FEED-FORWARD ACTIVATION AND FEED-BACK INHIBITION OF PYRUVATE KINASE TYPE L OF RAT LIVER

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It was reported from this laboratory that at least two types of pyruvate kinase were present in rat liver (Tanaka et al., 1967). These pyruvate kinases were named type L (liver) and type M (muscle), respectively. Liver was the only organ which contained both types of pyruvate kinase, and erythrocytes were the only cells other than liver cells which contained type L enzyme. Type M was widely distributed in various tissues. Liver type L enzyme might well be called a regulatory enzyme, since its quantity in liver was regulated by the diet or hormones and its activity was modulated through activation by phosphoenolpyruvic acid and inhibition by adenosine triphosphate (ATP). In 1966, Hess et al. reported that yeast pyruvate kinase was activated by fructose-1,6-diphosphate. Later, Taylor and Bailey (1967) reported that only pyruvate kinase extracted from liver was activated by fructose-1,6-diphosphate, whereas the enzyme from muscle was not. In this paper, it is reported that fructose-1,6-diphosphate activates the purified type L enzyme of rat liver at a low concentration of phosphoenolpyruvic acid and also reverses the inhibition of type L enzyme by ATP, that is, makes the enzyme insensitive to ATP inhibition, whereas fructose-1,6-diphosphate did not have any affect on the crystalline rat muscle pyruvate kinase. Similar results were obtained by Hess (1967).

Materials and Methods

The materials and methods used in the present experiments were the same to those given in our previous paper (Tanaka et al., 1967), unless otherwise mentioned. Pyruvate kinase was assayed by following the decrease of absorption at 340 mμ in the coupled reaction of the lactate dehydrogenase system with pyruvate kinase using a recording spectrophotometer. Type L pyruvate kinase was purified from livers of rats fed on a high carbohydrate diet. The partially purified preparation (specific activity of about 100), was mainly used in the experiments described in this paper, after it had been confirmed immunochemically that it was not contaminated with type M enzyme. Crystalline type M pyruvate kinase was isolated by the methods described in the same paper.

Results

Fig. 1 shows plots of the enzyme activities as a function of the concentration of phosphoenolpyruvic acid in purified muscle and type L pyruvate kinase in the absence (solid lines) and presence of 0.1 mM fructose-1,6-diphosphate (broken lines). As reported by Tanaka et al. (1965, 1966) and Taylor et al. (1967), the activity of type L enzyme was very low with concentrations of phosphoenolpyruvic acid below about 0.3 mM, and increased abruptly at concentrations above this level. This type of kinetics suggests that the enzyme is activated by the substrate itself (Dixon and Webb, 1958). However, in the presence of fructose-1,6-diphosphate, plots of enzyme activity as a function of phosphoenolpyruvic acid concentration with type L enzyme gave the usual Michaelis' curve, the maximum velocity was approximately doubled, and the Michaelis constant decreased to about one tenth (0.08 mM) of that (0.84 mM) in the absence of fructose-1,6-diphosphate. Fructose-1,6-diphosphate did not show any significant effects on type M pyruvate kinase. Similar results have been described by Taylor and Bailey (1967) who used crude liver pyruvate kinase.

As we reported previously (1967), type L enzyme was very much more sensitive to inhibition by ATP than type M enzyme. The apparent half inhibitory concentration of ATP for type L was 0.16 mM, which was about one twentieth of that for

type M (3.5 mM). Fig. 2 shows plots of the percentage inhibition by ATP as a function of the concentration of ATP in the absence and in the presence of fruc-

