Inducible Membrane-bound L-Lactate Dehydrogenase from Escherichia coli

PURIFICATION AND PROPERTIES*

MASAMITSU FUTAI and HIROMICHI KIMURA
From the Faculty of Pharmaceutical Sciences and Department of Botany, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

Membrane-bound L-lactate dehydrogenase was solubilized from the membranes of Escherichia coli and purified to homogeneity using conventional procedures. The enzyme had a pH optimum of 8.0, and a specific activity for L-lactate. Its apparent Km for L-lactate and maximal velocity were 1.2 \times 10^{-4} \text{ M} and 31 \text{ mmol of tetrazolium dye reduced/min/mg of protein}, respectively. It had a polypeptide molecular weight of 43,000 determined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. The average molecular weight of the purified enzyme after removal of detergent was estimated to be 480,000 by sucrose gradient centrifugation, suggesting that the enzyme was an oligomer in detergent-free solution. The purified enzyme was also an oligomer in the presence of Tween 80 or cholate. It contained about 1 mol of flavin mononucleotide/mol of polypeptide with molecular weight of 42,000. The fluorescence of the flavin in the purified enzyme was 5.3% quantum yield of that of FMN.

Specific antibody for this enzyme inhibited the enzyme activity and L-lactate-dependent uptake of oxygen in inverted membrane vesicles, suggesting that the purified enzyme was a primary dehydrogenase of the respiratory chain. The enzyme was induced by growing the cells on DL-lactate, in confirmation of an earlier report (Kline, E. S., and Mahler, H. R. (1965) *Ann. N. Y. Acad. Sci.* 119, 905-919). Cells grown aerobically on glycerol or anaerobically on glucose showed no appreciable activity of this enzyme or cross-reaction with other materials.

The respiratory chain of Escherichia coli has lately received much attention with recent increase in interest in energy metabolism in bacteria. Bacterial respiratory chains oxidize a wider range of substrates than mitochondria (1). Thus aerobically grown E. coli contain several different primary dehydrogenases which transfer electrons from their substrates to adjacent electron carriers; addition of the substrates of each of these dehydrogenases resulted in complete reduction of available cytochromes (2, 3). The sequence of electron flow has been studied biochemically and genetically (see Ref. 4 for review), although it is not yet completely established. A few dehydrogenases (5-9) and b-type cytochromes (9) of E. coli have been purified. However, for a better understanding of the assembly and function of the respiratory chain the properties of all the components in this multienzyme system should be known.

Earlier studies suggested that in E. coli L-lactate dehydrogenase is an inducible enzyme (10), as its specific activity in lysate of cells grown on DL-lactate was about 20-fold more than that of lysate of cells grown on glycerol or glucose. As shown below we have confirmed this observation and found that the specific activities of the enzyme in membrane fractions varied more than 100-fold depending on the growth conditions. It seemed interesting to purify this enzyme, because inducible enzyme may be useful in studies on the assembly of respiratory chain. The present paper describes the purification of L-lactate dehydrogenase. The homogeneous enzyme obtained was found to be a flavoprotein containing FMN as a prosthetic group, and several of the properties of the enzyme were examined. Using a specific antibody we found that this enzyme was located on the inner surface of cytoplasmic membranes of cells grown on DL-lactate.

**Experimental Procedures**

This section describes the purification of L-lactate dehydrogenase only. Other experimental procedures, including growth of bacteria, materials, and assay methods, are described in the miniprint supplement to this article.¹

Buffer A used during enzyme purification contained 1.0% Tween 80, 0.05 M Tris/HCl, pH 8.0, and 0.02% β-mercaptoethanol. All procedures were carried out at 0-4°C. Unless otherwise specified, centrifugation was done at 10,000 \times g for 20 min.

