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Properties of a Fructose-1,6-Diphosphate-Activated Lactate Dehydrogenase from *Acholeplasma laidlawii* Type A

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Acholeplasma laidlawii A possesses a nicotinamide adenine dinucleotide (NAD)-dependent L(+)-lactate dehydrogenase (LDH) which is activated specifically by low concentrations of fructose-1,6-diphosphate (FDP). Studies with partially purified enzyme show that the kinetic response to FDP is hyperbolic. The enzyme is inhibited by inorganic phosphate, adenosine triphosphate, and high concentrations of reduced NAD (NADH). Low activity is demonstrable in the absence of FDP at pH 6.0 to 7.2, but FDP is absolutely required in the region of pH 8. FDP causes an upward shift in the optimum pH of the enzyme, which is near 7.2 in tris(hydroxymethyl)aminomethane buffer. Activation of the enzyme by FDP is markedly affected by substrate concentration; FDP lowers the apparent K_m for pyruvate and NADH. The affinity of the enzyme for pyruvate is also influenced by H^+ concentration. The pyruvate analogue α-ketobutyrate serves as an effective substrate for the enzyme; when it is utilized, the enzyme is still activated by FDP. Reversal of the pyruvate reduction reaction catalyzed by the enzyme can be demonstrated with the 3-acetylpyridine analogue of NAD. The catalytic properties of the *A. laidlawii* enzyme and the known FDP-activated LDHs which occur among lactic acid bacteria are discussed.

During a study of the lactate dehydrogenases of mycoplasmas, it was found that *Acholeplasma laidlawii* type A possesses an L(+)-lactate dehydrogenase (LDH) (EC 1.1.1.27) which is activated by the glycolytic intermediate fructose-1,6-diphosphate (FDP; 11). The LDH from *A. laidlawii* has been studied further because it is essential for energy metabolism and has an important regulatory function in glycolysis.

Our use of FDP was prompted by the discovery by Wolin (19) that certain streptococcal LDHs are activated specifically by FDP. This activation by FDP appears to be uncommon and at present is known to occur only among members of the *Lactobacillaceae*, specifically in streptococci, where it has been found in every group examined thus far (2, 6, 17), in strains of *Lactobacillus bifidus* (16), and in *L. casei* (15). The FDP-activated LDHs of *A. laidlawii* and the lactic acid bacteria may have molecular similarities, since all have binding sites for the three ligands pyruvate, FDP, and reduced nicotinamide adenine dinucleotide (NADH). These LDHs may thus be useful in examining evolutionary relations between mycoplasmas and the lactic acid bacteria (11).

This report describes the partial purification of the FDP-activated LDH from *A. laidlawii* type A and results of studies on the catalytic and regulatory properties of this enzyme. In addition, we compare our findings on the properties of the *A. laidlawii* enzyme with available data on the FDP-activated LDHs from streptococci.

MATERIALS AND METHODS

Organisms and cultivation. *A. laidlawii* type A was grown under conditions described previously (10); 1% serum fraction (Difco) was added to the medium. The final glucose concentration was 0.5%.

Preparation of cell extract. Cells were harvested, washed once in 0.145 M sodium chloride-0.02 M sodium phosphate buffer (pH 7.5), and resuspended in the same buffer (cells were concentrated about 400-

to 600-fold). Mycoplasma cell suspensions in a 70% ethanol-ice bath were disrupted with a Biosonic II sonic oscillator at 100% power for six 90-s periods alternated with cooling for 3 min. Cell debris was removed by centrifugation at 80,801 × *g* for 30 min at 4 C. The supernatant fluid was stored at −20 C if not used immediately.

Enzyme assay. The NAD-dependent LDH was measured spectrophotometrically by recording the rate of oxidation of NADH at 340 nm with pyruvate as substrate. The standard assay system was composed of tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 7.2), 100 mM; sodium pyruvate, 0.5 mM; NADH, 0.15 mM; FDP, 1 mM; and water to a total volume of 1 ml. In certain studies phosphate buffer, glycine-hydrazine buffer, or 2-(*N*-morpholino) ethanesulfonic acid (MES)-NaOH buffer was substituted for Tris-hydrochloride buffer. Reactions were initiated by adding a suitable amount of enzyme preparation. One unit of enzyme activity is defined as the amount of enzyme which, under standard assay conditions, effects the oxidation of 1 μmol of NADH/min. In some experiments lactate oxidation was demonstrated by measuring reduction of the 3-acetylpyridine analogue of NAD (APNAD) at 365 nm (7). Cuvettes contained in a total volume of 1 ml: Tris-hydrochloride buffer (pH 8.0), 100 mM; L(+)- or D(−)-lithium lactate, 10 mM; APNAD, 2 mM. Rates were determined at 22 to 24 C in 1-cm cells with a Gilford 2000 recording spectrophotometer equipped with a Beckman DU monochrometer.

