PHOSPHOFRUCTOKINASE, A REGULATORY ENZYME IN PLANTS

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Glycolysis in muscle (e.g. Uyeda and Racker 1965), yeast (e.g. Ramaiah et al 1964) and ascites tumour cells (e.g. Freyer et al 1965) has been shown to be controlled at the point of phosphorylation of fructose-6-phosphate (F6P) by phosphofructokinase (PFK). The PFK from animals, plants and bacteria have been compared and all shown to have regulatory functions (Lowry and Passonneau 1964). As in other systems PFK in crude plant homogenates was inhibited by high levels of ATP but the inhibition could not be relieved by ADP, AMP or 3',5'-AMP. Higher levels of F6P and Pi (in parsley but not avocado) did however overcome the inhibition.

In this report PFK from carrots has been studied and the results of Lowry and Passonneau (1964) confirmed and extended. Citrate is shown to be very inhibitory, and acts synergistically with ATP. Increasing the magnesium concentration partially relieved the inhibitions of both high ATP and citrate. Pi would also relieve the inhibition of ATP, ADP and citrate.

EXPERIMENTAL

250 g of carrots were minced into 250 ml of 50 mM imidazole buffer pH 7.8 containing 40 g Polyclar AT, 2 mM EDTA and 57 mM 2-mercaptoethanol. The homogenate was ground with sand, filtered and centrifuged at 70,000 x g for 30 min. The supernatant was treated with saturated ammonium sulphate and the protein precipitating between 35 and 45 per cent saturation collected. This was taken up in 20 ml of 50 mM imidazole buffer pH 7.0. In this form

the enzyme was quite stable at 4°. The ammonium sulphate precipitation was essential to remove NADH oxidase activity which interfered with the assay.

The assay, which is based on that described by Uyeda and Racker (1965), is described in Table I.

RESULTS

The effect of F6P concentration on the rate is shown in Fig. 1. The curve is apparently not sigmoid as described for other systems but this may be due to activators such as ammonium ions which are present in the extract as well as in the auxillary enzymes. The K_m for F6P is 0.19 mM in the standard assay conditions.

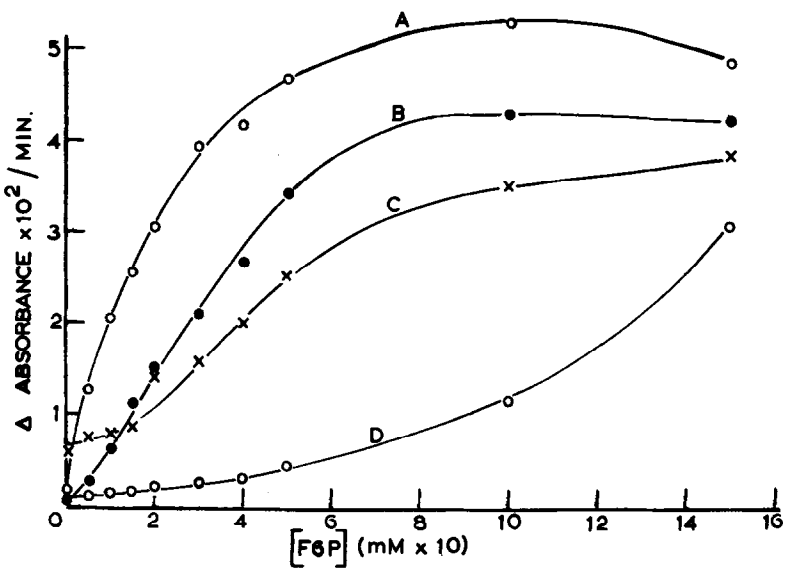

FIG. 1. The Effect of F6P Concentration on the Activity of PFK in the presence of (A) 1 mM ATP, (B) 1 mM ATP + 5 mM Citrate, (C) 5 mM ATP and (D) 5 mM ATP + 5 mM Citrate. The Assay Conditions are shown in Table I.

The effect of increasing the ATP concentration is shown in Fig. 2. The optimum ATP concentration is 1.0 mM but above this it becomes inhibitory. Almost complete inhibition occurs at 10 mM and 50 per cent at 5.7 mM. This curve is very similar to that described for rabbit muscle by Uyeda and Racker (1965).

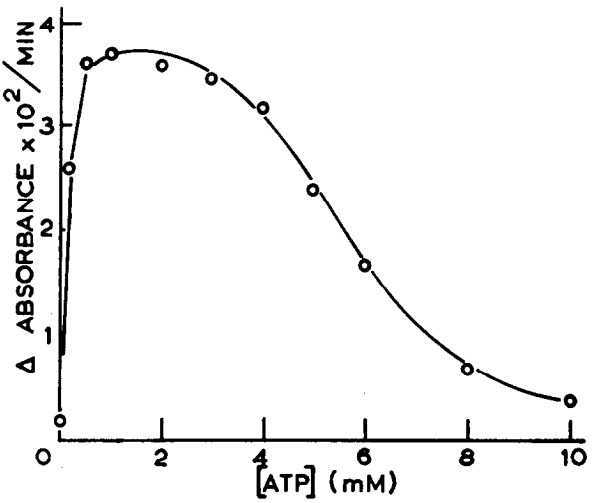

FIG. 2. The effect of ATP concentration on PFK activity.

