SOME KINETIC AND MOLECULAR PROPERTIES OF YEAST PHOSPHOFRUCTOKINASE

G.KOPPERSCHLÄGER, R.FREYER, W.DIEZEL and E.HOFMANN
Institute of Physiological Chemistry, Karl-Marx-University, Leipzig, Germany

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Yeast PFK had a sedimentation coefficient of 16.7 *S* both in the absence and in the presence of ATP, and did not dissociate even at very low protein concentrations. Sodium dodecyl-sulphate caused dissociation of the protein to subunits of 3.2 *S*.

The effects of pH on substrate affinities are described. In the presence of UTP, acting as non-inhibiting phosphate donor, the behaviour of the enzyme towards F-6-P was co-operative, with a Hill coefficient of 2.2.

1. Introduction

Owing to its allosteric properties, phosphofructokinase (PFK; EC 2.7.1.11) is considered an important enzyme in the regulation of metabolism. PFK preparations from various sources show similar properties: sigmoid behaviour towards F-6-P, hyperbolic behaviour with respect to the phosphate donor, response to effector molecules such as inorganic phosphate, adenine nucleotides, citrate, etc. However, PFK enzymes from different organisms have distinctive characteristics, especially in the inhibition by ATP, activation by 5′-AMP and 3′,5′-cyclic AMP, the effects of pH, sedimentation behaviour, etc.

This paper deals with some molecular and kinetic properties of yeast PFK. The protein has been studied by a number of workers [1–4]. Our results are in agreement with the recent study by Lindell and Stellwagen [4], and yield further information on the sedimentation, electrophoresis and kinetic properties of the enzyme.

2. Materials and methods

Yeast PFK was purchased from C.F.Boehringer GmbH, Mannheim. The specific activity was 100 units/mg. Enzyme activity was measured in a coupled assay using aldolase, triosephosphate isomerase and α-glycerophosphate dehydrogenase. Ammonium sulphate concentrations were less than 10 mM *. Dehydrogenation of NADH was followed spectrophotometrically with a Unicam SP 800.

Sedimentation experiments were performed with a Phywe U 60 L analytical ultracentrifuge at 50000 rpm and 20°C. Sedimentation in a density gradient was done according to the method of Martin and Ames [5], using yeast alcohol dehydrogenase as reference.

Electrophoresis was on agarose gels, in a 0.05 M phosphate buffer pH 8.1, in a field of 26 V/cm, for 1 hour. The protein band was located both enzymically and with the Amidoblack 10 B general protein stain. The enzyme stain consisted of the phosphate buffer (as used for the electrophoresis) containing the following reagents: 10 mM F-6-P; 3 mM ATP; 2 mM NAD; 6 mM $MgCl_2$; 6 mM Na-arsenate; 6 mM KCN; 6 mM K_2SO_4; 4 units/ml aldolase; 5 units/ml glyceraldehyde-3-phosphate dehydrogenase; 0.2 mg/ml MTT **; 30 μg/ml phenazine methosulphate.

* Sigmoid behaviour by the enzyme towards F-6-P has been shown in an assay using a pH-stat by Dr. I.Lorenz in our laboratory. Therefore, such behaviour was not an artefact of extraneous protein-protein interactions in the coupled assay.

