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Activation of Liver Pyruvate Kinase by Fructose 1,6-Diphosphate

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It has been reported by Hess, Haeckel & Brand (1966) that yeast pyruvate kinase (ATP-pyruvate phosphotransferase, EC 2.7.1.40) is stimulated by FDP.* This is apparently not a general property of pyruvate kinases, since in tests on extracts from *Lactobacillus fermentii* and several animal tissues stimulation by FDP was detected only with the enzyme from bovine heart muscle. Stimulation of rat-liver pyruvate kinase by very low concentrations of FDP is now reported, the pattern of the kinetics of stimulation supporting the view that this is an allosteric effect.

Preparation of enzyme extract. Rat liver was homogenized with 5 vol. of cold distilled water in a Waring Blendor and the homogenate centrifuged at 15000g for 1 hr. (MSE 18 centrifuge, 6 × 250 head, 12000 rev./min.). The protein of the supernatant fluid was fractionated by differential ammonium sulphate precipitation in the cold. The fraction precipitated by between 30% and 40% saturation with ammonium sulphate, which contained 50% of the total liver pyruvate-kinase activity, was redissolved in a small volume of water and stored at 4°. Crude extracts prepared in this way had a specific activity of about 1 unit/mg. of protein and were used without further purification (1 unit is defined as 1 μmole of substrate utilized/min. at 25°).

Assay. Pyruvate kinase was assayed by the method of Bücher & Pfeleiderer (1955). The reaction is coupled with lactate dehydrogenase to give oxidation of NADH₂, which is followed by measuring the change in *E*₃₄₀. Except where

* Abbreviations: FDP, fructose 1,6-diphosphate; PEP, phosphoenolpyruvate.

modified for a particular purpose the final reaction mixture contained: triethanolamine buffer, pH 7.5, 40mM; KCl, 70mM; NADH₂, 0.15mM; ADP, 1.0mM; MgSO₄, 8.0mM; lactate dehydrogenase [rabbit-muscle enzyme; Boehringer Corporation (London) Ltd., London, W.5], 10 units. The reaction, carried out at 25°, was started with PEP.

Results. The factors governing stimulation of pyruvate kinase by FDP are complex, the stimulated/unstimulated activity ratio, which may be as high as 40, depending not only on the FDP concentration but also on PEP concentration and pH. The interrelationship between FDP and PEP is best illustrated by the data showing the influence of each of these on the velocity-concentration curve for the other. In the absence of FDP the curve for initial velocity against PEP concentration is sigmoidal (Fig. 1), the plot of 1/v against 1/s curves upwards, the Hill plot gives a maximum slope of approximately 2 and half-maximal activity is attained at 0.6mM-PEP. In the presence of high concentrations of FDP (0.5mM), the response to PEP concentration is transformed to give a Michaelis-Menten curve. The reciprocal plot is now linear, the Hill plot has a slope of 1 and the half-maximal concentration of PEP is 60 μM, i.e. tenfold lower than in the absence of FDP.

The data for the converse relationship (varying FDP concentration at fixed concentrations of PEP) are less reliable, since by reason of the saturation characteristics of the system the most informative portions of the curves occur at low reaction rates and very low concentrations of FDP. Under these conditions measurements of reaction rate are imprecise and the presence of small quantities of

Fig. 1. Influence of FDP on the relationship between pyruvate-kinase activity and PEP concentrations. ●, No FDP; ○, 0.5 mM-FDP.

FDPase and aldolase in the crude enzyme may distort the results. However, the curves (not shown) demonstrate that at low concentrations of PEP the FDP stimulation curve is sigmoidal and that half-maximal stimulation occurs at about $0.5 \mu\text{M}$, quite significant stimulation being detectable below $0.1 \mu\text{M}$. With rising concentration of PEP the unstimulated activity increases (cf. Fig. 1), the effect of FDP is proportionately less and the sigmoidal nature of the curves is lost.

Changes in pH strikingly alter the response to FDP. A preparation that showed a rise of only 30% in response to 0.5 mM-FDP at pH 6.0 gave a 20-fold stimulation at pH 8.0. The pH optimum of the crude preparation used here was 7.0–7.5 in the presence of maximal concentrations of FDP and about 6.5 in its absence. The increase in stimulation ratios at high pH is due mainly to a rapid falling off in unstimulated activity above pH 7.5, stimulated activity being affected to a far smaller extent.

No corresponding relationship between FDP stimulation and the other substrate ADP could be detected. The curve for reaction velocity against ADP concentration, in the presence of 1 mM-PEP, has a Michaelis-Menten form and is unaffected by 0.5 mM-FDP.

Of other phosphorylated intermediates tested, glucose 6-phosphate, fructose 6-phosphate and 6-phosphogluconate stimulated pyruvate kinase, but at concentrations at least 100-fold higher than those required with FDP. Though these results could mean that these substances have some affinity for the FDP site they might also be explained by the presence of small quantities of FDP as an impurity. 2,3-Diphosphoglycerate and 2-phosphoglycerate were entirely without effect.

Tests on two preparations of muscle pyruvate

kinase, a purified rabbit-muscle pyruvate kinase [Boehringer Corporation (London) Ltd.] and the supernatant fluid from the centrifugation of a crude homogenate of rat muscle, indicated that FDP stimulation in these preparations is slight. In this latter preparation the PEP concentration curve gave a Michaelis-Menten form with K_m $5.5 \times 10^{-5} \text{ M}$, i.e. it closely resembled the PEP concentration curve obtained for liver pyruvate kinase in the presence of FDP.

Discussion. The main kinetic characteristics of the stimulation of pyruvate kinase by FDP are the homotropic co-operative interaction exhibited by both substrate (PEP) and effector (FDP), the abolition of the substrate-substrate interaction at high concentrations of FDP (heterotropic interaction) and the tenfold decrease in the apparent K_m for PEP. The loss of FDP homotropic interaction at high concentrations of PEP was less clearly demonstrated but may certainly be present. These are clearly the characteristics of an allosteric system (Monod, Wyman & Changeux, 1965). In terms of the Monod allosteric model these data indicate a 'positive K system', i.e. one in which both substrate and effector have differential affinities for the two hypothetical forms of the enzyme R and T.

The absence of stimulation of muscle pyruvate kinase by FDP should make it of interest to compare its structure and properties with those of the liver enzyme. It is possible that muscle pyruvate kinase *in situ* has allosteric properties similar to those of yeast and liver pyruvate kinase, but that these are lost consequent to changes in quaternary structure during extraction and purification.

The concentration at which FDP acts on liver enzyme is remarkably low, about 300-fold lower than is required for a comparable effect in yeast. However, the half-maximal concentration of FDP ($0.5 \mu\text{M}$) is higher than the concentration reported by Burch (1965) to be present in rat liver ($0.02 \mu\text{M}$). Since the high affinity of the liver enzyme for FDP is matched by a low intracellular concentration, it is probable that the FDP stimulation constitutes a switching mechanism for liver glycolysis, exerting a positive feed-forward effect as has been suggested for yeast by Hess *et al.* (1966).

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