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ROLE OF IONS IN THE REGULATION OF PORCINE LACTATE DEHYDROGENASE

EDGAR RIVEDAL and TORE SANNER

Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, Oslo 3 (Norway)

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Summary

Different ions affect the H_4 and M_4 isoenzymes of porcine lactate dehydrogenase (L-lactate:NAD^+ oxidoreductase, EC 1.1.1.27) in the same way, inhibiting the enzyme at low pyruvate concentrations, whereas at high pyruvate concentrations, the activities were enhanced. The inhibition was competitive with regard to pyruvate and NADH. The enhancement of the enzyme activity at high pyruvate concentration is due to the increase in the K_m value for pyruvate, implying that higher substrate concentrations are needed to obtain substrate inhibition. Sulphate behaved differently from the other ions. It inhibited in a noncompetitive manner with regard to pyruvate and did not activate the enzyme at high pyruvate concentration. The effect of ions increased with the size of the anion. The ionic strength was of less importance.

Introduction

The activity of most enzymes is affected by ions [1—7]. Relatively little is known about the mechanisms of these effects and they are in most cases assumed to be caused simply by increase in the ionic strength [1—4]. Recently, we have studied the inhibitory effects of ions on the NAD-linked isocitrate dehydrogenase and D-lactate dehydrogenase from the water mold *Blastocladiella emersonii* [8,9]. It was found that the inhibition increased with the thermochemical radius of the anions while the ionic strength seemed to be of less importance.

In the present investigation, we studied the effects of salts on porcine lactate dehydrogenase (L-lactat:NAD^+ oxidoreductase, EC 1.1.1.27). This enzyme was chosen because it was of interest to study whether the salt ion

inhibition is related to the size of the anions also for a mammalian enzyme, or if this is specific for enzymes from *B. emersonii*. Moreover, the lactate dehydrogenase isoenzymes from muscle and heart are claimed to have several different properties [10—13]. The question arises of whether the two isoenzymes are affected in the same way by ions.

Materials and Methods

Materials. Lactate dehydrogenase prepared from pig heart (H_4) and muscle (M_4) were purchased from Boehringer Mannheim (F.R.G.). The enzymes were purified electrophoretically and represent bands 1 and 5 respectively. NADH and pyruvate were obtained from Sigma Chemical Co. (St. Louis, MO). The salts used were of analytical grade.

Assay of enzyme activity. The activity was measured by the rate of decrease of absorption at 340 nm upon oxidation of NADH. A Gilford Model 2400 recording spectrophotometer with 1-cm cuvettes were used. The assay, unless otherwise specified, was made in 100 mM phosphate buffer (pH 6.3). The concentrations of the other substances present are given in the legends to the figures. All measurements were made at 25° C.

Results

The effects of ions on the H_4 isoenzyme of porcine lactate dehydrogenase are shown in Fig. 1. When the activity was measured at low substrate concentration, all the ions inhibited the enzyme (Fig. 1A). The inhibition increased with the atomic weight of the halide ions tested. KNO_3 inhibited to nearly the same

