INTERACTING BINDING SITES OF L-SPECIFIC LACTIC DEHYDROGENASE OF ESCHERICHIA COLI

E. M. Tarmy and N. O. Kaplan

From the Graduate Department of Biochemistry,
Brandeis University, Waltham, Massachusetts
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We have purified a DPNH dependent lactic dehydrogenase (LDH) from Escherichia coli B that differs markedly from the animal LDH's in many of its chemical and catalytic properties. In this communication we present evidence that this protein, which has an unusually high requirement for pyruvate, is a pyridine nucleotide-linked enzyme. We also describe some unanticipated kinetic properties.

Materials and Methods -- The details of the purification of this LDH will be published elsewhere. The enzyme was maintained in an active form in solutions containing 0.01 M $K\text{-}PO_4$ buffer, pH 7.5, plus 0.1 M 2-mercaptoethanol at 4°. The preparations used in these studies were free of DPNH oxidase and D- and L- lactate oxidases. The latter are probably flavoproteins (1).

The assay for enzyme activity was a modification of the animal LDH reaction mixture (2), in which the concentrations of DPNH and pyruvate were increased to 1 and 30 mM, respectively.

The stoichiometry of the reaction was determined by measuring DPNH oxidation spectrophotometrically and lactate production by the method of Barker and Summerson (3). The stereospecificity with regard to lactate was shown as described elsewhere (4).

The reverse of the physiological reaction was demonstrated in a reaction mixture containing 0.3 M Li D,L-lactate, 0.5 mM DPN and 0.1 M $K\text{-}PO_4$ buffer, pH 7.5, in 0.9 ml. The reaction was initiated by the addition of 0.1 ml E. coli LDH. Reduction of DPN at 340 mμ was followed.

A Cary Model 14 recording spectrophotometer was used for the kinetic studies. Because dilutions of the enzyme into 0.1 M $K\text{-}PO_4$ buffer, pH 7.5, were stable, it was possible to vary the pyruvate concentration over a wide range from 0.067 to 33 mM. Experiments were performed at two levels of DPNH and several pH's. The ionic strength was constant throughout these studies.

Results and Discussion -- Because of the apparently very low affinity of this LDH for its substrates, it was considered important to demonstrate that this enzyme catalyzes the following reaction:

$$\text{pyruvate} + \text{DPNH} + H^+ \rightleftharpoons \text{lactate} + \text{DPN}.$$

There was a stoichiometric correspondence between the rate of DPNH oxidation and that of lactate production for 10 min at 25°, demonstrating that the reduction catalyzed by the enzyme occurs as presented in the equation. Only the L isomer of lactate is formed, in agreement with the observation that L-lactate is excreted into the medium of growing cells.

The reversal of this reaction is exceedingly slow and difficult to demonstrate. A highly purified protein solution, which catalyzed the reduction of pyruvate at a rate of 300 μmoles per min under optimal conditions, was used. The L-lactate was oxidized at a rate of 20 mμmoles per min, which is less than 0.01% that of pyruvate reduction. In contrast, animal LDH's generally have a rate of lactate oxidation on the order of 10

to 20% that of pyruvate reduction (5).

As part of the comparison of this LDH with LDH's from other sources, we began kinetic studies to determine the K_m's for pyruvate and DPNH . We found that it has not been possible to determine these K_m's, because the enzyme does not follow Michaelis-Menten kinetics. With relatively low concentrations of pyruvate, plots of V vs. S are reproducibly sigmoidal, as illustrated in Fig. 1. Curves similar to the curve in Fig. 1 are obtained at other pH's and at a lower concentration of DPNH. Such kinetic behavior has not been observed for other LDH's (6). It

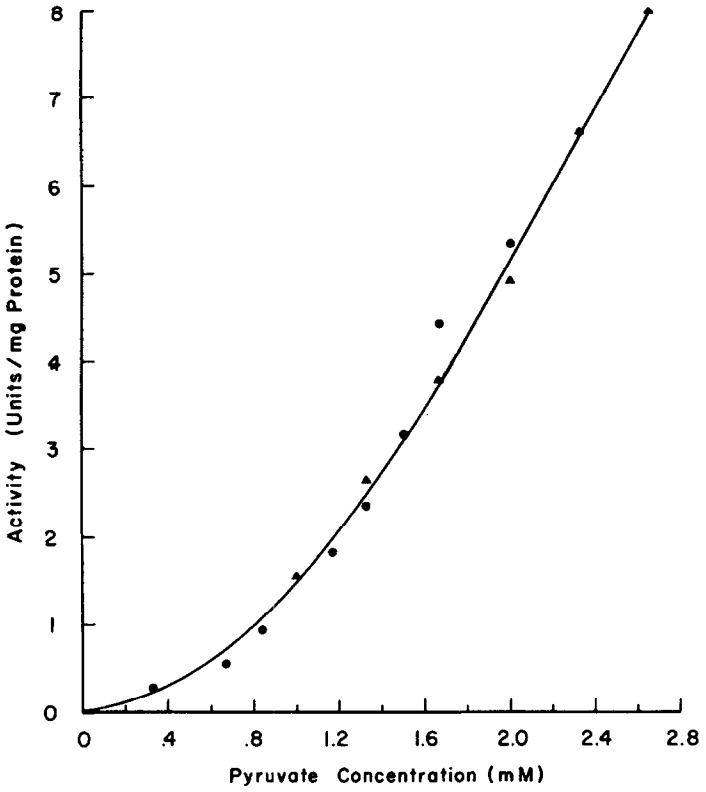

Fig. 1. Plot of LDH activity as a function of pyruvate concentration at pH 7.5, a DPNH concentration of 1 mM, and ionic strength 0.24 M. Under these conditions the apparent V_{max} is 29 units per mg protein and the enzyme is saturated at 20 mM pyruvate. The symbols represent experiments with two different dilutions of the enzymes.

is noteworthy that this enzyme is saturated at a pyruvate concentration 10 to 100 times higher than that necessary for animal LDH's (7).

The apparent order of the reaction with respect to pyruvate, n, was determined from Hill plots (8). The n values obtained were 2.0, 2.1, and 1.7 at pH's 7.5, 6.7, and 6.4, respectively. These data indicate that there are at least two interacting binding sites for pyruvate on the enzyme.

Since a number of proteins known to be controlled by unrelated metabolites or allosteric effectors display kinetic behavior analogous to that observed with this enzyme, it is reasonable to assume that some physiological compound might act as activator of E. coli LDH. A number of such reagents, including glycolytic intermediates, adenosine nucleotides, and cations, were added to the assay mixture or preincubated with the enzyme. However, none activates the enzyme.

The finding by Wolin (9) that fructose-1,6-diphosphate (F16P) activates LDH's from several species of Streptococcus is interesting in this regard. Wolin proposed that the level of F16P may regulate the activity of Streptococcus LDH. From our results it is clear that this control is not operative in E. coli because there is no F16P activation although, in the light of our kinetic findings, some metabolic control is possible.

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