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THE KINETIC EFFECTS OF OXALATE ON LIVER AND ERYTHROCYTE PYRUVATE KINASES

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SUMMARY

Oxalate dianion exerts a dual effect on allosteric liver and erythrocyte pyruvate kinases. In the absence of fructose 1,6 bisphosphate and at phosphoenolpyruvate concentrations lower than $K_{0.5s}$, oxalate apparently behaves as an allosteric activator. In the presence of fructose 1,6 bisphosphate and at higher phosphoenolpyruvate concentrations, oxalate is a powerful competitive inhibitor with respect to phosphoenolpyruvate. Such properties are consistent with the allosteric model of Monod-Wyman-Changeux for a substrate analogue. Inhibition constants of oxalate towards pyruvate kinase are in the same order of magnitude as blood oxalate concentration.

INTRODUCTION

During the recent past years, it was demonstrated that oxalate inhibits some pyruvate-metabolizing enzymes. Thus, pyruvate carboxylase (E.C. 6.4.1.1) is inhibited by oxalate with an apparent Ki of 5×10^{-5}M (1). Reed and Morgan (2) showed that oxalate is also a potent inhibitor of muscle pyruvate kinase (M type) (E.C. 2.7.1.40). This inhibition is competitive with respect to phosphoenolpyruvate, and the apparent Ki is very low : 6 μM. Oxalate likely reacts by its structural analogy with enolpyruvate. The second substrate, ADP, does not interfere with this inhibition mechanism.

The main isoenzyme of liver pyruvate kinase (L type) controls the balance between gluconeogenesis and glycolysis by its regulatory properties (3-4). The L type-pyruvate kinase is genetically different from the muscle enzyme, but the same gene controls the synthesis of tetrameric liver (L_4) and erythrocyte ($L_2L'_2$) pyruvate kinases (5) which, contrary to the muscle

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enzyme, are regulated by fructose 1,6 bisphosphate at physiological concentrations (3-6). Thus, we have checked the effect of oxalate on these two allosteric pyruvate kinases.

MATERIALS AND METHODS

Enzymes : L type pyruvate kinase was purchased from Sigma (rabbit liver pyruvate kinase, specific activity ca. 20 µmol per minute per mg of protein). Human erythrocyte pyruvate kinase was partially purified according to the previously described method (6). Its specific activity was 6 µmol per minute per mg of protein. No contaminating aldolase (E.C. 4.1.2.13), lactate dehydrogenase (E.C. 1.1.1.27) or enolase (E.C. 4.2.1.11) activities were detected.

Reagents : ADP, phosphoenolpyruvate (tricyclohexylammonium salt), NADH, fructose 1,6 bisphosphate and rabbit muscle lactate dehydrogenase were obtained from Boehringer, Mannheim.

Kinetic assays : The pyruvate kinase reaction was followed at 366 nm in an Eppendorf photometer, using the coupled assay involving lactate dehydrogenase. The assays were carried out at 27 $\pm$ 0.5°C, at the following concentrations : in a total volume of 3 ml, 50 mM triethanolamine (pH 7.6), 5 mM EDTA, 50 mM KCl, 10 mM $MgCl_2$, 0.8 mM ADP, 0.25 mM NADH, 18 units of lactate dehydrogenase and 0.05 to 0.07 units of liver or erythrocyte pyruvate kinase. Phosphoenolpyruvate and oxalate concentrations were varied as indicated in the legends of figures. Reaction was always started by adding ADP in order to avoid any absorbance change due to contamination of phosphoenolpyruvate by pyruvate.

RESULTS

The $K_{0.5s}$ of erythrocyte pyruvate kinase for phosphoenolpyruvate were 2.7×10^{-4}M (Hill coefficient : 2.40) in the absence of fructose 1,6 bisphosphate, and 3.0×10^{-5}M (Hill coefficient : 1.01) in the presence of 8×10^{-6}M fructose 1,6 bisphosphate. For liver pyruvate kinase, respective values were 2.8×10^{-3}M (Hill coefficient : 2.60), and 5.6×10^{-5}M (Hill coefficient : 1.07).

