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HUMAN PLATELET 6-PHOSPHOFRUCTOKINASE

RELATION BETWEEN INHIBITION BY Mg · ATP^{2-} AND COOPERATIVITY TOWARDS FRUCTOSE 6-PHOSPHATE AND INVESTIGATIONS ON THE FORMATION OF A TERNARY COMPLEX

JAN WILLEM N. AKKERMAN, GERTIE GORTER, JAN OVER, JAN J. SIXMA and GERARD E.J. STAAL

Dept. of Haematology, State University Hospital, Utrecht (The Netherlands)

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Summary

Human platelet 6-phosphofructokinase (EC 2.7.1.11) shows cooperativity towards Fru-6-*P* and is allosterically inhibited by high Mg · ATP^{2-} concentrations. No relation could be demonstrated between the cooperativity towards Fru-6-*P* and the inhibition by Mg · ATP^{2-}. Increasing the concentrations of Mg · ATP^{2-} only raised the apparent K_m values for Fru-6-*P*, but did not change the Hill constants. A possible formation of a Mg · ATP^{2-} : enzyme · Fru-6-*P* complex during catalysis was investigated. Our calculations suggest that such a ternary complex is indeed formed during the reaction.

Introduction

Platelet shape change, aggregation and especially release reaction are energy requiring processes [1,2]. Part of this energy is provided by glycolysis which is stimulated by the mentioned platelet functions [3,4]. Changes in the levels of glycolytic intermediates have indicated that phosphofructokinase plays an important regulatory role in the rat platelet [5,6]. We have recently confirmed this for human platelets (Akkerman, J.W.N., Gorter, G., Staal, G.E.J. and Sixma, J.J., unpublished results). More direct information about the regulatory role of this enzyme may be gained from investigations of the purified enzyme.

6-Phosphofructokinase (EC 2.7.1.11) catalyzes the conversion of Fru-6-*P* to Fru-1,6-P_2, coupled with the dephosphorylation of ATP to ADP. This catalysis depends on Mg^{2+}. In previous articles we have described a purification procedure and shown that the purified enzyme is activated strongly by sulphate [7,8]. Rather than ATP alone the Mg · ATP^{2-} complex is the substrate for the

enzyme, but high $Mg \cdot ATP^{2-}$ concentrations are inhibitory [9]. Free ATP^{4-} increases the allosteric inhibition by $Mg \cdot ATP^{2-}$ [9]. When ITP was used as a phosphate donor, free Mg^{2+} stimulated with an optimal effect at a ratio $[Mg^{2+}]/[Mg \cdot ITP^{2-}] \geqslant 1.0$ [9].

In this paper we report the interdependence between the allosteric inhibition by $Mg \cdot ATP^{2-}$ and the cooperativity towards Fru-6-*P*. A direct relationship between these phenomena was observed in phosphofructokinase from pig spleen [10], rat thymocytes [11] and rabbit muscle [12]. On the other hand, studies with phosphofructokinase from yeast [13,14], *Bacillus licheniformis* [15] and human erythrocytes [16] showed that the cooperativity towards Fru-6-*P* was not dependent on or parallel with, allosteric inhibition by $Mg \cdot ATP^{2-}$.

Layzer et al. [17] proposed that the reaction catalyzed by red blood cell phosphofructokinase takes place through a Ping Pong mechanism. In contrast Staal et al. [18] proposed the existence of a ternary complex for the same enzyme. Such a $Mg \cdot ATP^{2-} \cdot$ enzyme $\cdot$ Fru-6-*P* complex was also found for the enzyme from rabbit muscle [19]. Using the methods of Slater, Koster, Staal and Veeger [18,20—23] we investigated whether this complex was formed also during catalysis by the human platelet enzyme.

Materials and Methods

The nucleotide phosphates, added as sodium salts, glycolytic intermediates, cofactors and enzymes used for measurement of phosphofructokinase activity, were obtained from Boehringer-Mannheim. All other chemicals used were of analytical grade.

