Involvement of Lactaldehyde Dehydrogenase in Several Metabolic Pathways of *Escherichia coli* K12*

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Lactaldehyde dehydrogenase (E.C. 1.2.1.22) of *Escherichia coli* has been purified to homogeneity. It has four apparently equal subunits (molecular weight 55,000 each) and four NAD binding sites per molecule of native enzyme. The enzyme is inducible, only under aerobic conditions, by at least three different types of molecules, the sugars fucose and rhamnose, the diol ethylene glycol and the amino acid glutamate. The enzyme catalyzes the irreversible oxidation of several aldehydes with a $K_m$ in the micromolar range for a-hydroxyaldehydes (lactaldehyde, glyceraldehyde, or glycolaldehyde) and a higher $K_m$ in the millimolar range, for the a-ketoaldehyde methylglyoxal. It displays substrate inhibition with all these substrates. NAD is the preferential cofactor. The functional and structural features of the enzyme indicate that it is an isozyme of other *E. coli* aldehyde dehydrogenases such as glyceraldehyde phosphate dehydrogenase, glycolaldehyde dehydrogenase, or acetaldehyde dehydrogenase. The enzyme, previously described as specific for lactaldehyde, is thus identified as a dehydrogenase with a fairly general role in aldehyde oxidation, and it is probably involved in several metabolic pathways.

In bacteria, aldehyde oxidation is catalyzed by enzymes that may be classified in two general types according to their high or low substrate specificity (1). Enzymes such as succinate semialdehyde dehydrogenase (2) and malonate semialdehyde dehydrogenase (3) of *Pseudomonas* sp. belong to the high specificity type. Other aldehyde dehydrogenases from several species, including *Pseudomonas* sp. (4) or Acetobacter sp. (5), are rather nonspecific and have been assigned a more general role in the dissimilation of the intracellular or extracellular aldehydes in detoxication processes.

As stated by Kane (6), these enzymes with ambiguous catalytic functions cannot be considered multifunctional proteins according to the definition of Kirshner and Bisswager (7). Nevertheless, their involvement in multiple pathways is important in cellular metabolism and perhaps also in the evolution of diverse pathways.

In *Escherichia coli* K12, the lactaldehyde dehydrogenase was initially described as part of the L-1,2-propanediol metabolic pathway (8) in which it oxidizes lactaldehyde, formed after oxidation of the diol, by the enzyme propanediol oxidoreductase, to lactate. Later, when L-1,2-propanediol was identified as a product of fucose (9) and rhamnose (10) fermentation, it became apparent that the lactaldehyde dehydrogenase was involved in the oxidative pathway of these methylpentoses. Fucose and rhamnose are metabolized by two parallel pathways including an isomerization by specific isomerases (11, 12) and a phosphorylation by specific kinases (13, 14). Both pathways converge after the cleavage of fuculose 1-phosphate and rhamnulose 1-phosphate by the corresponding aldoalases, yielding, in each case, L-lactaldehyde and dihydroxyacetone phosphate (15, 15). Under anaerobic conditions, the lactaldehyde is reduced to 2,2-propanediol in a reaction that is reversed in the mutant cells able to use propanediol (9).

Under aerobic conditions, the lactaldehyde formed by the fermentation mechanism (9).

In another context, Caballero et al. (17) showed that the same lactaldehyde dehydrogenase was used to oxidize glycolaldehyde in *E. coli* mutants able to use ethylene glycol via glycolaldehyde.

