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THE INFLUENCE OF GLUCOSE 1,6-DIPHOSPHATE ON THE ENZYMATIC ACTIVITY OF PYRUVATE KINASE

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SUMMARY

1. The influence of Glc-1,6-P_2 on hepatic and red blood cell pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.1.40) is quite similar to that of Fru-1,6-P_2. The hexose diphosphates can replace each other in stimulating pyruvate kinase; after maximal stimulation by one of the compounds, the other is not capable of further stimulation.

2. The regulatory role of Fru-1,6-P_2 on the activity of pyruvate kinase is discussed in view of the results obtained.

INTRODUCTION

A large number of studies have been devoted to the regulation of pyruvate kinase (ATP: pyruvate phosphotransferase, EC 2.7.1.40), one of the key enzymes in glycolysis. It was found that there are two types[1], a M (muscle) type and a L (liver) type. It turned out that the L type exhibits allosteric properties allowing regulatory function. The glycolytic intermediate Fru-1,6-P_2 is able to stimulate the activity of the L type and changes the S-shaped relationship between substrate concentration and velocity into a normal hyperbolic one[2]. The [Fru-1,6-P_2] of the liver fluctuates with the nutritional state and, therefore, it was assumed that Fru-1,6-P_2 has a regulatory influence on pyruvate kinase. Furthermore, it was found that alanine inhibits the hepatic pyruvate kinase reaction[3], which can be of importance during gluconeogenesis. In our studies[4,5] on hepatic and erythrocytic pyruvate kinase it was found that the phosphorylated hexoses are able to stimulate the pyruvate kinase reaction and to overcome the ATP inhibition. In this study we compare the influences of Glc-1,6-P_2 and Fru-1,6-P_2 on the enzymatic reaction.

MATERIALS AND METHODS

Wistar rats, maintained on a normal diet and water *ad libitum*, were decapitated and the livers removed. Liver homogenates were prepared in 0.15 M NaCl containing

Abbreviation: PEP, phosphoenolpyruvate.

TABLE I

THE EFFECT OF GLC-1,6-P_2 (0.2 mM) AND FRU-1,6-P_2 (0.2 mM) ON THE ACTIVITY OF HEPATIC PYRUVATE KINASE IN (0.25 M) TRIS–HCl BUFFER (pH 7.5) WITH [PEP] = 0.5 mM AND [ADP] = 0.3 mM

Addition	*Activity (%)*
None	100
Glc-1,6-P_2	218
Fru-1,6-P_2	228

1 mM mercaptoethanol and centrifuged for 60 min at 100 000 × *g*. From the supernatant, the partially purified L type preparations were obtained according to the method of PASSERON *et al.*[6]. The preparations were finally dissolved in 0.25 M Tris–HCl buffer (pH 7.5) containing 1 mM mercaptoethanol. Red blood cell pyruvate kinase was isolated from human erythrocytes according the isolation procedure described previously[5]. The enzymatic activity was assayed by following the decrease of absorbance at 340 nm in the reaction coupled with lactate dehydrogenase as described by BÜCHER AND PFLEIDERER[7]. The activities shown are initial reaction rates.

ADP, PEP (phosphoenolpyruvate), NADH, Fru-1,6-P_2 and Glc-1,6-P_2 were obtained from Boehringer (Mannheim, Germany). Glc-1,6-P_2 was free of Fru-1,6-P_2, when analysed by the method of BÜCHER AND HOHORST[8]. All other reagents were of analytical grade purity.