**Step I** - A suspension of membranes (1.0 liter, 20 mg of protein/ml) from 450 g of wet cells (Escherichia coli ML008-325-dld3, L-lactate dehydrogenase-negative strain, Ref. 11) was mixed successively with 800 ml of 50% glycerol, 20 ml of 1.0% β-mercaptoethanol, and 10 ml of 1.0 M Tris/HCl, pH 8.0. Then 100 ml of 20% sodium cholate were added dropwise with stirring, followed by 132 g of solid ammonium sulfate. The mixture was stirred for 30 min and then centrifuged at

¹ Portions of this paper (including "Experimental Procedures," Figs. 3, 4, 6, 7, and 8 and Table II) are presented in miniprint following the References. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document 77M-485, cite author(s), and include a check or money order for $1.00 per set of photocopies.
100,000 × g for 3 h. The supernatant was brought to 0.05 M Tris/HCl, pH 8.0, by adding 1.0 M Tris/HCl, pH 8.0. The solution (about 1.9 liters) was mixed with 570 g of solid ammonium sulfate, stirred for 30 min, and centrifuged, and then the precipitate was dissolved in 500 ml of Buffer A.

Step 2 – The final solution of Step 1 (605 ml) was mixed with 55 g of Dextran T500, 60 g of Ficol, 40 g of polyethylene glycol 6000, and 95 ml of distilled water. The mixture was vigorously shaken on a rotatory shaker for 1 h and centrifuged. The mixture separated into three distinct phases on centrifugation. The second phase from the top was carefully collected by pipette and diluted 3-fold with Buffer A. The solution was made up to 40% saturation (12) with solid ammonium sulfate, stirred for 20 min, and centrifuged. The protein fraction formed a thin film on the top of the centrifuge tube. This fraction was dissolved in 50 ml of Buffer A.

Step 3 – The resulting solution was applied to a column of DEAE-cellulose (5 × 15 cm) equilibrated with Buffer A. The column was washed with a small amount of Buffer A, and eluted with a linear gradient formed by placing 300 ml of Buffer A containing 0.05 M NaCl in the mixing vessel and 300 ml of the same buffer containing 0.3 M NaCl in the reservoir. The flow rate was approximately 30 ml/h and fractions of 5 ml were collected. The dehydrogenase was eluted as a single peak in the middle of the gradient. Fractions containing more than 50% of the activity of the fraction with maximal activity were combined and glycerol was added at a final concentration of 20%. This fraction (120 ml) was frozen and stored at −80°C for further purification.

Step 4 – One-quarter of the frozen preparation (30 ml) was thawed and diluted 4-fold with Buffer A. This solution was then applied to a small DEAE-column (1 × 2 cm) previously equilibrated with Buffer A. The column was washed with a small portion of Buffer A and the enzyme was eluted with 0.5 M NaCl in the same buffer. The fraction containing most of the activity (about 1 ml, with about 80% of the total activity on the column) was applied to a column of Bio-Gel A-0.5 m (2 × 50 cm) previously equilibrated with 0.05 M NaCl in Buffer A. The flow rate was 10 ml/h and fractions of 2 ml were collected. The L-lactate dehydrogenase activity usually gave a single peak recovered in tubes 30 to 35. The purified enzyme was concentrated about 20-fold using a small DEAE-column as described above and was stored in 20% glycerol at −80°C until use. Tween 80 could be removed using the same small DEAE-column. The pure enzyme (1.0 mg) was applied to the column, the latter was washed extensively with 0.01 M Tris/HCl, pH 8.0, and then the enzyme was eluted with 2 ml of 0.5 M NaCl in the same buffer. This fraction contained less than 1 mol of Tween 80/mol of enzyme, assayed as described previously (19).

RESULTS
Activity of L-Lactate Dehydrogenase in Membranes Prepared from Cells Grown on Different Carbon Sources

Kline and Mahler (10) suggested that L-lactate dehydrogenase was inducible when *Escherichia coli* was grown on DL-lactate; the enzyme activity in cells grown on DL-lactate was about 20-fold higher than that of cells grown on glucose or glycerol. Thus, as this enzyme has been suggested to be one of the primary dehydrogenases in the respiratory chain of *E. coli* membranes (3), it was of interest to compare the specific activities of enzyme in membrane fractions from cells grown with different carbon sources. Fig. 1 shows that enzyme activity was highest in membranes prepared from *E. coli* ML308-205 grown on DL-lactate, and that no significant activity was detected in membranes from cells grown aerobically on glycerol or anaerobically on glucose. (The activities detectable in these cells were at least 100 times less than those in cells grown on DL-lactate.) L-Lactate-dependent uptake of oxygen of different membranes were proportional to their L-lactate dehydrogenase activities. None was detectable in membranes from cells grown anaerobically on glucose or aerobically on glycerol.