The enzyme has been partially characterized by Sridhara and Wu (8) as a NAD-dependent lactaldehyde dehydrogenase. In this report, we further characterize the homogeneous enzyme and compare it to other well-studied aldehyde dehydrogenases of *E. coli*. In view of the results presented here we propose to change the consideration of this enzyme as a specific lactaldehyde dehydrogenase to an aldehyde dehydrogenase of general function participating in several pathways.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—D,L-1,2-Propanediol, formaldehyde, acetaldehyde, and propionaldehyde were from Merck (Darmstadt, West Germany) and were purified by distillation. L-Lactaldehyde was synthesized by reacting D-threonine with ninhydrin according to the method of Zagaik et al. (18), and the product obtained was further purified by chromatography on a Whatman No. 3 (Chroma) filter paper with 1-propanol/ammonia (64, v/v) as solvent. The lactaldehyde concentration in the solution resulting from the elution of the corresponding spot was measured by the semicarbazone formation method (19). Glutamic y-semialdehyde was prepared by hydrolysis of D,L-pyrroline-5-carboxylic-acid-2,4-dinitrophenylhydrazone HCl as described by Mezel and Knox (20). Ethylene glycol was from Carlo Erba (Milano, Italy). Fucose, rhamnose, L-glyceraldehyde, glycolaldehyde, methylglyoxal, threonine, ninhydrin, and A1-pyroline-5-carboxylate-2,4-dinitrophenylhydrazone HCl and y-aminobutyric acid were from Sigma. Casamino acids were obtained from Difco (Detroit, MI). Hydroxylation of and gel electrophoresis reagents were purchased from Bio-Rad, except for acrylamide which was from Fluka (Buchs, Switzerland). Amphotyol are from Pharmacia (Uppsala, Sweden). The other chemicals were of the purest grade available.

**Bacterial Strains**—The parental strain used was an *E. coli* K12, strain E-15 (21) and is here referred to as strain 1. Strain 3 was a propanediol oxidoreductase constitutive mutant capable of growth on L-1,2-propanediol and derived from strain 1 after ethyl methanesulfonate mutagenesis (22). Strain 40, an L-1,2-propanediol-negative

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E. coli Aldehyde Dehydrogenase Serving Distinct Pathways

mutant lacking lactaldehyde dehydrogenase, was derived from strain 3 after N-methyl-N'-nitro-N-nitrosoguanidine mutagenesis (8). These three strains were kindly provided by E. C. C. Lin, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA. Strain JA-102, a spontaneous derivative of strain 3 obtained in our laboratory and previously referred to as strain EG3, was also used. Strain IA-720 (13) is an inducible superproducer of lactaldehyde dehydrogenase.

Growth Media and Preparation of Cell Extracts—Cells were grown and harvested as indicated previously (10). Extracts were prepared as described (24) except for the buffer, which was 10 mM sodium phosphate, containing 10 mM mercaptoethanol and 1 mM EDTA (buffer A). Since glutamate is not used as a sole carbon source by E. coli (25, 26) induction by glutamate was performed by growing the cells on glycerol to an absorbance of 3.0 measured at 420 nm; at that point the glutamate was added to a 12 mM concentration, the cells were incubated for 4 h at 37°C, and collected.

Lactaldehyde Dehydrogenase Purification—the enzyme was purified to homogeneity by a modification of the procedure described by Caballero et al. (17) consisting of the substitution of the last step (Ultrogel AcA-34 gel filtration) by hydroxylapatite column chromatography. To that end, the pooled fractions containing the enzyme after the affinity chromatography were concentrated and dialyzed against buffer A, and the preparation was loaded onto a column of hydroxyapatite (2 × 2 cm) that had been equilibrated and washed with 50 ml of the same buffer A. A linear gradient of 100 ml of buffer A ranging from 10 to 100 mM sodium phosphate at pH 7.3 was then applied. The dehydrogenase was eluted at a phosphate concentration of 30-40 mM. For routine procedures, the enzyme was obtained after a single pulse of 35 mM phosphate concentration.

Immunological Techniques—Antisera against lactaldehyde dehydrogenase were raised in New Zealand white rabbits, with the strain 3 purified enzyme as antigen. Purified lactaldehyde dehydrogenase (250 pg) in 300 μl of 10 mM sodium phosphate (pH 7.3) containing 150 mM NaCl was emulsified in an equal volume of Freund complete adjuvant, and the mixture was injected subcutaneously 2 and 4 weeks later. The rabbits were bled 1 week after the last booster injection. The globulin fraction was partially purified by 35% ammonium sulfate precipitation. Immunoelectrophoresis was performed as described by Laurell (27).

