KINETIC PROPERTIES OF HUMAN F_4 PHOSPHOFRUCTOKINASE

A poor regulatory enzyme

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1. Introduction

Phosphofructokinase (ATP:D-fructose 6-P-1 phosphotransferase, EC 2.7.1.11), one of the key enzymes of the glycolytic pathway, is an allosteric enzyme subjected to a complex regulation by various metabolites, i.e., fructose 1,6-P_2 [1], ATP [2] AMP and ADP [3], citrate [4], NH_4^+ [5] and SO_4^{2-} [6].

In addition to the already known muscle type (M-type) and liver type (L-type) forms [7], we have recently proven the existence of a third basic form of phosphofructokinase, called fibroblast (of F) type enzyme [8]. This newly discovered isozyme is predominantly expressed in fibroblasts, platelets, brain, lymphocytes and kidney. The homotetramer F_4 has been purified to homogeneity from human platelets [9].

The goal of this work was to characterize, on a kinetic point of view, human F_4 phosphofructokinase as compared to the other forms, L_4 and M_4.

2. Materials and methods

The substrates, auxiliary enzymes and coenzymes were from Boehringer-Mannheim or Serva-Heidelberg. Enzyme activities were measured at 30°C in a Gilford (Model 252) spectrophotometer.

2.1. *Purification of phosphofructokinase*

The homotetramers M_4 (from muscle), L_4 (from red cells) and F_4 (from platelets) were purified as in [9,10]. For red cells and platelets these homotetramers were first separated from the L–M and L–F hybrids by DEAE-cellulose chromatography [9,11]. The last step, was, in all the cases, an affinity chromatography on Dextran blue–Sepharose 4B column with elective elution by ADP and fructose 6-P [9,10].

2.2. *Kinetic studies*

Before use, phosphofructokinase and auxiliary enzymes were extensively dialyzed against a 50 mM Tris–HCl buffer (pH 8)/5 mM β-mercaptoethanol/ 1 mM $(NH_4)_2$ SO_4/0.01 mM fructose 1,6-P_2. The kinetic determinations were performed at pH 7 (in 50 mM glycyl-glycine buffer) and pH 8 (in 50 mM Tris–HCl buffer). In both cases the reaction mixture contained 1 mM $(NH_4)_2$ SO_4/0.5 mg/ml bovine albumin/5 mM dithiotreitol/100 mM KCl/0.5 mM Mg Cl_2/0.2 mM NADH/0.5 IU/ml aldolase/10 IU/ml triose phosphate isomerase/0.5 IU/ml glycerol-3-phosphate dehydrogenase. Activation by NH_4Cl was studied using the aforementioned reactive mixture slightly modified in that $(NH_4)_2SO_4$ was omitted and KCl was only 5 mM.

After 10 min incubation at 30°C, the reaction was started by $MgATP^{2-}$. The ATP/Mg^{2+} ratio was 1:3, Mg^{2+} being provided as the $MgCl_2$ salt. In the first experiments, in fact, we systematically employed 2 different ATP/Mg^{2+} ratios (1:3 and 1:1), and did not find any difference between the results obtained.

The kinetic behaviour towards fructose 6-P was studied over 0.025–4 mM, at 4 constant concentrations of $MgATP^{2-}$ (0.25, 0.5, 1 and 2 mM). The kinetic behaviour towards $MgATP^{2-}$ was determined over 0.01–3 mM, at a constant 1 mM fructose 6-P.

Activation by AMP and glucose 1,6-P_2 and inhibition by citrate were studied at a constant 0.5 mM fructose 6-P and 1 mM $MgATP^{2-}$. AMP was varied

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from 0.002–0.2 mM, glucose 1,6-P_2 from 0.001–0.2 mM and that of citrate from 0.1–1 mM.

3. Results

All the kinetic determinations were performed in triplicate, at both pH 7 and pH 8. We always found the same type of kinetic differences between the isozymes at pH 7 and pH 8, only more pronounced at pH 7. Since it is indeed known that the regulatory properties of phosphofructokinase are maximum at acidic pH [1–6], we shall only report here the results obtained at pH 7.

