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EFFECT OF TEMPERATURE UPON INHIBITION BY SUBSTRATE OF LACTATE DEHYDROGENASE ISOENZYMES FROM A POIKILOTHERM

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SUMMARY

The effect of temperature upon the inhibition by substrate (pyruvate and lactate) and the values of K_I for NAD^+ and pyruvate have been studied comparatively on lactate dehydrogenase (L-lactate : NAD^+ oxidoreductase, EC 1.1.1.27) isoenzymes purified from tissues of a poikilotherm (the snake *Bothrops neuwiedii*) and of a homeotherm (Ox).

Inhibition of the ophidian isoenzyme B_4 by high concentrations of pyruvate is suppressed at the upper limits of the temperature of the habitat. The data presented suggest that the temperature increment affects only the forces binding the pyruvate in the abortive ternary complex Enzyme–NAD^+–pyruvate.

INTRODUCTION

The effect of thermal changes upon the kinetic properties of the enzymes from poikilothermic organisms has been studied in several laboratories during the last five years [1–7]. The findings demonstrate some mechanisms through which biological adaptation to environmental conditions can be attained. Most observations indicate that maximal enzyme–substrate affinity [1–5] or allosteric properties [6] are exhibited at the temperature of the animal's habitat.

In a study of the effect of temperature upon regulatory enzymes from poikilotherms, we analyzed the phenomenon of substrate inhibition of lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase, EC 1.1.1.27) isoenzymes from a snake.

Everse and Kaplan [8] have recently proposed that lactate dehydrogenase isoenzyme 1 (B_4 or H_4) is a regulatory enzyme of a special type. It is regulated by its own oxidized substrate through the formation of an abortive ternary complex (enzyme–NAD^+–pyruvate). Most of the isoenzyme B_4 present in aerobic tissues would, under normal conditions, be unable to reduce pyruvate, which remains available for oxidation in the Krebs cycle.

We have studied comparatively the effect of temperature upon substrate inhibition of lactate dehydrogenase isoenzymes purified from tissues of a poikilotherm (*Bothrops neuwiedii*) and of a homeotherm (ox).

METHODS

Lactate dehydrogenase isoenzymes 1 (B_4) and 5 (A_4 or M_4) were isolated from heart and skeletal muscle of the snake *Bothrops neuwiedii* as previously described [7]. Bovine isoenzymes were purchased from Sigma (U.S.A.).

Enzyme assay

Determination of the enzymatic activity was performed by the method of Wróblewski and LaDue [9] for the direct reaction, and with the procedure described by Markert and Ursprung [10] for the reverse reaction. The temperature of the cuvette compartment was controlled with a water circulator. The reagent mixture was incubated in a water bath at the temperature of the assay for 15 min prior to starting the reaction by addition of the coenzyme.

Determination of K_i values

In order to determine the values of K_i for NAD^+ and pyruvate, formation of the abortive ternary complex was promoted by incubating isoenzyme B_4, NAD^+ and pyruvate for 30 min (the time it takes the complex to reach equilibrium [8]) at 10 °C or 35 °C. After that time, either NADH or L-lactate was added, and the velocity of the reaction recorded at the temperature of incubation.

The mixtures for the determinations of K_i for NAD^+ contained isoenzyme B_4, 0.2 mM sodium pyruvate, 0.1 M sodium phosphate buffer, pH 7.4, and the following final concentrations of NAD^+: 0, 0.02, 0.04, 0.08, 0.125, 0.250, 0.50 and 1.0 mM. The reaction was started by the addition of 0.075 and 0.15 mM NADH.

For the determinations of K_i for pyruvate, the enzyme was incubated with 1.5 mM NAD, 10 mM Tris buffer, pH 9.0, and 0, 0.025, 0.05, 0.075 and 0.10 mM sodium pyruvate, and the reaction was started with 10 and 40 mM L-lactate (lithium salt).

Values of K_i were calculated from Dixon's plots.

RESULTS

Inhibition by pyruvate

Fig. 1 represents curves obtained by plotting the percentage enzymatic activity against pyruvate concentration. The results at 10 °C and 35 °C indicate significant differences between the behavior of bovine and ophidian enzymes. The temperatures of 10 °C and 35 °C were chosen because these are, respectively, the mean lowest and highest temperatures usually recorded in the area in which *Bothrops neuwiedii* inhabits.

