An Induced Aliphatic Aldehyde Dehydrogenase from the Bioluminescent Bacterium, *Beneckea harveyi*

**PURIFICATION AND PROPERTIES***

(Received for publication, June 20, 1977)

**ANDREW L. BOGNAR AND EDWARD A. MEIGHENS†**

*From the Department of Biochemistry, McGill University, Montreal, Quebec H3G1Y6 Canada*

A NAD⁺-dependent aldehyde dehydrogenase, the activity of which induces at the same time as luciferase, has been purified from the bioluminescent bacterium *Beneckea harveyi*, and its chemical and physical properties have been investigated. The purification is accomplished in three steps resulting in an enzyme preparation that gives a single protein band on three different gel electrophoresis systems.

The molecular weight of the purified enzyme was estimated to be 120,000 by gel filtration. Sodium dodecyl sulfate-gel electrophoresis gave a molecular weight of 58,000 indicating that aldehyde dehydrogenase has a dimeric structure with subunits of similar molecular weight. The purified enzyme has a high specificity for long chain aliphatic aldehydes; the Michaelis constants for aldehydes decrease with increasing chain length as also observed for bacterial aldehyde dehydrogenases involved in the metabolism of hydrocarbons. The aldehyde specificity of the aldehyde dehydrogenase is similar to that of luciferase indicating that the functional role of the enzyme may be linked with the bioluminescent system.

The metabolism of long chain aliphatic aldehydes in bioluminescent bacteria is of particular interest since these compounds are substrates for the bioluminescent reaction catalyzed by luciferase (1-5). Recently, we have reported the presence of an aldehyde dehydrogenase in crude extracts of the bioluminescent bacterium, *Beneckea harveyi*, with a high specificity for long chain aliphatic aldehydes (6). Although aldehyde dehydrogenases and their role in metabolism have been studied in a wide variety of organisms, most of these enzymes either have a broad specificity or do not function with the long chain aliphatic aldehydes (7). Only in a few microorganisms have aldehyde dehydrogenases with such specificity been observed (8-10). In these cases, the enzyme is induced directly by growth in the presence of the aldehyde, its hydrocarbon or alcohol analogue, or is found constitutively in an organism which grows on these compounds.

† To whom correspondence should be addressed.

In contrast, the synthesis of aldehyde dehydrogenase in *B. harveyi* occurs without the addition of exogeneous long chain aliphatic compounds to the medium. The activity of aldehyde dehydrogenase in this bioluminescent bacterium may be under the same control as luciferase since low levels of activity are observed at early stages of bacterial growth followed by an increase in enzyme activity at the same time as the induction of luciferase (6). This result, as well as the similar specificity of aldehyde dehydrogenase and luciferase for long chain aliphatic aldehydes, suggests that the aldehyde dehydrogenase may be directly involved in the bioluminescent system of *B. harveyi*. In the present study, the purification of this enzyme and the characterization of some of its catalytic and structural properties are reported.

**EXPERIMENTAL PROCEDURES**

**Materials** — The commercial sources of reagents and chromatography media were the following: aldehydes, Aldrich; pyridine nucleotides and dithiothreitol, Sigma; guanidine hydrochloride, Mann ultrapure; mercaptopropanol, cyanogen bromide, diethanolcarboxl diimide, 6-aminohexanoic acid, acrylamide, and bisacrylamide, Eastman; DEAE-cellulose 23, Bio-Rad; Sepharose 4B, Pharmacia; Ultrogel ACA-44, LKB; Aquasieve II, Calbiochem. Protein molecular weight standards were obtained from Sigma and Worthington. Luciferase was purified according to the method of Gunsalus-Miguel et al. (11). Phosphate buffers were prepared by mixing appropriate amounts of 1.0 M NaH₂PO₄ and 1.0 M K₂HPO₄.

**Aldehyde Dehydrogenase Assay** — Aldehyde dehydrogenase activity was measured by the increase in absorbance with time at 340 nm after addition of an aliquot of the enzyme to 1.0 ml of 0.05 M phosphate buffer containing 0.002% dodecanal (1.0 × 10⁻⁴ M) and 1.5 × 10⁻⁴ M NAD⁺. Aldehyde stock solutions (0.2%) were prepared in either 2-propanol or dimethylformamide. The units of activity in micromoles/min were calculated using a molar extinction coefficient of 6220 (0.1%, 1 cm) for NADH at 340 nm (12).

