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DISTRIBUTION OF L- AND M-TYPE PYRUVATE KINASE BETWEEN PARENCHYMAL AND KUPFFER CELLS OF RAT LIVER

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SUMMARY

1. Parenchymal cells were isolated from rat liver by using EDTA + lysozyme or citrate. These cells contained only the L-type pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40).

2. Kupffer cells were isolated from rat liver by using pronase. The Kupffer cell preparation showed a ratio of M-type to L-type pyruvate kinase 10–20 times higher than the ratio in a total liver homogenate, suggesting that Kupffer cells only (probably) contain the M-type pyruvate kinase.

3. The results obtained suggest that gluconeogenesis is confined to the parenchymal cells of rat liver.

INTRODUCTION

Pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) catalyses the last step of glycolysis. Its activity is important in the regulation of the dynamic balance between gluconeogenesis and glycolysis in the liver. During glycolysis the enzyme must be active; during gluconeogenesis, on the other hand, activity leads to wastage of energy. In liver there are two types of pyruvate kinase[1]. The L-type pyruvate kinase shows an allosteric response to one of its substrates, phosphoenolpyruvate (PEP), and its activity is further influenced by glycolytic and gluconeogenic intermediates[2]. Fru-1,6-P_2 (ref. 3), Glc-1,6-P_2 (ref. 4) and other phosphorylated hexoses[5] increase the apparent affinity for the substrate PEP. On the other hand ATP acts as an allosteric inhibitor[3]. L-Alanine, a precursor of gluconeogenesis inhibits the L-type pyruvate kinase[6]. The abundance of this type of pyruvate kinase is regulated by the diet and by hormones[7].

The M-type pyruvate kinase is a non-allosteric enzyme as can be concluded from the Michaelis–Menten kinetics. From the properties of the two types of pyruvate kinase one could conclude that only the L-type is involved in the regulation of the

Abbreviation: PEP, phosphoenolpyruvate.

dynamic balance between glycolysis and gluconeogenesis. Since the liver contains different types of cells, it is possible that the two types of pyruvate kinase have a different localization. In order to investigate the localization differences of pyruvate kinases, we isolated Kupffer and parenchymal cells from rat liver.

MATERIALS AND METHODS

Type L pyruvate kinase was isolated from rat liver according to the isolation procedure described earlier[4]. Type M pyruvate kinase was isolated by the method of Passeron *et al.*[8]. Pyruvate kinase was assayed by following the decrease in absorbance at 340 nm in the coupled reaction with lactate dehydrogenase at room temperature according to Valentine and Tanaka[9]. The triethanol–HCl buffer (0.4 M, pH 7.5) was replaced by Tris–HCl buffer (0.25 M, pH 8.0). Parenchymal cells were obtained by a method described by Hommes *et al.*[10] and were centrifuged for 5 min at 50 × *g* and washed with the isolation medium except that EDTA and lysozyme were omitted. This procedure was repeated five times and the final precipitate was homogenized in 0.25 M Tris–HCl (pH 8.0) containing 1 mM mercaptoethanol. After centrifugation at 20 000 × *g* for 20 min the pyruvate kinase activity was measured. The final precipitate contained only parenchymal cells, as shown by light microscopy.

Kupffer cells were isolated by a method based on that described by Mills and Zucker-Franklin[11]. Wistar rats were anaesthesized with nembutal; the liver was perfused with 0.9% NaCl or Hank's balanced salt solution and after it became a light tan colour it was sliced into 3–5 mm fragments and placed in 10 ml Hank's balanced salt solution containing 0.25% pronase. After 30 min vigorous shaking at 37 °C the mixture was filtered and subsequently centrifuged for 5 min at 600 × *g*. The cells were resuspended by addition of Hank's balanced salt solution and recentrifuged. The procedure was repeated four times. The cells were then homogenized, centrifuged for 20 min at 20 000 × *g* and the pyruvate kinase activity was measured.

Pronase was obtained from Cal Biochem. Lysozyme, ADP, PEP, lactate dehydrogenase and NADH were obtained from Boehringer (Mannheim, Germany). All other reagents were of analytical grade purity.