The enzyme activity of cells grown anaerobically on glucose increased to the level in cells grown aerobically on DL-lactate, when the cells were washed and transferred to medium with DL-lactate and grown aerobically. The time required for full induction was about 1/4 of the generation time after transfer to the new medium. This induction was sensitive to chloramphenicol (data not shown). On the other hand essentially no activity was observed when cells were grown anaerobically on glucose plus DL-lactate. The specific activity of the enzyme in cells grown on DL-lactate alone was similar to that of cells grown on DL-lactate. Low but significant enzyme activity was observed in membranes from cells grown on glucose or succinate; the specific activities of the enzyme in membranes from these cells were 5.0 and 17.0, respectively, of that of cells grown on DL-lactate, and these activities also increased to the level of cells grown on DL-lactate when DL-lactate was added to the medium of glucose- or succinate-grown cells. In the experiments described above no appreciable activity was detected in the supernatant fraction obtained after removal of membranes. These results suggest that the enzyme is an inducible membrane protein. Thus it was of interest to purify this enzyme from the membrane of cells grown on DL-lactate and to characterize the mechanism of enzyme induction.

Fig. 1. Activities of dehydrogenases in membranes of *Escherichia coli* ML308-205 grown under different conditions. Membrane fractions were obtained by lysing cells grown aerobically (+O₂) on glucose (Glu), lactate (Lac), glycerol (Gly), or succinate (Suc), or anaerobically (−O₂) on glucose. L-Lactate, D-lactate, NADH, and succinate dehydrogenase were assayed and their specific activities are shown.

Solubilization and Purification of L-Lactate Dehydrogenase

Only a partial purification of this enzyme has been reported so far (10). In this work we solubilized this enzyme with 1.0% cholate and purified it to a homogeneous state as described
zyme. With L-lactate Michaelis-Menten kinetics were observed, the $K_m$ and maximal velocity being 1.2 x $10^{-4}$ M and 31

$\mu$mol of MTT reduced/min/mg of protein (at 23$^\circ$), respectively.Pyruvate was formed with stoichiometric reduction of MTT: 0.020 unit (micromoles of MTT reduced/min) of enzyme formed 0.016 $\mu$mol of pyruvate/min. With D,L-α-hydroxybutyrate the maximal velocity was the same as that with L-lactate, but the $K_m$ was 7.7 x $10^{-4}$ M. No appreciable oxidation of D-lactate was observed. Its rate of oxidation being less than 0.1% of that of L-lactate.

Optimum pH and Isoelectric Point—The optimum pH for the oxidation of L-lactate was between pH 8 and 9 (Fig. 3). On isoelectric focusing in polyacrylamide gel with 8 M urea (17) it gave only one densely staining band, suggesting that its isoelectric point was 8.3 (Fig. 2b). The formation of only one band also confirms the homogeneity of the purified enzyme.

Effects of Various Compounds on Enzyme—Among the metal ions tested, high concentrations of sodium and potassium ions activated the enzyme 2-fold (Table 2), whereas a high concentration of pyruvate (10 mM) almost completely inhibited it. N-Ethylmaleimide and oxamate had no effect.

Estimation of Molecular Weight—The molecular weight of the enzyme determined by gel electrophoresis in 7.5% polyacrylamide containing 0.1% sodium dodecyl sulfate (18) was 43,000. This value was obtained using subunits (α, β, γ, ε) of E. coli ATPase (38), bovine serum albumin, and cytochrome c as markers. Essentially the same value was obtained using 5% or 10% polyacrylamide. This value was confirmed using another gel system in the presence of sodium dodecyl sulfate (37). It is also supported by the content of flavin in the enzyme as discussed below. These procedures determined the polypeptide molecular weight of the enzyme after its denaturation with sodium dodecyl sulfate and β-mercaptoethanol.

Intrinsic membrane proteins often form oligomers or aggregates in aqueous solutions (for review see Ref. 38). Sucrose gradient centrifugation of the enzyme after removal of detergent gave an average molecular weight of 480,000 (Fig. 4c), assuming that the partial specific volume of the enzyme is the same as that of the standard protein used. This result suggests that the detergent-free enzyme was an oligomer of single polypeptide each with a molecular weight of 43,000.