**Protein Determinations** — The protein concentration was generally measured by the method of Lowry (18) using bovine serum albumin as a standard. Alternatively, the protein was determined by the biuret method (14). Similar results were obtained (±10%) by both methods.

**Dry Weight Measurements** — Aldehyde dehydrogenase was dialyzed exhaustively against distilled deionized water, lyophilized, and then dried under vacuum over phosphorus pentoxide. The amount of protein in the sample (1.2 mg) was determined by the biuret method and compared to the dry weight (1.25 mg).

**Amino Acid Analyses** — Weighed samples of aldehyde dehydrogenase, prepared as above for dry weight measurements, were hydrolyzed in vacuo in 6 N HCl for 24, 48, and 72 h at 110°. In some cases, an internal standard of norleucine was added initially to the HCl. After hydrolysis, the sample was dried on a flash evaporator, dissolved in citrate buffer, pH 2.2, and the amino acid composition...
Purification and Properties of Aldehyde Dehydrogenase

Enzyme Purification—The steps for the purification of aldehyde dehydrogenase from the bioluminescent bacterium, Beneckea harveyi, are summarized in Table I. A detailed description of each step in the procedure is given in the miniprint supplement immediately following this paper.1

The first step of purification involves DEAE-cellulose chromatography. The aldehyde dehydrogenase activity not only from the majority of the protein, but also from luciferase and NAD(P)H:flavin oxidoreductase activity. In the next step, the aldehyde dehydrogenase activity is precipitated with a relatively low concentration of ammonium sulfate (55% saturation), redissolved in a small volume of buffer and adsorbed directly onto NAD⁺-Sepharose. NAD⁺-Sepharose chromatography separates aldehyde dehydrogenase from the major part of the protein which does not bind to NAD⁺-Sepharose. The gel which was used in these experiments had a very low amount of bound NAD⁺ (not measurable spectrophotometrically, i.e. <1 μmol/g, dry weight, of gel) resulting in some leakage of aldehyde dehydrogenase activity prior to elution with NAD⁺. The use of a gel with a higher substitution of NAD⁺ required NaCl, as well as NAD⁺, for elution of the enzyme activity, giving a preparation of aldehyde dehydrogenase with a lower specific activity. Although the preparation eluted with NAD⁺ alone contained a few minor contaminants on gel electrophoresis, these contaminants, as well as the NAD⁺, are resolved from the aldehyde dehydrogenase activity in the final step of gel filtration. Due to the low capacity of the NAD⁺-Sepharose column, repeated attempts to adsorb the aldehyde dehydrogenase activity directly from the crude extract onto NAD⁺-Sepharose were unsuccessful.

Additional evidence for the homogeneity of the purified enzyme was obtained by polyacrylamide gel electrophoresis in Tris/glycine, pH 8.9, and phosphate, pH 7.0, under non-denaturing conditions. A single protein band was obtained in both cases (see miniprint supplement, Figs. 4S and 5S). In addition, it was shown that aldehyde dehydrogenase activity was directly associated with the single protein band in the phosphate gel (Fig. 5S).

Physical and Chemical Properties—The molecular weight of aldehyde dehydrogenase was estimated to be 120,000 by gel filtration on a column of Ultrigel Aca-44 calibrated with standards of known molecular weight assuming that the enzyme has the same shape and partial specific volume as the standard proteins. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the enzyme along with standards of known polypeptide molecular weight gave an estimate of 59,000 for the molecular weight of the polypeptide chains of aldehyde dehydrogenase. These results indicate that aldehyde dehydrogenase is a dimer with subunits of identical molecular weight.

The absorption spectrum of aldehyde dehydrogenase had

![Absorption spectrum](http://www.jbc.org/)

1 Some of the data (including Figs. 1S through 7S and additional Refs. 1 to 5) are presented in a miniprint at the end of this paper. Full size photocopies are available from the Journal of Biological Chemistry, 9505 Rockville Pike, Bethesda, Md. 20014. Request Document No. 77M-931, cite author(s), and include a check or money order for $1.00 per set of photocopies.