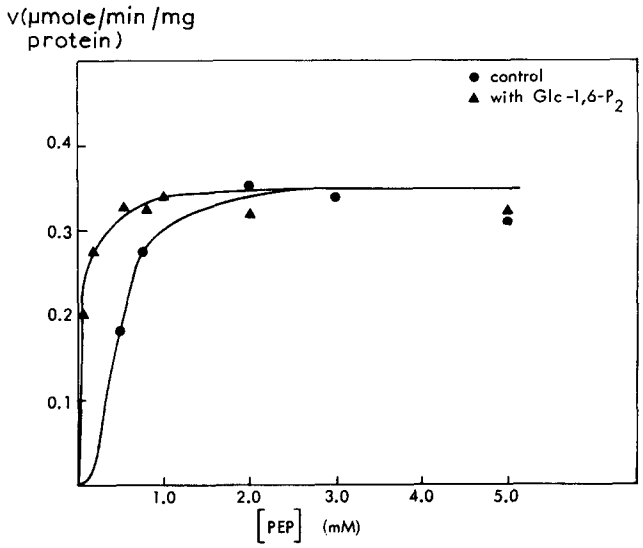

Fig. 1. The *v versus* [PEP] plot for hepatic pyruvate kinase at [ADP] = 0.3 mM, in the absence (●—●) and presence of 0.2 mM Glc-1,6-P_2 (▲—▲).

RESULTS

Table I shows that Glc-1,6-P_2 is able to stimulate the hepatic pyruvate kinase reaction. This stimulation is about equal to the stimulation obtained with Fru-1,6-P_2 (Table I), at least under these conditions.

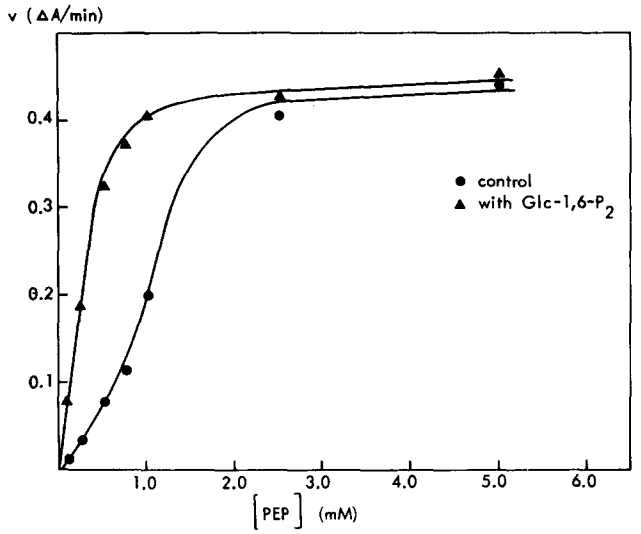

Fig. 2. The *v versus* [PEP] plot for red blood cell pyruvate kinase at [ADP] = 2 mM, in the absence (●—●) and presence of 0.2 mM Glc-1,6-P_2 (▲—▲), 20 μg of protein added.