F6P at higher concentrations will combat this inhibition, Fig. 1. With 5.0 mM ATP there is some oxidation of the NADH in the absence of F6P, which is probably due to an impurity in the ATP. At high ATP concentrations the F6P curve is distinctly sigmoid, which is typical of regulatory enzymes (Atkinson 1965).

Citrate is inhibitory and gives almost complete inhibition at a concentration of 10 mM. The inhibition by citrate is very similar to that of ATP and can be reversed by F6P (Fig. 1). ATP and citrate show a distinct synergistic inhibition (Fig, 1, Table I) which again can be partially reversed by F6P. DL isocitrate inhibits only 21 per cent at a concentration of 10 mM and succinate is not an inhibitor. A similar result has been found in muscle (Garland et al 1963) and in yeast (Salas et al 1965) although in this case isocitrate was as effective as citrate. The same results with ATP and citrate are obtained when 50 mM Hepes buffer (Good et al 1966) pH. 7.0 is used in place of imidazole.

TABLE I

ATP Conc. mM	Magnesium Conc. mM	Citrate Conc. mM	Other Additions at 5 mM	Rate Δ OD/Min.	Per Cent Optimal Rate
1.0	4.0	-	-	0.0330	(100)
5.0	4.0	-	-	0.0177	54
1.0	1.0	-	-	0.0162	49
5.0	20.0	-	-	0.0273	83
5.0	4.0	-	Pi	0.0293	89
1.0	4.0	-	ADP	0.0153	46
5.0	4.0	-	ADP	0.0058	17
1.0	4.0	-	AMP	0.0247	74
5.0	4.0	-	AMP	0.0139	42
1.0	4.0	5.0	-	0.0207	62
1.0	4.0	10.0	-	0.0040	12
5.0	4.0	5.0	-	0.0042	12
1.0	20.0	5.0	-	0.0293	88
1.0	20.0	10.0	-	0.0276	83
1.0	4.0	5.0	Pi	0.0259	79
1.0	4.0	5.0	ADP	0.0026	8
1.0	4.0	5.0	AMP	0.0124	38

The standard assay contained:- 4.0 mM magnesium chloride, 190 mM 2-mercaptoethanol, 0.2 mM F6P (sodium salt), 1.0 mM ATP, 0.1 mM NADH 7.2 units aldolase, 0.5 units α-glycerophosphate dehydrogenase and 1.6 units triose phosphate isomerase. The final volume was made up to 2.5 ml with 50 mM imidazole buffer pH 7.0 and all the reagents were made up in the same buffer and adjusted to pH 7.0. The reaction was started by the addition of 0.05 ml of the enzyme solution. The decrease in absorbance at 340 mμ was measured in a Hilger-Gilford recording spectrophotometer at room temperature.

The inhibition by ATP and citrate can not be reversed by AMP, ADP or 3',5'-AMP. ADP is an inhibitor and enhances the ATP and citrate inhibitions (Table I). AMP is less inhibitory, but again enhances the ATP and citrate inhibitions. Pi (as the sodium salt) partially reverses ATP and citrate inhibitions (Table I). These results are in complete contrast to those from adipose tissue where citrate inhibition is relieved by AMP, ADP and 3',5-AMP (Denton and Randle 1966). Raising the magnesium concentration to 20 mM also reverses the ATP and citrate inhibitions to a large extent. Similarly lowering the magnesium concentration to 1.0 mM in the standard assay causes a marked inhibition of the enzyme (Table I). The optimum magnesium concentration is 4.0 mM; at values in excess of this it becomes slightly inhibitory. Below 4.0 mM the rate falls off very rapidly as the magnesium concentration is

lowered and gives only 50 per cent of the optimal at 2.0 mM. It appears therefore that the magnesium:ATP ratio is very important in plant PFK as it is in brain PFK (Lowry and Passonneau 1965).

The influence of pH upon the ATP inhibition is very complex. The optimum pH in the standard assay is approximately 8.0. Below pH 7.0 ATP becomes more inhibitory, but 20 mM magnesium will restore the activity to a greater value than in the standard assay. Above pH 7.0 ATP is less inhibitory, but high magnesium will not restore the activity.

DISCUSSION

These results confirm that glycolysis is controlled in plants by PFK in a similar manner to yeast and animal tissues. The basic difference is that in plants the inhibition by ATP is not reversed by AMP or ADP. Pi appears to have this role in plants. The importance of Pi as a "coordinated stimulator of glycolysis" has been suggested before, because of its effect upon hexokinase and PFK (Uyeda and Racker, 1965).

The most important finding is that citrate is as an effective inhibitor as high concentrations of ATP. It is possible that citrate, and indeed also ADP, act by disturbing the ATP: magnesium ratio by magnesium chelation and the effect which is seen is ATP inhibition. However the fact that DL isocitrate is so much less inhibitory makes this less likely. The most important factor in PFK regulation in plants is therefore the concentration of citrate and ATP. It is possible that in plants other compounds such as the plant growth regulators are involved in enzyme regulation. So far only kinetin riboside has been tested and shown to have no effect.

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