Effect of pretreatment of the inner membrane with lecithin and mercaptoethanol on methyl mercury inhibition

Enzyme preparation	CH_3HgCl nM/mg protein	D-3-hydroxybutyrate activity nM substrat/min/mg protein
Inner membrane	–	460
Inner membrane	10	240
Inner membrane + lecithin	10	300
Inner membrane + 2 mercaptoethanol	10	420
Inner membrane + lecithin + 2 mercaptoethanol	10	460

obtained for methyl mercury concentration of 10 nM/mg protein. The enzyme is more sensitive than succinate deshydrogenase (20 nM/mg protein). Cytochrome oxydase is not inhibited in this range of concentrations, (17% inhibition for 4 mM/mg protein). The subsequent addition of pure lecithin and 2 mercaptoethanol (0.67 mM/mg protein) in the medium could not reverse the activity of 3 hydroxybutyrate deshydrogenase incubated with 10 nM/mg protein inhibitor. The effect of a pretreatment of the inner mitochondrial matrix with pure lecithin (0.67 mg/mg protein) and 2 mercaptoethanol (0.22 mM/mg protein) on the inhibitory action of methyl mercury on 3 hydroxybutyrate deshydrogenase is reproduced in the Table. The enzymatic activity of the control is not significantly stimulated by the addition of the reagents.

D-3-hydroxybutyrate deshydrogenase activity is partially protected by pretreatment with lecithin, however higher concentrations of the phospholipid do not produce a better action. Addition of an-SH reagent associated with the phospholipid prevents an inhibitory action of methyl mercury. The inhibition of methyl mercury on D-3-hydroxybutyrate deshydrogenase seems to imply both phospholipids and thiol groups as possible sites of action. Further studies are under way to elucidate the possible interaction of methyl mercury with lecithin and the relative stability of the binding to sulfhydryl groups of the enzyme[13].

Résumé. L'inhibition par le méthyl mercure de certains enzymes de la membrane interne de mitochondries de foie de rat, montre que la D-3-hydroxybutyrate deshydrogenase est très sensible. L'enzyme pré-incubée avec de la lecithine et du 2 mercaptoethanol est protégé.

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Demonstration of Methanol Dehydrogenase in Methanol Assimilating Yeasts

Methanol oxidation in bacteria has been investigated by several workers[1–3], and no evidence of a pyridine nucleotide-linked methanol dehydrogenase has been reported. OGATA et al.[4] described a methanol oxidation in yeast via an oxidase, but they did not detect a pyridine nucleotide-linked dehydrogenase. Their data suggests that a flavine nucleotide cofactor was involved in the oxidation. The present note describes the oxidation of methanol by cellular extracts of *Pichia pinus* and *Kloeckera sp.* 2201 utilizing pyridine nucleotide (NAD) or dichlorophenol indophenol (DCPIP) as electron acceptors.

Materials and methods. A 5-day-old slant culture of *Pichia pinus* NRRL YB-4025 and *Kloeckera sp.* 2201[4] was inoculated into respective flasks containing a sterile mineral salts medium composed of 1% NH_4NO_3, 0.1% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, vitamin mixture[4] and 2% methanol. Cultures were propagated for 4 days on a rotary shaker at 25C (250 RPM with 2″ throw). Cells were centrifuged and washed twice with 0.01 M potassium phosphate buffer, pH 7.0. Washed cells were resuspended in buffer and sonicated at 4 °C in ice-bath for 5 min (ten 30 sec intervals) with Branson Ultra Sonified (Branson Instruments, Conn. USA). Cell debris was removed by centrifugation at 20,000 $\times g$ for 10 min. The clear supernatant was used for enzyme assay.

Methanol dehydrogenase activity was assayed spectrophotometrically using a Zeiss Gilford instrument with an automatic chart recorder. The assay system (NAD-linked) contained potassium phosphate or *Tris*-HCl buffer, pH 7.0 and 8.5, 70 µmoles, NAD-1 µmole, enzyme (1 mg protein), and substrate, 20 µmoles. NAD reduction was measured at 340 nm. Specific activity was expressed as nanomoles of NAD or DCPIP[3] reduced per min per mg protein.

