EFFECT OF ENZYME CONCENTRATION ON THE KINETICS OF D-LACTATE DEHYDROGENASE FROM AEROBACTER AEROGENES

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SUMMARY

D-Lactate dehydrogenase of Aerobacter aerogenes deviated from Michaelis-Menten kinetics with respect to pyruvate at low enzyme concentrations, those used for normal spectrophotometric determination of activity. In a stopped flow spectrophotometric study the kinetics became Michaelis-Menten type when the enzyme concentration was raised to a level approaching the concentration in the cells.

INTRODUCTION

Kinetic studies of enzymes are limited by the type of equipment used for the measurement of activities. Spectrophotometers are used for the determination of enzyme activities including those of dehydrogenases. The use of standard spectrophotometers automatically places restriction on the upper and lower limits of enzyme concentration as well as substrate concentration.

Srere (1) pointed out that many enzymes are present in animal tissues and mitochondria at concentrations thousands fold higher than those used for normal in vitro study conditions and therefore may have properties different from those observed in vitro.

Wuntch, et al. (2) reported that substrate inhibition of mammalian lactate dehydrogenase observed at low enzyme concentrations disappeared when the enzyme concentration was raised to the level existing in the cells.

We have studied the kinetic properties of lactate dehydrogenase from Aerobacter aerogenes in a standard spectrophotometer as well as in a stopped flow one and have found that the non-linear Lineweaver-Burk

plots of velocity versus pyruvate concentration became linear when the enzyme concentration was raised above a critical level.

MATERIALS AND METHODS

A laboratory strain of Aerobacter aerogenes was grown in 3 l Fernbach flasks containing 2 l of medium consisting of 1.0% glucose, 0.3% proteose peptone and 0.8% K_2HPO_4. The flasks were incubated for 18 hours at 28°C without shaking.

D-Lactate dehydrogenase was purified approximately 400 fold following procedures similar to those used for the Escherichia coli enzyme (3). Details of the purification procedures and properties of the enzyme will be published elsewhere.

Reaction velocities at low enzyme concentrations were determined in a Unicam SP-700 spectrophotometer by measuring the rate of NADH oxidation at 340 mμ. The standard reaction mixture contained in a final volume of 3.0 ml, 0.1 M potassium phosphate buffer of pH 5.7, 5.0 mM potassium pyruvate, 0.15 mM NADH and enzyme. The reaction was initiated by the addition of enzyme and the absorbancy change at 340 mμ was followed at room temperature in silica cuvettes of 1.0 cm light path. One unit of enzyme was defined as the amount of enzyme that oxidized 1 μmole of NADH per minute.

For the determination of velocities at high enzyme concentrations a Durrum-Gibson stopped flow spectrophotometer was used. The reaction was initiated by mixing a solution of pyruvate and NADH with an enzyme solution. Both solutions were prepared in 0.1 M potassium phosphate (pH 7.0). The reaction cell held 0.15 ml of the mixture and had a light path of 2 cm. The change in transmittancy at 340 mμ was recorded on a Tektronix storage oscilloscope with an attached camera and was converted to absorbancy change.

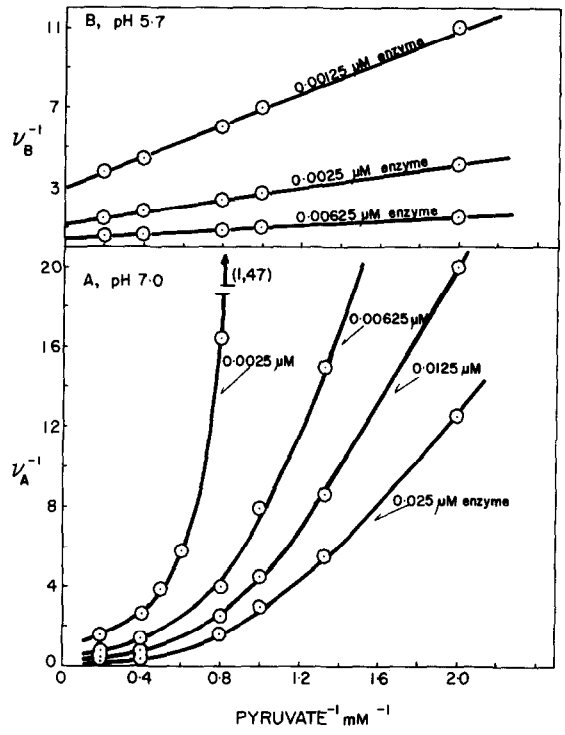

Fig. 1. Effect of pyruvate concentration on D-lactate dehydrogenase of A. aerogenes at different enzyme concentrations. Reaction velocities (V_A at pH 7.0 and V_B at pH 5.7) were determined as absorbancy change at 340 mμ per minute in a Unicam SP-700 spectrophotometer (1.0 cm light path). The reaction mixture contained in a total volume of 3.0 ml: 0.1 M potassium phosphate buffer (A: pH 7.0 and B: pH 5.7), potassium pyruvate, 0.15 mM NADH and enzyme.