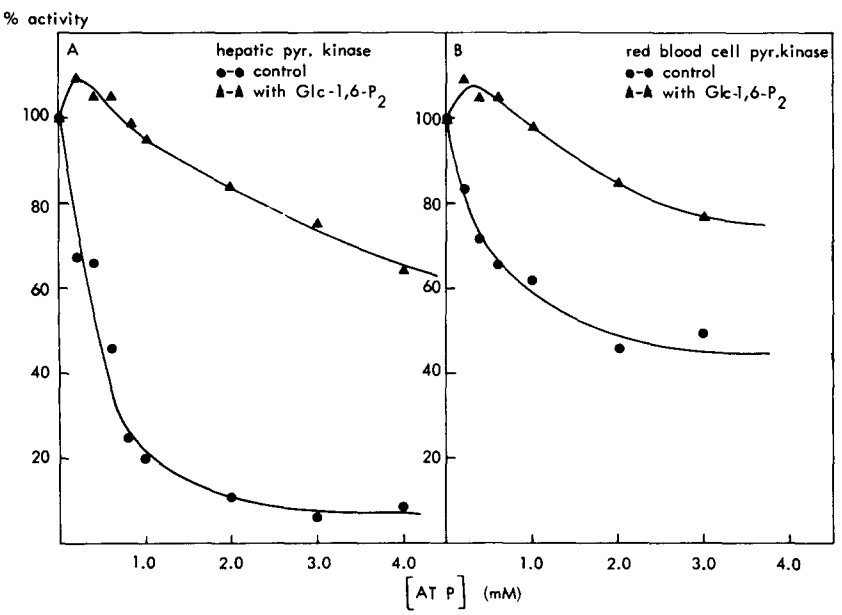

Fig. 3A. The ATP inhibition plots for hepatic pyruvate kinase at [PEP] = 0.5 mM and [ADP] = 0.5 mM. ●—●, control; ▲—▲, with Glc-1,6-P_2 (0.2 mM).

Fig. 3B. The ATP inhibition plot for red blood cell pyruvate kinase at [PEP] = 0.5 mM and [ADP] = 0.5 mM; ●—●, control; ▲—▲, with Glc-1,6-P_2 (0.2 mM), 20 μg protein added.

Glc-1,6-P_2, not only stimulates the pyruvate kinase reaction, but is also able to transform the S-shaped curve into a normal hyperbolic one (Fig. 1). These results are quite similar to the results obtained with the ligand Fru-1,6-P_2 (refs. 1, 2). At pH 5.9 the effects of Fru-1,6-P_2 disappear. The same results were obtained with Glc-1,6-P_2; no stimulation occurs at all. Fig. 2 shows the influence of Glc-1,6-P_2 on the red blood cell pyruvate kinase reaction at pH 7.6. These results are similar to the results obtained with the hepatic pyruvate kinase.

Similar to Fru-1,6-P_2, Glc-1,6-P_2 is able to reverse the ATP inhibition on the pyruvate kinase reaction (Fig. 3A). This is also the case with red blood cell pyruvate kinase (Fig. 3B).

It is clear from these data that the effects of Glc-1,6-P_2 and Fru-1,6-P_2 are quite similar. In liver both ligands have a synergistic effect only at low ($<50\ \mu$M) concentration (Fig. 4). Fig. 5 shows that with the red blood cell pyruvate kinase at

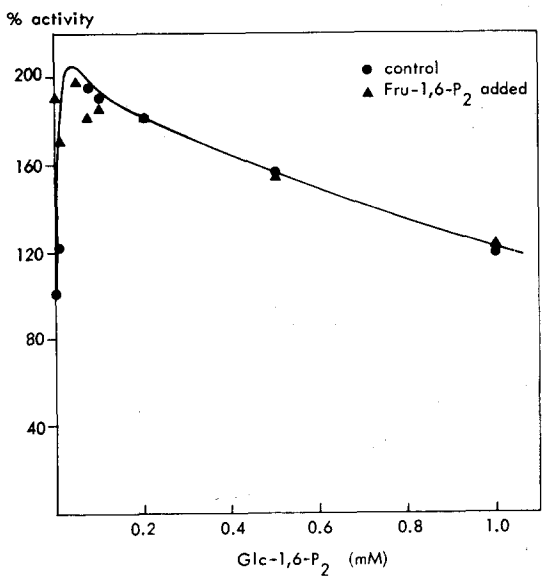

Fig. 4. The *v versus* [Glc-1,6-P_2] for hepatic pyruvate kinase in the presence and absence of Fru-1,6-P_2; ●—●, control; ▲—▲, with Fru-1,6-P_2 (0.2 mM), [PEP] = 0.5 mM and [ADP] = 0.3 mM.

500 μM, Glc-1,6-P_2 Fru-1,6-P_2, does not further stimulate, but that in the absence of Fru-1,6-P_2 half-maximal stimulation by Glc-1,6-P_2 is obtained at 60 μM.

At a [Fru-1,6-P_2] of 50 μM the hepatic pyruvate kinase is practically fully stimulated; however, at 10 μM the stimulation is already about 90% of the maximal obtainable. For Glc-1,6-P_2 the concentration necessary for full stimulation is about 50 μM; at a concentration of 10 μM the stimulation is about 60% of maximal obtained. For the red blood cell pyruvate kinase the situation is somewhat different. At 10 μM of Fru-1,6-P_2 the reaction is fully stimulated but for Glu-1,6-P_2 the pyruvate kinase reaction is still not fully stimulated at a concentration of 200 μM.