The enzyme also sediments more rapidly than catalase ($M_r$ = 240,000) in sucrose gradient centrifugation in the presence of 1.0% Tween 80 or 1.0% cholate (Fig. 4, a and b). Apparent molecular weight ranging from 320,000 to 360,000 was obtained from the centrifugation. Essentially the same results were obtained by gel filtration through Bio-Gel A-0.5m equilibrated with buffer containing Tween 80 or cholate. These values are rough estimates of the molecular weight of the oligomers in detergent solution as discussed previously (39). However, the value obtained by the centrifugation with cholate could not be explained by binding of unusual amount of the detergent to the enzyme. The amount of cholate bound to the enzyme was estimated by the method of Clarke (40). On centrifugation no peak of radioactivity of [3H]cholate was found corresponding to that of the enzyme. The upper limit of cholate bound to the enzyme could not be established by assuming that the height of the peak of radioactivity is the same magnitude as the scatter of the data. If the enzyme bound 20 mol of cholate/mol of polypeptide of molecular weight 43,000, there should be a peak of 2,700 cpm (shown by the vertical bar in Fig. 4b).

However, less than this amount of cholate must have been bound, because the deviation of the peak region was less than

1 The abbreviation used is: MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide.
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Fig. 2. a, polyacrylamide gel electrophoresis of Escherichia coli L-lactate dehydrogenase in the presence of 0.1% sodium dodecyl sulfate. Approximately 10 μg of purified enzyme were applied to 7.5% polyacrylamide gel. After electrophoresis the gel was stained with Coomassie brilliant blue. A picture and densitometric tracing of the gel are shown. b, isoelectric focusing of E. coli L-lactate dehydrogenase in urea-polyacrylamide gel. Gels containing Ampholine (pH 3 to 10) were used. The gels were stained, scanned, and sliced for pH determination as described under "Experimental Procedures."

2,700 cpm. Thus the sum of the molecular weight of the detergent portion (409 [molecular weight of cholate] × 20) and polypeptide (43,090) is much lower than the value expected from the centrifugation (Fig. 4b). It was also estimated by the same procedure that less than 200 molecules of cholate bound to catalase. These results suggest that lactate dehydrogenase was an oligomer even in the presence of some detergents.

Properties of Enzyme as Flavoprotein

Visible Absorption Spectrum of Enzyme - The absorption spectrum of the enzyme in the visible region had peaks at around 460 nm and 380 nm, suggesting that the enzyme was a flavoprotein (Fig. 5). On addition of L-lactate, the peak at 460 nm disappeared with appearance of a shoulder at around 425 nm and a broad peak at 340 nm. The enzyme was also reduced with dithionite as shown in Fig. 5. The reduced form was maintained at room temperature for 30 min even under aerobic conditions. The molar extinction coefficient of the oxidized enzyme at 460 nm was 7.80 mM-1 cm-1, calculated assuming that the molecular weight was 43,000.

Fluorescence of Enzyme - Fluorescence (excited at 460 nm and measured at 520 nm) due to the flavin was observed and its quantum yield at pH 7.0 was 5.3% of that of FMN, assuming that 1 mol of enzyme contained 1 mol of flavin. It was constant between pH 9 and 6, but increased on lowering the pH further. The fluorescence at pH 3.0 was 20 times that at pH 7.0.

Determination of Flavin in Enzyme - The flavin could be extracted with 10% trichloroacetic acid or acid ammonium sulfate, suggesting that it was noncovalently bound to the enzyme. Characters of the flavin extracted from the enzyme with acid ammonium sulfate were examined. Its absorption spectrum in the visible region was identical with that of FMN (data not shown). Its pH-fluorescence curve was the same as that of standard FMN (Fig. 6). Moreover, its Rf values on paper and thin layer chromatography (29, 30) were the same as those of FMN.

The flavin extracted from L-lactate dehydrogenase was also identified as FMN by a test with bacterial luciferase which does not react with FAD (33). An extract whose flavin concentration was determined to be 2.4 × 10^-4 M from its absorbance was shown to contain 2.6 × 10^-6 M FMN by the luciferase test. Using four different procedures the enzyme was shown to contain about 1 mol of FMN/polypeptide of Mr = 43,000. The values of the content of FMN obtained by the luciferase test, assay after converting FMN to lumiflavin, absorption (at 460 nm), and fluorescence were 0.90, 0.72, 0.83, and 0.90 mol/mol of enzyme, respectively.

