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COMPLEX KINETICS OF HUMAN LEUKOCYTE AND PLATELET PYRUVATE KINASES

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Summary

In the presence of SH group protectors, human leukocyte and platelet pyruvate kinases demonstrate biphasic kinetics with respect to the phospho*enol*pyruvate substrate.

SH group oxidation by oxidized glutathione reveals positive cooperativity kinetics for purified preparations of leukocyte and platelet pyruvate kinases. Complete reversal of the phenomenon may be obtained by incubation for several hours in dithiothreitol.

This communication illustrates the existing relationships between enzyme conformation, the redox state of the SH groups, and the observed kinetics.

Three principal forms of pyruvate kinase, L, M_1 and M_2 were demonstrated in man by kinetic, electrophoretic and immunological studies.

M_2 type pyruvate kinase found in leukocyte and platelet and L type pyruvate kinase found in erythrocyte can be entirely distinguished by electrophoresis [1]; their kinetic properties and in particular their mechanism of substrate binding [2] are different.

This communication shows that the pyruvate kinase of all three blood cells demonstrate biphasic kinetics with respect to phospho*enol*pyruvate, thus suggesting negative cooperativity. We have also shown that the kinetics are related to the redox state of the SH groups of leukocyte and platelet pyruvate kinase.

The kinetic study was performed on crude extracts of leukocyte and platelet as well as purified samples. The method of blood cell separation was that described previously [1]. Leukocyte and platelet pyruvate kinase were highly purified by ammonium sulfate precipitation, followed by column chromatography with CM- Sephadex and elution with phospho*enol*pyruvate (Kahn, A., unpublished data). Specific activity is 220 I.U./mg of proteins for leukocyte

pyruvate kinase and 130 I.U./mg of proteins for platelet pyruvate kinase.

Enzyme activity was measured at 30°C with a Zeiss PMQII spectrophotometer. The reaction mixture (final volume 3 ml) contained 100 mM Tris · HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 4 mM phospho*enol*pyruvate, 4 mM ADP, 0.1 mM $NADH_2$, 1 mM dithiothreitol and 20 units of lactate dehydrogenase. Final protein concentration was adjusted to 2 mg/ml with bovine serum albumin. Before use, the crude or purified enzymes were dialyzed against a buffer containing 200 mM Tris · HCl (pH 7.6), 1 mM EDTA, 1 mM ϵ-aminocaproic acid, 1 mM dithiothreitol and 500 mM sucrose.

Complex kinetics were showed for a number of M_2 [3—6] and L [7] type pyruvate kinases by various investigators, not however in any consistent manner. This complex character seems to appear as a result of experimental conditions: Mg^{2+} [6] or EDTA concentrations or degree of purity [3] or according to the presence of an inhibitor such as ATP for erythrocyte pyruvate kinase [7] or different amino acids [8,9]. We chose to conduct all our experiments with constant concentrations of EDTA (1 mM) and $MgCl_2$ (10 mM). With 10 mM $MgCl_2$ van Berkel [6] did not observe any plateau. The purification of platelet and leukocyte pyruvate kinase was performed in the presence of EDTA (1 mM) and β-mercaptoethanol (1 mM).

In a medium containing 1 mM of dithiothreitol (or mercaptoethanol), and a constant concentration of Mg^{2+} (10 mM) and EDTA (1 mM) we demonstrated biphasic kinetics with respect to phospho*enol*pyruvate on a great number of crude extracts of leukocyte and platelet and highly purified preparations (Figs. 1 and 2): Before the plateau (cups in the curve) the Hill coefficient is equal to 0.6 and, after the plateau, to 1.9 (insert Figs. 1 and 2). The plateau always occurred at the same phospho*enol*pyruvate concentrations. This suggests

