, C. G. (1958) in: Methods of Biochemical Analysis (Glick, D., ed.) Vol. 6, pp. 63–67, Interscience Publishers, New York.
[11] Sulebele, G. A. and Rege, D. V. (1968) Enzymologia 12, 320–334.
[12] Shanmugam, K. T. and Berger, L. R. (1969) Arch. Mikrobiol. 69, 206–215.
[13] Heim, W. G., Appleman, D. and Pyfrom, H. T. (1955) Science 122, 693–694.
[14] Tolbert, N. E. (1971) Ann. Rev. Plant Physiology, 22, 45–74.
[15] Avers, C. J. and Federmann, M. (1968) J. Cell. Biol. 37, 555–559.
[16] Fukui, S. personal communications.