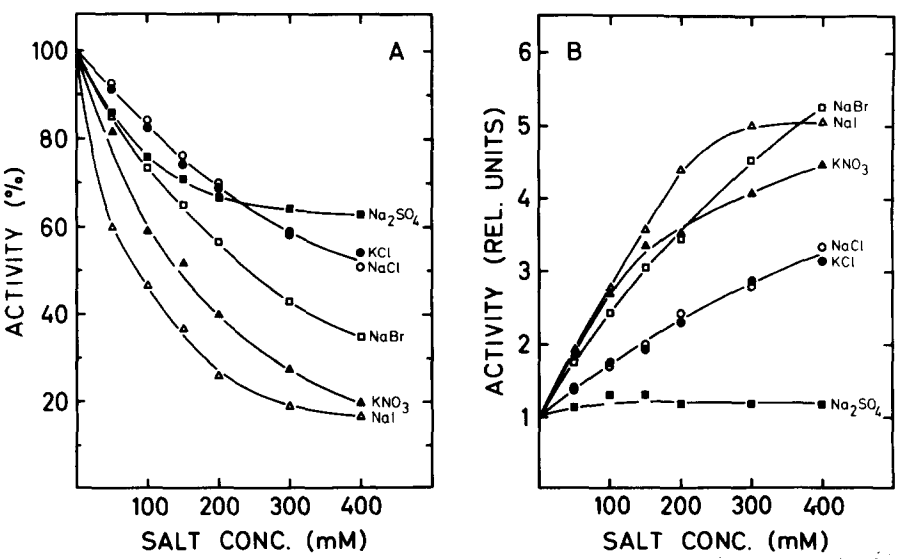

Fig. 1. Effect of different ions on the activity of the H_4 isoenzyme. (A) Inhibition of enzyme activity by ions at low substrate concentrations (0.05 mM pyruvate and 0.025 mM NADH. (B) Enhancement of activity by ions at high substrate concentrations (25 mM pyruvate and 0.1 mM NADH).

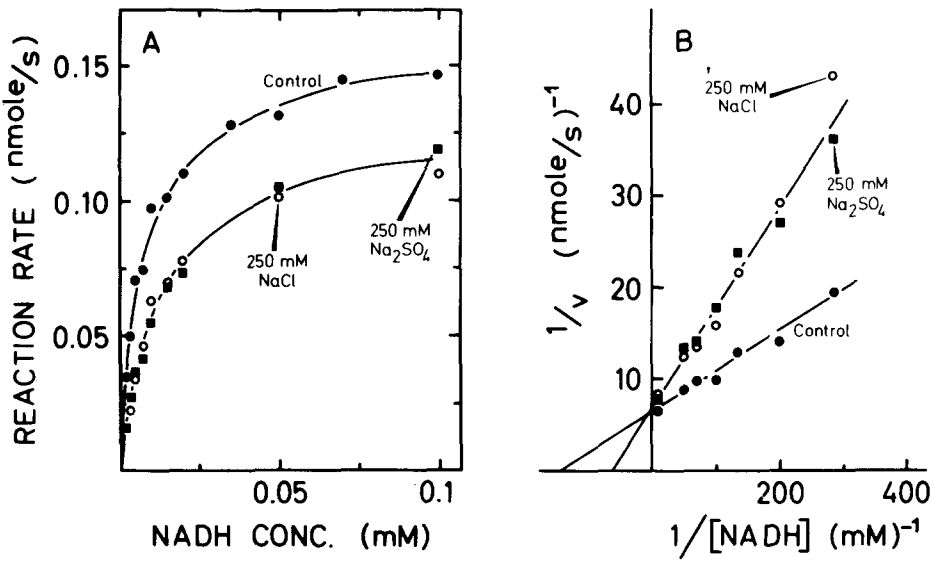

Fig. 2. Effect of NaCl and Na_2SO_4 on the NADH saturation curve for the H_4 isoenzyme. (A) Enzyme activity as a function of NADH concentration in the presence of 250 mM NaCl or 250 mM Na_2SO_4. (B) Double-reciprocal plot of the data shown in Part A. The activity was measured with 0.05 mM pyruvate.

extent as NaI. The inhibition was the same whether sodium or potassium salts were used. Surprisingly, with Na_2SO_4 the inhibition levelled off at approx. $\frac{2}{3}$ of full activity. With the exception of Na_2SO_4, the inhibition increased with the thermochemical radius of the anions in the same way as previously found for NAD-linked isocitrate dehydrogenase and D-lactate dehydrogenase from *B. emersonii* [8,9].

When the effect of ions was determined at high substrate concentration, all the ions, except Na_2SO_4, enhanced the activity (Fig. 1B). With NaI, NaBr and KNO_3, the activity increased by a factor of about 4 when the ions were present at 250 mM. On a molar basis, the efficiency of the ions to enhance the activity increased with the size of the anion. In separate experiments, it was found that K_2SO_4 affected the enzyme in the same way as Na_2SO_4.

Fig. 2A shows the effect of NaCl and Na_2SO_4 on the saturation curve for NADH. In this experiment, the same low pyruvate concentration was used as in Fig. 1A. The concentrations of the ions were adjusted to give equal inhibition. It is apparent from the double-reciprocal plot (Fig. 2B) that both NaCl and Na_2SO_4 inhibit the enzyme in a competitive manner with regard to NADH.

