END-PRODUCT INHIBITION OF YEAST PHOSPHOFRUCTOKINASE BY ATP [1]

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Some relation of the phosphofructokinase (PFK) reaction to the Pasteur effect has been suspected for over twenty years, even after the emphasis on the control of glycolysis became centered on the phosphate and/or phosphate acceptor. Indirect evidence for this relation has been claimed for reconstructed systems (Aisenberg and Potter, 1957) and for yeast (Lynen et al., 1959; although not supported by Maitra and Steinberg, 1963), ascites tumor (Lonberg-Holm, 1959), and muscle (Park et al., 1961; Newsholme and Randle, 1961). Direct evidence for inhibition of PFK by excess ATP and its counteraction by several metabolites has been recently reported for Fasciola hepatica and muscle PFK (Mansour and Mansour, 1962; Passonneau and Lowry, 1962). Nothing was known on the PFK of yeast (Negelein, 1936), where the Pasteur effect was originally found. In addition there was a possibility that it could be less unstable than the muscle enzyme.

The data presented below show that yeast PFK is inhibited by ATP competitively with fructose-6-phosphate

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(F6P), in such a way that within the normal range of physiological concentrations the activity of the enzyme decreases with the increase of ATP concentration and increases with the square of F6P concentration. It is suggested that ATP may be considered as the end product of the pathway whose first irreversible step is the PFK reaction, and that end-product inhibition by ATP can act as a feed-back control in glycolysis.

EXPERIMENTAL

The effect of the concentration of ATP on yeast PFK activity [3] at a given concentration of F6P is shown in Fig. 1. If ATP is substituted by GTP, there is no inhibition by excess nucleotide within the same range.

Within a wide range of ATP concentrations, the effect of the concentration of F6P corresponds to second order kinetics. As shown in Fig. 2, the double reciprocal plot gave parabolic lines with respect to the concentration of F6P (A), and straight lines with respect to the square of the latter (B). The reaction becomes first order with respect to F6P at very high F6P/ATP ratios and over wider ranges if ATP is substituted by GTP (Fig. 3A). Under these conditions, the double reciprocal plots give parallel lines (Fig. 3).

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[3] Yeast PFK was obtained by extracting dried brewers yeast (Anheuser and Busch, "for enzyme work") with 6 volumes of 0.1 M $KHCO_3$ - 1 mM CH_3-CH_2SH - 1 mM EDTA. The fraction precipitated between 40 and 60 per cent saturation of $(NH_4)_2SO_4$ was dialyzed against several changes of 0.05 M potassium phosphate - 5 mM $MgCl_2$ - 1 mM CH_3-CH_2SH - 1 mM EDTA, pH 7.5. This PFK preparation is free of ATPase and is stable within the assay conditions in this work.

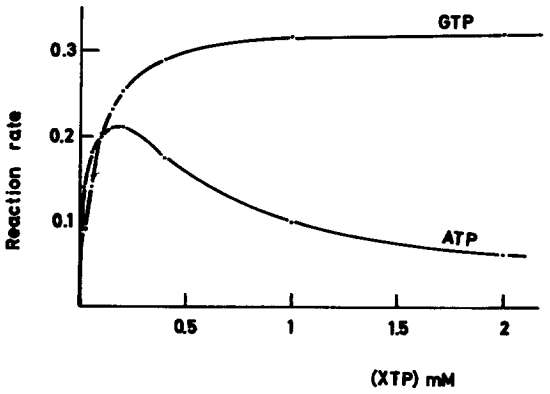

Fig. 1. Yeast phosphofructokinase activity with ATP and GTP, at 0.9 mM fructose-6-P.

PFK activity was assayed by following the decrease in optical density at 340 mμ in the presence of excess aldolase, triose phosphate isomerase, and L-α-glycero-phosphate dehydrogenase (Boehringer), 0.1 mM DPNH, 0.05 M Tris pH 7.5, 5 mM CH_3-CH_2SH, 10 mM $MgCl_2$, F6P (obtained by treating glucose-6-P with glucose phosphate isomerase and assuming the concentration of F6P in the equilibrated mixture to be 1/4 of the total ester), and ATP or GTP as indicated in the figures. The reaction was started by the addition of the PFK preparation. Rate is expressed as μmoles of substrate transformed per min per mg protein.