3.1. *Kinetics towards fructose 6-P*

Figure 1 and table 1 show that the maximal cooperativity was observed for M_4 (Hill coefficient ~3–4) and L_4 (Hill coefficient 1.4–3.4) while the positive homotropic interactions were by far less pronounced for F_4 (Hill coefficient <1.5).

Cooperativity strongly depended on ATP concentration for L_4, slightly for F_4 and not at all for M_4. In the same way, $K_{0.5}$ for fructose 6-P increased with $MgATP^{2-}$ concentration for L-type and F-type enzymes, but not for muscle-type phosphofructokinase. The F-type isozyme exhibited a higher apparent affinity for fructose 6-P than the other forms.

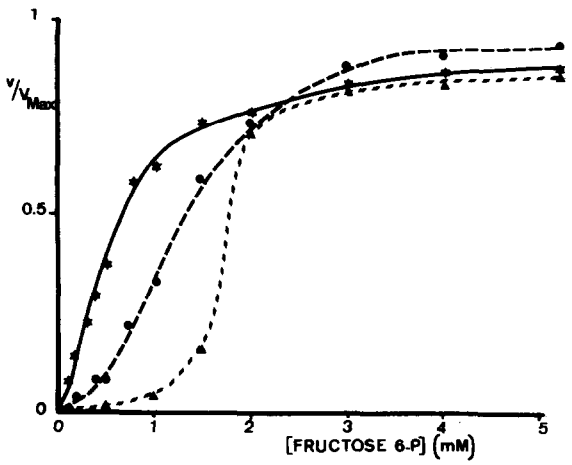

Fig.1. Kinetics towards fructose 6-P; $MgATP^{2-}$, 0.5 mM (pH 7). (–▲–▲–) M_4 phosphofructokinase; (–●–●–) L_4 phosphofructokinase; (★–★) F_4 phosphofructokinase. V_{max} was appreciated from a Lineweaver-Burk plot at high fructose 6-P concentration. For further details, see section 2.

3.2. *ATP inhibition (fig.2)*

ATP acts as a substrate of the reaction, but it

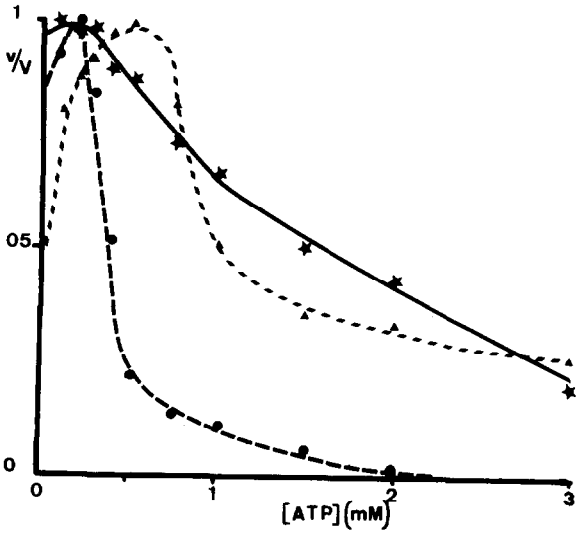

Fig.2. ATP inhibition; fructose 6-P, 1 mM (pH 7). The results are expressed as a fraction of the maximal activity measured. See legends to fig.1.