Interaction of oxalate with erythrocyte pyruvate kinase : In fig. 1, initial velocity is plotted versus oxalate concentration in the absence of fructose 1,6 bisphosphate. At low phosphoenolpyruvate concentration (below the $K_{0.5s}$), oxalate behaved as an activator. When phosphoenolpyruvate concentration increased, a slight inhibition by oxalate was observed. In contrast, in the presence of saturating levels of fructose 1,6 bisphosphate (8 µM), a strong inhibition by oxalate became evident. It was purely competitive with respect to phosphoenolpyruvate (fig. 2). Replots of the slopes versus oxalate concentration were linear over a concentration range eight times the apparent inhibition constant (8 µM oxalate). A similar Ki for oxalate was found for muscle pyruvate kinase (2). The second substrate, ADP, was without effect in the physiological concentration range (0.2 to 0.8 mM).

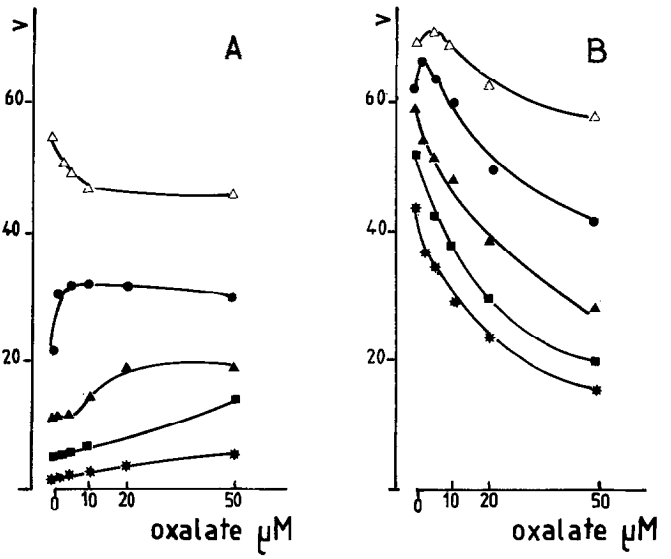

Figure 1 : Influence of oxalate on initial velocity v (expressed as millimicromoles of product formed per minute in the assay) of erythrocyte pyruvate kinase in the absence, A, and in the présence, B, of 8 µM fructose 1,6 bisphosphate ; phosphoenolpyruvate concentrations were ✱ : 4.4 x 10^{-5}M ; ■ : 8.8 x 10^{-5}M ; ▲ : 13.2 x 10^{-5}M ; ● : 22 x 10^{-5}M ; △ : 44 x 10^{-5}M.

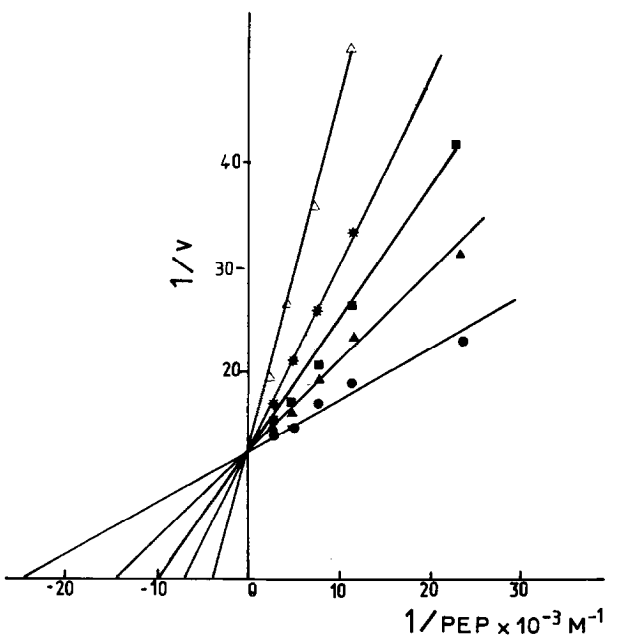

Figure 2 : Double reciprocal plot of the influence of oxalate on the initial velocity of fructose 1,6 bisphosphate activated erythrocyte pyruvate kinase reaction. Velocity v is expressed as micromoles of product formed per minute in the assay ; the oxalate concentrations were ● : 0 ; ▲ : 5 µM ; ■ : 10 µM ; ✱ : 20 µM ; △ : 50 µM.

