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M-TYPE PYRUVATE KINASE OF LEUCOCYTES: AN ALLOSTERIC ENZYME

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SUMMARY

1. The influence of pH and amino acids on the activity of pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.1.40) from leucocytes were studied.

2. The data can be explained by the model of Monod, Wyman and Changeux. It is proposed that this model is also valid for the other various types of pyruvate kinase.

INTRODUCTION

It is generally accepted that the mammalian glycolytic enzyme pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.1.40) exists in two different forms[1]: L(liver) type and M(muscle) type. L-type pyruvate kinase is present in liver, erythrocytes and kidney[2] and the enzymatic activity of this form is allosterically modulated by phosphoenolpyruvate (PEP), K^+, Fru-1,6-P_2, Glc-1,6-P_2, phosphorylated hexoses, ATP and alanine[3–7]. The M-type is present in muscle, brain, heart, liver, kidney and leucocytes[2]. The M-type was called a non-allosteric enzyme as could be concluded from the Michaelis–Menten kinetics. From the differences in kinetic behaviour between the M- and L-type it was concluded that the metabolic changes in the cell affect the M-type pyruvate kinase less than the L-type. Recently Jiménez de Asúa *et al.*[8] showed that the M-types from liver and muscle can be inhibited by amino acids. From these inhibition studies they concluded that the M-type pyruvate kinase exists at least in two different forms. It was subsequently discovered by Sols (personal communication) that the glycolytic intermediate Fru-1,6-P_2 is able to reverse the inhibition of the liver M-type by alanine.

Since the M-type of pyruvate kinase is found only in Kupffer cells[9,10] and the M-type occurs also in the leucocytes[2], we intended to investigate more closely the kinetic behaviour of the leucocyte enzyme. This is of special interest in the question as to what extent the leucocyte enzymes are related to the Kupffer cell enzymes, in connection with the use of leucocyte enzymes in metabolic liver disease.

Abbreviation: PEP, phosphoenolpyruvate.

MATERIALS AND METHODS

Leucocytes were isolated according to the method described by Wyss *et al.*[11]. Finally, the cells were sonicated for 1 min at 21 kHz, and centrifuged for 10 min at 10 000 × g_{max}. In the supernatant, pyruvate kinase activity was assayed by following the decrease in absorbance at 340 nm in the coupled reaction with lactate dehydrogenase at room temperature, according to the method of Valentine and Tanaka[12]. The triethanol–HCl buffer (0.4 M, pH 7.5) was replaced by Tris–HCl buffer (0.25 M, pH 7.5 or 8.5 as indicated in the figures). For the assay at pH 5.9, a 0.1 M Tris–maleate buffer was used.

ADP, PEP, NADH and Fru-1,6-P_2 were obtained from Boehringer (Mannheim, Germany). L-Alanine was obtained from Merck (Darmstadt, Germany). L-Proline, L-tryptophan, L-phenylalanine, L-glutamate, DL-valine and L-threonine were obtained from British Drug Houses Ltd. All other reagents were of analytical grade purity.

RESULTS

Fig. 1 shows the dependence of the leucocyte pyruvate kinase activity on the PEP concentration at an ADP concentration of 2 mM and pH 7.5 in various conditions. The activity in the absence of alanine shows, in agreement with Campos *et al.*[13], normal Michaelis–Menten kinetics at this pH. From the Hill plot (insert Fig. 1) an n value of 1.1 can be calculated, suggesting no cooperative interaction between

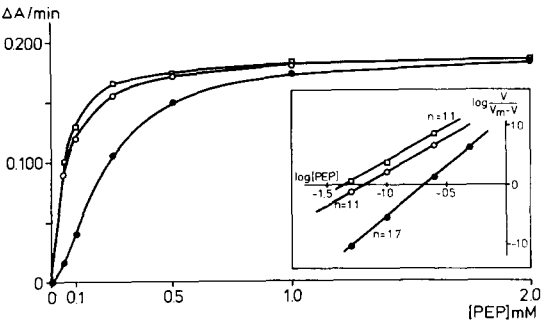

Fig. 1. ΔA *vs* [PEP] plot of M-type leucocyte pyruvate kinase at pH 7.5 with [ADP] = 2 mM in various conditions. □—□, control; ●—●, with 1 mM alanine; and ○—○, with 1 mM alanine and 0.5 mM Fru-1,6-P_2. Added protein, 84 μg.

