SALT-INDUCED ALTERATION OF D(-) LACTATE DEHYDROGENASE FROM POLYSPHONDYLIUM PALLIDUM

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Two types of pyridine nucleotide-linked L(+) lactate dehydrogenases (LDH's) have been found in animal tissues. Their somatic distribution has been correlated with the age of the animal (Cahn et al., 1962; Markert and Ursprung, 1962) and the function of the tissue (Dawson et al., 1964). The enzyme designated "H" (heart) type exhibits marked substrate inhibition at pyruvate concentrations greater than 3×10^{-4} M, whereas the "M" (muscle) type is only slightly inhibited by 1×10^{-2} M pyruvate. Although related, these two proteins are distinct as shown by a number of criteria (Pesce et al., 1964).

In this note, we are reporting a DPN^+-linked LDH, specific for D(-) lactate, from the cellular slime mold Polysphondylium pallidum, which can be induced to lose its native characteristic of substrate inhibition upon incubation in appropriate salt solutions.

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Materials and Methods--Vegetative cells of P. pallidum were grown in axenic medium (Sussman, 1963) to a density of 1-2 X 10^7 cells/ml. After harvesting by centrifugation at 240 g for 10 minutes, they were resuspended in dilute salt solution (10 mM NaCl, 10 mM KCl and 3 mM $CaCl_2$) (Bonner, 1947) and recentrifuged. After decantation the cells in the pellet were ruptured by freezing and thawing and the supernatant solution diluted one to four in 0.01 M triethanolamine-HCl buffer (pH 6.8). The protein concentration of this solution was found to be 5 mg/ml by the method of Lowry et al. (1951). Enzyme activity was measured at 25° by following the decrease in the 340 mμ absorption band of DPNH in a Zeiss spectrophotometer. The assay buffer was 3 ml of 0.1 M triethanolamine-HCl buffer (pH 6.8) and the initial DPNH concentration 1.5 mM. All salts used in this study were of reagent grade. $NaClO_4$ was obtained by neutralizing concentrated perchloric acid with sodium hydroxide. Sodium citrate solution was brought to neutrality with citric acid.

Results--In attempting an ammonium sulfate fractionation of the crude extract it was found that incubation in 30% saturated ammonium sulfate resulted in the loss of the phenomenon of substrate inhibition. Upon prolonged incubation at 4° the enzyme exhibited further changes in its catalytic properties--first, an increase in K_m and later, a lowering of the observed V_{max} together with a further increase in K_m. The degree of reversibility upon dialysis depended on the length of time of incubation. Form II could always be converted back to I, the native type. These changes are summarized in Fig. 1.

The effectiveness of different salts in promoting these changes was examined by making a one to ten dilution of the enzyme in a 0.1 M (final concentration) salt solution and incubating

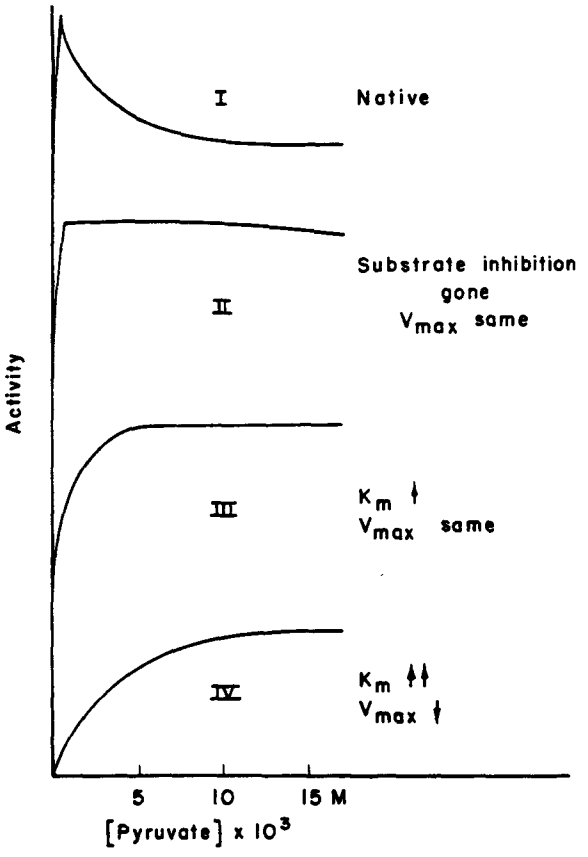

Fig. 1. P. pallidum LDH after incubation in 30% saturated $(NH_4)_2SO_4$. The top curve shows the kinetics observed with the native enzyme; II, one day after the solution was made 30% saturated with $(NH_4)_2SO_4$; III, after 7 days; and IV, after 12 days. There was some variation in the time course with different experiments, but this sequence was always observed.

for 5 minutes at room temperature. The state of the enzyme in regard to its catalytic properties is expressed by giving the ratio of the initial reaction velocity at 3×10^{-4} M to that at 1×10^{-2} M pyruvate (Table I). The effectiveness of the salts was found to follow the lyotropic series but only within each valence group. In contrast to the salting-in effects of anions in that series, in these experiments divalent anions were more effect-

Table I. Effectiveness of Different Salts in Altering *P. pallidum* LDH

The enzyme was dialyzed for 12 hr against 0.01 M triethanolamine-HCl buffer (pH 6.8), diluted 1:10 in salt solution and incubated for 5 min at 25°. 0.05 ml of this mixture was assayed. The final concentration of added salt in the cuvet was 1.7 mM. The control was diluted with water. The rate of reaction is given as the $(\Delta O.D._{340\ m\mu}/min) \times 10^3$

