NADP SPECIFIC DIHYDROXYACETONE REDUCTASE FROM *DUNALIELLA PARVA*

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1. Introduction

The oxidation of glycerol to dihydroxyacetone by certain organisms, particularly those of the genus *Aerobacter* has long been known [1], and is the basis for the commercial production of dihydroxyacetone [2]. NAD^+ dependent glycerol dehydrogenase was partially purified from *Aerobacter aerogenes* [3–7] and *Escherichia coli* [8]. $NADP^+$ was ineffective as a substitute for NAD^+. The activity was maximal with glycerol, 1, 2-propanediol or 2, 3-butanediol [4, 7].

Several mammalian tissues contain $NADP^+$ dependent glycerol dehydrogenases which catalyze the interconversion of glyceraldehyde and glycerol [9]. D-glyceraldehyde is much preferred over dihydroxyacetone.

In the present communication we demonstrate the presence of a new enzyme, an NADP dependent dihydroxyacetone reductase in cell free extracts of *D. parva*. The enzyme catalyzes the reaction:

$$\text{Dihydroxyacetone} + \text{NADPH} + H^+ \rightleftharpoons \text{Glycerol} + \text{NADP}^+$$

2. Methods

D. parva was cultured as previously described [10]. Cell free extracts of *D. parva* were obtained by osmotic bursting of the algae [11]. Logarithmic phase algae were harvested and the cells were washed with 1.5 M NaCl, 10 mM Tris, pH 7.5 at room temp. After centrifugation the pellet was diluted 1/30 with 5 mM Tricine, pH 7.5, and allowed to stay for 15 min at 4°. Broken cells were removed by centrifugation and the cell free extract was assayed for dihydroxyacetone reductase as described below.

In the assay $NADP^+$ reduction or NADPH oxidation was followed in a Cary 16 recording spectrophotometer at 340 nm. The reaction was carried out in 1 cm light path cuvettes at room temp. The standard assay mixture for glycerol oxidation contained: 20 mM tricine-glycine pH 9.0, 0.05 mM $NADP^+$, 2.8 M glycerol and cell free extract of *D. parva* corresponding to about 50 μg chlorophyll in a total volume of 3.0 ml. The reaction was initiated by the addition of the glycerol. The standard assay mixture for dihydroxyacetone reduction contained: 20 mM Tricine, pH 7.5, 0.1 mM NADPH, 5 mM dihydroxyacetone, and cell free extract of *D. parva* corresponding to about 50 μg chlorophyll in a total volume of 3.0 ml. The reaction was started by the addition of the dihydroxyacetone. The activity of the enzyme is given as the rate of $NADP^+$ oxidation per mg chlorophyll per hr. One mg chlorophyll corresponds to about 5×10^8 *D. parva* cells.

Chlorophyll was assayed following the method of Arnon [12].

3. Results and discussion

Table 1 shows the requirements for dihydroxyacetone reductase activity by a cell free extract of *D. parva*. It is clear that in either direction there is an absolute requirement for all the components used. NAD^+ and NADH were inactive as substitutes for $NADP^+$ and NADPH. The rate of both glycerol oxidation and dihydroxyacetone reduction was proportional to the amount of cell free extract added. However no activity

Table 1
Requirements for NADP dependent dihydroxyacetone reductase activity by cell free extracts of *D. parva*.

Dihydroxyacetone	Reduction	Glycerol	Oxidation
Reaction mixture	NAD(P)H oxidation	Reaction mixture	NAD(P) reduction
	(μmoles × mg chl^{-1} × hr^{-1})		(μmoles × mg chl^{-1} × hr^{-1})
Complete	45	Complete	42
−NADPH	0	−$NADP^+$	0
−*D. parva* extract	0	−*D. parva* extract	0
−Dihydroxyacetone	3	−Glycerol	0
−NADPH, + NADH	4	−$NADP^+$, + NAD^+	0

Assay and experimental procedure were that described under methods.

of $NADP^+$ dependent dihydroxyacetone reduction was observed in cell free extracts of another green alga *Euglena gracilis*.