Immunological Studies on L-Lactate Dehydrogenase in Membranes of E. coli

Properties of Antibody against L-Lactate Dehydrogenase - The antibody was prepared against purified enzyme using the Freund adjuvant. It is interesting that this enzyme seemed to be more antigenic than n-lactate or glycerol-3-phosphate dehy-
L-lactate dehydrogenase from E. coli membranes—As shown above the activity of L-lactate dehydrogenase varied more than 100-fold depending on the conditions of growth of the cells. Confirmation that this large variation was due to variation in the amount of enzyme protein in the membranes was obtained by subjecting membranes from cells grown under different conditions to immunodiffusion. As described above, a distinct precipitin line was observed only between the antibody and membranes (16 mg/ml) from cells grown on DL-lactate (Fig. 1a). Weak precipitin lines were observed between antibody and membranes (32 mg of protein/ml) from cells grown on glucose or succinate, but not with membranes (32 mg of protein/ml) of cells grown aerobically on glyceral or anaerobically on glucose (data not shown). Using different concentrations of membranes it was estimated that cross-reacting materials in membranes from cells grown on succinate or glucose was less than 20% of that in membranes from cells grown on DL-lactate. In these experiments membranes were pretreated with 1.0% Triton X-100 and immunodiffusion was carried out in agarose containing the same detergent. Thus nonspecific precipitation of the enzyme during immunodiffusion was avoided. These results suggest that the change in activity of the enzyme described above (Fig. 1) was due to change in the amount of enzyme molecules in the membranes.

The antibody caused concentration-dependent inhibition of the purified enzyme. 1 unit of enzyme being inhibited 80% by 4 mg of antibody (Fig. 8a). In contrast, the immunoglobulin fraction from rabbits before immunization had no effect on the activity and did not form any precipitin line with the enzyme in the immunodiffusion test. Excess antibody (10 mg) had no effect on pure DL-lactate dehydrogenase or succinate and glycerol-3-phosphate dehydrogenase in the membranes.

To confirm that the purified enzyme was a primary dehydrogenase in the respiratory chain of E. coli we tested the effect of the antibody on the membrane-bound enzyme from cells grown on DL-lactate. Addition of the antibody (0.8 mg) to membrane vesicles prepared by French press (containing 1 unit of enzyme) caused 80% inhibition of L-lactate-dependent reduction of MTT and oxygen uptake (Fig. 8b). As L-lactate-dependent respiration would be inhibited by inhibition of L-lactate dehydrogenase, this suggests that the purified enzyme is a true component of the respiratory chain. Moreover, as membrane vesicles prepared by French press are known to be inverted (15), this result also suggests that L-lactate dehydrogenase is localized on the inner surface of the cytoplasmic membranes. It is noteworthy that for 50% inhibition more antibody was required with pure enzyme than with the membrane-bound enzyme.

DISCUSSION

In this work we solubilized L-lactate dehydrogenase and purified it to a homogeneous state. Conventional techniques were used for the purification but Tween 80 was added to all buffers. In enzyme purification we used aqueous phase partitioning (Step 2), a technique found successful in purification of membrane-bound phospholipase A (41). This technique removed a major contaminant that could not be removed by the other procedures tested. The technique could be used with large batches of material, giving good purification (about 2.5-fold), although not so good as that of phospholipase A (41).

The homogeneity of the enzyme was examined by polyacrylamide gel electrophoresis in the absence of sodium dodecyl sulfate and isoelectric focusing in polyacrylamide gel containing urea. In both cases the enzyme gave only one strong band on the gel. These bands were concluded to be L-lactate dehydrogenase because the activity and the protein bands were eluted in the same position from a column of DEAE-cellulose or Bio-Gel A-0.5m. The identity of the bands with enzyme was confirmed by analyzing membranes from cells grown with different carbon sources. No protein band corresponding to that of pure enzyme could be detected in cholate extracts from cells grown aerobically on glyceral or anaerobically on glucose (data not shown).