Enzyme Assays—The aldehyde dehydrogenase activity was assayed spectrophotometrically at 25°C by following the absorbance at 340 nm (NADH formation) in a mixture (1 ml) that consisted of 1 mM glyceraldehyde, 2.5 mM NAD, and 100 mM sodium glycine buffer at pH 9.5. For the specificity studies, the concentrations of the different substrates were changed as indicated. The kinetic determinations were performed with six different concentrations of substrates bracketing those indicated in Table V for each aldehyde. The initial velocities obtained during the first 30 s of reaction were determined. The kinetic constants were obtained by linear regression analysis of the data plotted according to Lineweaver and Burk.

The concentration of proteins in cell extracts and purified preparations was determined by the method of Lowry et al. (28), modified by Benzandoum and Weinstein (29) to avoid EDTA interference.

Cell Electrophoresis—Acrylamide gel electrophoresis in dissociating conditions was performed according to Laemmli (30), and the gels were stained with Coomassie Brilliant Blue (R-250) or by silver stain (31, 32). Where indicated, the gels were stained for lactaldehyde dehydrogenase activity by incubation in a reaction mixture that contained (per liter) 100 mg of glycine (brought to pH 9.5 by 0.5N HCl) and 0.3 mmol of l-lactate, 5 mmol of NAD, 300 μg of mouse blue tetrazolium, and 0.1% of phenazine methosulfate.

Isoelectric Focusing—Isoelectric focusing was carried out on 5% acrylamide plates (120 × 10 × 1.5 mm) containing 7.5% Pharmalytes at pH 3-10 (7) for 6 h at a constant power of 8 watts. Solutions of 0.1 M HSO4 and 0.1 M NaOH were employed to soak the electrodes. The system was liquid refrigerated with a Multiphor apparatus (LKB).

Before staining, the gel was soaked for 1 h with gentle shaking in a mixture containing 10% (v/v) acetic acid and 10% (v/v) acetate buffer adjusted to pH 8.5 with concentrated solutions of NaOH. A third stage mutant (strain JA102), able to grow on ethylene glycol, showed an increase over the semi-constitutive level of enzyme in the extracts, the height of the rocket deviated from linearity. In addition, lactaldehyde dehydrogenase was undetectable by western blotting under anaerobic conditions. In the absence of inducing nutrients, e.g. growth on glycerol or succinate, the cells displayed semi-constitutive levels of the enzyme which, in the presence of fucose, rhamnose, or glutamate, increased 3- to 4-fold. In contrast to lactaldehyde dehydrogenase, both products of glutamate metabolism, were unable to act as inducers. In mutant cells (strain 3) able to grow on propylene glycol, the enzyme was also induced by this product. A third stage mutant (strain JA102), able to grow on ethylene glycol, showed an increase over the semi-constitutive basal level of enzyme, and a 3-fold induction of this level when grown on ethylene glycol.

The relationship between specific activity and rocket height was a nearly constant value of 11 to 12, except for the glucose culture in which, owing to the low concentration of enzyme in the extracts, the height of the rocket deviated from linearity. In addition, the negative mutant strain 40, lacking lactaldehyde dehydrogenase, showed no detectable cross-reacting material. None of the conditions tested induced the synthesis of the enzyme in this strain. It should be pointed out that, like the wild type strain, mutant strain 40 although using glutamate as a sole nitrogen source, cannot use it as a sole carbon source.

Preparations of lactaldehyde dehydrogenase, purified by the procedure described previously by Caballero et al. (17), contained a contaminant protein that appeared as a faint band of higher electrophoretic mobility when stained by Coomassie Brilliant Blue (Fig. 1) but not when stained by enzyme activity (not shown). Addition of several protease inhibitors to the purification procedures did not prevent the presence of the contaminant band, suggesting that its origin was not proteolytic cleavage of the enzyme. Replacement of the Ultrogel AcA-34 column chromatography, the last step in the original purification procedure, by a hydroxyapatite column chromatography allowed us to eliminate the contaminant from the enzyme. This modified procedure resulted in an overall yield of 17%, 134-fold purification, and a preparation with a specific activity of 14.7 units/mg (Table II).