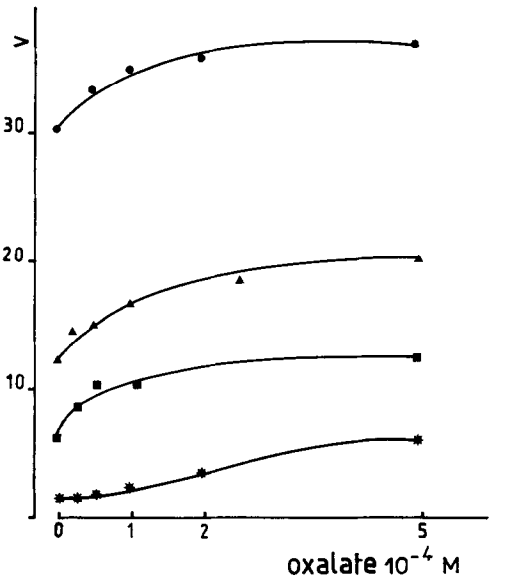

Figure 3 : Influence of oxalate on initial velocity v (expressed as millimicromoles of product formed per minute in the assay) of liver pyruvate kinase without fructose 1,6 bisphosphate ; phosphoenolpyruvate concentrations were ✱ : 0.5 x 10^{-3}M ; ■ : 0.83 x 10^{-3}M ; ▲ : 1.33 x 10^{-3}M ; ● : 2.5 x 10^{-3}M.

Interaction of oxalate with liver pyruvate kinase : In the absence of fructose 1,6 bisphosphate, the affinity of the enzyme for phosphoenolpyruvate is low (the $K_{0.5s}$ is about ten times higher than for erythrocyte pyruvate kinase). It seems obvious that this pyruvate kinase is at least partially present under the phosphorylated form described by Engstrom (7). However, at low but saturating concentrations of fructose 1,6 bisphosphate (8 µM), the apparent K_M decreases to 5 x 10^{-5}M, proving that the enzyme is not desensitized. In the absence of fructose 1,6 bisphosphate, an activating effect of oxalate could not be detected in the oxalate concentration range used for the erythrocyte enzyme. But, with oxalate concentrations above 2 x 10^{-5}M (fig. 3), an activation was observed when phosphoenolpyruvate concentration was lower than the $K_{0.5s}$. In the presence of 8 µM fructose 1,6 bisphosphate, oxalate behaved as a strong competitive inhibitor with respect to phosphoenolpyruvate (fig. 4). Replot of slopes versus oxalate concentrations was linear and yielded an apparent inhibition constant of 2.7 x 10^{-5}M. ADP concentration had no effect.

DISCUSSION

Two possible artefacts have to be at once ruled out. The first, a strong decrease of free Mg^{++} concentration due to the formation of magne-