Results and discussion. Cell-free extracts of both yeast cultures grown on a methanol containing medium oxidized methanol via NAD-linked methanol dehydrogenase. The Table shows the activity of methanol dehydrogenase at pH 7.0 and at pH 8.5. In the case of *Pichia pinus*, the methanol oxidation rate utilizing NAD or DCPIP at pH 8.5 was greater than that at pH 7.0, whereas, in *Kloeckera sp.* 2201, NAD-linked activity was found to be greater at pH 7.0. OGATA et al.[4] described methanol oxidation via methanol oxidase in *Kloeckera sp.* 2201, but no dehydrogenase activity using NAD, NADP, or DCPIP was reported. Lack of NAD or DCPIP-linked activity in the *Kloeckera sp.* 2201 cell-extract of OGATA

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Specific activity of methanol dehydrogenase in cell-free extracts of methanol-utilizing yeast

Culture	System	Type of electron acceptor	Cells grown	
			Methanol	Glucose
Pichia pinus	pH 7.0	NAD (at 340 nm)	32[a]	0
	pH 8.5	NAD (at 340 nm)	72	0
	pH 7.0	DCPIP (at 600 nm)	621	0
	pH 8.5	DCPIP (at 600 nm)	870	0
Kloeckera sp. 2201	pH 7.0	NAD (340 nm)	80	0
	pH 8.5	NAD (340 nm)	64	0
	pH 7.0	DCPIP	50	0
	pH 8.5	DCPIP	435	0

[a] Specific activity in nmoles/min/mg protein.

et al. may be due to differences in procedures employed in the preparation of the cell-free extract. OGATA et al. prepared the cell-extract by grinding cells in a mortar with alumina for 5 h at 4 °C, while in the present report, cell-extracts were prepared by sonication.

The results reveal that methanol dehydrogenase activity was inducible since dehydrogenase activity was not found in cultures propagated on glucose. In summary, an inducible methanol dehydrogenase has been detected in cellular extracts of 2 methanol assimilating yeasts, *Pichia pinus* and *Kloeckera sp.* 2201. They were found to be linked to either NAD or DCPIP. Nicotinamide adenine dinucleotide phosphate did not serve as an electron acceptor. This is the first report describing NAD-linked methanol dehydrogenase activity in yeast[5].

Zusammenfassung. Eine induzierbare Methanoldehydrogenase wurde in Zellextrakten von 2 Methanol assimilierenden Hefen, *Pichia pinus* und *Kloeckera sp.* 2201, festgestellt, die entweder von NAD oder DCPIP abhängig waren. Nicotinamid Adenin-Dinukleotidphosphat diente jedoch nicht als Elektronen-Akzeptor.

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Metabolism in Porifera IV. Biosynthesis of the 3β-Hydroxymethyl-A-nor-5α-Steranes from Cholesterol by *Axinella verrucosa*

Sponges contain a great variety of sterols, which vary from one species to another[1]; knowledge of this subject has increased rapidly in recent years[2,3]. Besides conventional sterols, unusual sterols have been found. For example, aplysterol and 24(28)-didehydroaplysterol, having the cholesterol nucleus and side-chains in which an 'extra' carbon atom is attached at C-26[4], are the major sterol components in the family Verongidae[3]. Modifications of the sterol nucleus have also been found[5]. The total sterol content of *Axinella polypoides* is a mixture of 19-nor-stanols, which combine the unusual 19-nor-cholestanol nucleus with conventional saturated and Δ^{22}-unsaturated C7 (24-nor), C_8, C_9 and C_{10} side-chains[6], while *Axinella verrucosa*, in which the usual sterols are also absent, contains stanols with a 3β-hydroxymethyl-A-nor-5α-cholestane nucleus carrying C_8, C_9 and C_{10} side-chains (**1–6**)[7].

On the other hand, there is little information about the origin of sterols in sponges. We have recently shown by tracer experiments that *Verongia aerophoba* does not incorporate mevalonate into aplysterol[8]. We now report the conversion of [4-^{14}C]-cholesterol into 3β-hydroxymethyl-A-nor-5α-steranes by *A. verrucosa*. The sponge was also fed with [1-^{14}C]-acetate, and the radioactivity in the fatty acids and stanols was measured.

The labelled substrates were fed to the sponge maintained in well-aerated sea water at 14 °C by addition of aqueous (acetate) and ethanolic (cholesterol) solutions to the aquaria. Sterols were recovered from the light petroleum extract of the lyophilized tissues, while fatty acids were obtained from the subsequent chloroform-methanol extract by saponification, and then purified, after conversion into methyl esters, by chromatography on silica followed by distillation at 250 °C (experimental details are given in reference[8]). The light petroleum extract, after addition of carrier cholesterol in the case of the cholesterol incubations, was chromatographed on silica[9].

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