The curves for bovine isoenzymes follow the pattern repeatedly demonstrated by many authors for mammalian lactate dehydrogenases [11, 12]. At 35 °C, the differences between A_4 and B_4 isoenzymes are significant. At a temperature close to the physiological, the two extreme isoenzymes from a homeotherm show distinct sensitivity to inhibition by substrate.

The study with the isoenzymes from snake tissues showed a marked inhibition of lactate dehydrogenase B_4 by high concentrations of pyruvate at 10 °C. At this temperature, the difference between A_4 and B_4 is that usually described for the same enzymes from homeotherms at higher temperatures.

When determinations of enzymatic activity were performed at 35 °C, snake

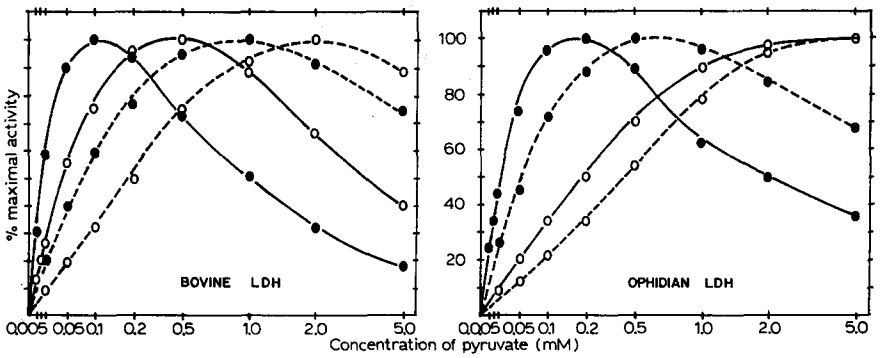

Fig. 1. Effect of pyruvate concentration upon the activity of bovine (left) and ophidian (right) lactate dehydrogenase (LDH) isozyme A_4 (– – –) and B_4 (———) determined at 10 °C (●) and 35 °C (○). The initial reaction velocity, expressed as a percentage of the maximal activity, is plotted against the concentration of pyruvate. The final concentration of NADH was 0.150 mM, that of phosphate buffer, pH 7.4, was 0.1 M, and those of pyruvate were 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mM. Each point represents the average of six determinations.

lactate dehydrogenase B_4, as well as A_4, were not affected by high substrate concentrations (Fig. 1). At the habitat's highest temperatures, the phenomenon of pyruvate inhibition disappears.

Although the results are not represented in the figure, curves at 20 °C were also determined. In all cases, they were intermediate between those obtained at 10 °C and 35 °C.

The modifications of the K_m values and of optimal substrate concentrations produced by thermal change were those already described [7].

Inhibition by lactate

When the reverse reaction (lactate→pyruvate) was studied, the curves of the percentage of enzymatic activity against lactate concentration were identical for bovine and ophidian enzymes (Fig. 2). There was no inhibition of lactate dehydrogenase A_4

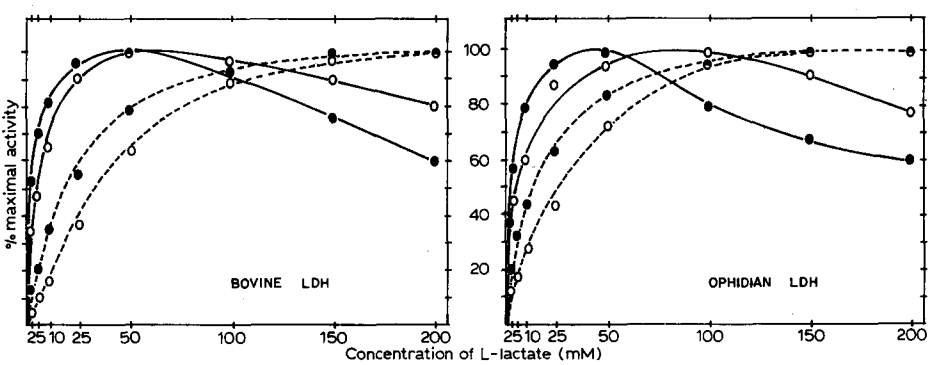

Fig. 2. Effect of lactate concentration upon the activity of bovine (left) and ophidian (right) lactate dehydrogenase (LDH) isozyme A_4 (– – –) and B_4 (———), determined at 10 °C (●) and 35 °C (○). The initial reaction velocity, expressed as a percentage of the maximal activity, is plotted against the concentration of lactate. The final concentration of NAD^+ was 1.0 mM, that of Tris, pH 9.0, was 10 mM, and those of L-lactate were 2, 5, 10, 25, 50, 100, 150 and 200 mM. Each point represents the average of six determinations.

