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# ALDEHYDE DEHYDROGENASE FROM PHOTOHETEROTROPHICALLY GROWN *RHODOSPIRILLUM RUBRUM*

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Abstract

Extracts from cells of *Rhodospirillun rubrum* cultured photoheterotrophically under anaerobic conditions exhibit an $NAD^+$– dependent, soluble aldehyde dehydrogenase which catalyses the dehydrogenation of acetaldehyde to acetate (aldehyde: $NAD^+$ oxidoreductase. EC 1.2.1.3). The enzyme is $NAD^+$ specific with the aldehydes tested, no activity is observed with $NADP^+$ or in the absence of $NAD^+$. Sulfhydryl reagents markedly increase the aldehyde dehydrogenase activity. For initial reaction rates of acetaldehyde dehydrogenation the optimum activity of the enzyme is between pH 9.0 to 9 . 5, with an apparent Michaelis constant for acetaldehyde of $10^{-5}$ M. No requirements for potassium or orthophosphate are apparent. Extracts from cells cultured anaerobically on ethanol, as the sole carbon source, exhibit five to ten times the specific aldehyde dehydrogenase activity compared to cell cultured on malate plus glutamate. This increase in the specific activity of aldehyde dehydrogenase preparations from cells cultured on ethanol is inhibited by proflavin or chloramphenicol. The evidence suggests that the aldehyde dehydrogenase in *R. rubrum* is an inducible enzyme.

## Introduction

When *Rhodospirillum rubrum* is cultured photoheterotrophically in the absence of $O_2$ with ethanol as the sole organic carbon source, alcohol dehydrogenase activity can be detected in cell free extracts from such cultures (Chaudhary, 1971; Chaudhary &Frenkel, 1971). When such extracts are incubated with ethanol, in the absence of molecular oxygen, little if any acetaldehyde is formed, instead acetate accumulates in the reaction mixture. The search for aldehyde dehydrogenases in such extracts, utilizing acetaldehyde as substrate, revealed the presence on an $NAD^+$– dependent aldehyde dehydrogenase (Aldehyde : $NAD^+$ oxidoreductase. EC 1.2.1.3) (Chaudhary & Frenkel, 1971). Thus far, there appears to be no detailed report in the literature on aldehyde dehydrogenases in *R. rubrum.* However, Krasil'nikova (9176) reported the presence of aldehyde dehydrogenase activity in some other members of *Rhodospirillaceae*.

## Materials and Methods

A strain of *Rhodospirillum rubrum* (courtesy of Dr. J. Bolton, Department of Physical

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Table 1. Partial purification of an $NAD^+$ – dependent aldehyde dehydrogenase from *Rhodospirillum rubrum* grown photoheterotrophically on ethanol.

| Step | Enzyme activity (units/mg protein) (substrate. acetaldehyde) | + 1.6 mM DL-dithiothreitol |
|---|---|---|
| 1. Sonic extract centrifuged 15 min at 20,000 x g Supernatant | 0.048 | 0.19 |
| 2. Supernatant # 1 centrifuged 90 min at 60,000 x g, resulting | | |
| a. Supernatant | 0.14 | 0.56 |
| b. Sediment | 0.0 | – – |
| 3. Protamine sulfate added to fraction 2a. Centrifuged 15 min at 20,000 x g Resulting supernatant | 0.17 | 0.68 |
| 4. Ammonium sulfate fractionation. Sediment from 35-45% saturation, resupended and dialyzed | 0.34 | 1.36 |

* Enzyme unit is defined as the amount of enzyme which produces 1 $\mu$ mole NADH per minute (Steinman & Jakoby, 1967).

Chemistry, University of Minnesota, USA.) was subjected to further selection to yield a strain growing more repidly anaerobically in light with ethanol as its sole carbon source (strain Et-1). This strain was maintained anaerobically on liquied media containing malate plus glutamate (Hickman & Frenkel, 1959); the cultures were incubated at 30°C and illuminated with incandescent lamps at an intensity of about $10^5$ ergs $cm^{-2}S^{-1}$ Cells actively growing on this medium were inoculated into culture flasks to which ethanol (2.6 ml of 95% ethanol per liter of culture medium) had been added through a millipore filter (.22 micron). The flasks were continuously gassed with 5% $CO_2$ in Helium, incubated at 30°C and illuminated as indicated above.