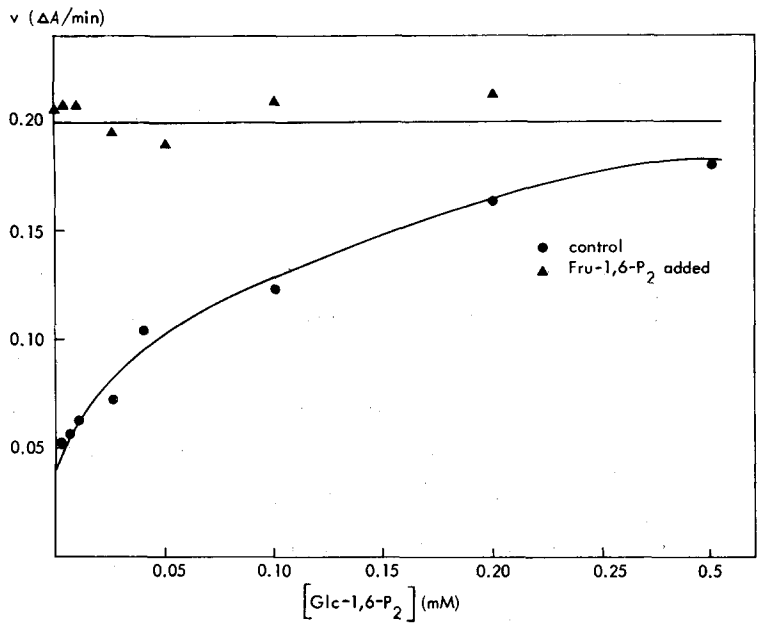

Fig. 5. The *v versus* [Glc-1,6-P_2] for red blood cell pyruvate kinase in the absence and presence of Fru-1,6-P_2; ●—●, control; ▲—▲, with Fru-1,6-P_2 (0.5 mM). [PEP] = 0.5 mM and [ADP] = 0.5 mM, 20 μg of protein added.

DISCUSSION

From the data presented it is clear that the effects of Fru-1,6-P_2 and Glc-1,6-P_2 are quite similar. The concentration of Fru-1,6-P_2 in the liver fluctuates during feeding and fasting (5–20 μM)[9]. However, the [Glc-1,6,P_2] remains constant at about 15 μM[10]. At this concentration of Glc-1,6-P_2 the hepatic pyruvate kinase is more than 80% stimulated, while at 5 μM Fru-1,6-P_2 pyruvate kinase is already stimulated for 50%. This means that Fru-1,6-P_2 cannot have any regulatory function on hepatic pyruvate kinase. For red blood cell pyruvate kinase it was shown that Fru-1,6-P_2 has already a fully stimulating effect at 10 μM, while for Glc-1,6-P_2 the concentration has to be more than 200 μM. However, the [Glc-1,6-P_2] in the red blood cell is 300 μM[11]. According to BARTLETT[11], the red blood cell [Fru-1,6-P_2] is 200 μM. With these concentrations the pyruvate kinase in the erythrocyte is fully stimulated. This means that the enzyme is in the R state[5]. For the red blood cell it is rather doubtfull if a regulation of pyruvate kinase is necessary. The rate of glycolysis is governed by the activities of hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) and phosphofructokinase (ATP: D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), in which the concentrations of adenine nucleotides also play an important role[1,5,12,13].

Although there will be some wasting of ATP during the gluconeogenesis due to cycling[9], the pyruvate kinase reaction must be inhibited in order to increase the PEP concentration which is necessary for the gluconeogenesis. However, this regulation cannot be done by a fluctuating Fru-1,6-P_2 concentration of the liver.

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