Analytical methods. Fructose-1,6-diphosphate was determined by the method of Roe and Papadopoulos (12). Assays to determine whether FDP disappeared during the enzyme reaction were carried out on standard assay mixtures (containing 0.5 μmol of FDP), on controls where the enzyme reaction was stopped immediately by addition of 5% trichloroacetic acid, and on controls where enzyme, pyruvate, or FDP was omitted.

Protein was determined by the method of Lowry et al. (9) with crystalline bovine serum albumin as a standard or by the method of Warburg and Christian as described by Layne (8).

Polyacrylamide gel electrophoresis. The number of proteins in the partially purified enzyme preparation was estimated by polyacylamide gel electrophoresis. Electrophoresis in 7.5% gels was carried out as described previously (11); gels were stained with 0.25% Coomassie blue and destained in 7% acetic acid.

Chemicals. Cofactors, substrates, analogues, hydrogen ion buffers, and calcium phosphate gel were purchased from Sigma Chemical Co. (St. Louis, Mo.); L(+)- and D(−)-lithium lactate were obtained from Calbiochem (San Diego, Calif.).

RESULTS

Partial purification of the enzyme. All procedures were carried out at 4 C except Sephadex gel filtration, which was at 7 C.

Step 1, heat treatment. Samples (5 ml) of cell extract were added to preheated Corex tubes (Corning; 15 by 110 mm) and heated at 65 C for 4 min in a water bath. The tubes were quickly cooled in an ice bath and centrifuged at 30,000 × *g* for 30 min.

Step 2, Sephadex gel chromatography. The clear supernatant fluid from step 1 was applied to a Sephadex G-200 column (2.6 by 34 cm) that had been equilibrated with 10 mM potassium phosphate buffer, pH 6.2 (4.3 ml of supernatant fluid was applied to the column for the results summarized in Table 1). The column was eluted with the same buffer, and 5-ml fractions were collected. The activities and protein contents of the fractions were determined, and those fractions with the highest specific activity were pooled (generally in the region of fractions 12 to 17).

Step 3, purification with calcium phosphate gel. Fifty microliters of a calcium phosphate gel suspension in water (49 mg [dry weight]/ml) were added for each 5 ml of pooled Sephadex fraction, and the mixture was held at 4 C for 10 min with occasional gentle mixing. The mixture was then centrifuged (27,000 × *g* for 10 min), the supernatant was removed, and the pellet was washed once with distilled water. The enzyme was extracted from the gel by resuspending the pellet in 0.1 M potassium phosphate buffer, pH 7 (1 ml of buffer for each 5 ml of Sephadex pool); elution was carried out for 10 min with occasional mixing. The mixture was centrifuged, the supernatant fluid was collected, and the extraction was repeated when necessary. Usually the bulk of the specific activity was found in the first extract supernatant fluid, and it was not necessary to pool the supernatant fluids. It should be recognized that different preparations may vary in the amount of gel required for adsorption of the enzyme. The amount can be determined readily by the fractional adsorption method of Ochoa (3). The extract was immediately frozen in small samples and stored at −20 C. Typical results of the

TABLE 1. *Purification of NAD-dependent L(+)-lactate dehydrogenase from A. laidlawii*

Fraction	Purification step	Protein (mg)	Enzyme activity (U)	Specific activity (mU/mg)
	Crude extract	215	5.86	27.2
1	Heat denaturation supernatant	72	4.77	66.2
2	Sephadex G-200 eluate	9.8	3.15	321
3	Calcium phosphate gel eluate	1.75	1.36	777

purification procedure are summarized in Table 1.

Cell extracts could be stored at −20 C for many weeks with no detectable loss in activity. The Sephadex and calcium phosphate-purified preparations were stable at −20 C for several weeks with only slight loss of activity; however, upon thawing, the calcium phosphate-purified enzyme lost approximately one-third of its activity within 5 h at 4 C. This deterioration was not affected by addition of dithioreitol (2 mM), but the gradual loss was reduced to about 12% in 6 h by addition of glycerol (50% final concentration). Consequently, the purified enzyme routinely was diluted with 50% glycerol in distilled water after thawing. Experiments using purified enzyme were performed as rapidly as possible and after preliminary studies with crude preparations had been carried out. However, results were essentially identical whether crude or purified enzyme preparations were used.