Table 1
Kinetics towards fructose 6-P

Isozymes / $MgATP^{2-}$ (mM)	M-type		L-type		F-type	
	$K_{0.5}$ (mM)	Hill coeff.	$K_{0.5}$ (mM)	Hill coeff.	$K_{0.5}$ (mM)	Hill coeff.
0.25	1.2	3.0	0.9	1.4	0.27	0.8
0.5	1.8	3.6	1.4	2.0	0.62	1.2
1	1.3	3.9	1.5	2.7	0.84	1.3
2	2.0	3.5	2.3	3.4	1.2	1.5

The figures given in this table represent the results of typical experiments. Similar results were obtained by using a ATP/Mg^{2+} ratio of 1:1 (instead of 1:3). Hill coefficient and $K_{0.5}$ for fructose 6-P were determined from a Hillplot; maximal velocity was calculated from a Lineweaver-Burk plot

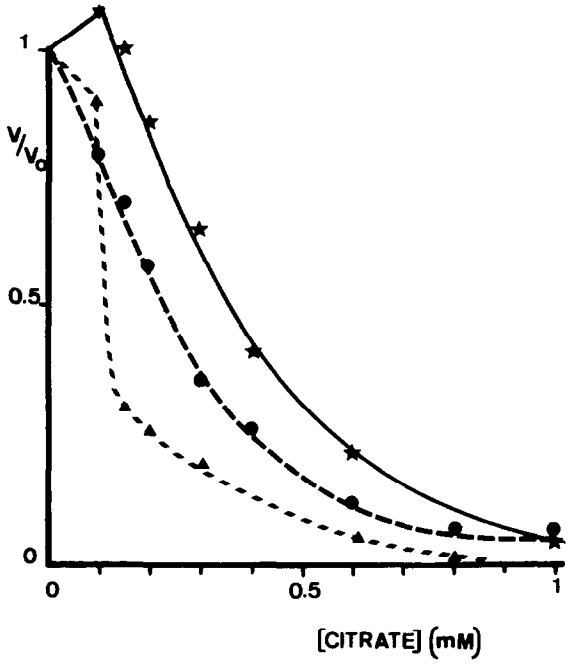

Fig.3. Citrate inhibition; fructose 6-P, 0.5 mM; $MgATP^{2-}$, 1 mM (pH 7). See legends to fig.1,2.

becomes inhibitor as its concentration rises [2]. It is known for several years that L_4 isozyme is more inhibited than M_4 [7]. We show here that it is also more inhibited than F_4. Compared to M_4, ATP inhibition of F_4 began at lower ATP concentration and was of a similar extent.

3.3. *Citrate inhibition (fig.3)*

As expected [4], the isozyme which was the most strongly inhibited by citrate was muscle-type phosphofructokinase, followed by liver-type enzyme. F_4 was the least inhibited form.

3.4. *Glucose 1,6-P_2 activation (fig.4)*

Since it is not possible to study fructose 1,6-P_2 activation using the coupled assay with aldolase, triose phosphate isomerase and glycerol phosphate dehydrogenase, we studied the influence of glucose 1,6-P_2 on the different phosphofructokinase isozymes.

Glucose 1,6-P_2 has been shown to be able to activate phosphofructokinase in a similar fashion to fructose 1,6-P_2 [12–14]. Activation by glucose 1,6-P_2 was maximum for M_4 phosphofructokinase and intermediate for L_4. F_4 phosphofructokinase was totally insensitive to glucose 1,6-P_2.

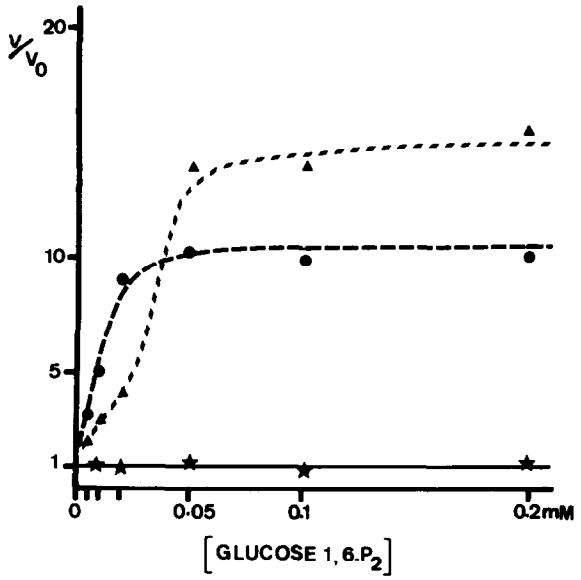

Fig.4. Glucose 1,6-P_2 activation; fructose 6-P, 0.5 mM; $MgATP^{2-}$, 1 mM (pH 7). See legends to fig.1–3.