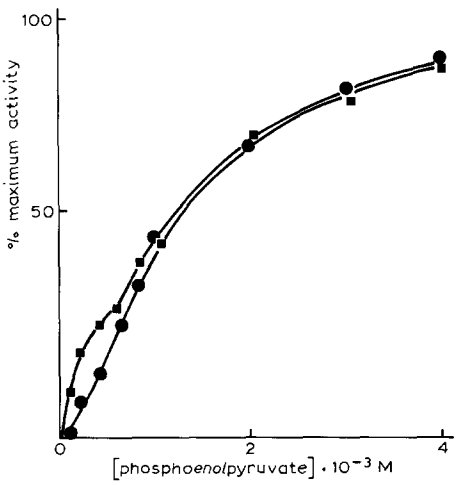

Fig. 1. Influence of the oxidized glutathione and dithiothreitol on the phospho*enol*pyruvate curve of the crude and purified leukocyte pyruvate kinase at pH 7.6 with 4 mM ADP. ●, the activity vs. [phosphoenolpyruvate] plots of the crude and purified leukocyte pyruvate kinase after incubation for 12 h at 4°C with 2.5 mM oxidized glutathione; ■, the activity of the reduced leukocyte pyruvate kinase or the oxidized leukocytes pyruvate kinase after incubation for 3 h at 4°C, with 10 mM dithiothreitol (superimposed curves).

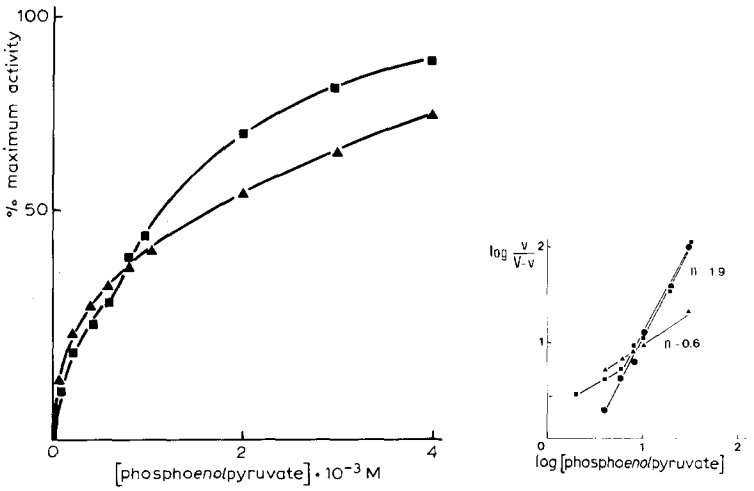

Fig. 2. Influence of the oxidized glutathione and dithiothreitol on the phospho*enol*pyruvate curve of the crude platelet pyruvate kinase. ▲, the activity of the platelet pyruvate kinase after incubation for 12 h at 4°C with 2.5 mM oxidized glutathione; ■, the activity of the reduced platelet pyruvate kinase or the oxidized platelet pyruvate kinase after incubation for 3 h at 4°C, with 10 mM dithiothreitol (superimposed curves). The inset is the Hill plot of the values obtained in Figs. 1 and 2. The calculated Hill coefficients (*n*) are indicated. ●, highly purified leukocyte and platelet pyruvate kinase in the presence of oxidized glutathione; ▲, crude platelet pyruvate kinase in the presence of oxidized glutathione; ■, leukocyte and platelet pyruvate kinase (crude extracts and purified preparations) in the presence of dithiothreitol.

that the complex kinetics obtained were probably not the result of a mixture of two or more isozymes or polymorphic forms of leukocyte and platelet pyruvate kinase, although this hypothesis cannot be completely excluded. The complex kinetics may be explained by negatively cooperative interactions, in accordance with Levitski and Koshland [10] and Teipel and Koshland [11]. Recently Ibsen [9] proposed with K_3M chicken isozyme a more elaborate mechanism calling on joint sequential concerted process to explain a biphasic curve with respect to phospho*enol*pyruvate. This enzyme behaves as though it contained three active subunits and only one less active subunit. It would spontaneously convert to the R conformation after only one of the three K subunits is converted by phospho*enol*pyruvate. An analogous hypothesis might account for the pyruvate kinase of leukocyte and platelet. However, M_2 type pyruvate kinase is not a hybrid of L and M_1 type pyruvate kinase because no partial antigenicity was found between M_2 pyruvate kinase and L pyruvate kinase [3,12]. According to the active and sequential isozymic transition from M_2 f to M_1 isozyme proposed by Marie et al. [12], the M_2 type pyruvate kinase of leukocyte could be an heterotetramer (m_3m').

