Biochimica et Biophysica Acta, 350 (1974) 237—239

BBA Report

BBA 61290

ALCOHOL DEHYDROGENASES OF *EUGLENA GRACILIS*, STRAIN Z

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(Received March 12th, 1974)

Summary

Cell-free extracts from heterotrophically grown *Euglena gracilis* were assayed for alcohol dehydrogenase activities by the reduction of NAD with various alcohols as substrates. Highest rates were obtained with isopropanol and cinnamyl alcohol. Isopropanol was oxidized at about twice the rate of ethanol. Mercaptoethanol inhibited isopropanol dehydrogenase activity but had no effect on ethanol oxidation.

Ethanol is an excellent carbon source for heterotrophic growth of various strains of *Euglena gracilis* [1,2]. To our knowledge, little effort has been made to study the metabolism of alcohols in these organisms. Hurlbert and Rittenberg [3] detected an NAD-specific alcohol dehydrogenase (alcohol: NAD^+ oxidoreductase, EC 1.1.1.1) but no NADP activity in cell-free extracts of *Euglena gracilis* var. *bacillaris*, and Danforth and Hunter [4] found an NADP-dependent ethanol oxidation but no NAD activity in the same organism Both NADP- and NAD-specific alcohol dehydrogenases, particle-associated and soluble, have been reported in a related organism, *Astasia longa* [5]. In view of these reports, we considered it of interest to determine if enzymes of alcohol metabolism are inducible in *Euglena gracilis* or if their presence might be related to growth conditions or nature of the carbon source.

Euglena gracilis, strain Z, was grown axenically at room temperature in modified Cramer—Myer medium [2] with either 1% ethanol or 0.06 M acetate (pH 5) as sole carbon sources. Cultures were aerated during growth, and cells were harvested by centrifugation at late log phase. Packed cells were washed once with 0.066 M phosphate buffer, pH 7.4, containing 1 mM EDTA. Frozen, packed cells were thawed in an equal volume of 0.066 M

buffer and sonicated for two 10-s periods at maximum intensity on ice. The broken cells were centrifuged at 40 000 × *g* for 30 min and the resulting supernatants were frozen, thawed and centrifuged again for 30 min to remove additional particulate material. Extracts prepared from cells broken with the French pressure cell or by grinding with Alumina in a mortar produced the same results. Alcohol dehydrogenase activities were assayed by spectrophotometric measurement of NAD reduction at 340 nm. A reference cuvette containing 1 mM NAD or NADP, 0.01 M pyrophosphate buffer, pH 8.5 and enzyme in a total volume of 3 ml was used to zero the spectrophotometer, and reduction of pyridine nucleotide was followed in the test cuvette containing the same ingredients plus 0.1 M alcohol.

Only NAD-specific alcohol dehydrogenase activities were detectable in extracts from either ethanol or acetate grown cells using ethanol, isopropanol or propanol as substrates. Specific activities of ethanol dehydrogenase activities were the same in green cells grown on acetate or ethanol or in bleached (chloroplastless) mutants grown on ethanol. The pH optima of the ethanol dehydrogenase activity was 8.5 with sodium pyrophosphate buffer, pH 8, in phosphate. Table I shows the specific activities of alcohol dehydrogenase activities in acetate grown cell extracts using a variety of alcohols as substrates. The highest activity was found with isopropanol, and this activity was consistently at least twice that obtained with ethanol. Both ethanol

TABLE I

SUBSTRATE SPECIFICITY OF *EUGLENA GRACILIS* ALCOHOL DEHYDROGENASES

All alcohols were assayed at concentrations of 0.1 M or saturation. Soluble, acetate-grown *Euglena* extract containing 2 mg protein was used in these assays as described in the text.

Alcohol	Specific activity (nmoles NAD reduced·min^{-1}·mg^{-1} protein)
2-Propanol (isopropyl alcohol)	48.2
3-Phenyl-2-propen-1-ol (cinnamic alcohol)	20.2
1-Pentanol (*n*-amyl alcohol)	19.4
Ethanol	13.3
Propanol	8.2
2-Pentanol (*sec*-amyl alcohol)	3.4
2-Octanol	1.5
2-Methyl-2-propanol (*tert*-butyl alcohol)	1.2
2-Phenylethanol (phenethyl alcohol)	1.2
Methanol	1.2

and isopropanol activities were inhibited approximately 50% by $6.6 \cdot 10^{-6}$ M *p*-chloromercuribenzoate, but neither activity was affected by 10^{-5} M iodoacetamide. However, isopropanol dehydrogenase activity was inhibited by 10^{-2} M mercaptoethanol and ethanol activity was not affected (Fig. 1).

We are aware of no reports of a specific isopropanol dehydrogenase in the literature, and the experiments described in this report suggest that such an enzyme may exist in *Euglena gracilis*.

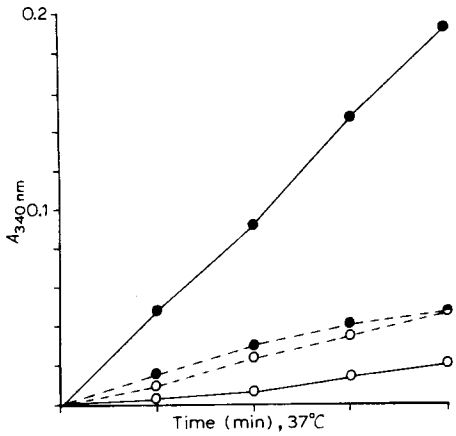

Fig.1. Effects of 2-mercaptoethanol on ethanol- (- - -) and isopropanol-dependent reduction (——) of NAD. ●, no mercaptoethanol; ○, 0.1 M mercaptoethanol. Cell-free *Euglena* extract was dialyzed over night at 4 °C against 2 l 10^{-3} M pyrophosphate buffer, pH 8.5. Reactions were catalyzed by 1.25mg protein in a volume of 3 ml as described in the text.

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