Activation of the lactate dehydrogenase by FDP. The activation of the *A. laidlawii* LDH by FDP is shown in Fig. 1. It can be seen that the kinetic response to increasing concentrations of FDP is hyperbolic, that a concentration of FDP as low as 1 μM produces an observable activation, and that a maximal rate is obtained with 35 μM FDP under these assay conditions. Hyperbolic curves similar to the curve in Fig. 1 were observed at other pHs as well (not shown). This hyperbolic response to FDP by the LDH from *A. laidlawii* differs completely from the sigmoidal responses observed with the known FDP-activated LDHs (2, 6, 17, 19). The K_m for FDP of the *A. laidlawii* LDH is approximately 2.4 μM. This K_m value is less than one-tenth of the analogous $M_{0.5}$ values (the concentration of activator required for 50% of its maximal effect [1]) reported for several of the known streptococcal LDHs (2, 17) and is less than the value we estimated for the *S. cremoris* LDH from the data of Jonas et al. (6).

Utilization of FDP. All of the FDP was recovered after oxidation of approximately 0.2 μmol of NADH. We conclude that FDP is not utilized in this reaction and that it serves only as an activator of the lactate dehydrogenase.

Specificity for FDP as the activating ligand. The activation of the LDH appears to be highly specific for FDP. None of the following compounds substitute for FDP when examined in the standard assay system at concentrations of 1 mM: fructose, fructose-1-phosphate, fructose-6-phosphate, glucose, glucose-6-phosphate, ribose, ribose-5-phosphate, glycerol, or glyceraldehyde-3-phosphate. A combination of

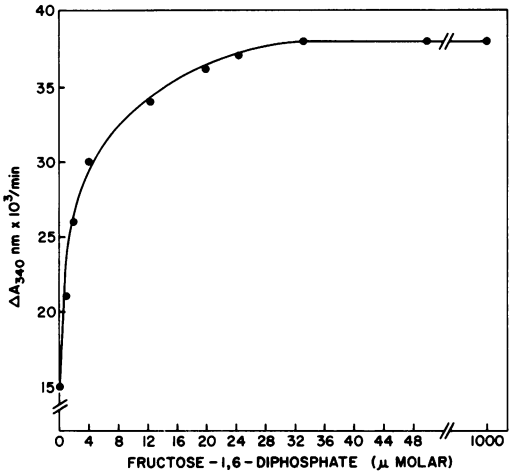

FIG. 1. *Dependence of A. laidlawii LDH activity on FDP. The standard assay was used except that the FDP concentration was varied as indicated. Reactions were initiated by adding 0.84 μg of fraction 3 protein (Table 1).*

fructose-1-phosphate and fructose-6-phosphate (final concentrations of 1 mM each) also failed to activate the enzyme. Inorganic phosphate at concentrations from 1 to 100 mM did not activate the enzyme when substituted for FDP in the standard assay.

Substrate stereospecificity and coenzyme specificity. The enzyme is specific for the L(+)-stereosiomer of lactic acid. As reported previously, only L(+)-lactic acid is formed from pyruvate (11). This was confirmed under conditions, described below, where it was possible to reverse the reaction and obtain oxidation of L(+)-lactate only and not D(−)-lactate.

The purified enzyme is specific for NADH, and no coenzyme activity can be demonstrated when reduced nicotinamide adenine dinucleotide phosphate is substituted for NADH in the standard assay. The rate of NADH oxidation decreases rapidly with increasing concentrations of NADH above 0.2 mM under the standard assay conditions (Fig. 2). (This inhibitory effect can also be seen in the reciprocal plots of NADH activity shown in Fig. 8.)

Effect of pH. The effect of pH on the reaction rate depends markedly on the presence or absence of FDP (Fig. 3). In the presence of FDP, reaction rates are increased in the pH range from 6.5 to 8.0, and the optimal pH is shifted upward. Furthermore, it can be seen that FDP is absolutely required for activity from pH 7.5 to 8.0. At pH 6.0, FDP is slightly inhibitory. MES buffer was utilized so that the effect of pH could be examined in the presence of a single buffer that did not influence reaction rates greatly.

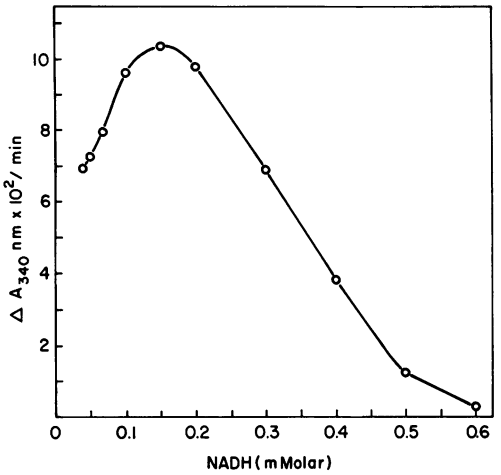

FIG. 2. *Effect of NADH concentration on the rate of the reaction catalyzed by A. laidlawii LDH. The standard assay was used except that NADH was varied as indicated. Reactions were initiated by adding 1.7 μg of fraction 3 protein (Table 1).*