The criteria for homogeneity of the enzyme preparation was based on isoelectric focusing (not shown) and polyacrylamide gel electrophoresis in the presence of SDS (Fig. 1). Only one band was observed in the gels when applying either

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1 The abbreviation used is: SDS, sodium dodecyl sulfate.
Enzyme activities and immunological quantification of lactaldehyde dehydrogenase of E. coli grown aerobically on different carbon sources

Specific activities of lactaldehyde dehydrogenase were determined under standard conditions in crude extracts of wild type strain and the indicated mutant strains. Immunoelectrophoresis was performed in a gel containing 0.4% (v/v) of the specific globulins prepared as indicated under "Experimental Procedures." Except where indicated, 50 µg of extract was applied to the plate and the height of the Laurell rockets in mm measured after staining with Coomassie Brilliant Blue.

| Carbon source   | Strain 1 Activity | Strain 3 Activity | Strain JA-102 Activity | Strain 40 Activity |
|-----------------|-------------------|-------------------|------------------------|-------------------|
|                 | Rocket mm         | Activity munits/mg| Rocket mm             | Activity munits/mg|
| Glucose         | 40                | 40                | 200                    | 790               |
| Glycerol        | 80                | 80                | 220                    | NR*               |
| CAA*            | 220               | 175               | 800                    | 280               |
| Glycerol + glutamate | 175           | 200                | 26'                    | 27 NR*            |
| Fucose          | 360               | 28                | 800                    | 790               |
| Rhamnose        | 340               | 27                | 280                    | 25'               |
| Propanediol     |                   |                   |                        |                   |
| Ethylene glycerol |                  |                   |                        |                   |

* Not relevant.
* Casein hydrolysate.
* 20 µg of extract applied.

Up to 25 µg of protein (Coomassie Brilliant Blue stain) or 1 µg of protein (silver stain).

Ultrigel Aca-34 column chromatography allowed a molecular weight estimate of 220,000 (Fig. 2). The mobility of the enzyme on SDS-polyacrylamide gel electrophoresis yielded a subunit molecular weight of 55,000. Therefore, the enzyme appears to be a tetramer of equivalent weight subunits.

In a pH gradient ranging from 3.0 to 6.0, the enzyme was focused as a single band at pH 4.6 when stained either for activity or with Coomassie Brilliant Blue.

The ultraviolet absorption spectrum of lactaldehyde dehydrogenase in 10 mM sodium phosphate buffer (pH 7.0) showed a λmax at 278 nm, and a 280/260 nm ratio of 1.54 which suggested a very low NAD content. However, a small amount of coenzyme was probably present because, when the enzyme was treated with charcoal, the 280/260 nm ratio rose to 1.82, the λmax remaining at 278 nm. The enzyme was then complexed with NAD by incubating at 23 °C for 10 min with increasing concentrations of this cofactor. Titration of the enzyme with NAD was followed at 360 nm, a characteristic absorption band of the NAD-aldehyde dehydrogenase complexes (33). There was a linear increase in absorbance with the addition of up to approximately 4 mol of NAD/mol of protein; further addition of coenzyme did not increase the absorbance (Fig. 3). At saturation, the λmax shifted to 272 nm and the 280/260 nm ratio fell to 1.15.

The amino acid composition of the enzyme is shown in Table III. The results are the average of 24, 48, and 72 h
ments were performed for each substrate. Michaelis constant for the coenzyme NAD was 0.28 mM when the substrate was lactaldehyde, or methylglyoxal. Substrate inhibition by NAD was not observed with any of the aldehydes.

Taking 100% for the activity on glycolaldehyde, the substrates: 1 mM for glycolaldehyde, 0.1 mM for L-lactaldehyde, 0.5 mM for L-glyceraldehyde, and 2 mM for methylglyoxal. Taking 100% for the activity on glycolaldehyde, the substrates at 0.5 mM concentration gave relative activities as indicated in Table IV.