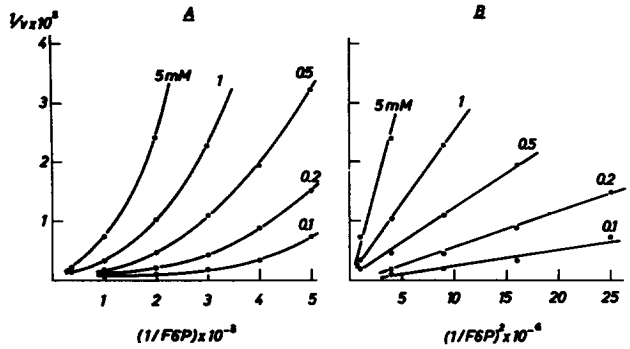

Fig. 2. Effect of the concentration of fructose-6-P on phosphofructokinase activity at the concentrations of ATP-Mg indicated in the figure, in millimoles/l. Other assay conditions as in Fig. 1.

No appreciable effect on the activity of yeast PFK (at inhibitory levels of ATP) was observed with Pi, cyclic--3',5'-AMP, AMP, ADP, or FDP (using in the latter case the

pyruvate kinase-lactic dehydrogenase method) within the range of concentrations used by Passonneau and Lowry (1962).

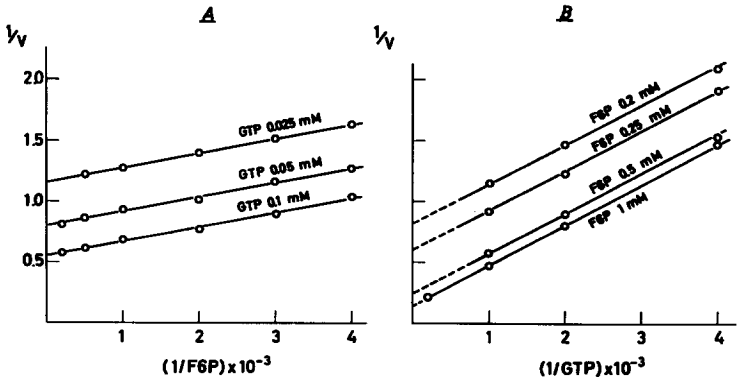

Fig. 3. Kinetics of the phosphofructokinase reaction in non-inhibitory conditions. Assay conditions as in Fig. 1, at the concentrations of GTP and F6P indicated in the figure.

DISCUSSION

The strong inhibition of yeast PFK by excess ATP, but not by excess GTP (see Fig. 1), suggests that the enzyme has a regulatory binding site for ATP different from the substrate site. Competition with F6P further suggests that occupancy of the regulatory site by ATP interferes with occupancy of the sugar substrate site. Moreover, the second order kinetics with respect to F6P which accompany inhibition could depend on a second regulatory site for F6P interfering with that for ATP, or simply involve a dimer in which occupancy of a bridging ATP regulatory site could be competitive with those of F6P in two active sites. Second order kinetics with respect to F6P is shown also by muscle PFK (Viñuela and Sols, 1963). The second order kinetics in relation to some

regulatory inhibitions (Changeux, 1961; Gerhart and Pardee, 1962) may have a general significance. As far as mechanism, perhaps in terms of polymerized enzymes. For metabolic regulation, possibly in terms of increased sensitivity of the regulable enzyme to changes in metabolite levels.

The kinetics of yeast PFK in non-inhibition conditions suggests that the two substrates, F6P and ATP, are not present simultaneously on a given active site (Alberty, 1953). Similar kinetics have been observed with a muscle PFK preparation desensitized to ATP inhibition, over a wide range of ATP and F6P concentrations (Viñuela and Sols, 1963).

ATP may be considered as the end product of the glycolytic pathway after the hexosemonophosphate cross-road. This is the pathway that specifically leads to ATP production. Its first irreversible step is the PFK reaction. Inhibition of PFK by ATP would then be a case of end-product inhibition in the sense well established for biosynthetic pathways (Moyed and Umbarger, 1962), with the additional novelty that in this case the end product is also a substrate of the sensitive enzyme. The fact that PFK activity decreases with increasing ATP within the physiological range of ATP and F6P concentrations in fermenting yeast suggests that this end-product inhibition by ATP can act as a feed-back control mechanism in normal yeast glycolysis. In aerobiosis, the increased yield of ATP per mole of hexosemonophosphate would further brake the PFK reaction. Such a feed-back control could adjust the rate of the ATP-producing glycolytic pathway to the overall rate of net disposal of ATP, without piling up of intermediates (for an illustration see Holzer, 1961).

Passonneau and Lowry (1962) have emphasized the large increase in PFK activity in muscle that may be expected when the ATP concentration falls. Of possible greater significance is the fact that the inhibition of PFKs by ATP is competitive with the square of the F6P concentration. This property opens a way for automatic, drastic forcing of PFK activity by the large increase in F6P formation that occurs in stimulated muscle, before any marked fall in the ATP level.

Further work on the mechanisms of metabolic regulation of yeast and muscle PFKs is in progress.

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