*Enzyme Preparation*

After incubating the cultures for 36 to 48 hours, the cells were harvested by centrifugation, washed once with 0.1 M Na-glycylglycine pH 8.0 (Gg-buffer). The resulting cell

pastes were suspended in equal volumes of 0.2 M Na-glycyglycine containing 10 m M cysteine (Gg-cysteine buffer), final pH 8.0, and were sonicated at 1° – 3°C for 6 min. in a 10 KHz Raytheon magnetostrictive oscillator. The supensions thus obtained were centrifuged for 15 min. at 20,000 x g. The resulting supernatant was centrifuged for 90 min. at 60,000 x g. To the supernatant thus obtained, protamine sulfate was added gradually to yield a 0.1% solution. This preparation, after it was incubated at 1°C for one hour, was centrifuged for 15 min. at 20,000 x g. The resulting supernatant was fractionated with purified ammonium sulfate, the sediment formed at the 35-45% saturation level yielding the highest aldehyde dehydrogenase activity. The sediment thus obtained was redissolved in 0.1 M Gg-cysteine buffer and dialyzed against 0.1 M Gg-buffer (pH 8.0) at 1°C. (Table 1).

*Enzyme Assay*

The enzyme catalyzed reactions were followed by measuring the increases in absorbancy at 340 nm with a Cary Model 14 spectrophotometer in the presence of selected aldehydes (Table 2). The reaction mixtures contained the partially purified and dialyzed

**Table 2. Rates of dehydrogenation of selected aldehydes catalyzed by a partially purified $NAD^+$ – dependent aldehyde dehydrogenase from *Rhodospirillum rubrum* cultured photoheterotrophically on ethanol.**

| Substrate | conc. mM | Enzyme activity units/mg protein | Realtive Rates |
|---|---|---|---|
| acetaldehyde | 1.0 | 1.80 | 100 |
| glyceraldehyde | 1.0 | 0.16 | 9 |
| | 3.0 | 0.32 | 18 |
| benzaldehyde | 1.0 | 0.49 | 27 |
| fromaldehyde | 1.0 | 0.00 | 0 |
| † acetaldehyde | *0.3 | 2.00 | 100 |
| † glyceraldehyde-3-phosphate | 0.3 | 0.16 | 8 |

Reaction mixture:Enzyme, 0.15 mg protein per ml., 1.6 mM DL-dithiothreitol, 10.5 mM $NAD^+$ in 60 mM Na-glycylglycine, pH 9.3 t=25°C.

*For comparison with glyceraldehyde-3-phosphate, the activity with 0.3 mM acetaldehyde is taken as 100%

† Addition of $10^{-3}$M potassium or orthophosphate had no effect on the enzyme activity.

enzyme preparation (0.15 mg protein per ml final volume) in 60 mM Gg-buffer PH 9.3; plus 0.5 mM $NAD^+$, with or without 1.6 mM DL-dithiothreitol. The reactions were started by the addition of 0.05 ml of 20 mM acetaldehyde (final conc. 0.33 mM in a final volume of 3.0 ml), or by the addition of other aldehydes (Table 2); the reaction mixtures were incubated at 25°C for 10 minutes. Acetaldehyde disappearance and acetate formation were monitored by paper chromatography (Jones *et al.*, 1953). Protein determinations were carried out by the method of Lowry *et al.* (1951). The effect of pH on the enzyme activity was studied with glycylglycine or Tris buffers of appropriate pH (Fig I).

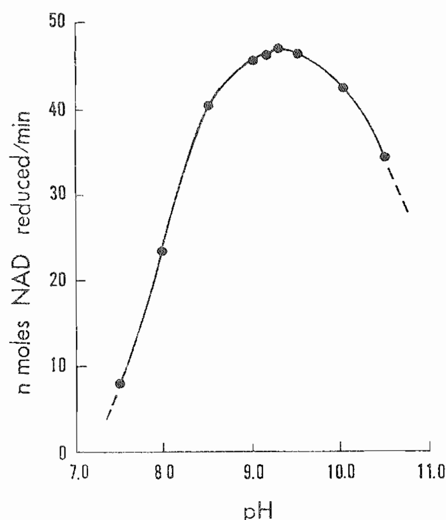


Fig. 1. The effect of pH on the activity of the soluble aldehyde dehydrogenase from *Rhodospirillum rubrum*.
Reaction mixture: 60 mM glycylglycine, $Na^+$ salt; 0.5 mM $NAD^+$; 0.33 mM acetaldehyde; enzyme preparation 0.14 mg protein/ml. t = 25°C.
Enzyme acticity at pH 9.3 = 0.32 units / mg protein.
(Enzyme unit defined in foot note for table 1).
The use of Tris buffers at the same pH values yielded comparable results.

Gel electrophoresis on acrylamide gels (Fig. 2) were carried out after Davis (1964). The staining of the dehydrogenase(s) was carried out by a modification of the method described by Scandalios (1969).

*Adaptive Nature of the Enzyme*

In order to study the nature of the increase of aldehyde dehydrogenase activity, on transfer of the malate grown *R. rubrum* cells to a medium containing ethanol, either proflavin (35 mg/L) or chloramphenicol (100 mg/L) were added to the culture media (Aurich & Eitner, 1977) as detailed in Table 5.