This enzyme differs from NADH-dependent lactate dehydrogenase (42) in its location, sensitivity to oxamate, and other properties. The pyridine nucleotide enzyme appears to be directed toward the production of lactate (43), while the present enzyme is probably a primary dehydrogenase in the respiratory chain for two reasons: (a) antibody against it inhibited L-lactate-dependent oxygen uptake in membrane vesicles, and (b) membranes lacking this enzyme could not take up oxygen on addition of L-lactate. This enzyme also differs from membrane-bound DL-lactate dehydrogenase (5, 6) in its sensitivity to inhibitors, its flavin prosthetic group, molecular weight, and other properties. Moreover, the specific activity of L-lactate dehydrogenase did not change significantly under different conditions, whereas L-lactate dehydrogenase seemed to be inducible.

The molecular weight of the purified enzyme after removal of detergent was estimated to be 480,000 by centrifugation on a linear sucrose gradient. The enzyme contained 1 mol of flavin/mol of polypeptide (M_r = 43,000). Thus, the detergent-free enzyme may be an oligomer of active flavoprotein. It must be noted that the enzyme existed as oligomers even in the presence of Tween 80 or cholate. Determination of the more accurate molecular weight of those oligomers is planned. The purified enzyme was less sensitive to antibody against L-lactate dehydrogenase than membrane-bound enzyme. For 50% inhibition the purified enzyme required about 4-fold more antibody than the membrane-bound enzyme. Thus, the purified enzyme may be less accessible to antibody than the enzyme in membranes. This finding agrees with the suggestion that the purified enzyme was oligomers even in the presence of detergents.

We have shown recently that in detergent-free buffer, purified L-lactate dehydrogenase of Escherichia coli formed a mixture of dimers and trimers which could be dissociated into monomers by Triton X-100 (44). Formation of such oligomers after removal of detergents seems to be one of the major
properties of intrinsic membrane proteins (38). The interaction of hydrophobic portions of monomer molecules was suggested to be important in formation of oligomers (38, 40). As shown previously (44), detergent-free D-lactate dehydrogenase was activated by phospholipids or detergents. Thus it should be interesting to examine the properties of L-lactate dehydrogenase after removal of the detergents; studies on these properties are in progress.

In this study, L-lactate dehydrogenase was shown to be an inducible membrane-bound enzyme. When cells were cultured under conditions giving low enzyme activity, little or no material cross-reacting with antibody was detectable in the membranes or cytoplasmic fractions of the cells. Moreover, the polypeptide corresponding to the enzyme could not be detected in membranes from cells in which enzyme activity was low. Our results suggested that L- or D-lactate or one of their metabolites together with oxygen was required for induction of the enzyme, but more work is necessary on the mechanism of induction and the nature of the inducer.

Little is known about the biogenesis of enzymes in bacterial cytoplasmic membranes. The respiratory chain complex with fairly well characterized components, such as the present enzyme, should be useful in studies on this problem. This enzyme seems to be fairly abundant in the membranes of cells grown on lactate. It constituted about 3% of the total membrane protein judging from the specific activities of purified and membrane-bound enzyme. This relative abundance of the enzyme, but more work is necessary on the mechanism of induction and the nature of the inducer.

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REFERENCES
5. Futai, M. (1973) Biochemistry 12, 2466-2474
33. Watanabe, T., and Nakamura, T. (1972) J. Biochem. (Tokyo) 72, 647-653
Lactate Dehydrogenase from E. coli Membranes

M. L. Fleischer and J. L. Demple

Department of Biochemistry, University of Chicago, Chicago, Illinois

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This paper describes the purification and properties of lactate dehydrogenase from E. coli membranes. The enzyme was purified by a combination of fractionation and affinity chromatography. The enzyme was then used in a number of experiments to study its kinetic properties and to determine the mechanism of its catalytic activity.

Table I

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Fig. 1 shows the activity profile of lactate dehydrogenase in the presence of various concentrations of NADH and NAD. The enzyme was most active at NADH concentrations of 0.5 to 2.0 mM and 0.1 mM for NAD. The optimal pH for the reaction was pH 7.0.

Fig. 2 shows the effect of varying the substrate concentration on the reaction rate. The Michaelis-Menten constant (Km) was estimated to be 0.1 mM for lactate and 0.05 mM for NADH.

Fig. 3 shows the effect of temperature on the reaction rate. The enzyme was most active at temperatures ranging from 30 to 40°C. At temperatures below 30°C, the activity decreased, and above 40°C, it was completely inactivated.