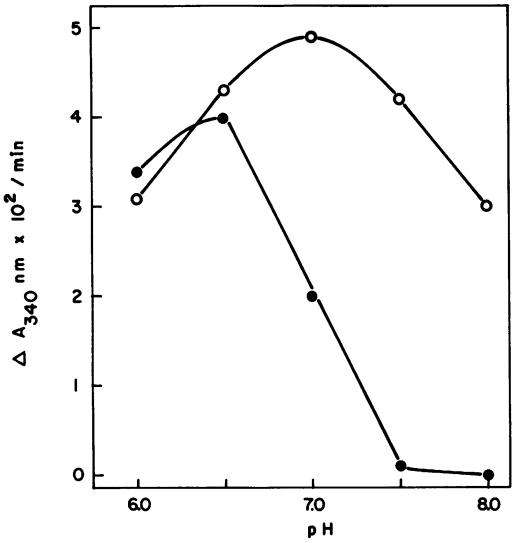

FIG. 3. *Effect of FDP on the pH profile of the reaction catalyzed by A. laidlawii LDH. The standard assay was used except that 100 mM MES-NaOH buffer was substituted for Tris buffer at the pH values indicated and FDP was added as shown. Symbols: ●, no FDP; ○, 1.0 mM FDP. Reactions were initiated by adding 21 μg of crude extract protein.*

The reaction rate in MES buffer at the optimum pH of 7.0 in the presence of FDP (Fig. 3) is approximately 5% less than that obtained in Tris buffer at its optimum of pH 7.2 (not shown).

Effect of pyruvate on activation by FDP. It was found that activation of the enzyme by FDP is a function of pyruvate concentration as well as pH. Pyruvate saturation curves revealed that at low concentrations of pyruvate the reaction rate is very low in the absence of FDP and the activation by FDP is maximal (Fig. 4, curves A and B, scale numbered below the abscissa). After a maximal velocity is reached, inhibition by pyruvate becomes evident both in the presence and absence of FDP. Finally the enzyme becomes insensitive to activation by FDP at pyruvate concentrations of approximately 5 mM and above.

It should be noted that the pyruvate saturation curve obtained in the absence of FDP displays sigmoid kinetic behavior (Fig. 4, curve C, scale numbered above the abscissa) if reaction rates from a range of low concentrations of substrate (from 0.1 mM pyruvate, the lowest concentration at which reaction rates could be measured accurately, to 1.0 mM), are plotted on an appropriately chosen scale, as emphasized by Atkinson (1). The curve for the pyruvate response obtained with FDP is hyperbolic when plotted on either scale (plot omitted). The reaction rates used to plot curve C were obtained with a second, freshly thawed sample of the enzyme preparation to reduce any possible effects of enzyme deterioration on the shape of the curve.

At pH 8, the shape of the pyruvate response

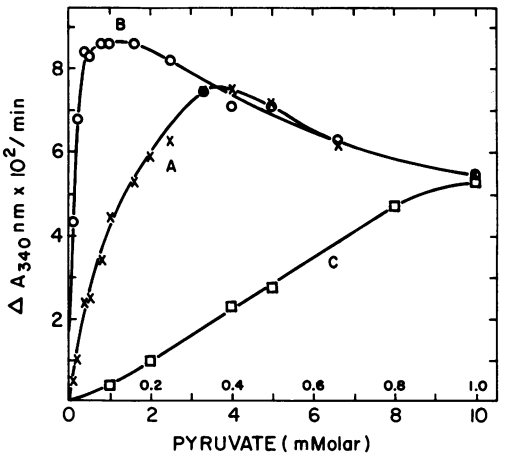

FIG. 4. *Activation of A. laidlawii LDH by FDP as a function of pyruvate concentration. The standard assay was used except that FDP and pyruvate were added as indicated. A, no FDP (×); B, 1.0 mM FDP (○); C, no FDP (□). Reaction rates from a series of reactions with low concentrations of pyruvate were plotted on a separate scale (above abscissa). Reactions were initiated by adding 1.7 μg of fraction 3 protein (Table 1). Reaction rates for curve C were obtained with a second, freshly thawed sample of the enzyme preparation.*

curve obtained in the absence of FDP is clearly sigmoidal, and the conversion by FDP of the kinetic response curve from a sigmoidal shape to a hyperbola is pronounced (Fig. 5). A marked change in the sensitivity of the control of enzyme function also occurs at pH 8. Fructose diphosphate activates over a broader range of pyruvate concentrations, and no inhibition is observed below a concentration of 15 mM pyruvate (slight inhibition which becomes evident in both curves at 20 mM pyruvate is not shown). The sigmoidal pyruvate saturation curve obtained at pH 8 is suggestive of cooperative binding of pyruvate at low H^+ concentration.

At pH 6.0 (MES buffer), the pyruvate response curve is sharply hyperbolic in the presence or absence of FDP (not shown); as already noted (Fig. 3), FDP is slightly inhibitory at this pH.

