INHIBITION OF PEA-SEED PHOSPHOFRUCTOKINASE BY PHOSPHOENOLPYRUVATE

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Recent work has indicated that phosphofructokinase (PFK) may be an important regulatory enzyme in the control of glycolysis. Much of the evidence for this hypothesis has been provided by the effect of a number of metabolites and intracellular constituents on PFK activity. Passonneau and Lowry (1964) found that PFK from sheep brain and liver was inhibited by ATP, citrate and Mg^{++} and was stimulated by ADP, fructose 1,6-diphosphate (FDP), AMP, 3',5'-AMP, NH_4^+, K^+ and inorganic orthophosphate. Similar results were reported by Uyeda and Racker (1965) and these authors also found that 1 mM phosphoenolpyruvate (PEP) inhibited rabbit muscle PFK by 41%.

Less work has been carried out on the properties of PFK from the tissues of higher plants. Lowry and Passonneau (1964) reported the inhibition of parsley PFK by ADP and ATP and Dennis and Coultate (1966) found carrot PFK was inhibited by ADP, ATP and citrate. Dennis and Coultate (1967) subsequently showed that PFK from Brussels sprout leaves was inhibited by citrate and ATP and stimulated by inorganic phosphate.

The present communication describes the strong inhibition of PFK from pea seeds by PEP. Depending on the enzyme assay con-

ditions employed, 50% inhibition of PFK activity was obtained with PEP concentrations ranging from 2 μM to 14 μM. The possible significance of this phenomenon in the control of carbohydrate metabolism is discussed.

EXPERIMENTAL

The enzyme extract containing PFK was prepared as follows: pea seeds were ground and extracted with ether as described previously (Turner, 1957). Defatted pea powder (50 g) was suspended in 150 ml of an aqueous solution of pH 8.2 containing 50 mM $NaHCO_3$, 4 mM NaF and 5 mM $MgCl_2$ and extracted for 2 hr. on a mechanical roller. After centrifuging at 10,000 x g and subsequently at 30,000 x g, the supernatant was treated with saturated $(NH_4)_2SO_4$, pH 8.3. The fraction precipitating between 28% and 35% saturation was taken up in 40 ml 50 mM imidazole buffer, pH 7.6, and dialysed for 2 hr. against 5 mM imidazole buffer, pH 7.6. The enzyme preparation contained approximately 5 mg protein/ml. All operations were carried out at 2°.

PFK activity was assayed by coupling the production of FDP with the oxidation of NADH through aldolase, triose phosphate isomerase and α-glycerol phosphate dehydrogenase. The enzyme assay mixtures (total volume 3 ml) contained 60 μmoles imidazole buffer, pH 7.6, 1.5 μmoles fructose 6-phosphate (F6P), 4.8 μmoles $MgCl_2$, 0.42 μmole NADH, 5 μg α-glycerol phosphate dehydrogenase, 25 μg aldolase, 0.4 μg triose phosphate isomerase and 0.02 ml enzyme preparation (containing 100 μg protein). After equilibration at 25° for 8 min., the reaction was started by the addition of 0.75 μmole ATP. The decrease in extinction at 340 mμ was followed in a Beckman model DU spectrophotometer equipped with a Gilford automatic sample changer and recorder.

RESULTS

The inhibitory effect of increasing concentration of PEP on pea-seed PFK is shown in Figure 1. The enzyme was inhibited 50% by 14 μM PEP and when the concentration of PEP was increased to 30 μM the inhibition was 95%.

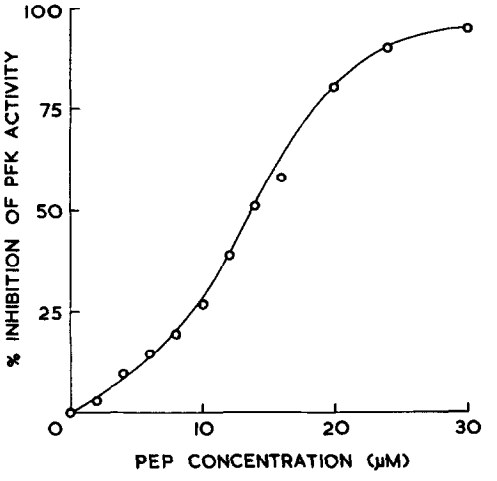

Figure 1. The effect of increasing PEP concentration on the activity of pea-seed PFK. The conditions of enzyme assay are described under Experimental.

In separate experiments preliminary observations were made on the effects of other substances on the inhibition of pea-seed PFK by PEP. The inhibition was increased by the addition of ATP, ADP, AMP and citrate and was decreased by F6P, $MgCl_2$ and inorganic phosphate. In the absence of PEP, PFK was inhibited by ATP, ADP and citrate and stimulated by inorganic phosphate.

Decreasing the concentrations of F6P and $MgCl_2$ in the enzyme reaction mixtures increased the sensitivity of the pea-seed PFK to inhibition by PEP. With enzyme reaction mixtures contain-

ing 0.6 μmole F6P and 2.4 μmoles $MgCl_2$, 5.2 μM PEP inhibited the reaction by 50%. When F6P and $MgCl_2$ were further reduced to 0.36 μmole and 1.44 μmoles respectively, 50% inhibition of PFK activity was obtained with 2 μM PEP.

DISCUSSION

The present investigation has shown that pea-seed PFK is strongly inhibited by very low concentrations of PEP. Under appropriate enzyme assay conditions 50% inhibition of PFK activity was obtained with PEP concentrations as low as 2 μM. The enzyme from animal tissues appears to be less sensitive to PEP. Uyeda and Racker (1965) observed PEP inhibition of rabbit muscle PFK but considerably higher concentrations of PEP were required to give a comparable degree of inhibition. These authors found that inhibitions of 41% and 66% were given by PEP concentrations of 1 mM and 2 mM respectively. Calf-lens PFK was inhibited 8% in the presence of 2.5 mM PEP (Lou and Kinoshita, 1967).

The pronounced inhibition of pea-seed PFK by PEP suggests that this phenomenon may be of significance in the control of carbohydrate metabolism in plant tissues. PEP is the substrate for pyruvate kinase, an enzyme later in the glycolysis sequence, and any accumulation of PEP may strongly inhibit the phosphorylation of F6P. It is possible that the rate of glycolysis may be substantially influenced by a control system involving the interaction of PFK and pyruvate kinase and their substrates and products. The present investigation has shown that pea-seed PFK is inhibited by ADP. If such a control system operates, the two substrates of pyruvate kinase (ADP and PEP) may inhibit PFK. Conversely ADP, a product of the PFK reaction, may increase pyruvate kinase activity as ADP is a substrate for this reaction.

Hess, Haeckel and Brand (1966) found that FDP stimulated yeast pyruvate kinase. If this stimulation occurs with the pyruvate kinase of higher plants then FDP, the other product of the PFK reaction, may also increase pyruvate kinase activity. Moreover, the ATP produced in the pyruvate kinase reaction is a potential substrate for PFK and, at higher concentrations, a potential inhibitor.

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