**Table 3. Coenzyme specificity of the aldehyde dehydrogenase from *Rhodospirillum rubrum* with acetaldehyde as electron donor.**

| Coenzyme | Coenzyme conc. mM | Relative rates |
|---|---|---|
| $NAD^+$ | 0.4 | 100 |
| $NADP^+$ | 0.4 | 0 |

Reaction mixture: Enzyme, 0.5 mg protein per ml., acetaldehyde 0.33 mM in 60 mM Na-glycylglycine pH 9.3. t = 25°C.

## Results and Discussion

Aldehyde dehydrogenases which catalyze the dehydrogenation of acetaldehyde have been reported from a number of microganisms (Nakayama, 1960, 1961; Jakoby, 1963; Heydeman & Azoulay, 1963; von Tigerstrom & Razell, 1968 a, b; Krasil' nikova, 1972, 1976). The characteristics of the aldehyde dehydrogenase isolated from *R. rubrum* in many ways are similar to those reported for the aldehyde dehydrogenase isolated from *Pesudomonas aeruginosa* by von Tigerstrom and Razell (1968a). They are similar with respect to their solubilities, their relative activities with different aldehydes tested, and their activation by sulfhydryl reagents. In the case of the *R. rubrum* preparations, with acetaldehyde as electorn donor, DL-dithiothreitol (.16 mM) increased their activities three to four fold (Table 1). Rates of dehydrogenation of several aldehydes by the partially purified enzyme perparations from *R. rubrum* are given in Table 2. Highest

**Table 4. Levels of aldehyde dehydrogenase in extracts of *Rhodospirillum rubrum* cultured photohetrotrophically on malate or ethanol.**

| High speed supernatant (fraction 2a, table 1) from cells cultured on | Enzyme activity (units/mg Protein) | |
|---|---|---|
| | | + 1.6 mM DL–dithiothreitol |
| Malate | 0.016 | 0.06 |
| Ethanol | 0.15 | 0.60 |

Reaction mixture: Enzyme, 0.45 mg protein per ml., acetaldehyde 0.33 mM, 0.5 mM $NAD^+$ in 60 mM Na-glycylglycine, pH 9.3.t = 25°C.

activities were observed with acetaldehyde as electron donor, comparable to the relative activities with different substrates of the *Peseudomonas* enzyme of von Tigerstrom & Razell (1968a). With the *R. rubrum* preparations no activity could be detected with formaldehyde as electron donor in the presence of NAD+ (Table 2). Also, with the electron donors examined, we have found no evidence thus far that NADP+ can act as an electron acceptor in aldehyde dehydrogenations catalyzed by the *R. rubrum* enzyme (Table 3). The *R. rubrum* enzyme may be classified as an aldehyde dehydrogensase: EC 1.2.1.3 (International Union of Biochemistry, 1978/79, p. 62; or as an aldehyde: NAD+ oxidoreductase; ibid. p. 530). The *R. rubrum* enzyme differs from preparations obtained by Krasil'nikova (1972) from *Chloropseudomonas* in that the enzyme obtained from the latter organism, wnen acting on short chain aldehydes, is specific for NADP+ as coenzyme.

**Table 5. Effects of addition of selected inhibitors of messenger RNA formation on adaptive increase of aldehyde dehydrogenase in photohetrophically grown cultures of *Rhodospirillum rubrum***

| Carbon source and other additions in the medium | Additions at the time of harvest | Relative activity in cell extracts (fraction 2, Table 1) |
|---|---|---|
| Malate | – | 12 |
| Ethanol | – | 100 |
| Ethanol + Proflavin (35 mg/L of culture medium) | – | 10 |
| Ethanol | Proflavin (35 mg/L of culture medium) | 92 |
| Ethanol + chloramphenicol (100 mg/L of culture medium | – | 12 |
| Ethanol | Chloramphenicol (100 mg/L of culture medium). | 100 |

* *R. rubrum* cells cultured for 30 hours on a medium containing malate were harvested under axenic conditions and about 2 gm transferred to each of the experimental flasks having 750 ml of culture medium per flask. The medium contained 0.25% malate or 0.25% ethanol plus additions as indicated in the Table. After 24 hours the cells were harvested and cell extracts assayed for aldehyde dehydrogenase. Reaction mixture as under Table 4.

The pH optimum for the *R. rubrum* aldehyde dehydrogenase preparations with acetaldehyde as electron donor (initial rates) was found to be between pH 9.0 to 9.5 (Fig 1). This may be comparable to the pH optima reported Krasil'nikova (1976) for enzyme preparations from *Rhodospeudomonas vridis* (pH 9.0) and for preparations from two strains of *Rhodopseudomonas palustris* (pH 9.7) with acetaldehyde as electron donor and NAD+ as coenzyme.