The activity of the enzyme is evidently influenced by H^+ concentration (Fig. 3) as well as by the concentration of pyruvate, FDP, NADH, and other factors (described below). Comparison of the pyruvate saturation curves (without FDP) obtained at pH 8 (Fig. 5) and pH 7.2 (Fig. 4) shows that increasing H^+ concentration changes the pyruvate saturation curve from sigmoidal to hyperbolic, with a decrease in the concentration of pyruvate required for half maximal velocity (this observation is supported also by a hyperbolic curve at pH 6.0, not shown). This suggests that H^+ influences activity, at least in part, by increasing the affinity of the enzyme for pyruvate. Other manifestations of change in enzyme activity produced by H^+ are onset of inhibition at a lower pyruvate concentration at pH 7.2 (Fig. 4) than at pH 8 (Fig. 5) and the very narrow range of FDP activation at pH 7.2 compared to the broad range at pH 8. Also, at pH 6.0, FDP does not activate, but rather is inhibitory.

During these studies it was observed that reaction rates obtained in Tris buffer at pH 7.2 in the absence of FDP are slightly curved (rates decrease) but become linear when the pyruvate concentration reaches about 5 mM. (The reaction rates for the nonlinear curves were estimated by drawing straight lines through the initial portion of the slightly curved recorder traces.) Interestingly, this shift occurs in the same concentration region where inhibition begins to appear (Fig. 4, curve A). Inclusion of FDP in the reaction mixture results in a linear reaction rate even at the lowest concentrations of pyruvate that produce a measurable reaction. Slightly curved reaction rates are also observed in Tris buffer at pH 8.0 in the absence of FDP, but the effect of pyruvate concentration differs from that seen at pH 7.2; reaction rates are linear at low concentrations (about 0.7 mM and below) where the pyruvate response curve is nearly flat (Fig. 5), but become nonlinear at about 1 mM pyruvate and above. These shifts from linear to nonlinear reaction rates in Tris buffer are dependent on pyruvate and H^+ concentration, but their occurrence cannot be satisfactorily explained at present. Linear rates are obtained with α-ketobutyrate in Tris buffer, and all reaction rates observed in MES or HEPES buffer are linear.

FDP activation with α-ketobutyrate as substrate. The pyruvate analogue, α-ketobutyrate, serves as an effective substrate for the enzyme; when the analogue is utilized, activation by FDP still occurs (Fig. 6). The response

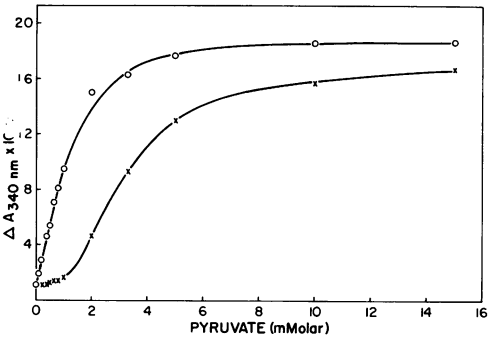

FIG. 5. *Activation of A. laidlawii LDH by FDP as a function of pyruvate concentration at pH 8. The standard assay was modified by using tris buffer at 8; FDP and pyruvate were added as indicated. Symbols: ×, no FDP; O, 1.0 mM FDP. Reactions were initiated by adding 118 μg of crude extract protein.*

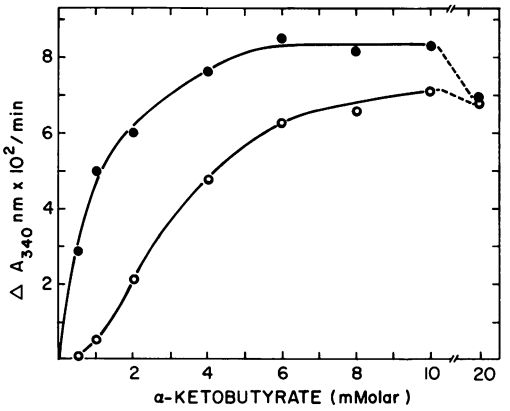

FIG. 6. *Activation of A. laidlawii LDH by FDP as a function of α-ketobutyrate concentration. The standard assay was modified by substituting varying concentrations of α-ketobutyrate for pyruvate. FDP was added as indicated. Symbols: O, no FDP; ●, 1.0 mM FDP. Reactions were initiated by adding 21 μg of crude extract protein.*

curves obtained at pH 7.2 are shifted on the horizontal axis, and the curve obtained without FDP is more clearly sigmoidal than the analogous pyruvate curve obtained at pH 7.2. These results suggest that α-ketobutyrate fits the binding sites for pyruvate but that the enzyme has a lower affinity for α-ketobutyrate. In contrast to the results with pyruvate, reaction rates in the absence of FDP are linear, further suggesting differences in the fit of this substrate to the enzyme site.