Salt Conc. (M)		Rate Pyruvate Conc. 3×10^{-4} M	Rate Pyruvate Conc. 1×10^{-2} M	Ratio	pH of Incubation Mixture
Control		26	6	4.3	6.6
NaF	0.1	23.5	8	2.9	6.9
NaCl	.1	23.5	9.5	2.5	6.5
NaBr	.1	25.5	11	2.3	6.3
NaI	.1	20.5	16	1.5	6.4
Na_2SO_4	.1	21.5	15.5	1.4	6.8
$Na\text{-}PO_4$	.1	20.5	16	1.3	7.0
NaSCN	.1	21.5	20	1.1	7.0
$NaClO_4$	.1	22	22	1.0	7.0
Na_3citrate	.1	17	21.5	0.9	7.0

ive than monovalent ones and trivalent citrate was the most effective of all. This suggests that in this case charge effects are superimposed on the salting-in effects that have been associated with the binding of anions to the peptide bond (Robinson and Jencks,1963). Further experiments showed that these effects increased with either higher salt concentrations or more dilute enzyme preparations. Substitution of one cation for another did not affect the ratios as long as the anion concentration remained constant. Those cations tested were Li^+, Na^+, K^+, NH_4^+, Cs^+, Mg^{++}, Ca^{++}, and Ba^{++}. A control experiment indicated that the amount of salt transferred from the incubation to the reaction mixture

did not affect the reaction itself, i.e., the native enzyme retained its catalytic properties when assayed in buffer containing the amount of salt transferred from any of the reaction mixtures.

It was found, however, that after salt treatment the enzyme diluted in the assay mixture began gradually to recover its native properties in the course of the reaction. Dilution of the enzyme in the assay buffer alone for an equivalent period of time did not reverse the alteration until substrates were added (Fig. 2). Neither pyruvate nor DPNH alone could reverse the alteration. DPN^+ and/or lactate were also ineffective.

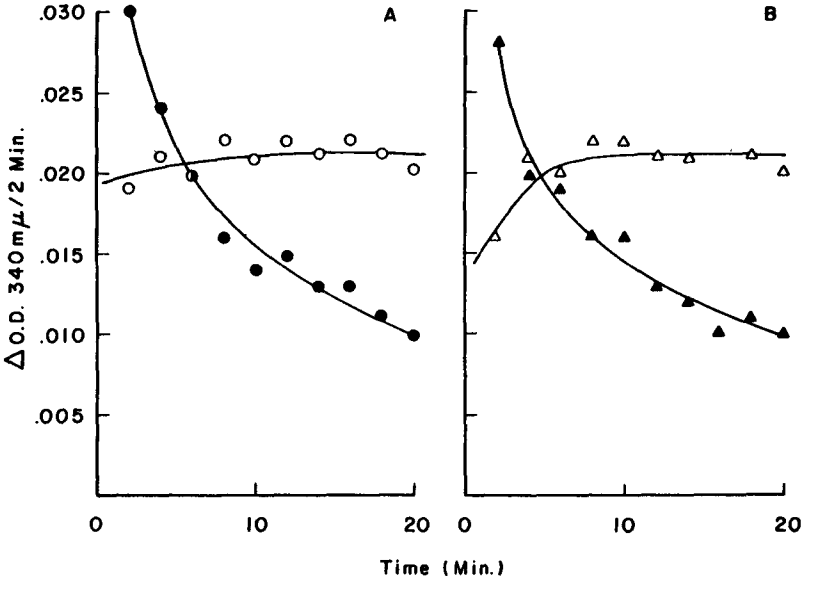

Fig. 2. Change of catalytic behavior of salt-treated P. pallidum LDH during assay. A. Partial recovery of native properties during assay under standard conditions. ○: 3×10^{-4} M Na pyruvate; ●: 1×10^{-3} M Na pyruvate. The incubation mixture was a one-to-ten dilution of enzyme in 0.4 M NaSCN. 0.05 ml of this mixture was used in the assay. B. Partial recovery of native properties after 20-minute incubation in assay buffer following salt treatment as in A. Abscissa indicates time from adding of substrates. The lower initial rate with 3×10^{-4} M pyruvate is caused by the additional dilution of the enzyme during the 20-minute incubation in buffer. △: 3×10^{-4} M Na pyruvate; ▲: 1×10^{-2} M Na pyruvate.

An additional observation is worth noting. Although the observed V_{max} of the altered enzyme(II) is the same as that of the native enzyme(I) for the reaction between pyruvate and DPNH, the observed V_{max} for the reverse reaction between DPN^+ and lactate is reduced more than half after alteration.

At this time we cannot say whether substrate binding induces the native conformation of the enzyme or stabilizes this form in an equilibrium mixture. We did find, however, that 3×10^{-4} M sodium oxamate (a competitive inhibitor of pyruvate reduction by this enzyme) and 1.5 mM DPNH together protected the enzyme against alteration by a 5-minute incubation in 0.1 M $(NH_4)_2SO_4$ (Table II).

Table II. Protection of Native Properties of P. pallidum LDH by DPNH and Oxamate

The conditions were the same as for Table I. The incubation mixture was 0.1 M in $(NH_4)_2SO_4$. The concentration of DPNH in the incubation mixtures was 1.5 mM and of Na oxamate, 3×10^{-4} M.

Treatment	Ratio
Control	3.7
$(NH_4)_2SO_4$	1.0
$(NH_4)_2SO_4$ + DPNH and oxamate	3.8
$(NH_4)_2SO_4$ + DPNH	1.0
$(NH_4)_2SO_4$ + oxamate	1.0

In conclusion, we have found that exposure to certain salt solutions can cause this D(-) LDH to lose its property of being inhibited by high pyruvate concentrations. This effect is antagonized by the substrates pyruvate and DPNH acting in concert. It is hoped that further exploration of this phenomenon will reveal the basic mechanisms of both the alteration and the protection by substrates.

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