Further information was obtained in experiments where the effect of ions was determined with increasing pyruvate concentrations (Fig. 3). The effect of NaCl and Na_2SO_4 on the saturation curve for pyruvate is shown in Fig. 3A. These ions inhibited the activity at low pyruvate concentrations, whereas at higher pyruvate concentrations (where substrate inhibition became apparent) the activity was enhanced in the presence of NaCl. From the double-reciprocal plot (insert), it follows that at low pyruvate concentrations NaCl inhibited the activity in a competitive manner with regard to pyruvate, whereas the inhibition by Na_2SO_4 was noncompetitive.

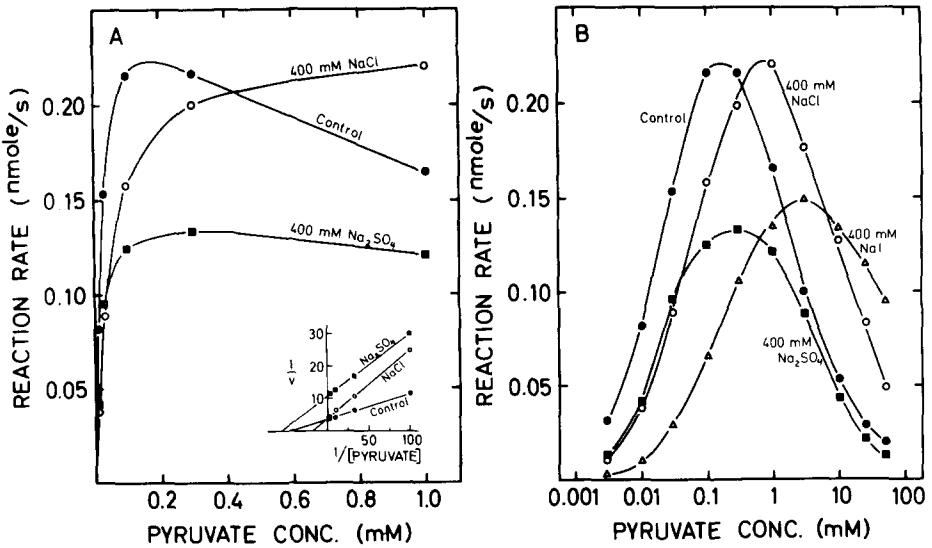

Fig. 3. Effect of different ions on the pyruvate saturation curve for the H_4 isoenzyme. (A) Enzyme activity as a function of pyruvate concentration in the presence of 400 mM NaCl or 400 mM Na_2SO_4. The insert shows double reciprocal plot of the data. (B) Saturation curves for pyruvate in the absence and presence of ions. The pyruvate concentration is given in a logarithmic scale. All experiments were made with 0.1 mM NADH.

Fig. 3B shows activity curves where the pyruvate concentrations were varied by a factor of more than 10 000. Bell-shaped curves were obtained in the semilogarithmic plot both in the absence and presence of ions. The maximum activity obtained in the presence of 0.4 M NaCl was the same as in the control, but the curve was displaced to higher pyruvate concentrations. The curve obtained in the presence of Na_2SO_4 was also very similar to that of the control. In this case, however, the amplitude of the curve was reduced. These results are in accordance with the data in part A which show that Na_2SO_4 reduced V while it had only a small effect on K_m, and that NaCl increased K_m without affecting V. The results obtained with NaI were more complex. Thus, it is evident, in this case, there had been an increase in K_m as well as a decrease in the maximum obtainable reaction rate.

A large difference exists between H_4 and M_4 isoenzymes, especially with regard to substrate inhibition [10—13]. It was therefore of interest to compare the ion inhibition for the two isoenzymes. Fig. 4A shows that also the inhibition of the M_4 isoenzyme increases with the size of the ions. It was not possible to inhibit the M_4 isoenzyme more than approx. 50% with Na_2SO_4. The insert shows that NaCl inhibited in a competitive manner with regard to pyruvate while the inhibition with Na_2SO_4 was noncompetitive. Fig. 4B shows the activity as a function of the pyruvate concentration in the absence and presence of ions. Bell shaped curves were obtained in a semilogarithmic plot also with the M_4 isoenzyme. The amplitude of the curve was reduced in the presence of Na_2SO_4, while in the presence of KNO_3 the curve was also displaced to higher pyruvate concentrations. It is thus apparent that the M_4 isoenzyme is affected by ions in the same way as the H_4 isoenzyme.