Effect of the concentration of FDP on the apparent K_m for pyruvate and NADH. Experiments were performed to determine whether activation by FDP resulted in a change in the apparent affinity of the enzyme for pyruvate, NADH, or both. As shown in Fig. 7 and 8, the apparent Michaelis constants (K_m) for both pyruvate and NADH decrease with increasing concentrations of FDP with no affect on V_{max}. Thus, activation by FDP appears to involve an increase in affinity of the enzyme for both its substrate and cofactor.

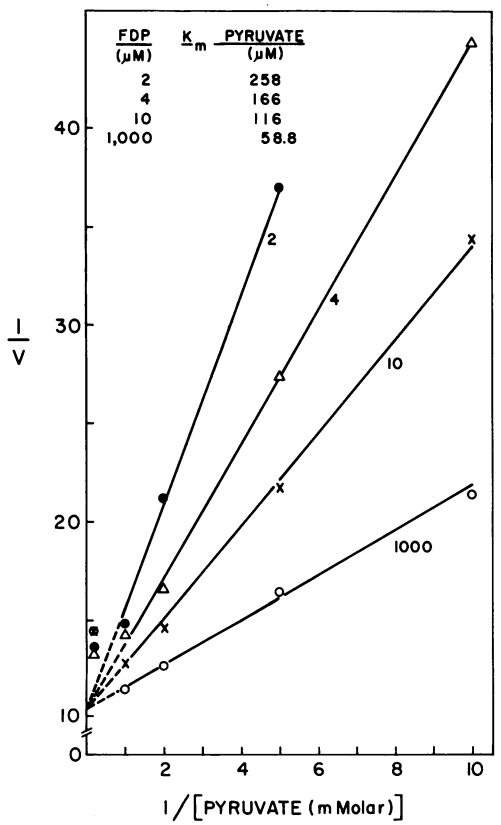

FIG. 7. *Effect of FDP on the K_m of the A. laidlawii LDH for pyruvate. The standard assay was used except pyruvate and FDP were varied as indicated. Numbers below the curves are concentrations of FDP (micromolar). Reactions were initiated by adding 1.7 μg of fraction 3 protein (Table 1).*

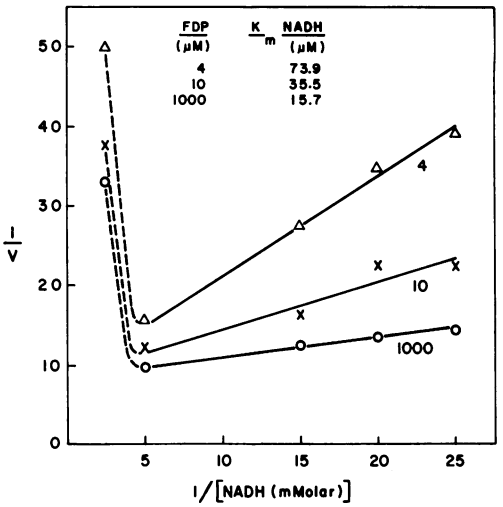

FIG. 8. *Effect of FDP on the K_m of the A. laidlawii LDH for NADH. The standard assay was used except NADH and FDP were varied as indicated. Numbers below the curves are concentrations of FDP (micromolar). Reactions were initiated by adding 1.7 μg of fraction 3 protein (Table 1). Marked inhibition at the highest concentration of NADH is evident.*

Inhibition by ATP. Addition of adenosine triphosphate (ATP) to the standard assay resulted in inhibition. The results illustrated in Fig. 9 show that ATP inhibits LDH activity by competing with NADH.

Effect of inorganic phosphate. As already noted, inorganic phosphate does not substitute for FDP in the standard assay; in fact, 100 mM phosphate is slightly inhibitory. When attempts were made to assay activity in phosphate buffer, no activity, or only extremely low activity, could be demonstrated at pH 7.5 and 8.0. At pH 6.0 and 6.5 in the absence of FDP, activity equal to approximately half that found with the standard assay is obtained. However, no activation by FDP could be demonstrated at any pH between 6.0 and 8.0; indeed FDP was slightly inhibitory at pH 6.0. The effect of inorganic phosphate on the catalytic activity of the enzyme was examined by adding phosphate to the standard assay or to an MES-buffered assay mixture. It was found that addition of increasing concentrations of inorganic phosphate produced a marked inhibition of enzyme activity (Table 2). It can be seen that inhibition requires rather high concentrations of phosphate relative to the substrate and activator concentrations.

