TWO INTERCONVERTIBLE FORMS OF YEAST PHOSPHOFRUCTOKINASE WITH DIFFERENT SENSITIVITY TO ENDPRODUCT INHIBITION [1]

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Activation and inhibition of phosphofructokinase (PFK) preparations from several animal tissues by a number of metabolites has been recently reported. The very complicated kinetics observed have led to the suggestion of a variety of regulatory binding sites in PFK (see Passonneau and Lowry, 1963). Inhibition by excess ATP-Mg has been recently characterized in this laboratory, with a yeast preparation, as endproduct inhibition that gives second order kinetics with respect to fructose-6-P in inhibitory conditions (Viñuela et al., 1963). Mansour and Mansour (1962) reported evidence for an activating system stimulated by cyclic 3',5'-AMP with preparations from a liver fluke. More recently, Mansour (1963), has observed what seems to be a direct activation of heart PFK by cyclic 3',5'-AMP.

We report here the identification of two forms of yeast PFK, their interconversion, and an effect of cyclic 3',5'-AMP on a conversion enzyme.

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EXPERIMENTAL

Incubation of a crude yeast extract in the presence of fluoride leads to the disappearance of the marked sensitivity of the PFK to inhibition by ATP (Table I). Crude extracts incubated with and without fluoride as in Table I were additive when assayed together for PFK activity, at both concentrations of ATP. This seemed to rule out changes during the preincubation in the level of some metabolite that could affect the activity of the enzyme with high ATP, and suggested that some change in the enzyme itself was involved. This change may appear as an activation of the enzyme when tested with a high concentration of ATP. This "activation" can be prevented by EDTA, and it does not take place with gel filtered (Sephadex G-25) or dialyzed extract. Some decrease in PFK sensitivity to ATP took place in the dialyzed extract, when ATP and Mg^{++} were added to the preincubation mixture. This effect of preincubation with ATP-Mg was markedly enhanced by the addition of cyclic 3',5'-AMP (Table I). When a crude extract "activated" by incubation with fluoride is dialyzed, sensitivity of the PFK to inhibition by ATP reappears. These results suggested that in yeast PFK can exist in two forms, and that there are two enzymic activities able to catalyze the interconversion. One of these activities could be a kinase. Inhibition of the other activity by fluoride is consistent with the hypothesis that it could be a phosphatase. Cyclic 3',5'-AMP seems to activate the kinase. No direct effect of cyclic 3',5'-AMP on the activity of yeast PFK has been detected at concentrations during the assay up to 0.1 mM.

TABLE I

"Activation" of yeast PFK in extracts

A crude extract was obtained by grinding commercial pressed baker's yeast (Danubio) with alumina (Alcoa A-301), diluting with 10 volumes of 50 mM potassium phosphate, pH 7.5, 0.1 mM EDTA, 1 mM ethanethiol, and elimination of the insoluble residue by centrifugation at 20,000 x g for 15 minutes. Dialysis, when indicated, was carried out against several changes of 50 mM potassium phosphate, pH 7.5, 0.1 mM EDTA, 5 mM $MgCl_2$. 0.1 ml of extract (containing 0.7 mg protein) was incubated at room temperature for 20 minutes in a total volume of 0.25 ml with additions as indicated.

PFK activity was assayed with 20 µl aliquots of the incubated mixtures in a total volume of 2 ml in the presence of excess aldolase, triose phosphate isomerase, and L-α-glycerophosphate dehydrogenase (Boehringer), 0.1 mM NADH, 5 mM ethanethiol, 50 mM imidazole, pH 7.5, 5 mM $MgCl_2$, 0.2 mM fructose-6-P (obtained by treating glucose-6-P with glucose phosphate isomerase until equilibrium), and 0.05 mM or 0.5 mM ATP, as indicated. The reaction was started by the addition of the preincubated extract and followed at room temperature by the decrease in optical density at 340 mµ. Blanks without fructose-6-P were carried out in parallel. Activity is expressed as mµmoles of substrate transformed per minute per mg protein.

Extract	Preincubation additions	PFK activity 0.05 mM ATP	0.5 mM ATP
Undialyzed	None	64	16
	20 mM NaF	86	153
	20 mM NaF and 0.1 M EDTA	70	17
Dialyzed	None	70	12
	20 mM NaF	74	12
	20 mM NaF and 1 mM ATP-Mg	65	30
	20 mM NaF, 1 mM ATP-Mg, and 2 mM cyclic 3',5'-AMP	67	89

The above hypothesis has been partially confirmed by the successful separation of PFK from an "activating enzyme", as shown in Table II. The fluoride requirement indicates that the presumed phosphatase catalyzing the

TABLE II

Separation of PFK and its "activating" enzyme

A crude extract prepared as in Table I was fractionated with ammonium sulfate. The fraction precipitated between 40 and 60% saturation was dissolved in 10 mM magnesium acetate, 1 mM ethanethiol, 0.1 mM EDTA, adjusted to pH 7.5, and dialyzed against the same medium. The dialyzed preparation was adjusted to pH 6 and fractionated with acetone between +2 and -8° C. The fractions collected between 5 and 15% and between 35 and 50% acetone were dissolved in 25 mM potassium phosphate, pH 6.5, 1 mM ethanethiol, 0.1 mM EDTA. Their protein content was 58 and 18 mg per ml, respectively.