RESULTS AND DISCUSSION

The D-lactate dehydrogenase from A. aerogenes was similar to those from E. coli (4) and Butyribacterium rettgeri (5) in many properties.

In acid pH ranges (pH 5.2 and 5.7) the enzyme activity showed a normal Michaelis-Menten type kinetics with respect to pyruvate concentration, while at a pH value of 7.0 or higher the activity-pyruvate plots deviated from the normal kinetics resulting in curved Lineweaver-Burk plots (Fig. 1). The activity-NADH plots were always linear in the double reciprocal form.

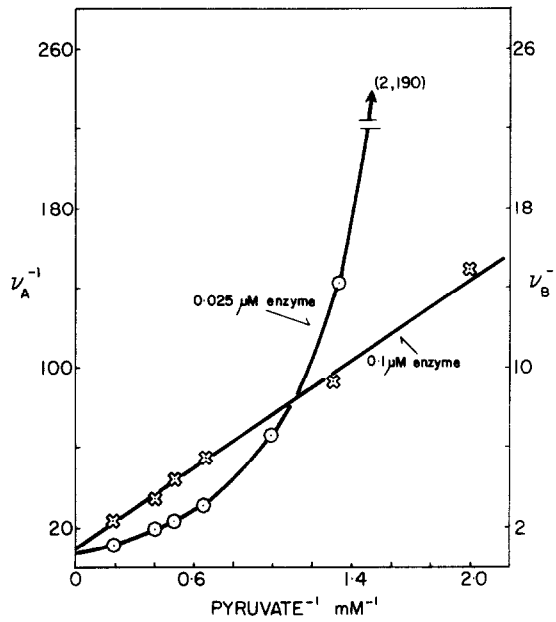

Fig. 2. Effect of high enzyme concentration on the Lineweaver-Burk plot of velocity versus pyruvate concentration. Reaction velocities (V_A with 0.025 μM enzyme and V_B with 0.1 μM enzyme) were determined in a Durrum-Gibson stopped flow spectrophotometer at 340 mμ as described in Materials and Methods and expressed as absorbancy change per second. NADH, 0.15 mM; pH, 7.0 (0.1 M potassium phosphate); enzyme and pyruvate, as indicated.

When the enzyme concentration was raised the double reciprocal activity-pyruvate plot became increasingly linear (Fig. 1) and finally at the enzyme concentration of 0.1 μM using a stopped flow spectrophotometer, the plot became perfectly linear (Fig. 2). Further increase in the enzyme concentration by 10 fold did not affect the nature of the Michaelis-Menten type kinetics.

Under the conditions of our experiments there was no initial lag of reaction when studied in the stopped flow spectrophotometer (Fig. 3) in contrast with the E. coli enzyme (4).

The purified D-lactate dehydrogenase from A. aerogenes has a specific activity of 1000 units/mg protein. Since 1,000 g

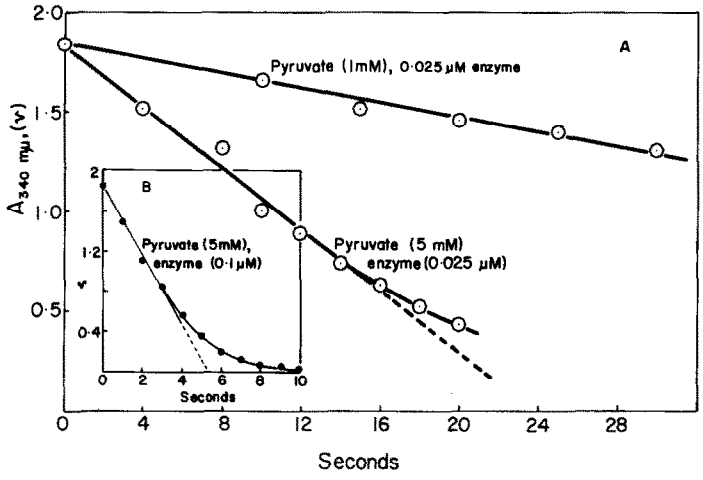

Fig. 3. Linearity of lactate dehydrogenase reaction with time in stopped flow experiments. The conditions were the same as in Fig. 2.

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packed wet cells, after sonication, released 31,500 units of the enzyme, the concentration of enzyme in A. aerogenes cells is higher than 31.5 mg/l, the value calculated assuming that 1,000 g cells occupy 1 l of volume. The molecular weight of enzyme determined by sucrose gradient centrifugation was 110∿140,000. The minimum concentration of enzyme in the cells is calculated as $31.5 \times 10^{-3}/140{,}000$, i.e., 2.25×10^{-7} M or 0.225 μM.

It is therefore concluded that the lactate dehydrogenase of A. aerogenes shows normal Michaelis-Menten kinetics with respect to pyruvate when the enzyme concentration used approaches the concentration in the cells.

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