activity at lactate concentrations as high as 200 mM. Beef and snake isoenzymes were equally affected by temperature. At 10 °C, the activity with 200 mM lactate was 60% of the maximal for both B_4 isoenzymes, while it was 77% for the ophidian isoenzyme and 80% for that of beef at 35 °C.

The values of K_m for lactate of isoenzyme B_4 from both species were not affected by the change of temperature.

K_i for NAD^+ and pyruvate

The enzyme–NAD^+–pyruvate complex responsible for the inhibition by substrate, can be dissociated by the addition of NADH or lactate to the medium [8]. This property has been utilized to determine the K_i values for NAD^+ and pyruvate.

The results are presented in Table I. The values of K_i for NAD^+ remained the same at 10 °C or 35 °C and were practically identical for beef and snake isoenzyme.

TABLE I

VALUES OF K_i FOR NAD AND PYRUVATE OF LACTATE DEHYDROGENASE B_4*

K_i for NAD:	Beef enzyme (mM)	Snake enzyme (mM)
At 10 °C	0.085	0.090
At 35 °C	0.095	0.110
K_i for Pyruvate:		
At 10 °C	0.025	0.025
At 35 °C	0.031	0.115

* See text for methodological details.

Dixon's plots of the results for pyruvate are shown in Fig. 3. It can be noted that the inhibition by pyruvate of the reverse reaction is of the competitive type. The values of K_i for pyruvate of the bovine isoenzyme B_4 were not significantly affected by temperature, while the values of the ophidian isoenzyme showed a 4.6-fold increase between 10 °C and 35 °C. This last result agrees with those presented with the curves of pyruvate inhibition (Fig. 1). It confirms the reduction of ophidian B_4 sensitivity to pyruvate inhibition when temperature increases.

It appears that the thermal increment affects the binding of pyruvate in the ternary complex of snake lactate dehydrogenase B_4, but not that of the coenzyme.

DISCUSSION

The effects of thermal change upon the inhibition by pyruvate of lactate dehydrogenases from the snake *Bothrops neuwiedii* reported here are comparable to those previously obtained by Cowey et al. [13] with the unique lactate dehydrogenase from tissues of the plaice and by Somero [14] with crude extracts of skeletal muscle from the mudsucker fish. The degree of inhibition is clearly reduced when the temperature increases. For the isoenzyme B_4 of the snake there is no inhibition by concentrations of pyruvate as high as 5 mM at the upper limits of the habitat's temperature. There was