Activity with NADP was very low (one-tenth of that obtained with NAD) and exhibited a $K_m$ of 6.25 mM. The Michaelis constant for the coenzyme NAD was 0.28 mM when the substrate was glycolaldehyde and a very close value, 0.12 mM, at 0.5 mM concentration gave relative activities as indicated in Table IV. Substrate inhibition was very strong with lactaldehyde, diminishing progressively with glycolaldehyde, glyceraldehyde, or methylglyoxal. Substrate inhibition by NAD was not observed with any of the aldehydes.

The enzyme was inhibited by p-hydroxy mercuribenzoate (50% inhibition at 1.5 mM) and by some cations added as its chlorides (50% inhibition at 10 mM for Mn$^{2+}$, 5 mM for Ca$^{2+}$, 0.40 mM for Cu$^{2+}$, and 0.25 mM for Zn$^{2+}$). No inhibition was found with MgCl$_2$ up to 25 mM concentration.

### Table III

Amino acid composition of lactaldehyde dehydrogenase of E. coli

| Amino acid          | Frequencies in native enzyme | Frequencies in subunit |
|---------------------|-----------------------------|------------------------|
| Aspartate           | 155.7                       | 35.6                   |
| Threonine$^a$       | 90.0                        | 22.5                   |
| Serine$^a$          | 62.0                        | 15.5                   |
| Glutamic acid       | 224.0                       | 56.0                   |
| Proline             | 75.8                        | 18.9                   |
| Glycine             | 172.0                       | 43.0                   |
| Alanine             | 206.0                       | 51.5                   |
| Valine$^a$          | 130.3                       | 32.6                   |
| Methionine          | 8.0                         | 2.0                    |
| Isoleucine$^a$      | 116.2                       | 29.0                   |
| Leucine             | 121.2                       | 30.3                   |
| Tyrosine$^a$        | 45.0                        | 10.5                   |
| Phenylalanine       | 59.6                        | 14.9                   |
| Histidine           | 28.8                        | 6.7                    |
| Lysine              | 84.8                        | 21.2                   |
| Arginine            | 96.8                        | 24.2                   |
| Tryptophan$^a$      | 9.6                         | 2.4                    |
| Cysteine$^a$        | 6.0                         | 1.5                    |

$^a$ Extrapolated to zero time hydrolysis.

$^b$ Maximum value.

$^c$ Values obtained from 24 h hydrolysis with tryptamine and p-toluensulfonic acid.

hydrolyses, except where noted. Residues in the native enzyme are expressed as mol/220,000 g of protein. Assuming that the enzyme four polypeptide subunits are identical, their composition is indicated in the last column and expressed as mol/55,000 g of protein.

Several substrates at concentrations ranging between 0.01 and 10 mM were used to determine the substrate specificity of our enzyme preparation. The enzyme was active with α-hydroxaldehydes and an α-ketoaldehyde; it did not oxidize other aldehydes significantly (Table IV). The reversibility of the reaction catalyzed by lactaldehyde dehydrogenase was studied with glycose and lactate as substrates at concentrations between 10 and 200 mM and NADH as cofactor at concentrations ranging from 0.1 to 2 mM. The assay was conducted under standard conditions as indicated for the reaction of oxidation with pH buffer systems between pH 6.5–9.5. No activity was found under any of these conditions. Thus, the aldehyde oxidative reaction catalyzed by lactaldehyde dehydrogenase is irreversible.

To further characterize substrate specificity, kinetic experiments were performed for each substrate. Michaelis constants and maximal velocities are listed in Table V. Since both $K_m$ values and maximal velocities varied with substrates, values of "kinetic power" of the metabolic conversion ($V/K_m$) as defined by Keleti and Welch (34) are also listed for easy comparison of the possible physiological role of the different substrates. These values range between 2.5 and 190.

Activity with NADP was very low (one-tenth of that obtained with NAD) and exhibited a $K_m$ of 6.25 mM. The Michaelis constant for the coenzyme NAD was 0.28 mM when the substrate was glycolaldehyde and a very close value, 0.12 mM, when the substrate was lactaldehyde.