The apparent Michaelis constant of acetaldehyde for the *R. rubrum* enzyme was extrapolated to an approximate $K_m \div 10^{-5}$ M compared to an apparent $k_m = 10^{-4}$ M for acetaldehyde for the $NADP^+$ dependent aldehyde dehydrogenase from *Acetobacter* sp. (Nakayama, 1960)

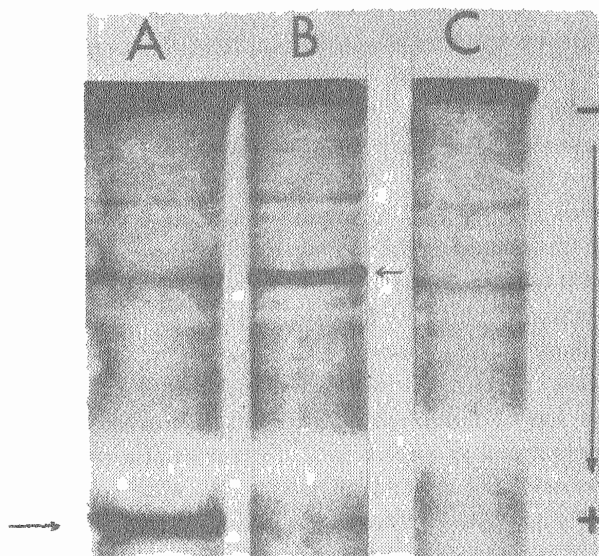


Fig. 2. Polyacrylamide gel electrophoresis of bacterial extract fractionated by high speed centrifugation.
20 µg protein per tube in 60 mM Tris, pH 9.3.
(aldehyde dehydrogenase activity: 0.56 units per mg protein
Anionic gel system with a running pH of 9.3 (1.25 - 2.5 ma per tube at 24 to 37 volts/cm) $t=3^{o}C$.
Leading buffer front marked by bromphenol blue
Staining mixture 0.5 mM $NAD^+$
0.1 mM 5-methylphenazinium methyl sulfate
0.3 mM Nitroblue tertrazolium
in 60 mM Tris, pH 9.0

A Staining mixture plus 10 mM ethanol
B. Staining mixture plus 0.2 mM acetaldehyde.
C Staining mixture only

Glyceraldehyde-3-phosphate served as a poor substrate for the aldehyde dehydrogenase examined here (Table 2); reaction rates with this substrate were not increased by the addition of $10^{-3}$ *M* orthoposphate to the reaction mixtures. Glyceraldehyde-3-phosphate dehydrogenase activity in extracts of *R. rubrum* were reported by Anderson & Fuller (1967). The relationship of this enzyme activity to the one described here will require further elucidation. Also, for the dehydrogenation of acetaldehyde, the *R. ruburm* preparation showed no requirments for orthophosphate or potassium ions compared to the enzyme prepared from *Pseudomonas* (von Tigerstrom & Razell, 1968a).

An analysis of the deproteinized reaction mixtures by filter paper chromatography provided evidence that the products of the enzyme catalyzed reaction with acetaldehyde and $NAD^+$ as substrates resulted in the formation of acetate and NADH.

Gel electrophoresis on acrylamide gels (Davis, 1964) in the presence of acetaldehyde, upon staining (Scandalios, 1969), revealed one major band due to the presence of an aldehyde dehydrogenase (Fig. 2B); in the presence of ethanol, the activity of an induced alcohol dehydrogenase (Chaudhary, 1971) was observed (Fig. 2A).

Bognar & Meighen (1978) have reported the induction of an aliphatic $NAD^+$ dependent aldehyde dehydrogenase in *Beneckea harveyi*. This enzyme, however, markedly differs from the *R. rubrum* enzyme, in that the *Beneckea* dehydrogenase shows no activity with acetaldehyde as electron donor. Von Tigerstrom & Razell (1968a) have referred to the aldehyde dehydrogenase of *Pseudomonas aeruginosa* as an "induced enzyme". The specific activities which they measured in the supernatant of cell extracts subjected to 31, 700 x g for 45 minutes was 65 times higher in extracts from cells cultured on ethanol when compared to those cultured on acetate. In *R. rubrum* we observed a five to ten fold increase in specific activities of the aldehyde dehydrogenase when cells were cultured on ethanol compared to cells cultured on malate plus glutamate (Table 4). The increase in the specific aldehyde dehydrogenase activity from cells cultured on ethanol could be inhibited almost completely by the addition either of 35 mg/l proflavin or of 100 mg/l chloramphenicol (Aurich & Eitner, 1977) to the media containing ethanol (Table 5) which strongly suggests that we are dealing with an inducible enzyme.

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