RESULTS

In Fig. 1 are plotted the activities of the isolated L- and M-type pyruvate kinases *versus* the PEP concentration at fixed [ADP] and pH 8.0. The isolated M-type pyruvate kinase follows the Michaelis–Menten kinetics, and is not influenced by Fru-1,6-P_2. In contrast, the L-type pyruvate kinase shows a sigmoidal curve, which can be converted into a hyperbolic curve by adding 0.5 mM Fru-1,6-P_2. From the curves obtained with the isolated L-type it can be concluded that Fru-1,6-P_2, at least under our assay conditions, stimulates the enzyme activity at 1 mM PEP by a factor of 11.0 ± 0.3 ($n = 6$). From these results (Fig. 1) it is possible to calculate the activity ratio of the L- and M-types. It was found that in crude liver homogenates at 1 mM PEP an average activation of 7.1 was obtained by adding 0.5 mM Fru-1,6-P_2. From this it was calculated that the L-type contributes about 61% to the overall pyruvate kinase activity prior to Fru-1,6-P_2 addition.

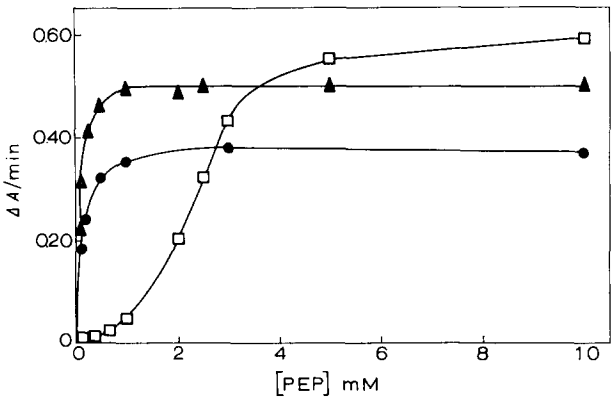

Fig. 1. The *v vs* [PEP] plot of the partly purified L- and M-type pyruvate kinase at [ADP] = 2 mM. in presence and absence of Fru-1,6-P_2 (0.5 mM). □—□, L-type pyruvate kinase; ▲—▲, L-type pyruvate kinase in the presence of Fru-1,6-P_2; ●—●, M-type pyruvate kinase in the absence or presence of Fru-1,6-P_2.

Fig. 2 shows the pyruvate kinase activity of the homogenate of isolated parenchymal cells, tested under the same conditions as in Fig. 1. From this plot we conclude that only the L-type pyruvate kinase is present in parenchymal cells. Also, when parenchymal cells were isolated with citrate (27 mM) in Hank's balanced salt solution instead of EDTA and the lysozyme-containing medium (described in Materials and Methods) the same result was obtained.

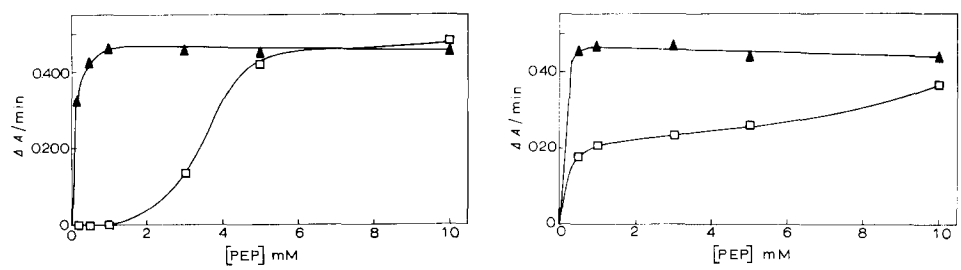

Fig. 2. The *v vs* [PEP] plot of pyruvate kinase activity in the parenchymal cell homogenate in the absence and presence of Fru-1,6-P_2 (0.5 mM). □—□, without Fru-1,6-P_2; ▲—▲, with Fru-1,6-P_2.

Fig. 3. The *v vs* [PEP] plot of pyruvate kinase activity in the Kupffer cell homogenate in the absence and presence of Fru-1,6-P_2 (0.5 mM). □—□, without Fru-1,6-P_2; ▲—▲, with Fru-1,6-P_2.