Reversal of the reaction. The *A. laidlawii* lactate dehydrogenase is unable to catalyze the

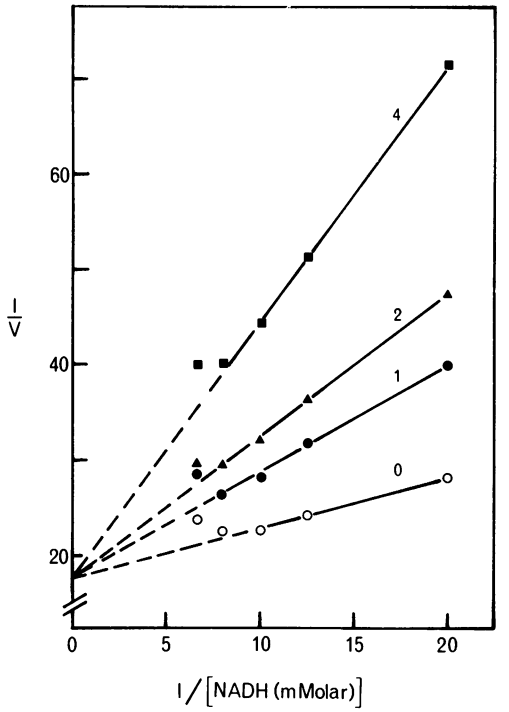

FIG. 9. *Inhibition of A. laidlawii LDH activity by ATP. The standard assay was used except NADH was varied as indicated, and ATP was added. Numbers above the curves are concentrations of ATP (millimolar). Reactions were initiated by adding 160 μg of crude extract protein.*

oxidation of lactate to pyruvate with NAD under conditions where this reaction can usually occur (11). Assay in the presence of hydrazine at alkaline pH (0.1 M glycine-hydrazine buffer, pH 9.2) also failed to provide conditions for lactate oxidation. Reversal can be demonstrated by utilizing APNAD as the cofactor (7) and providing a pH of 8 (Table 3). Under these conditions L(+)-lactate is oxidized, but the reaction is slow in comparison to pyruvate reduction with NADH, and a considerably greater enzyme concentration is required to obtain a measurable rate. FDP (1 mM) very slightly increases the rate of the reverse reaction with APNAD.

DISCUSSION

Wolin discovered that LDHs from several streptococci specifically require FDP for activity and suggested that FDP might be an allosteric activator for these enzymes (19). Fructose diphosphate-activated LDHs have now been found in two lactobacilli (15, 16) as well as in all 10 of the species of streptococci that have been examined (2, 6, 17). The LDHs from *L. bifidus*

TABLE 2. *Inhibition of the A. laidlawii A LDH by inorganic phosphate*[a]

Phosphate concn (mM)	Inhibition (%) in:	
	Tris-hydrochloride buffer[b]	MES buffer[c]
0	0	0
5	13	ND[d]
10	17	ND
25	54	ND
50	74	71

[a] Dibasic sodium phosphate was added at the concentrations shown to the standard tris-hydrochloride-buffered assay or to an MES-buffered assay mixture. The pH of the phosphate solutions was adjusted with 1 N HCl.
[b] pH 7.2.
[c] pH 7.0.
[d] Not done.

TABLE 3. *Lactate oxidation with the 3-acetyl-pyridine analogue of NAD*[a]

Substrate	Cofactor	Specific activity[b]
L(+)-Lactate	APNAD	420
D(−)-Lactate	APNAD	0
L(+)-Lactate plus D(−)-lactate	APNAD	402
L(+)-Lactate	NAD	0
Pyruvate	NADH	1032

[a] See Materials and Methods for descriptions of the assay conditions.
[b] Specific activity is defined as micromoles of APNAD reduced or NADH oxidized per minute per milligram of fraction 2 protein.

(16) and *L. casei* (15) require Mn^{2+}, in addition to FDP, for activation and thus differ from both the streptococcal and the *A. laidlawii* FDP-activated LDHs.

The kinetic properties of several of the streptococcal enzymes have been studied in detail (2, 5, 6, 17). All of the FDP-activated LDHs are similar in being specific for L(+)-lactate and NADH, but the streptococcal LDHs may differ from one another in specific properties such as concentration of FDP required for maximal activation, kinetic response to pyruvate, occurrence of activity in the absence of FDP, inhibition by inorganic phosphate or ATP, optimal pH, ability to oxidize lactate, and effect of activator, substrates, and inhibitors on heat inactivation of the enzyme.

Our results show that the FDP-activated L(+)-LDH from *A. laidlawii* generally resembles the LDHs from streptococci and that it possesses a combination of unusual catalytic

properties: a kinetic response to pyruvate that is influenced by H^+ even in the absence of FDP, a hyperbolic kinetic response to increasing concentrations of FDP, and the ability to utilize α-ketobutyrate as a substrate. The affinity for pyruvate of the *A. laidlawii* LDH appears to be influenced both by FDP and H^+ concentration, but whether a relationship exists between the activity of FDP and H^+ is not yet clear.

