FDP-ACTIVATION OF YEAST PYRUVATE KINASE[1]

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In previous work we have found that under the steady-state conditions of a fully activated glycolysis the pyruvate kinase reaction is greatly displaced from equilibrium (Hess et al., 1962, 1965). On the basis of this finding we have postulated that the pyruvate kinase reaction is one of the control points of the glycolytic pathway. This hypothesis has further been substantiated by analysis of the mechanism of glycolytic oscillation in S. carlsbergensis, which revealed a cross over point at the pyruvate kinase reaction in intact cells (Hommes, 1964) and in a cell-free extract (Hess et al., 1966) as well as a high sensitivity of the oscillation toward titration with yeast pyruvate kinase (Hess et al., 1965).

These findings led us to the assumption that a specific metabolite does control the reaction of pyruvate kinase. In a general study of the properties of yeast pyruvate kinase we have found that fructose diphosphate

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(FDP) is a potent activator of pyruvate kinase, thus, explaining the rigid control imposed on this enzyme during transient and steady-state conditions of glycolysis (Hess et al., 1965). We here report on our findings.

Material and methods. Yeast pyruvate kinase has been prepared from brewers` yeast (KRONENBRAUEREI, Dortmund), which was first carefully dried overnight at 30° C on filter paper. The dry material was extracted by glycerol (25 %, pH 8.0). The crude extract was treated with ethanol (10 %) and nucleic acid (Nukleinsäure I A, Zellstofffabrik Waldhof, Mannheim-Waldhof, Nr. 12 220, 10 %), added consecutively at a pH of 5.0. The resulting precipitate was centrifuged. The supernatant was kept in the cold overnight, until a white, coarse precipitate was formed, containing the total activity of phosphoglycerate kinase, and centrifuged off. The supernatant was treated with ammonium sulphate to 1.2 M, pH 6.2. The sediment was discarded and a second ammonium sulphate precipitation at 1.9 M was carried out, yielding the main fraction of pyruvate kinase in the precipitate. This taken up in 1 M ammonium sulphate solution and processed through a Sephadex G 200 column (80 x 3,5 cm, flow speed 15 ml per hour). A large protein peak was isolated containing pyruvate kinase with a specific activity of 30 to 60 µmoles per min per mg protein, which was used for kinetic studies. In some experiments assays were carried out with the same result using a crude extract of brewers yeast or a cell-free extract of S. carlsbergensis obtained according to (Hess et al., 1966). According to the Sephadex-filtration the molecular weight of the purified pyruvate kinase preparation was approximately 200 000.

Protein determinations were carried out by the Biuret method.

FDP was assayed by the enzymic test using aldolase, α -glycerophosphate dehydrogenase and DPNH as indicator system. The activity of pyruvate kinase was usually determined by coupling with lactate dehydrogenase as an indicator enzyme. In some experiments the rate of disappearance of phosphoenolpyruvate (PEP) was assayed at 240 mμ in a spectrophotometer in order to exclude interference of FDP with the lactate dehydrogenase system. No influence of fructose diphosphate on the latter enzyme was found. In the crude extract a breakdown of FDP to phosphoenolpyruvate was avoided by addition of iodoacetate (2.5 mM) and arsenate (5 mM). Neither compound interfered with the pyruvate kinase reaction. PEP, ATP, ADP, FDP and LDH were purchased from Boehringer & Söhne GmbH. Mannheim-Waldhof. FDP was passed through a Sephadex G 10 column before use.

EXPERIMENTAL RESULTS AND COMMENTS

On addition of FDP a 20-50 fold activation of pyruvate kinase can be recognized in a routine optical test. A detailed analysis of this activation revealed a relation between the concentration of FDP and the activity as demonstrated with the log plot of figure 1 where two portions can be distinguished, pointing to a change in the activation mechanism with higher FDP level. Half maximal activation is found with a concentration of 1.5×10^{-4} M FDP depending on the assay conditions. From the upper slope of the plot an order of $n \sim 2.0$ is computed, suggesting a multipoint activation of the allosteric type. From such experiments we have learned that the FDP activation is "competitive" with PEP, which also operates with an S-shaped

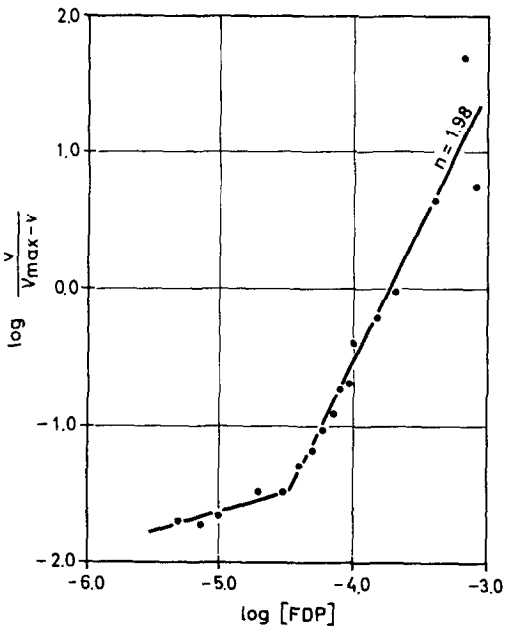

Fig. 1 Pyruvate kinase activity as a function of the concentration of the activator.
abscissa: $[M]$
assay conditions: Triethanolamine-HCl (0, 2 M), Mg SO_4 (5 mM), PEP (0, 25 mM), ADP (0, 3 mM), DPNH (0, 25 mM), LDH 80 I.U. (= international units = μMol x min^{-1}), PK 2 I.U.
Final volume 2 ml, final pH 6.5 (exp. III/5/60).