** 2-(4′,5′-dimethyl-thiazolyl-(2′))-3,5-diphenyl-tetrazolium bromide.

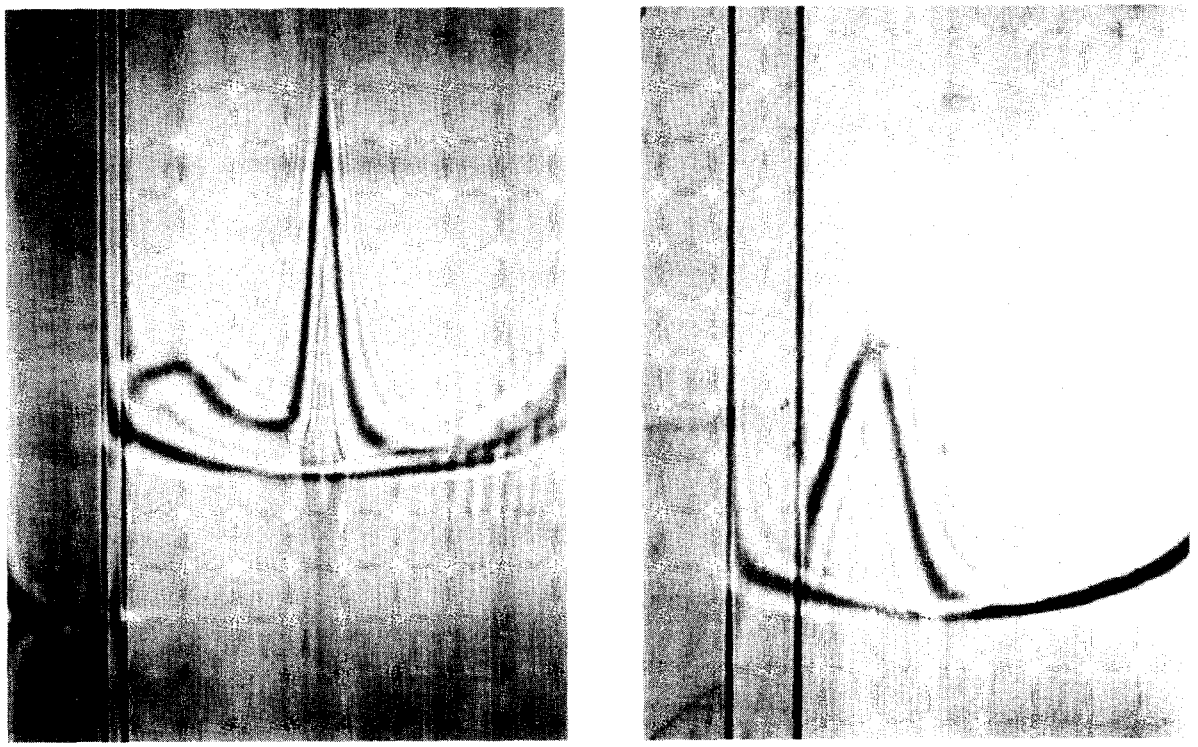

Fig. 1. Sedimentation pattern of yeast PFK. (a) without and (b) with Na-dodecyl-sulphate. Photographs were taken after 25 min (a) and after 30 min (b).

3. Results and discussion

The sedimentation pattern showed a sharp, symmetrical peak and a slower component, which was presumably an enzymically inactive contaminant (fig. 1a). In 0.1 M phosphate buffer pH 8.0, in the presence of 0.2 mM FDP, the $S_{20,w}$ of the main component was 16.7 *S*, the total protein concentration being 4 mg/ml. According to Lindell and Stellwagen [4], the dependence of sedimentation coefficient on protein concentration was that expected for a single molecular species. Even at much lower concentrations (10 μg/ml or less), sedimentation in gradients 5–20% of sucrose, does not suggest dissociation of yeast PFK to enzymically active subunits. The sedimentation coefficient at these low concentrations was 14.8 *S*. The high recovery of enzymic activity from these experiments (> 80%) suggests that irreversible dissociation or denaturation could be ignored. The presence of 5 mM ATP and 10 mM $(NH_4)_2SO_4$ did not change the sedimentation pattern significantly in experiments with high or low protein concentration. In contrast, PFK from skeletal muscle [6,7] and from heart [8] showed sedimentation behaviour which was dependent on protein concentration and on the presence of effectors. This indicates that the yeast and mammalian enzymes differ in their molecular properties. Incubation of yeast PFK with 5 mM sodium dodecyl-sulphate at pH 10 dissociated the 16.7 *S* entity to a molecular species of 3.2 *S* (fig. 1b), with concomitant loss of activity. The experiments of Lindell and Stellwagen [4] showed that this species may be composed of subunits of 1.65 *S*.

Electrophoresis of yeast PFK on agarose gave a single diffuse band with slight trailing (fig. 2a). The mobility was estimated as 1.6 times that of PFK from rabbit muscle (fig. 2b). Both yeast and muscle PFK were found to bind blue dextran (obtained from Pharmacia, Uppsala) without alteration to the enzymic activity. No such dye binding was observed