The activity of the *A. laidlawii* LDH is influenced by the concentrations of pyruvate, FDP, and H^+. This enzyme displays a sigmoidal pyruvate saturation curve only at high pH in the absence of FDP; upon addition of FDP or with an increase in H^+ concentration its kinetic response becomes hyperbolic. In contrast, the D(−)-LDHs from *Escherichia coli* (13, 14) and *Butyribacterium rettgeri* (18) exhibit sigmoidal pyruvate saturation curves over a range of pH values; these LDHs appear to be allosteric enzymes with pyruvate acting at an activator site distinct from the catalytic site for pyruvate. The activities of these D(−)-LDHs appear to be controlled by the concentrations of pyruvate and H^+ (14, 18). Recently, Brown and Wittenberger described similar sigmoidal pyruvate saturation kinetics for the FDP-activated LDH from *S. mutans* (2). The *S. mutans* LDH is under even more stringent metabolic control than are the D(−)-specific LDHs because of additional regulation by FDP. The LDH from *S. mutans* was the first microbial L(+)-specific enzyme described as having a sigmoidal pyruvate rate curve; the *A. laidlawii* L(+)-LDH as this feature only under the specific conditions described above. Mammalian muscle L(+)-LDH isozyme 5 also displays a sigmoidal pyruvate saturation curve, but this LDH is activated by several citric acid cycle intermediates (4).

The *A. laidlawii* LDH is unusual in being able to utilize α-ketobutyrate as a substrate and in being activated by FDP even when utilizing the analogue. In contrast, the *E. coli* (14), *B. rettgeri* (18), and *S. mutans* (2) LDHs cannot utilize α-ketobutyrate as a substrate; rather, this analogue appears to act as an allosteric activator of these LDHs, changing the pyruvate saturation curve from sigmoidal to hyperbolic, except that with the *B. rettgeri* LDH the analogue is inhibitory near saturating substrate concentrations (18).

The *A. laidlawii* and the three bacterial LDHs described are generally similar in that they catalyze the essentially unidirectional reduction of pyruvate with NADH and are inhibited by ATP. Considered together, they illustrate gradations of metabolic control that can operate on the pyruvate catalytic site.

The FDP-activated LDHs from *A. laidlawii* and the streptococci share one or more specific characters. For example, the enzymes from *A. laidlawii*, *S. mutans* (2), and *S. cremoris* (6) are all inhibited by ATP, and in common with *S. bovis* 444 (19) and *S. cremoris* (6), the *A. laidlawii* enzyme can display some activity in the absence of FDP. The *A. laidlawii* LDH is unstable during various purification procedures and shares this property with *S. bovis* LDH; both enzymes are also similar in catalyzing the essentially unidirectional reduction of pyruvate (19). At first the *A. laidlawii* LDH was considered unique in being inhibited by inorganic phosphate, but recently this property was found to be characteristic of the enzyme from *S. cremoris* (6). Although the *A. laidlawii* and *S. cremoris* LDHs share some properties, they differ in others, for example, in ability to oxidize lactate and in optimum pH.

A property of the *A. laidlawii* LDH not yet described for any of the streptococcal LDHs is the marked inhibition of activity by high concentrations of NADH. What may prove to be a most significant difference between the *A. laidlawii* enzyme and the known streptococcal LDHs is the kinetic response to increasing amounts of FDP. The kinetic response of the *A. laidlawii* enzyme to FDP is hyperbolic. The LDHs from at least two other acholeplasmas are also activated by FDP; one produces a hyperbolic response to FDP while the other appears to have a sigmoidal response to FDP (Neimark, unpublished results). In contrast, a sigmoidal response to FDP is displayed by all of the streptococcal FDP-activated LDHs that have been studied (2, 6, 17, 19). The sigmoidal response to the activator was interpreted as indication that the streptococcal LDHs may have two or more cooperative binding sites for FDP (1, 2, 17). It is possible that streptococcal LDHs with a hyperbolic response to FDP remain to be discovered, since members of only a few streptococcal groups have been examined. As already mentioned, variation in the kinetic response to pyruvate has been found among streptococci, where enzymes from various groups appear to have either one or at least two cooperative binding sites for pyruvate (17).

The *A. laidlawii* LDH appears to function essentially as a pyruvate reductase; its activity is influenced by FDP, pyruvate H^+ and NADH concentration and is inhibited by ATP and inorganic phosphate. These properties suggest that this FDP-activated LDH, by controlling pyruvate metabolism, has an important role in regulating the carbohydrate metabolism of *A. laidlawii*.

It remains to be established whether the occurrence of similar regulatory LDHs among the acholeplasmas and the *Lactobacillaceae* results from convergent evolution or a true phylogenetic relationship.

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