0.05 ml aliquots of the 5-15 fraction were incubated at room temperature for 15 minutes in a total volume of 0.5 ml containing 10 mM potassium phosphate, pH 6.5, 5 mM $MgCl_2$, 1 mM ethanethiol, and other additions as indicated. After this incubation, PFK activity was assayed in 5 to 50 µl aliquots with 0.5 mM fructose-6-P and 1 mM ATP as indicated in Table I

Preincubation additions	PFK activity
None	11
0.1 ml 35-50 fraction	11
0.1 ml 35-50 fraction, 20 mM NaF, 1 mM ATP-Mg, and 0.1 mM cyclic 3',5'-AMP ("complete system")	220
Complete system, minus 35-50 fraction	15
Complete system, minus ATP-Mg	13
Complete system, plus 0.1 M EDTA	11
Complete system, minus NaF	9

reverse conversion still accompanies one or both of the above enzymes.

The two forms of PFK, insensitive and sensitive to inhibition by ATP, may be designated as a and b respectively because of the resemblance to the muscle phosphorylase system (Krebs and Fischer, 1962; Parmeggiani and Morgan, 1962). The effect of the concentration of ATP on the activity of these

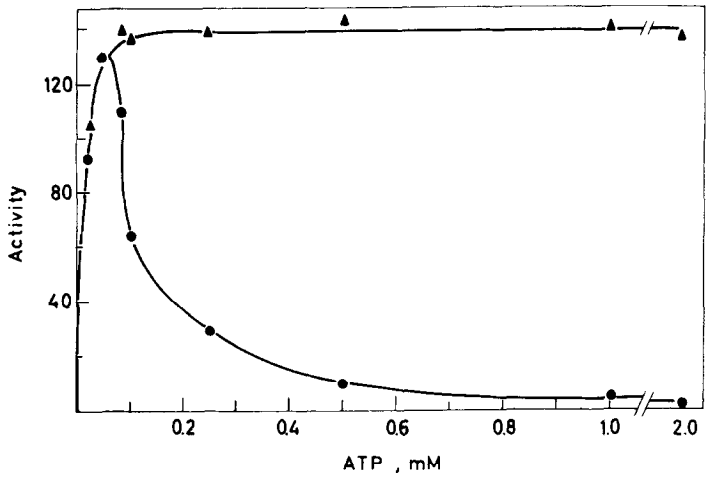

Fig. 1. Effect of the concentration of ATP on the activity of PFK a and b

A 40-60 ammonium sulfate fraction of yeast extract was dissolved in 25 mM potassium phosphate, pH 7.5, 0.1 mM EDTA, 1 mM ethanethiol, and 5 mM $MgCl_2$, and dialyzed against the same medium. To obtain the a form, this preparation was incubated with 1 mM ATP-Mg, 0.1 mM cyclic 3',5'-AMP, 20 mM NaF, 5 mM $MgCl_2$, and 10 mM potassium phosphate, pH 6.5, at room temperature for 15 minutes. The b form is the same preparation similarly incubated with $MgCl_2$ and phosphate only. After these treatments PFK activity was assayed in aliquots of 5 µl for PFK a and up to 50 µl for PFK b, as described in Table I with 0.5 mM fructose-6-P and ATP as indicated in the figure. ▲ , PFK a; ● , PFK b.

two forms of PFK at pH 7.5 is shown in Fig. 1. [3] Sensitivity of yeast PFK to inhibition by citrate (M.L. Salas et al., 1964) also disappears when converted to the a form.

Preliminary observations indicate that although in resting yeast PFK occurs essentially in the b form, significant proportions of the a form are present in growing yeast.

[3] Inhibition of yeast PFK by ATP decreases with the pH in the physiological range.

DISCUSSION

A form of PFK insensitive to endproduct inhibition can make possible a high glycolytic rate in the presence of high concentrations of the endproducts. This possibility may be important for certain biosynthetic processes in growing yeast.

The parallelisms in regulatory mechanisms between yeast PFK and muscle phosphorylase are striking. In muscle, citrate can inhibit PFK (Parmeggiani and Bowman, 1963; Garland et al., 1963) and phosphorylase b (M. Salas et al., 1964). A simultaneous "activation" of both enzymes in a given tissue would be very efficient for a rapid mobilization of glycogen.

Cyclic 3',5'-AMP has not yet been estimated in yeast. It has been identified in E. coli, where marked changes in its concentration have been found in relation with the availability of an external source of energy (Makman and Sutherland, 1963). The possibility that cyclic 3',5'-AMP could act as a chemical signal for the parallel adjustment in certain tissues of phosphorylase and PFK to maximal efficiency is a problem of considerable physiological interest.

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