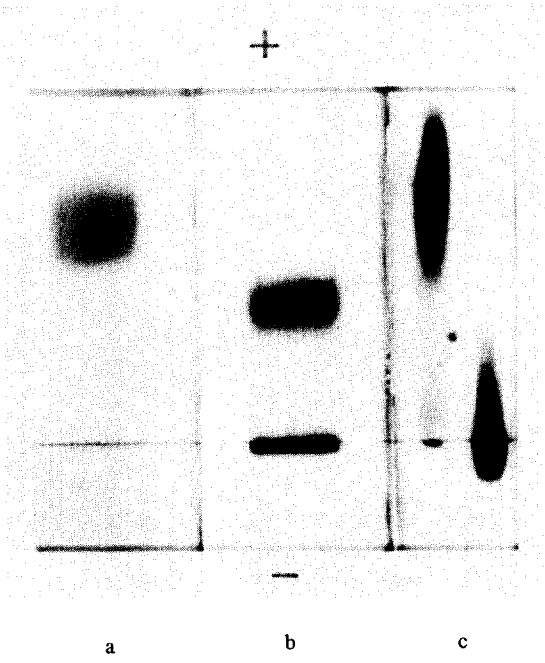

Fig. 2. Agarose-gel electrophoresis of PFK. (a) yeast PFK, (b) muscle PFK, (c) yeast PFK with (right) and without (left) dextran-blue. Located by enzymic reaction.

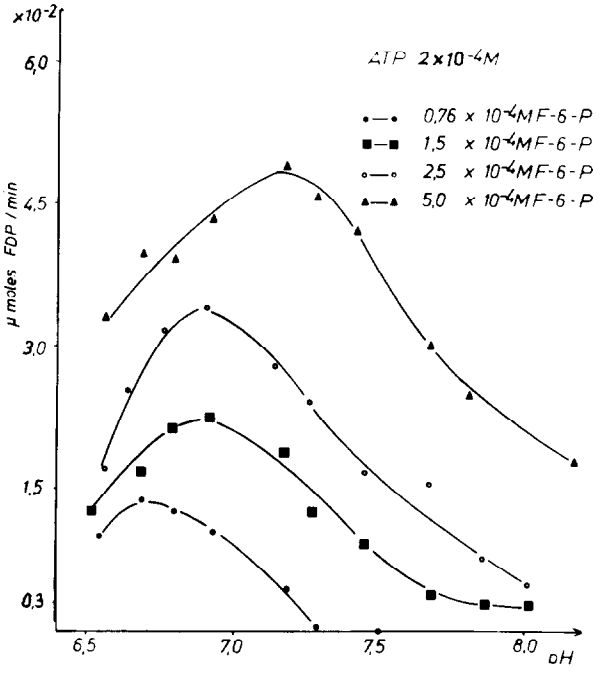

Fig. 3. Change of pH optimum with varying F-6-P concentrations (ATP 2×10^{-4} M).

with certain other proteins tested, namely yeast hexokinase and alcohol dehydrogenase. The dye complex with yeast PFK had a mobility near zero, the apparent cathodic migration being due to electro-osmosis (fig. 2c).

Yeast PFK showed kinetic properties that differed from those of the mammalian enzyme. The pH optimum of yeast PFK was shifted to higher pH on increasing the concentration of F-6-P (fig. 3). This change could be related to allosteric effects by hydrogen ions. On decreasing the pH, the $[S]\ \frac{1}{2}V_{max}$ decreased (fig. 4), indicating an increased affinity for F-6-P. The V_{max} was also affected. In some experiments, acidification produced a decrease in co-operativity towards F-6-P, the Hill coefficient falling from 2.7 to 1.6. Lindell and Stellwagen [4] have shown that the pH optimum was shifted to lower pH on increasing the ATP concentration. Experiments in our laboratory confirmed this finding, and suggest that the affinity of ATP for the catalytic sites increased, and for the inhibitory sites decreased, on lowering the pH. Decrease in inhibition is probably the predominant effect on acidification.

The pH dependence of yeast PFK contrasts with that of the mammalian enzyme. For the latter, there was a decrease in the affinity towards F-6-P [9], while the inhibition by ATP [10] increased on lowering the pH. Thus, increase in ATP concentration altered the optimum to higher pH values [11].

There is a general problem of whether the sigmoid behaviour towards F-6-P is merely the result of the complex interactions of the enzyme with ATP, acting both as substrate and as allosteric inhibitor. Increasing the ATP concentration decreased the affinity for F-6-P, with both yeast and mammalian PFK. However, the Hill coefficient towards F-6-P remained approximately constant between 2.0 and 2.5 [9]. In place of ATP, other phosphate donors – UTP, GTP, ITP and CTP – did not show an inhibitory effect on yeast PFK [1,2,4]. With ITP as one of the substrates, the enzyme showed hyperbolic behaviour towards F-6-P [4], which suggests that the sigmoid behaviour towards F-6-P depends on the inhibitory effect of ATP. Contradicting this conclusion, in the presence of UTP, which is not inhibitory, the enzyme showed