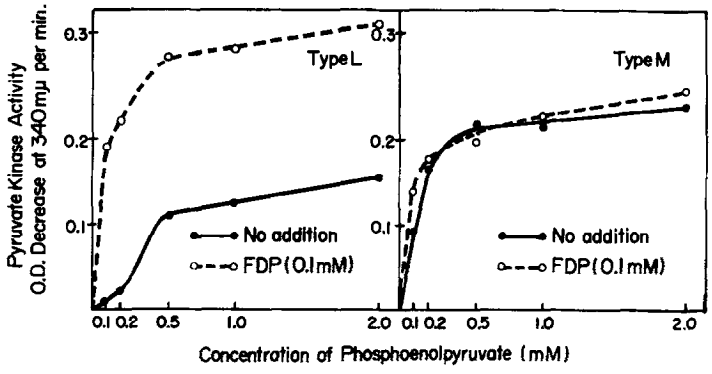

Fig. 1. Pyruvate kinase activity as a function of phosphoenolpyruvic acid concentration.

Pyruvate kinase activity is expressed as decrease of optical density at 340 mμ per minute. The enzyme was assayed by the lactate dehydrogenase method (Tanaka et al., 1967) in the absence (solid line) and in the presence of 0.1 mM of fructose-1,6-diphosphate (broken line). The composition of the standard assay mixture was as follows: Tris (hydroxymethyl)-aminomethane 50 μmoles, $MgSO_4$ 5 μmoles, KCl 100 μmoles, NADH 0.2 μmoles, lactate dehydrogenase 2 units, ADP 0.2 μmoles and the indicated amounts of phosphoenolpyruvic acid in a total volume of 1.0 ml. The left figure shows the results for the liver type L (specific activity: 100), and the right figure for crystalline type M pyruvate kinase.

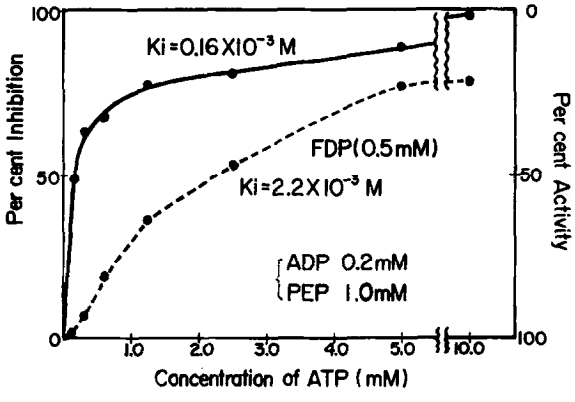

Fig. 2. Effect on ATP inhibition of type L pyruvate kinase of fructose-1,6-diphosphate.

The enzyme was preincubated with the assay mixture (Tanaka et al., 1967) containing the indicated amounts of ATP alone or together with fructose-1,6-diphosphate (0.5 mM). Then the reaction was started by addition of phosphoenolpyruvic acid (1 mM). The solid line shows the results obtained in the absence of fructose-1,6-diphosphate and the broken line shows those in the presence of fructose-1,6-diphosphate.

tose-1,6-diphosphate (0.5 mM). In these experiments, 1.0 mM of phosphoenolpyruvic acid was used, since activation of fructose-1,6-diphosphate was not significant at this concentration, and the enzyme activities measured in the presence and absence of fructose-1,6-diphosphate were taken as 100 per cent activity for each curve. As clearly shown in Fig. 2, the apparent half inhibitory concentration was increased about ten fold by addition of fructose-1,6-diphosphate. This effect of fructose-1,6-diphosphate in overcoming ATP inhibition was very clearly observed at about 1 mM ATP, which has been reported to be a physiological concentration of ATP.