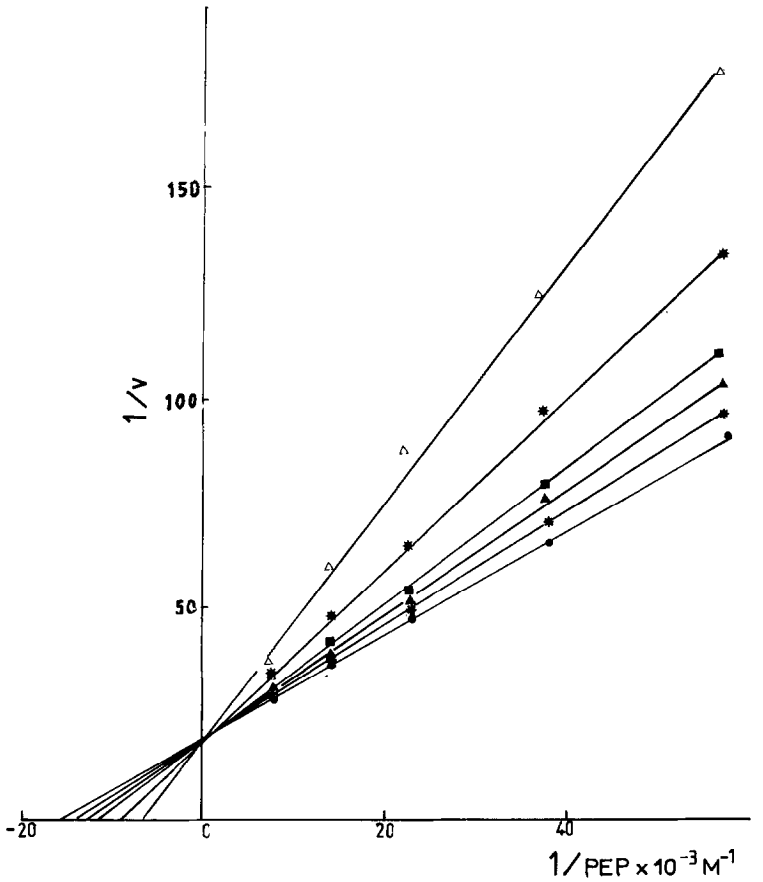

Figure 4 : Double reciprocal plot of the influence of oxalate on the initial velocity of fructose 1,6 bisphosphate activated liver pyruvate kinase reaction. Velocity v is expressed as micromoles of product formed per minute in the assay ; the oxalate concentrations were ● : control ; ✸ : 2 μM oxalate ; ▲ : 5 μM oxalate ; ■ : 10 μM oxalate ; ✸ : 20 μM oxalate ; △ : 50 μM oxalate.

sium-oxalate complex, is not relevant since the product of magnesium and oxalate concentrations was always largely lower than the solubility product of the complex. We have checked that the second artefact, i.e. inhibition of added lactate dehydrogenase by oxalate (8), did not occur under our assay conditions.

The dual effect of oxalate on allosteric pyruvate kinase is clearly explained in the R $\rightleftharpoons$ T equilibrium model of Monod-Wyman-Changeux (9). In the absence of the physiological allosteric activator (fructose 1,6 bisphosphate) and at low concentration of phosphoenolpyruvate, oxalate induces a

T ⟶ R transition, and oxalate binding to one substrate site enhances the binding of phosphoenolpyruvate to the other sites : oxalate then acts as an allosteric activator. When the enzyme is predominantly under the R state (at a high phosphoenolpyruvate concentration or in the presence of fructose 1,6 bisphosphate), oxalate behaves as a competitive inhibitor with respect to phosphoenolpyruvate. The effect of oxalate is then similar to the one observed with muscle pyruvate kinase which may be considered as blocked under the R state.

This dual effect of oxalate is more obvious on erythrocyte pyruvate kinase than on the commercial liver enzyme : it is likely that the latter is predominantly under the phosphorylated form (7), and higher oxalate concentrations are necessary to give similar effects in the absence of fructose 1,6 bisphosphate.

The normal blood oxalate concentration was estimated to be 2 to 3×10^{-5}M (10), and red blood cells are freely permeable to oxalate (11). Preleminary experiments on human erythrocytes showed that incubations with 10^{-4}M oxalate modified the steady state concentration of glycolytic intermediates as observed in erythrocytes from patients with pyruvate kinase deficiency (12). Thus, in vivo, oxalate may modify the actual velocity of pyruvate kinase, especially when blood oxalate concentration is raised under pathological conditions.

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