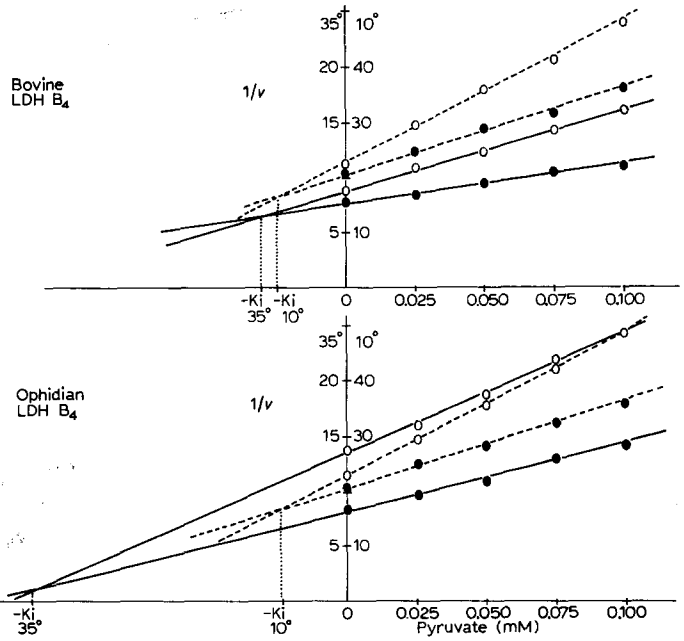

Fig. 3. Dixon's plot of the inhibition by pyruvate of the reaction lactate → pyruvate catalyzed by bovine (upper graph) and ophidian (lower graph) lactate dehydrogenase (LDH) isoenzyme B_4 determined at 10 °C (– – –) and 35 °C (———). Isoenzyme B_4, 10 mM Tris buffer, pH 9.0, and 1.5 mM NAD were incubated for 30 min with 0, 0.025, 0.050, 0.075, and 0.10 mM pyruvate. After that time, 10 mM (○) or 40 mM (●) L-lactate was added. The reciprocal of the initial velocity is plotted against the concentration of pyruvate. Each point represents the average of six determinations.

a significant difference with the same enzyme from a homeotherm (ox) studied simultaneously in the same conditions.

It is interesting that inhibition by high concentrations of lactate on the reverse reaction is identical, at a given temperature, for bovine and ophidian isoenzymes.

It has been pointed out [8] that, while pyruvate inhibition of the direct reaction regulates the functioning of lactate dehydrogenase B_4 as an "aerobic" isoenzyme, the inhibition by lactate of the reverse reaction would be meaningless from the physiological point of view.

The thermally-induced changes in the sensitivity of lactate dehydrogenase B_4 to inhibition by pyruvate suggest a mechanism of biological adaptation. As Hochachka and Somero [15] indicated, the activity of poikilotherms generally increases with temperature. On the other hand, the oxygen tension of body fluids falls at higher temperatures. This situation would cause an increase in the usage of anaerobic glycolysis as the temperature rose.

The isoenzyme B_4 of the snake *Bothrops neuwiedii* appears particularly suited to favor an aerobic type of metabolism at low temperature and to function without restrictions in anaerobiosis when the environmental temperature increases.

The findings presented demonstrate that the modulation by pyruvate of lactate dehydrogenase B_4 activity in the snake is strikingly affected by thermal changes. The molecular bases for this phenomenon are unknown. From our data, however, it can

be assumed that the stability of the abortive ternary complex enzyme–NAD^+–pyruvate is highly reduced by temperature increments and that only the forces binding the pyruvate in the complex are affected to a significant degree.

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REFERENCES

1 Hochachka, P. W. and Somero, G. N. (1968) Comp. Biochem. Physiol. 27, 659–668
2 Hochachka, P. W. and Lewis, J. K. (1970) J. Biol. Chem. 245, 6567–6573
3 Baldwin, J. and Hochachka, P. W. (1970) Biochem. J. 116, 883–887
4 Moon, T. and Hochachka, P. W. (1971) Biochem. J. 123, 695–705
5 Hochachka, P. W. and Lewis, J. K. (1971) Comp. Biochem. Physiol. 39B, 925–933
6 González, A. M., González, F., Golf, S. and Marcus, F. (1972) J. Biol. Chem. 247, 6067–6070
7 Gerez de Burgos, N. M., Burgos, C., Gutiérrez, M. and Blanco, A. (1973) Biochim. Biophys. Acta 315, 250–258
8 Everse, J. and Kaplan, N. O. (1973) Adv. Enzymol. 37, 61–133
9 Wróblewski, F. and LaDue, J. S. (1955) Proc. Soc. Exp. Biol. Med. 90, 210–213
10 Markert, C. L. and Ursprung, H. (1962) Develop. Biol. 5, 363–381
11 Plagemann, P. G. W., Gregory, K. F. and Wróblewski, F. (1961) Biochem. Z. 334, 37–48
12 Kaplan, N. O., Everse, J. and Admiraal, J. (1968) Annu. N.Y. Acad. Sci. 151, 400–412
13 Cowey, C. B., Lush, I. E. and Knox, D. (1969) Biochim. Biophys. Acta 191, 205–213
14 Somero, G. N. (1973) Comp. Biochem. Physiol. 44B, 205–209
15 Hochachka, P. W. and Somero, G. N. (1973) Strategies of Biochemical Adaptation, p. 59, W. B. Saunders Co. Philadelphia