In the materials so far examined pyruvate kinase type L has only been found in liver and erythrocytes. Therefore it was of interest to know whether the pyruvate kinase of erythrocytes showed the same kinetic properties as those of liver type L enzyme. Pyruvate kinase type L in a fresh hemolyzate and also in a partially purified enzyme preparation was not activated by fructose-1,6-diphosphate, though this pyruvate kinase was neutralized completely by antibody against liver type L enzyme but not by that against type M. This suggests that there must be two forms of type L pyruvate kinase, one of which is activated by fructose-1,6-diphosphate, and the other which is not. Table 1 shows that crystalline type L enzyme, which was sensitive to fructose-1,6-diphosphate at zero time, looses its sensitivity with time of incubation at 37°C in very dilute solution. Aliquots of the incubated enzyme solution were taken at the times indicated in the table, and then the enzyme activity was assayed in the presence and absence of fructose-1,6-diphosphate. Before incubation at 37°C, the enzyme activity was almost undetectable, if it was assayed in the absence of fructose-1,6-diphosphate. Then during the incubation, the enzyme activity assayed even in the absence of fructose-1,6-diphosphate was gradually recovered with time. In contrast, the enzyme activity measured in the presence of fructose-1,6-diphosphate did not show any significant change during incubation. After 120 min.incubation, the enzyme activity in the absence of fructose-1,6-diphosphate reached the same level of the activity as in the presence of fructose-1,6-diphosphate. In other words, type L enzyme was completely

desensitized toward activation by fructose-1,6-diphosphate. It must be noted here that no change in immunological properties of the enzyme was observed, after complete desensitization, and the kinetic properties of the desensitized type L enzyme became quite similar to those of the type M enzyme. The half inhibition concentration of ATP was also about the same as for the type M enzyme.

Fructose-1,6-diphosphate	Pyruvate kinase activity O.D. change at 340 mμ per minute			
	Incubation time (minutes)			
	0	30	60	120
Not added	0.02	0.16	0.42	0.50
Added	0.43	0.52	0.58	0.50

Table 1. Desensitization of pyruvate kinase type L of rat liver.

Crystalline pyruvate kinase type L was dissolved in 0.15 M KCl solution containing 1 mM of EDTA, pH 7.5, at a concentration of 5 units per ml. This diluted enzyme solution was incubated at 37°C without shaking. Aliquots of enzyme were taken at the indicated times, and assayed in the absence and presence of fructose-1,6-diphosphate (0.2 mM). The assay mixture in this experiment was the same as for Fig. 1, except that the concentrations of phosphoenolpyruvic acid and ADP were 0.05 mM and 0.5 mM, respectively.

Discussion

It was found that pyruvate kinase activity in rat liver is regulated in two ways. The quantities of type L and type M pyruvate kinase fluctuate under various physiological and pathological conditions, as described previously by Tanaka et al. (1967). The enzyme activity of type L pyruvate kinase of rat liver is regulated by feed-forward activation by phosphoenolpyruvic acid or fructose-1,6-diphosphate, and by feed-back inhibition by ATP. This feed-forward activation and feed-back inhibition counteract each other. It is suggested that the reaction rate of pyruvate kinase in rat liver was regulated by the levels of ATP, fructose-1,6-diphosphate and phosphoenolpyruvic acid in the liver cells. It should be noted here, that type L pyruvate kinase from erythrocytes was not activated by fructose-1,6-diphosphate. One clue to elucidate this difference in the molecular basis of the reactions is the fact that crystalline liver type L enzyme is desensitized to fructose-1,6-di-

phosphate on incubation at 37°C in very dilute solution. Studies on the resensitization of the desensitized enzyme are in progress in our laboratory. Though the optimal conditions and mechanism of resensitization are still unknown, there is a possibility that interconversion between the sensitive and nonsensitive forms of type L enzyme may occur in liver cells, but not in erythrocytes. This could be another type of mechanism regulating pyruvate kinase type L activity in liver.

Summary

Crystalline type L pyruvate kinase was activated by fructose-1,6-diphosphate. The Michaelis constant for phosphoenolpyruvic acid decreased to about one tenth and the maximum velocity was doubled by the presence of fructose-1,6-diphosphate. The activity of type M pyruvate kinase was not influenced significantly by fructose-1,6-diphosphate. The sensitivity to ATP inhibition of type L enzyme was decreased very markedly by fructose-1,6-diphosphate. Liver type L enzyme became completely insensitive to fructose-1,6-diphosphate activation after 120 minutes incubation at low concentration at 37°C.

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