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concentration / activity relationship. The log plot of PEP activation is given on figure 2. Again an order of an $\sim$ 2.0 is calculated. FDP activation is not competitive with ATP or ADP. The activation of FDP is dependent on the magnesium level, and maximum activation is observed at 2.5 mM magnesium. Since within the limits of the determination FDP could quantitatively be recovered after macroscopic interaction with pyruvate kinase, the activation reaction is considered to be of catalytic nature.

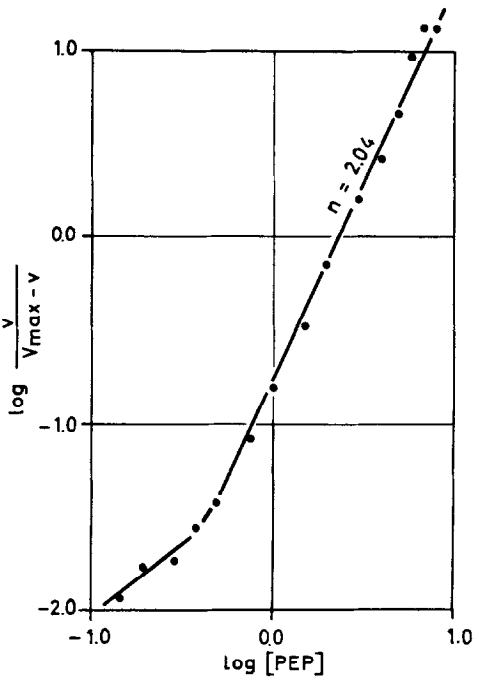

Fig. 2 Pyruvate kinase activity as a function of the concentration of phosphoenolpyruvate.
abscissa: [mM]
assay conditions: Triethanolamine-HCl (0, 2 M), Mg SO_4 (10 mM), KCl (5 mM), ADP (5 mM), DPNH (0, 25 mM), LDH 80 I.U., PK 3 I.U.
Final volume 2 ml, final pH 6.5 (exp. IV/18/24).

A study of pyruvate kinases of other (ascites tumor cells, human erythrocytes, rat skeletal muscle, bovine hypophysis, Lactobacillus fermentii) showed that only yeast and bovine heart pyruvate kinase can be activated by FDP, and points to the specificity of the control site of these enzymes.

The activation of FDP can be counteracted by ATP as demonstrated in figure 3 where successive activation by FDP and inhibition by ATP

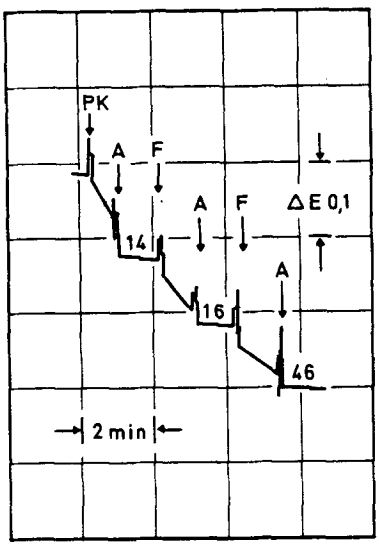

Fig. 3 Spectrophotometric record of DPNH (Photometer Eppendorf, 366 mu) of the pyruvate kinase (PK)-lactate dehydrogenase (LDH) system. Numbers indicate control ratios. Assay conditions: Triethanolamine-HCl (0, 2 M), Mg SO_4 (5 mM), PEP (1 mM), ADP (1 mM), DPNH (0, 25 mM), LDH 80 I.U., PK 2, 4 I.U. Final volume 2 ml, final pH 6.5. Consecutive additions (final concentrations): ATP (A) 3 mM, FDP (F) 1 mM, ATP 5 mM, FDP 3 mM, ATP 8 mM (exp. II/162/C 2).

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is recorded in an optical test. The inhibition by ATP is not specific and can also be observed with ITP, CTP, UTP, TPN and citrate. Other control metabolites like DPN or cyclic AMP were not effective.

The reversible inhibition and activation of the activity of pyruvate kinase demonstrate the wide control range, at which FDP and ATP can operate. Control ratios (the ratio of the activated/inhibited rate) between 15 and 160 are observed and well above the control ratios

so far reported, for instance in case of the control of oxidative phosphorylation (Chance et al., 1955). The high control ratio observed under physiological concentration fulfill the criteria of a flip-flop-enzyme operative as a control point of glycolysis. The significance of such a flip-flop operation for the mechanism of glycolytic oscillation is obvious: While ATP can be considered as the damping component, FDP is the gain component of the glycolytic control network. Both components operate alternatively and thus produce, in cooperation with the other phospho-kinases of glycolysis, a pulsed glycolytic turnover (Hess et al., 1966).

Since FDP is acting in this case not only eventually to supply substrate to pyruvate kinase, but also to activate pyruvate kinase by an enzymatic control mechanism, we choose to distinguish the latter function by the term forward control or to accept the engineering jargon "feed-forward" control. In this case, the activity of pyruvate kinase increases with the FDP concentration so the effect of the control upon the rate is positive, rather than negative and, thus, a full description of the control is afforded by the term "positive feed-forward". It is assumed that such a device may play an important role in controling transient states in metabolism.

Because of the remarkable activation of pyruvate kinase by FDP as well as of the sigmoidal PEP - substrate activity curve yeast pyruvate kinase must be considered as an allosteric protein. The mechanism of the activation is under investigation.

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