The Substrate Constant for Dissolved Molecular Oxygen of Methanol-assimilating Yeasts

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INTRODUCTION

The pathway of methanol metabolism by some yeast species has been elucidated recently. It is initiated by an inducible alcohol oxidase, a flavoprotein producing formaldehyde and hydrogen peroxide from methanol and oxygen (Tani *et al.*, 1972; Fujii & Tonomura, 1972; Sahm & Wagner, 1973). Catalase is induced together with alcohol oxidase (Roggenkamp, Sahm & Wagner, 1974). Both enzymes may be contained within peroxisome-like microbodies, which are present in large amounts during growth on methanol, but not during growth on ethanol or glucose (van Dijken *et al.*, 1975).

Most aerobic micro-organisms have respiration rates which are constant over a wide range of oxygen concentrations. Diminished respiration rates are only observed at concentrations below approximately 0.2 mg O₂/l. Apparently, the substrate constant for dissolved oxygen is of this order of magnitude. Preliminary chemostat experiments with methanol-assimilating yeasts suggested that in yeast cultures with methanol as the sole source of carbon and energy, higher oxygen concentrations might be needed to avoid oxygen limitation of the growth rate than in cultures with other carbon sources.

In the present investigation the substrate constant for molecular oxygen, defined as the oxygen concentration (mg O₂/l) at which the respiration rate is half the maximum, is determined for resting yeast cells grown on and respiring with methanol or ethanol as the sole source of carbon and energy.

METHODS

Strains. The yeast strains studied were kindly supplied by the Centraal Bureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands, unless stated otherwise. Strains used were *Candida boidinii* Ramirez CBS2428 and strain B isolated from soil; *Hansenula capsulata* Wickerham CBS1993; *Hansenula philodendra* van der Walt & Scott CBS6075, *Kloeckera* sp. strain 2201 (isolated and described by Ogata, Nishikawa & Ohsugi, 1969); *Pichia trehalophila* Phaff, Miller & Spencer CBS5361; *Torulopsis nemodendra* van der Walt *et al.* CBS6280.

Growth of cultures. The growth medium consisted of (g l⁻¹): methanol or ethanol, 2; ammonium chloride, 0.38; sodium sulphate, 0.1; trace elements and vitamins according to Middelhoven (1969); potassium phosphate buffer, 0.1 M, pH 6.0. With *H. capsulata*, *H. philodendra* and *T. nemodendra*, yeast extract (5 g/l methanol medium) was added to increase the growth rate. The yeast strains were precultivated at 30 °C in conical flasks. Young cultures (1 ml) were inoculated into 100 ml medium in a 300 ml conical flask. Incubation took place at 30 °C in a rotary shaker (Gallenkamp) at 200 rev./min. The growth period varied with the

Fig. 1. The oxygen uptake by resting *Candida boidinii* strain B cells grown with methanol or ethanol as the sole source of carbon and energy. The oxygen uptake by resting cells respiring endogenously or with growth substrate was recorded. A and B, methanol-grown cells; C and D, ethanol-grown cells. Cell densities and other conditions were (g l^{-1}): A, yeast 0.19, methanol 1.0; B, yeast 3.8, respiring endogenously; C, yeast 0.6, ethanol 1.0; D, yeast 3.5, respiring endogenously.

strain and the carbon source. All cells were harvested in the exponential growth phase when no more than 50 % of the maximum cell yield had been attained, as indicated by extinction measurements. The cultures were chilled and centrifuged. The yeast cells were washed three times with ice-cold 0.1 M-potassium phosphate buffer pH 6.0 and used within 2 h for respirometer experiments. Cell density was determined as the dry weight of the culture, or by comparison of the E_{623} with a standard curve.

Oxygen uptake. The rate of oxygen uptake was determined with Biological Oxygen Monitor model 5301 (Yellow Springs Instruments Co. Inc.) coupled to a 100 mV potentiometer. The rates of both endogenous and exogenous substrate respiration were measured in 5 ml air-saturated 0.1 M-potassium phosphate buffer pH 6.0. The substrate concentration was 1 g l^{-1} , which was sufficient to consume 95 % of the oxygen in 5 to 15 min at 30°C .

RESULTS AND DISCUSSION

Oxygen uptake by ethanol-grown cells, respiring either with ethanol substrate or endogenously, is independent of O_2 concentration down to the limit of sensitivity of the instrument (about 0.15 mg/l O_2) or less (Fig. 1, curves C and D; only data for *C. boidinii* strain B are shown since other strains behaved similarly). Thus, as with other organisms, the substrate constant for O_2 was very low. This was also observed with methanol-grown yeasts respiring endogenously (curve B), except in the case of *H. capsulata*.

With methanol as substrate, the oxygen uptake became dependent upon the residual O_2 concentration at a much higher concentration (Fig. 1, curve A). This could not have been due to the accumulation of inhibitory dissimilation products such as formaldehyde, as oxygen uptake continued after the yeast suspension had been re-saturated with air. Reciprocal

Table 1. *The substrate constants for oxygen of methanol-assimilating yeast strains grown in methanol medium*

Resting yeast cells grown in methanol medium were suspended in buffer. The oxygen uptake of cells respiring endogenously or with methanol was measured and recorded. The calculation of the substrate constant is described in Results and Discussion.

Yeast species	Strain number	K_{O_2} (mg l ⁻¹)	
		Endogenous respiration	Respiration with methanol
<i>Candida boidinii</i>	CBS2428	≈ 0.15	1.0
	Strain B	≈ 0.15	1.3
<i>Hansenula capsulata</i>	CBS1993	0.7	0.6
<i>Hansenula philodendra</i>	CBS6075	≈ 0.15	0.8
<i>Kloeckera</i> sp.	2201	≈ 0.15	0.8
<i>Pichia trehalophila</i>	CBS5361	≈ 0.15	0.5
<i>Torulopsis nemodendra</i>	CBS6280	≈ 0.15	0.7

plots of the rate of O_2 consumption versus O_2 concentration are linear. Values for the substrate constant K_{O_2} calculated from the regression lines are listed in Table 1. Substrate constants for oxygen of the various yeast strains were high for cells respiring with methanol, and very low for cells (except *H. capsulata*) respiring endogenously.

It appears that methanol-assimilating yeast species show at least two types of respiration: one endogenously or with ethanol as the substrate, and one with methanol as the substrate. Oxygen uptake due to the former type proceeded at a constant rate at O_2 concentrations down to about 0.15 mg l⁻¹. Respiration with methanol as the substrate showed substrate constants for oxygen between 0.5 and 1.3 mg O_2 /l. Possibly these high values are due to the involvement of alcohol oxidase (a flavoprotein) in the primary reaction between oxygen and methanol. Nevertheless, we found that respiration on methanol is inhibited by 50 to 80 % by 0.2 mM-potassium cyanide, a potent inhibitor of cytochrome oxidase-mediated respiration. This suggests that terminal respiration in methanol-assimilating cells is partly mediated by cytochrome oxidase and partly by the system responsible for the high substrate constant for O_2 . Attempts to inhibit respiration on methanol with salicyl hydroxamate, an inhibitor of the cyanide-resistant respiration of *Candida lipolytica* (Henry, Hamaide-Deplus & Nyns, 1974) and *Neurospora crassa* (Edwards, Rosenberg & Maroney, 1974), were unsuccessful as the compound was non-toxic to the strains studied at concentrations < 5 g l⁻¹.

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