Owing to substrate inhibition, a different optimum concentration was obtained for the oxidation of each of the substrates: 1 mM for glycolaldehyde, 0.1 mM for L-lactaldehyde, 0.5 mM for L-glyceraldehyde, and 2 mM for methylglyoxal. Taking 100% for the activity on glycolaldehyde, the substrates at 0.5 mM concentration gave relative activities as indicated in Table IV. Substrate inhibition was very strong with lactaldehyde, diminishing progressively with glycolaldehyde, glyceraldehyde, or methylglyoxal. Substrate inhibition by NAD was not observed with any of the aldehydes.

The enzyme was inhibited by p-hydroxy mercuribenzoate (50% inhibition at 1.5 mM) and by some cations added as its chlorides (50% inhibition at 10 mM for Mn$^{2+}$, 5 mM for Ca$^{2+}$, 0.40 mM for Cu$^{2+}$, and 0.25 mM for Zn$^{2+}$). No inhibition was found with MgCl$_2$ up to 25 mM concentration.

### Table IV

Activity of lactaldehyde dehydrogenase relative to activity with glycolaldehyde

All assays were in 100 mM sodium glycine buffer (pH 9.5) containing 2.5 mM NAD; 0.5 mM aldehyde was employed.

| Substrate          | Activity$^a$ % |
|--------------------|----------------|
| Glycolaldehyde$^a$ | 100            |
| L-Glyceraldehyde$^a$ | 100           |
| L-Lactaldehyde     | 39             |
| Methyglyoxal       | 14             |
| Succinic semialdehyde | 0          |
| Glutamic γ-semialdehyde | 0     |
| Propionalddehyde   | 0              |
| Acetaldehyde       | 0              |
| Formaldehyde       | 0              |

$^a$ Activity is based on the activity obtained with 0.5 mM glycolaldehyde which was taken as 100%.

### Table V

Kinetic properties of lactaldehyde dehydrogenase

| Substrate        | $K_m$ mM | V units/mg | V/$K_m$ |
|------------------|----------|------------|---------|
| Glycolaldehyde$^a$ | 0.38     | 19.0       | 50.0    |
| L-Glyceraldehyde$^a$ | 0.15     | 12.0       | 80.0    |
| L-Lactaldehyde$^a$ | 0.04     | 8.0        | 150.0   |
| Methylglyoxal$^a$ | 1.00     | 2.2        | 2.5     |

$^a$ Coenzyme concentrations: 0.05–0.5 mM.

$^b$ Substrate concentrations: 0.025–0.5 mM.

$^c$ Substrate concentrations: 0.015–0.1 mM.

$^d$ Substrate concentrations: 0.5–2 mM.

$^e$ Coenzyme concentrations: 0.05–0.5 mM. Glycolaldehyde and L-lactaldehyde were used at saturating concentrations, 1 and 0.1 mM, respectively.
The first hint indicating that lactaldehyde dehydrogenase is involved in several pathways in E. coli cells was the induction of this enzyme in the presence of molecules metabolized by different pathways, such as fucose, rhamnose, ethylene glycol, or glutamate. Fucose and rhamnose would not be expected to produce different inducer molecules since their metabolism is similar. L-1,2-Propanediol, an intermediate metabolite of these sugars, might also yield the same inducer molecule. However, ethylene glycol is clearly different in view of the fact that, although it uses the same enzymes as those that metabolize L-1,2-propanediol, the intermediate metabolites are different (23). Thus, the control machinery for the expression of lactaldehyde dehydrogenase would appear to recognize two different inducer molecules.

With regard to glutamate, it is difficult to think of any relationship between its metabolism and those of the other molecules capable of inducing the enzyme. Consequently, there may be a third, unknown inducer molecule. The multiple metabolic roles of glutamate (35-37) do not permit us at present to suggest which of the related pathways produces the inducer. Nevertheless, it is very unlikely that induction of lactaldehyde dehydrogenase by glutamate could be related to its use as nitrogen source, since strain 40, unable to induce the enzyme, behaves in this respect as wild type E. coli. It is also of interest that, although in E. coli cells glutamate is decarboxylated to \( \gamma \)-aminobutyric acid, a compound not further metabolized in these cells (38), or it is reduced to glutamic \( \gamma \)-semialdehyde (35), these products of the glutamate metabolism do not induce lactaldehyde dehydrogenase.