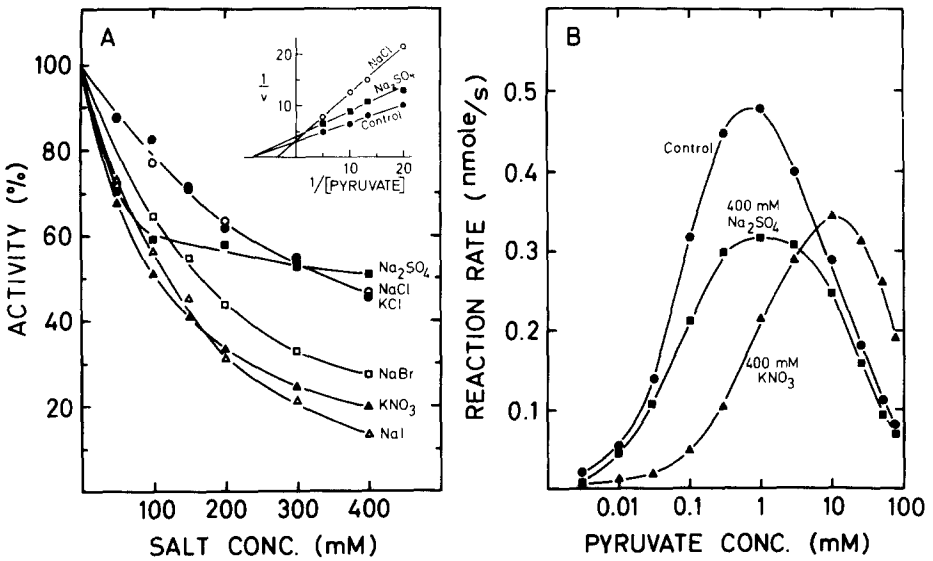

Fig. 4. Effect of different ions on the activity of the M_4 isoenzyme. (A) Inhibition of enzyme activity by different ions at low substrate concentrations (0.25 mM pyruvate and 0.025 mM NADH). The insert shows double reciprocal plot of the enzyme activity for different pyruvate concentrations in the absence and presence of ions. (B) Effect of ions on the saturation curve for pyruvate. The pyruvate concentration is given in a logarithmic scale. The activity was measured with 0.1 mM NADH.

The present experiments were carried out at relatively low pH. In separate experiments, we obtained, in principle, the same results at pH 7.4. However, since K_m for pyruvate increases with increasing pH, it is more difficult to obtain a clear illustration of the effects studied, especially with the muscle enzyme, if the experiments were performed at higher pH.

Discussion

The results show that the activity of the heart (H_4) and muscle (M_4) isoenzymes of porcine lactate dehydrogenase were inhibited by ions at low pyruvate concentrations. The inhibition was competitive with regard to pyruvate for all the ions except sulphate, which inhibited in a noncompetitive manner. The ions enhanced the enzyme activity at high pyruvate concentrations. This enhancement is due to the increase in K_m for pyruvate implying that higher substrate concentrations are needed in the presence of salts to obtain substrate inhibition. Sulphate has also been found to affect D-lactate dehydrogenase from *B. emersonii* different from the other ions [9].

In previous studies on the heart and muscle isoenzymes from lactate dehydrogenase, much attention has been paid to demonstrate differences between the isoenzymes [10—13]. The present data demonstrate that considering the higher pyruvate concentrations needed for M_4 isoenzyme compared to the H_4 isoenzyme, they are affected by ions in the same manner. It is of particular interest that in the case of the present enzyme as well as with isocitrate dehydrogenase, the ions seem to have rather specific effects on kinetic parameters. Thus, in both cases, a small competitive effect was observed with

regard to the coenzyme, whereas the major effect was caused by a strong competitive effect with the main substrate.

Recently, we found, with NAD-linked isocitrate dehydrogenase and D-lactate dehydrogenase from *B. emersonii*, that the thermochemical radius of the anions seemed to determine the degree of inhibition [8,9]. The ionic strength was of less importance. This inhibition pattern is confirmed in the present study.

Acknowledgement

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