Fig. 3 shows the pyruvate kinase activity versus [PEP] of the Kupffer cell homogenate. This plot is completely different from the one obtained with parenchymal cells (Fig. 2). The activity increment at 1 mM PEP, due to 0.5 mM Fru-1,6-P_2, in Fig. 3 is 2.2 fold, which indicates that the M-type is enriched in the homogenate. We can calculate that the L/M ratio in this Kupffer cell preparation is 0.135 (compare the value of 1.56 in a crude liver homogenate), indicating a relative increase of the M-type contribution of at least 10 fold. The Fru-1,6-P_2 stimulations obtained with different preparations of crude liver, parenchymal cell and Kupffer cell homogenates are summarized in Table I.

TABLE I

RELATIVE ACTIVITIES OF L- AND M-TYPES OF PYRUVATE KINASE

For the measurement of activity see Fig. 1 and Materials and Methods.

Source of homogenate	*n*	*Fru-1,6-P_2 stimulation at 1 mM PEP*	*Ratio of activities* L-/M-type pyruvate kinase*
Whole rat liver	6	7.1	1.56
Parenchymal cells	6	∞	∞
Kupffer cells	6	1.7–2.4	0.07–0.16

* Fru-1,6-P_2 absent.

Since pronase and lysozyme are proteolytic enzymes, a possible criticism exists: these enzymes might preferentially digest one of the two types of pyruvate kinase. Therefore we incubated the isolated L- and M-types from rat liver with either lysozyme (0.05%) or pronase (0.25%). This concentration of lysozyme did neither affect the L- nor the M-type activity, even after 2 h incubation. That no preferential digestion by lysozyme occurred, could also be concluded from the fact that when citrate was used instead of lysozyme in the isolation of parenchymal cells, the same results were obtained. Pronase on the other hand affected the L-type in the presence of Fru-1,6-P_2 to the same extent as the M-type. When isolated L-type was incubated with pronase, the activation of the reaction was increased by the addition of Fru-1,6-P_2. Therefore, the activity ratio of L to M-type pyruvate kinase for Kupffer cells (as shown in Table I) may be overestimated. However, it is likely that during the isolation procedure, pyruvate kinase is protected from the added proteolytic enzymes by the cell membrane.

DISCUSSION

The data presented show that only the parenchymal cells of rat liver contain the L-type pyruvate kinase and it is highly suggestive that the Kupffer cells only contain the M-type. It is generally accepted that the L-type is involved in the regulation of glycolysis and that the enzyme can be switched off during gluconeogenesis. It is, therefore, likely that gluconeogenesis is also located solely in the parenchymal cells. Tanaka *et al.*[2] already in 1967 mentioned the occurrence of L- and M-type pyruvate kinase in liver and noticed a linkage between the L-type pyruvate kinase and glucokinase (EC 2.7.1.12). Their data also suggested a linkage between the M-type with hexokinase (EC 2.7.1.1). They concluded "that there may be two kinds of glycolysis pathway in the liver. One of these is catalysed by hexokinase and type M pyruvate kinase and might be called the basal pathway. The other is catalysed by glucokinase and type L pyruvate kinase and is a regulatory pathway. The rate of the basal pathway would not be influenced by dietary and hormonal conditions, and it would meet the minimum demands of the cell. The rate of the regulatory pathway would fluctuate in response to dietary and hormonal conditions and it would meet special demands of the cell". We agree with this parallelism, since Sapag-Hagar *et al.*[12] showed that glucokinase is present in parenchymal cells and hexokinase is virtually restricted to the non-parenchymal tissue. In the present paper it is shown that the L-type pyruvate kinase

is confined to the parenchymal cell. Therefore, it is clear now that the two pathways, distinguished by Tanaka *et al.*[2], are even localized in two different liver cell types. The parenchymal cell responds to the metabolic changes of the liver, whereas the Kupffer cells lack such a possibility.

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Recently, Crisp and Pogson[13] showed that also in mouse liver the L-type pyruvate kinase is confined to the parenchymal cell.

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