Besides the multiple induction pattern of lactaldehyde dehydrogenase, the participation of the enzyme in diverse metabolic processes is also supported by its broad substrate specificity oxidizing not only \( \alpha \)-hydroxyaldehydes but also \( \alpha \)-ketoaldehydes. Even though the kinetic power (\( V/K_\text{m} \)) of the latter is considerably lower than that for the \( \alpha \)-hydroxyaldehydes, we should point out that it is of similar order of magnitude than the kinetic power of many other substrate-envelope systems for which a metabolic role has routinely been accepted (39-41).

The nearly constant relationship between specific activity and height of the rocket indicates that the differences observed in the enzymatic activity are due to changes in the synthesis of the enzyme rather than to modifications in the activity of the enzymatic protein present in the cell.

In a mutant defective for this enzyme (strain 40), the absence of both activity and lactaldehyde dehydrogenase protein under any inducing conditions suggests that all aldehyde oxidative activities serving the different pathways are a function of the same enzymatic protein.

The purification to homogeneity of the enzyme has allowed us to examine some of its structural features and to distinguish it from other well-known aldehyde dehydrogenases acting on \( \alpha \)-hydroxyaldehydes in E. coli. Thus, the molecular weight of 220,000 and the composition of four subunits of 55,000 of lactaldehyde dehydrogenase are clearly different from those of glyceraldehyde phosphate dehydrogenase (33) which has a molecular weight of 144,000 and four subunits of 36,000. The amino acid compositions also show differences such as the contents of lysine, aspartate (including asparagine), threonine, and methionine which are lower in lactaldehyde dehydrogenase and the contents of glutamate, arginine, proline, and isoleucine which are higher.

Three isozymes, A, B and C, of glycolaldehyde dehydrogenase have been described by Morita et al. (42) in E. coli B. An important difference between those aldehyde dehydrogenases and lactaldehyde dehydrogenase is the reversibility of the reaction which has not been observed with lactaldehyde dehydrogenase. According to Morita et al. and another report (43), these enzymes seem not to use NAD as a coenzyme. Although lactaldehyde dehydrogenase has some features similar to those of isozyme A, such as metal ions inhibition and broad specificity of substrates, and displays some activity with NADP, an identity with this isozyme cannot be established.

Acetaldehyde dehydrogenase, another well-characterized enzyme in E. coli, is a CoA-dependent enzyme (44). In contrast, lactaldehyde dehydrogenase is incapable of oxidizing acetaldehyde either in the presence or absence of CoA.

To our knowledge, after the report of Sridhara and Wu (8) on lactaldehyde dehydrogenase, no other study of this enzyme has been published. Our results on the analysis of mutant strain 40 identify the enzyme reported here with that studied by Sridhara and Wu. Although the limited molecular characterization of lactaldehyde dehydrogenase carried out by these authors does not permit a good comparison, the molecular weight of 100,000 found by them for the native enzyme seems to correspond to a dimeric composition rather than to the tetrameric composition resulting from our molecular weight determination. In view of the NAD titration described in this report, we are inclined to consider the tetrameric form that corresponds to the native enzyme.

Substrate inhibition and uncertainty of substrate concentrations, owing to the origin of the aldehydes, may explain the differences between the relative activities obtained by Sridhara and Wu (8) and those presented in this report. The almost undetectable activities on glyceraldehyde and methyglyoxal (they did not assay glycolaldehyde) observed by these authors led them to the erroneous conclusion that the enzyme was highly specific for lactaldehyde.

The information summarized above leads to two conclusions. First, the enzyme described in this report is clearly different from other aldehyde dehydrogenases described in E. coli. And second, aldehyde dehydrogenase role is, most likely, the oxidation of diverse aldehydes produced by different pathways of cellular metabolism.

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E. coli Aldehyde Dehydrogenase Serving Distinct Pathways

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