---

**Table I**

<table>
<thead>
<tr>
<th>Purification of aldehyde dehydrogenase</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purification step</td>
<td>Volume</td>
</tr>
<tr>
<td>ml</td>
<td>mg</td>
</tr>
<tr>
<td>Lysate supernatant</td>
<td>550</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>400</td>
</tr>
<tr>
<td>Chromatography</td>
<td>NH₄SO₄, fractionation (0 to 55% saturation)</td>
</tr>
<tr>
<td>NAD⁺-Sepharose</td>
<td>60</td>
</tr>
<tr>
<td>Chromatography</td>
<td>Concentration by aqueadice</td>
</tr>
<tr>
<td>Gel filtration</td>
<td>11.4</td>
</tr>
</tbody>
</table>

a Volume of sample after the purification step.

b Protein concentrations were determined by the method of Lowry (13).

c Activity was determined as described under “Experimental Procedures” using 0.002% dodecanal in the assay.

---

1 Some of the data (including Figs. 1S through 7S and additional Refs. 1 to 5) are presented in a miniprint at the end of this paper. Full size photocopies are available from the Journal of Biological Chemistry, 9505 Rockville Pike, Bethesda, Md. 20014. Request Document No. 77M-931, cite author(s), and include a check or money order for $1.00 per set of photocopies.
Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified aldehyde dehydrogenase. The enzyme was applied to a 10% polyacrylamide gel in 0.1% sodium dodecyl sulfate, 0.01 M phosphate, pH 7.0, electrophoresed at 8 mA/gel at room temperature and stained for protein as described under "Experimental Procedures." The bottom of the gel indicates position of the tracking dye, an A_{280}/A_{260} ratio of 1.7 in phosphate, pH 7.0, with no absorption in the visible region of the spectrum. A specific absorption coefficient of 0.75 (0.1%, 1 cm) at 280 nm was calculated for aldehyde dehydrogenase using the biuret method to measure the protein concentration. The amount of protein determined by the biuret or Lowry methods (13) using bovine serum albumin as a standard was in close agreement with the dry weight of aldehyde dehydrogenase (98 and 106%, respectively), indicating that nonprotein components are not present in significant amounts.

**Amino Acid Composition**—The amino acid composition of aldehyde dehydrogenase is given in Table II. The total weight of amino acid residues agrees within 10% of the dry weight of the analyzed sample. An apparent partial specific volume of 0.732 ml/g was calculated from the amino acid composition (21). No similarity in amino acid composition was found between aldehyde dehydrogenase and luciferase (19), both of which catalyze reactions involving the oxidation of aliphatic aldehydes in this bioluminescent bacterium.

**Enzyme Specificity**—The specificity of aldehyde dehydrogenase for aldehydes and pyridine nucleotides is given in Table III. The purified enzyme catalyzes the reduction of NAD{\textsuperscript+} in the presence of aliphatic aldehydes with more than three carbons, whereas no activity is detected with propionaldehyde, acetaldehyde or a variety of aromatic aldehydes. The enzyme activity with aldehydes from butanal to tetradecanal is relatively constant with somewhat higher activities obtained with hexanal and heptanal. The specificity of the enzyme for long chain aliphatic aldehydes is reflected primarily in the decrease in $K_{m}$ as the chain length of the aldehydes is increased. The $K_{m}$ for aldehydes decreases about 1.5-fold for the addition of each ethylene group up to octanal and then more sharply (3- to 5-fold for each methylene group) at longer chain lengths.

The specificity for aliphatic aldehydes is somewhat different than previously reported for this enzyme in crude extracts. We have now reinvestigated the aldehyde dehydrogenase activity in crude extracts and found the lower activities reported earlier (6) were due to the low NAD{\textsuperscript+} concentrations ($7 \times 10^{-5} M$) as well as incomplete solubilization of aldehyde stock solutions at higher concentrations in 0.1% Triton X-100. The difficulty in using detergents for preparation of aldehyde stock solutions has also been shown by Lebeault et al. (8). These problems have been avoided in the present investigation by using higher NAD{\textsuperscript+} concentrations (1.5 mM) and by preparation of the stock aldehyde solutions in isopropyl alcohol or dimethylformamide.