the binding sites of PEP, at least under these conditions. In the presence of alanine (1 mM) a curve is obtained which is slightly sigmoidal and has the same V as in the absence of alanine. Under these conditions a Hill coefficient of 1.7 can be calculated. However, addition of Fru-1,6-P_2 (0.5 mM) converts the sigmoidal curve into a hyperbolic one (n = 1.1). From this plot it can be concluded that Fru-1,6-P_2 overcomes the alanine inhibition, which is accompanied by a loss of the cooperative interaction between the PEP binding sites. These properties resemble those of the L-type pyruvate kinase. Therefore we investigated the influence of pH, since pH affects the al-

losteric properties of the L-type pyruvate kinase. In Fig. 2, leucocyte pyruvate kinase activity *versus* PEP concentration at an ADP concentration of 2 mM and pH 8.5 is plotted. With the normal activity curve no significant cooperativity is found (n = 1.2). In the presence of alanine (1 mM) an n value of 1.8 is obtained, whereas the $K_{0.5}$ for PEP is increased from 0.2 mM at pH 7.5 to 0.75 mM at this pH. Fru-1,6-P_2 (0.5 mM) is still able to overcome the alanine inhibition and lowers the n value from 1.8 to 0.9. At pH 5.9 no inhibition of alanine (1 mM) could be observed (not shown) and the $K_{0.5}$ for PEP in the presence or absence of alanine (1 mM) is 0.05 mM. The same value is obtained when alanine (1 mM) *plus* Fru-1,6-P_2 (0.5 mM) are present.

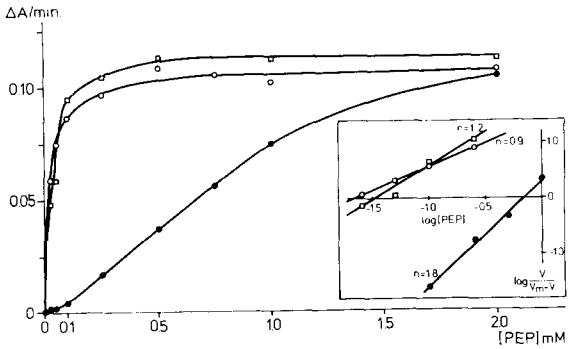

Fig. 2. ΔA *vs* [PEP] plot of M-type leucocyte pyruvate kinase at pH 8.5 with [ADP] = 2 mM in various conditions. □—□, control; ●—●, with 1 mM alanine; and ○—○, with 1 mM alanine and 0.5 mM Fru-1,6-P_2. Added protein, 60 μg.

Fig. 3 shows the influence of the alanine concentration at 0.5 mM PEP and ADP concentration of 2 mM at pH 7.5 on the pyruvate kinase activity before and after addition of 0.5 mM Fru-1,6-P_2. The curve obtained in the absence of Fru-1,6-P_2 is sigmoidal. Addition of Fru-1,6-P_2 overcomes the alanine inhibition, even at high concentrations of alanine (5–10 mM).

It is known that alanine does not inhibit the muscle M-type pyruvate kinase, but that phenylalanine inhibits this enzyme[8]. Fig. 4 shows the influence of phenylalanine on the leucocyte pyruvate kinase in the absence and presence of Fru-1,6-P_2.

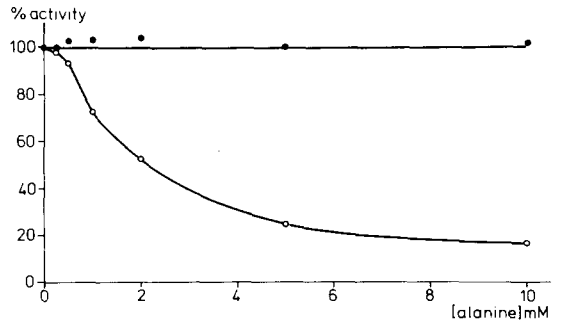

Fig. 3. The influence of alanine on the activity of M-type leucocyte pyruvate kinase at [PEP] = 0.5 mM, [ADP] = 2 mM and at pH 7.5. ○—○, control; and ●—●, the addition of Fru-1,6-P_2 (0.5 mM).

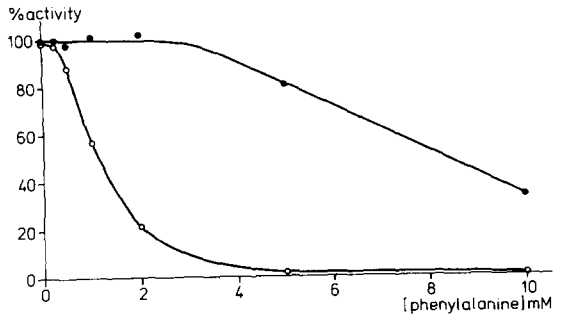

Fig. 4. The influence of phenylalanine on the activity of M-type leucocyte pyruvate kinase at [PEP] = 0.1 mM, [ADP] = 2 mM and at pH 7.5. ○—○, control; and ●—●, the addition of Fru-1,6-P_2 (0.5 mM).