3.5. *AMP activation (fig.5)*

As in [3], we found that AMP was a strong activator of L_4 and M_4 enzymes. By contrast, F_4 phosphofructokinase was practically insensitive to AMP.

3.6. *Influence of NH_4^+*

Under the conditions reported in section 2, maximal activation by NH_4Cl was 12–15-fold for both L_4 and M_4 phosphofructokinases and only 3-fold for F_4; it was obtained for approximately the same NH_4Cl concentration (30 mM).

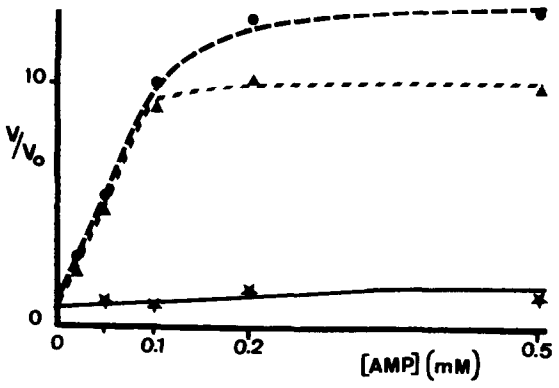

Fig.5. AMP activation; fructose 6-P, 0.5 mM; Mg ATP^{2-}, 1 mM (pH 7). See legends to fig.1–4.

4. Discussion

This work describes for the first time some kinetic characteristics of F_4 phosphofructokinase which represents the third, newly discovered basic form of phosphofructokinase in man. Its main kinetic features are: practically hyperbolic kinetic for fructose 6-P (except at high ATP concentration); absence of activation by AMP and glucose 1,6-P_2; slight activation by NH_4^+; slight inhibition by ATP and citrate.

These studies were made with F_4 phosphofructokinase purified from human platelets. We could therefore ask whether this unexpected behaviour was due to the environment or to particular properties of these cells. We also studied the kinetics of enzyme from human fibroblasts and some malignant tissues which have been proven to contain a high amount of F_4 form [8] and we found the same kinetic properties, in particular hyperbolic kinetics towards fructose 6-P (experiments not shown). Therefore we think that these properties are intrinsic of the F_4 isozyme.

Non-allosteric phosphofructokinases have already been described in some microorganisms. A non-allosteric enzyme in normal *Lactobacillus plantarum* has been shown in [15]. In *E. coli*, the main species of phosphofructokinase are allosteric, but mutants with non-allosteric enzymes have been described [16,17].

However, in mammals no example of such a type of phosphofructokinase is known so far. It cannot be said that F_4 is a 'non-allosteric' enzyme, since it is regulated by citrate, ATP and exhibits slight positive homotropic effects towards fructose 6-P at high ATP concentration. Its regulatory properties, however, seem clearly less developed than those of M_4 and L_4 enzymes since it is less regulated by almost all the allosteric effectors studied here than these latter phosphofructokinases; it is in this way that it can be compared to the non-allosteric enzymes from the aforementioned microorganisms.

It is assumed that phosphofructokinase plays an essential role in regulation of glycolysis, and it is worth noting that the regulatory forms are expressed in cells in which this metabolic pathway has to be accurately regulated, e.g., muscle and liver. By contrast, the isozyme F_4 is found in relatively undifferentiated cells (e.g., fibroblasts or malignant cells), or in cells in which glycolysis is not known to play a major physiological role (e.g., platelets or lymphocytes).

In conclusion, besides the 2 well characterized muscle-type and liver-type forms of phosphofructokinase, which exhibit complex regulatory properties, a third basic form exists in man (and probably in mammals). This third isozyme can be regarded as a poor regulatory enzyme as compared to the other phosphofructokinase forms.

Acknowledgements

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