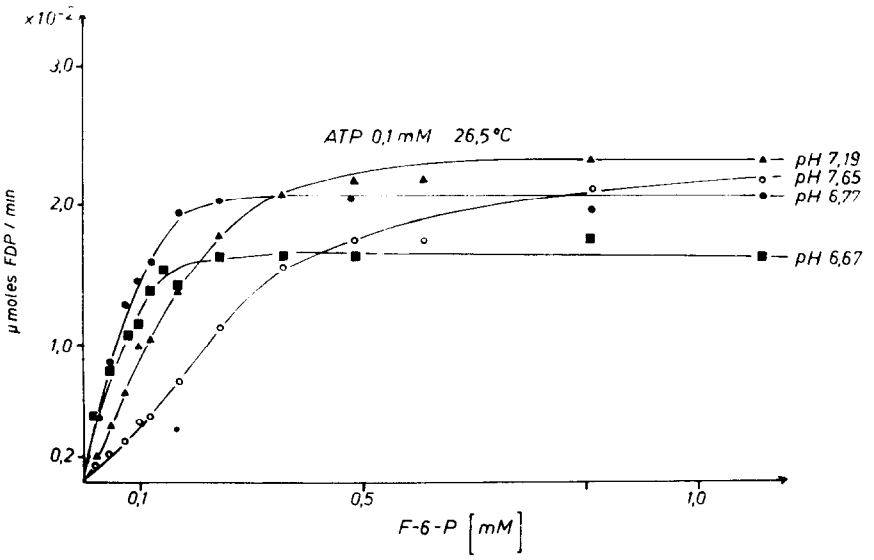

Fig. 4. Dependence of PFK activity from F-6-P concentration at different pH values (ATP: 1×10^{-4} M).

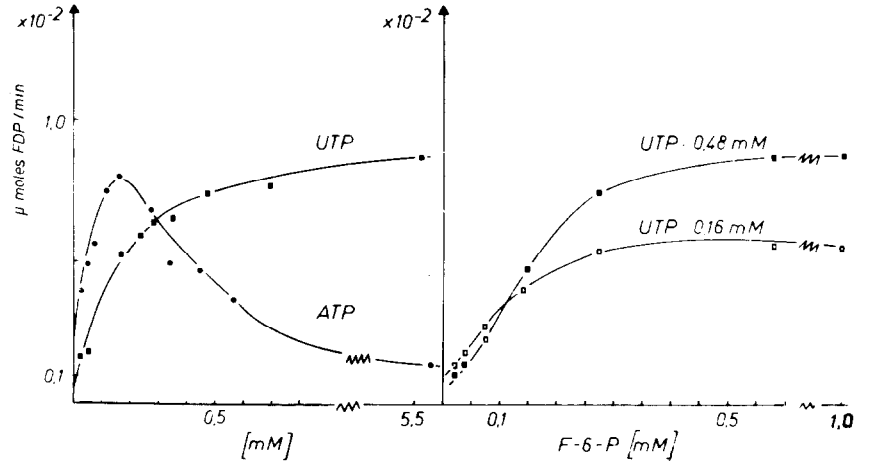

Fig. 5. Effect of ATP and UTP (pH 7.25; 0.25 mM F-6-P); dependence of activity on F-6-P concentration at two UTP concentrations (pH 7.0).

sigmoid behaviour towards F-6-P (fig. 5). With either UTP or ATP the Hill coefficient with respect to F-6-P was approximately 2.2, varying with different enzyme preparations.

5′-AMP activated the enzyme, and depending on the pH, increased the affinity for F-6-P, resulting in pseudo-hyperbolic characteristics, although the Hill coefficient remained unaltered. This confirms the results of Atkinson, Hathaway and Smith [12].

Blangy, Buc and Monod [13] found that ATP did not have an inhibitory effect on PFK from *E. coli*, and did not influence the affinity of this enzyme for F-6-P. Nevertheless the enzyme showed co-operative behaviour towards F-6-P. Therefore, such co-opera-

tivity cannot depend on the inhibitory effects produced by the phosphate donor. It seems that co-operativity with F-6-P is due to the properties of the protein itself. However, substrate affinity and strength of co-operativity are governed by regulatory sites occupied by ATP, citrate and 5′-AMP. Ammonium sulphate abolishes, at least with ascites-tumor-PFK, co-operativity both between the F-6-P and between the regulatory and catalytic sites [9].

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