Fig. 4 shows the effect of pH on the reaction rate. The enzyme was most active at pH 7.0 and showed a decrease in activity at pH values lower than 6.0 and higher than 8.0.

Fig. 5 shows the effect of 2-deoxy-D-glucose on the reaction rate. The enzyme activity was inhibited by 2-deoxy-D-glucose at concentrations above 10 mM.

The enzyme was stable at pH 7.0 and 4°C for at least 2 weeks. It was inactivated by heating at 50°C for 15 minutes.

The enzyme was found to be a dimer, as determined by gel filtration and ultracentrifugation.

The enzyme was found to be stable in the presence of a number of metal ions, including Co2+, Ni2+, and Mn2+.

The enzyme was found to be active in the presence of a number of divalent cations, including Mg2+, Ca2+, and Mn2+.

The enzyme was found to be active in the presence of a number of anions, including Cl-, Br-, and I-.

The enzyme was found to be active in the presence of a number of detergents, including SDS, Triton X-100, and CHAPS.

The enzyme was found to be active in the presence of a number of inhibitors, including iodoacetate, p-chloromercuribenzoate, and lactic acid.

The enzyme was found to be active in the presence of a number of activators, including NADH, ADP, and ATP.

The enzyme was found to be active in the presence of a number of electron donors, including glycerol, glucose, and lactate.

The enzyme was found to be active in the presence of a number of electron acceptors, including NAD, ADP, and ATP.
**L-Lactate Dehydrogenase from E. coli Membranes**

**Fig. 7(a)** Immuno diffusion pattern of immunoglobulins to L-lactate dehydrogenase. Immunodiffusion was carried out in agarose gel containing 0.1% Triton X-100, 0.05 M Tris-HCl, pH 8.0, and 0.05 M NaCl. Immunoglobulin B (0.5 mg per well) was placed in the center well and various fractions were placed in the peripheral wells. (1) Purified L-Lactate Dehydrogenase (0.0 mg per well) in 0.05 M Tris-HCl, pH 8.0 containing 1.0% Tween 80. (2) Membranes from cells grown at 37°C. (3) Membranes from cells grown at 0°C. (4) Membranes from cells grown in glucose. (5) Membranes from cells grown on galactose. Membrane preparations (1 mg protein/ml) were suspended in 0.05 M Tris-HCl, pH 8.0 containing 1.0% Triton X-100. Cells were grown aerobically in top atmosphere sacrificed. Samples of about 1 ml were placed in the wells. Immunoglobulin B from bovine serum albumin did not form a precipitin line.

**Fig. 7(b)** Purity of lactate dehydrogenase of the immunoprecipitate formalin between antibody and antigen to the lactate extract. Immunoglobulin B (0.1 mg per well) and lactate extract (0.2 mg per well) from cells grown at 37°C lactate were bounded in 0.05 M Tris-HCl, pH 8.0 at 4°C for 2 hours. The immunoprecipitate formed was collected by centrifugation at 5,000 g for 10 min. It was washed three times with 0.05 ml saline of 0.05 M Tris-HCl, pH 8.0 containing 0.05 M NaCl and 1.0% Tween 80. Each fraction containing 0.05 mg protein was dissolved in 0.5 ml distilled water containing 0.16 M sodium dodecyl sulfate and subjected to electrophoresis. Gels were stained with Coomassie Blue and scanned. Band corresponding to Immunoglobulin B (heavy chain) and light chain B) is above.

**Fig. 8** Effect of the antibody on purified L-lactate dehydrogenase and membrane vesicles prepared by French press. (a) Purified enzyme (0.05 mg/ml) was incubated with different amounts of antibody for 30 min at 25°C or at 0°C with 20 mg/ml. Under conditions in the absence of antibody ( ) or in the presence of L-lactate dehydrogenase ( ) and L-lactate dehydrogenase ( ), the activity of the control is expressed in percentage of the activity of the control enzyme. The control without L-lactate dehydrogenase ( ). The data are the mean of triplicate determinations. As a control 0.1% Triton X-100 was added to the same medium. The activity of the control was only about 10% of the activity of the control.
Inducible membrane-bound L-lactate dehydrogenase from Escherichia coli.  
Purification and properties.
M Futai and H Kimura


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