The specificity of aldehyde dehydrogenase for pyridine nucleotides is also given in Table III. The enzyme has maximum activity with NAD{\textsuperscript+}, with only 3 to 5% of this activity with AcPyAD and NADP{\textsuperscript+}, and no activity with NMN. The lower activities for AcPyAD and NADP{\textsuperscript+} are not due to differences in $K_{m}$ since AcPyAD and NADP{\textsuperscript+} have comparable $K_{m}$ values and NADP{\textsuperscript+} apparently has a much lower $K_{m}$ than NAD{\textsuperscript+}.

**DISCUSSION**

A number of aldehyde dehydrogenases from microorganisms grown on long chain hydrocarbons show a similar specificity for long chain aliphatic aldehydes to the Beneckea...
The enzyme. Two aldehyde dehydrogenases, inducible by growth on hydrocarbons, are found in *Pseudomonas aeruginosa*, one of which is soluble and the other membrane bound (10). Both enzymes have a similar specificity to the *B. harveyi* enzyme for aliphatic aldehydes and the soluble enzyme shows a decrease in $K_m$ for aldehydes with increasing chain length. The $K_m$ values for aldehydes, however, are 10 to 100 times higher than those reported in our study and decrease in a relatively uniform fashion, only 1.2- to 1.5-fold for the addition of each methylene group between butanal and tetradecanal. A similar specificity and $K_m$ dependence for aldehydes has also been observed for constitutive membrane-bound aldehyde dehydrogenase found in the marine bacterium, *Alcaligenes sp.* (9). This latter strain was believed to have adapted to growth on hydrocarbons released into the ocean by a nearby oil refinery. In the yeast, *Candida tropicalis*, the specificity of the aldehyde dehydrogenase for long chain aliphatic aldehydes is based on an increase in the maximum velocity with aldehyde chain length rather than a change in $K_m$ (8). In fact, the $K_m$ for aldehydes actually increases with increasing chain length for this aldehyde dehydrogenase.

The organism listed above can grow on hydrocarbons and thus contain a defined system for hydrocarbon metabolism, consisting of a hydroxylase and long chain alcohol and aldehyde dehydrogenases (9). In contrast, *B. harveyi* cannot grow on hydrocarbons, and other enzyme constituents in this system, such as a long chain alcohol dehydrogenase, have not been detected in this organism. The aldehyde dehydrogenase activity in *B. harveyi* is induced without the addition of exogeneous hydrocarbon derivatives by a yet unknown mechanism, increasing at the same stage of cell growth as the luciferase activity (6). Double label experiments (22) have confirmed that the increase in activity of luciferase is due to protein synthesis (22) and have shown that other polypeptides are also specifically synthesized during this period, including a polypeptide that is present in a partially purified preparation of aldehyde dehydrogenase.

The enzymic basis for the in vivo synthesis of the aldehyde required for the bioluminescent reaction has not yet been demonstrated. Cline and Hastings (24) have shown that at least two activities are involved in the production of the aldehyde or functionally equivalent factor (aldehyde factor) of the bioluminescent reaction in *B. harveyi*. We have investigated the possibility that aldehyde dehydrogenase could be used to produce aldehyde by attempting to reverse the reaction catalyzed by the enzyme. No decrease in the absorbance of NADH could be obtained in the presence of ATP (+ Mg$^{2+}$), aldehyde dehydrogenase and long chain aliphatic acids or their acyl coenzyme A derivatives.

A definitive role can not be assigned to aldehyde dehydrogenase in this organism at the present stage of study. The limited aldehyde specificity of this enzyme, having no activity with aromatic and short chain aldehydes, would make it of little use as a general detoxifier to remove aldehydes from the cell (7). It appears that the function of this enzyme may be to specifically remove the aldehyde (or aldehyde factor) produced as substrate for the bioluminescent reaction. It will be of interest therefore to determine what specific role aldehyde dehydrogenase plays in the bioluminescent system.

**REFERENCES**


Additional references can be found on p. 450.
An induced aliphatic aldehyde dehydrogenase from the bioluminescent bacterium, Beneckea harveyi. Purification and properties.

A L Bognar and E A Meighen


Access the most updated version of this article at http://www.jbc.org/content/253/2/446

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/253/2/446.full.html#ref-list-1