In the absence of Fru-1,6-P_2 a sigmoidal curve is obtained, while Fru-1,6-P_2 can only overcome this inhibition completely up to 2 mM phenylalanine. At high phenylalanine concentration this restoration of activity is impaired.

In Table I the influences of various amino acids on the activity of the leucocyte pyruvate kinase are summarized. When these results are compared with the effects of amino acids on the activity of the liver and muscle M-type[8], it follows that the M-type of leucocytes is very similar to the liver M-type. With valine, threonine and proline, added in a final concentration of 1 mM, no inhibition is found and addition of Fru-1,6-P_2 has no effect. Increasing this concentration to 5 mM results in a striking inhibition, suggesting that these compounds act also as allosteric inhibitors, while the restoration of activity by Fru-1,6-P_2 is dependent on the amino acid used. With valine, proline and alanine the activity is fully restored, whereas with phenylalanine, threonine and tryptophan the activity is only restored to the extent of 81–86%.

TABLE I

THE INFLUENCE OF VARIOUS AMINO ACIDS ON THE LEUCOCYTE M-TYPE PYRUVATE KINASE AT [PEP] = 0.1 mM, [ADP] = 2 mM and pH 7.5 IN THE ABSENCE AND PRESENCE OF Fru-1,6-P_2 (0.5 mM).

Addition	*Concentration (mM)*	*% of activity*	
		−Fru-1,6-P_2	*+Fru-1,6-P_2*
L-Phenylalanine	1	57	101
	5	2	81
L-Valine	1	100	105
	5	48	97
L-Proline	1	101	101
	5	41	100
L-Tryptophan	1	93	97
	5	34	86
L-Glutamate	1	107	102
	5	93	92
L-Alanine	1	38	102
	5	20	99
L-Threonine	1	100	98
	5	65	86

DISCUSSION

The similarity in inhibition by amino acids of the activity of the M-type of leucocytes and the M-type of liver pyruvate kinase makes it very likely that these types are quite similar. Moreover, Fru-1,6-P_2 is able to overcome the amino acid inhibition of the M-type of leucocytes, as has also been shown for the liver M-type pyruvate kinase (A. Sols, personal communication). We have shown previously[9] that the M-type of liver is confined to the Kupffer cells. The similarities described here between leucocyte and Kupffer cell pyruvate kinase support the common reticulo endothelial origin of these cell types, as suggested earlier (*cf.* ref. 14).

From the presented kinetic data it must be concluded that the M-type of leucocytes can show cooperative interaction between the binding sites for PEP. The curves obtained in the presence of the allosteric inhibitor alanine are sigmoidal and are very similar to the normal activity curves obtained with the L-types of liver[5,15] or erythrocytes[16]. At pH 5.9 the latter enzymes obey Michaelis–Menten kinetics with respect to PEP and cannot be activated by Fru-1,6-P_2. At pH 7.5 these enzymes have a sigmoidal response to PEP and this response is transformed into a normal hyperbolic relationship by the addition of Fru-1,6-P_2[15,16]. At pH 8.5 these effects are more pronounced[8]. The results obtained with the M-type of leucocytes are quite similar, but the presence of alanine (1 mM) is required. The inhibition curves of alanine and of phenylalanine show that these compounds act as allosteric inhibitors. When 5–10 mM phenylalanine is present, Fru-1,6-P_2 is not able to overcome the inhibition, which makes it clear that phenylalanine and Fru-1,6-P_2 act as allosteric antagonists. These properties are in complete agreement with the two-state R$\rightleftharpoons$T model of Monod *et al.*[17], applied originally to the allosteric L-type liver pyruvate kinase by Rozengurt *et al.*[15] and extended to human erythrocyte L-type pyruvate kinase by Staal *et al.*[16].

For the leucocyte enzyme we can state that this enzyme is normally in the R state, while alanine and phenylalanine are able to convert this R form into the T form. The effect of Fru-1,6-P_2 can be explained by a simple reconversion of the T state to the R state. It is likely that this model is also valid for the liver M-type pyruvate kinase, whereas the allosteric inhibition by phenylalanine on the activity of muscle M-type pyruvate kinase[18] indicates that this model may even be extended to the muscle M-type pyruvate kinase. Probably, the differences in kinetic behaviour of the various isoenzymes of pyruvate kinase are the result of a different allosteric constant (L). This L value is different from zero for the various L-types and zero for the various M-types, at least at physiological pH and in the absence of modifiers. It is likely that this difference in L value is the consequence of structural differences between the various pyruvate kinase isoenzymes.

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