The second result of this paper concerned the influence of SH group oxidizing agents on the kinetics with respect to phospho*enol*pyruvate. Preparations of leukocyte and platelet pyruvate kinase containing 1 mM dithiothreitol, 10 mM $MgCl_2$, 1 mM EDTA were incubated at 4°C for 12 h with 2.5 mM oxidized glutathione. The controls without oxidized glutathione incubated in the same conditions showed always the kinetic with a plateau (described above). With oxidized glutathione, some kinetic modifications appeared: crude ex-

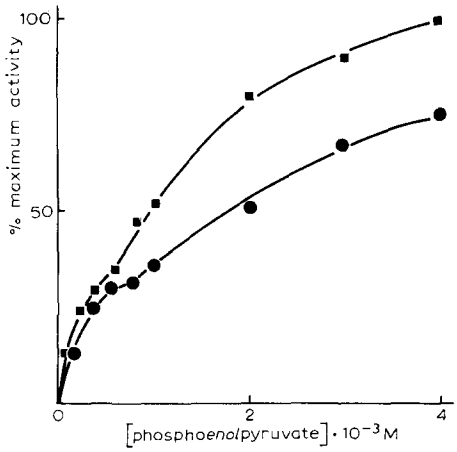

Fig. 3. Influence of the oxidized glutathione and dithiothreitol on the phospho*enol*pyruvate curve of the mixing of crude leukocyte and platelet pyruvate kinase. ●, the activity of the mixing after incubation for 12 h at 4°C with 2.5 mM oxidizing glutathione; ■, the activity of the oxidizing mixing after incubation for 3 h at 4°C with 10 mM dithiothreitol.

tracts of leukocyte and purified pyruvate kinase of leukocytes and platelets demonstrated an allosteric kinetics with positive cooperativity (Fig. 1). The Hill coefficient is equal to 1.9. However, when the platelet sample was taken from crude extracts the saturation plot (Fig. 2) looks qualitatively like a Michaelis-Menten curve, although the double reciprocal plot is concave downward (not shown) and the Hill coefficient is less than 1 (insert of Figs. 1 and 2). In their paper Levitski and Koshland [10] described this situation as being compatible with negative cooperativity. The mixture of crude extracts of platelets and leukocytes did not modify the kinetic of every enzyme. In the presence of oxidized glutathione the kinetics remained biphasic (Fig. 3).

The influence of SH group-oxidizing agents on the modification of kinetics with respect to *Penol*pyruvate kinase [13], rat liver L pyruvate kinase [14] and rat liver M_2 pyruvate kinase [8] was previously described by van Berkel et al. [8]. SH group oxidation led to an enzyme conformation which showed lower affinity for phospho*enol*pyruvate with the same maximal activity. Our experience with leukocyte and platelet pyruvate kinase has shown that oxidation of the thiol groups equally resulted in a decrease in affinity for phospho*enol*pyruvate. Herein, this was due to a change from biphasic kinetics to positive cooperativity kinetics. The phenomenon was completely reversed by incubation for 4 h in dithiothreitol (10 mM).

It was shown for glyceraldehyde-3-phosphate dehydrogenase [15] that formation of an intra-chain disulfide bond by oxidation, considerably modified allosteric interactions, this being an illustration of the relationship between structure and function. The same type of mechanism may be advanced for leukocyte and platelet M_2 pyruvate kinases.

Complex kinetics with respect to phospho*enol*pyruvate were mostly demonstrated for M_2 type pyruvate kinase in normal or cancerous tissues [4]. With foetal tissues [5] or M_1 type pyruvate kinase, there are no examples of biphasic

kinetics in literature. This would tend to support the hypothesis of a complex structure for M_2 type pyruvate kinase as related to the occurrence of complex kinetic. The change in kinetics produced in particular by oxidized glutathione has eluded all physiological interpretation up to now, since concentrations used are much greater than those detected in intracellular medium.

The kinetics with respect to ADP, of leukocyte and platelet pyruvate kinase (ten crude extracts and highly purified preparations) were michaelian with a Hill coefficient equal to 1. The kinetics were not influenced by the SH group redox state.

The other kinetic properties of leukocyte and platelet pyruvate kinase (pH curve, inhibition by ATP and amino acids) were similar to those described by van Berkel and Koster [16] for leukocyte pyruvate kinase and were comparable to the kinetic properties of M_2 type pyruvate kinase.

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