The K_m values for glycerol and $NADP^+$ were determined at pH 9.0 and found to be 1.4 M and 15 μM, respectively. The K_m values for dihydroxyacetone and NADPH at pH 7.5 were 0.8 mM and 4 μM, respectively.

The dependence of the rate of dihydroxyacetone reductase on the pH of the assay medium is presented in fig. 1. It can be seen that dihydroxyacetone reduction proceeds best around pH 7.5, while the reverse reaction of glycerol oxidation has an optimum pH around 9.0.

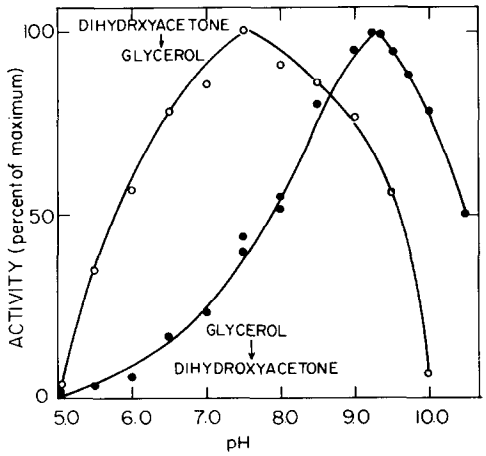

Fig. 1. pH dependence of the rate of glycerol production and breakdown. Assays as described in Methods. The following buffers were used at 30 mM over the following pH ranges: tricine, pH 5.0 – 7.5; tricine-glycine, pH 7.5 – 9.0; and CAPS (cyclohexylaminopropane sulfonic acid, Sigma), pH 9.0 – 10.5. 100% activity refers to 45 μmoles NADPH oxidized per mg chlorophyll per hr.

Table 2 summarizes the results concerning the specificity of the enzyme toward its substrate. As shown, the activity was by far maximal with glycerol with the next best substrate possessing only 12% of the activity with glycerol. The reverse reaction of NADPH oxidation was very poor (less than 5% of the activity with dihydroxyacetone) with the following substrates: D, L-glyceraldehyde, dihydroxyacetonephosphate, D, L-ribulose, sedoheptulose and methylglyoxal.

D. parva produces and accumulates high amounts of free glycerol within the cells [13, 14] and this glycerol serves as the major osmotic regulator of the cell [14]. The metabolic pathway for glycerol synthesis in *Dunaliella* is still obscure. It was recently suggested that an NAD^+ dependent glycerol phosphate dehydrogenase participates in glycerol synthesis in *D. parva* [13]. However, the activity of the latter enzyme of *D.*

Table 2
Substrate specificity of dihydroxyacetone reductase.

Substrate	Relative activity
Glycerol	1.00
2, 3-Butanediol	0.12
1, 2-Propanediol	0.10
iso-Erythritol	0.09
meso-Erythritol	0.04
1, 2-Ethanediol	0.03
D-sorbitol	0
D-mannitol	0

The reaction mixture contained: 20 mM tricine-glycine, pH 9.0, 0.15 mM NADP, cell-free extract of *D. parva* corresponding to 80 μg chlorophyll in a total volume of 3 ml and optimal concentrations of the indicated substrates (between 2.0 – 2.5 M). Activity of substrates is expressed relative to the activity with glycerol.

parva is very weak in comparison to that of the $NADP^+$ dependent dihydroxyacetone reductase described herein. The equilibrium constant of the dihydroxyacetone reductase reaction [7], and the high affinity of the enzyme for dihydroxyacetone and low affinity for glycerol tend to promote the synthesis and accumulation of large amounts of glycerol. It is, therefore, reasonable to suggest that the dihydroxyacetone reductase described herein plays a major role in the path of glycerol synthesis in *D. parva*.

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