Human platelet phosphofructokinase was partially purified as described previously [7]. The enzyme activity was measured by coupling the formation of Fru-1,6-P_2 to the α-glycerophosphate dehydrogenase reaction. The oxidation of NADH was followed at 340 nm in a Perkin-Elmer 124 spectrophotometer at 25° C. The assay mixture contained in a final volume of 3 ml: 0.2 M Tris $\cdot$ HCl (pH 8.1); 6 mM KCl; 0.05 ml dialyzed auxiliary enzymes (fructose diphosphate aldolase, 10 mg/ml; triosephosphate isomerase, 2 mg/ml; glycerophosphate dehydrogenase, 2 mg/ml); 0.2 mM NADH (disodium salt) and the concentrations of substrates of the reaction catalyzed by phosphofructokinase as indicated in Results. $MgCl_2$ was present at concentrations of $[Mg_{total}] = 3 \times [\text{nucleotide phosphate}_{total}]$. The levels of the $Mg \cdot ATP^{2-}$ complex were calculated with the aid of a stability constant of 20 000 M^{-1} which has been determined for a medium which resembles the assay mixture used by us [24,25]. The $Mg \cdot ITP^{2-}$ complex was assumed to have an identical stability constant [26]. Complex formation between Mg^{2+} and Fru-6-*P* and between K^+ and ATP^{4-} or ITP^{4-} was considered negligible because of the low stability constants of these complexes [25,27]. The formation of $HATP^{3-}$, $Mg \cdot HATP^-$ and similar complexes of ITP^{4-} was neglected since these complexes represent less than 1% of the total nucleotide content at pH 8.1 [28].

The reaction was started by adding 0.02 ml purified human platelet phosphofructokinase. A unit of enzyme activity is defined as the amount of enzyme catalyzing the formation of 1 μmol of Fru-1,6-P_2 per min at 25° C. The various

purified phosphofructokinase preparations that were tested (n = 5) had a spec. act. of about 7 units/mg protein, as measured at 4 mM Fru-6-*P*, 0.4 mM ATP_{total} and 5 mM $MgSO_4$. The protein content was assayed according to Lowry et al. [29] using crystalline bovine serum albumin as a standard.

The influences of ATP and ITP were not disturbed by sodium effects and corrections were not necessary.

Results

Relation between inhibition by Mg · ATP^{2-} and cooperativity towards Fru-6-P

Phosphofructokinases isolated from different sources are characterized by sigmoidal velocity curves with respect to Fru-6-*P* and allosteric inhibition at high Mg · ATP^{2-} concentrations. Both the cooperativity towards Fru-6-*P* and the inhibition by Mg · ATP^{2-} are most pronounced at neutral pH. Unlike other types of phosphofructokinase the platelet enzyme still exhibits these properties to a large extent when the pH is raised to 8.1 on condition that bivalent anions such as sulphate are absent [7,8]. Fig. 1A illustrates the inhibition by Mg · ATP^{2-} at pH 8.1. The inhibition is counteracted by Fru-6-*P*. The sigmoidal velocity curves for Fru-6-*P* (Fig. 1B) illustrate the cooperativity towards this substrate. At rising Mg · ATP^{2-} concentrations and therefore increasing Mg · ATP^{2-} inhibition the curves seem to become more sigmoidal.

From these data it may be concluded that the inhibition by Mg · ATP^{2-} influences the kinetics towards Fru-6-*P* and vice versa. We investigated this relationship by comparing the velocity curves for Fru-6-*P* as measured in the presence and in the absence of inhibition by Mg · ATP^{2-}.

It is generally assumed that the inhibition at high Mg · ATP^{2-} levels involves an inhibitory site which is highly specific for this nucleotide. At pH 8.1 the platelet enzyme also shows this specificity [7]. When Mg · ITP^{2-} was used as a phosphate donor no inhibition was observed (Fig. 2A). This suggested that Mg · ITP^{2-} only acted on the catalytic site. Involvement of both inhibitor and catalytic site (using ATP) could thus be separated from participation of the catalytic site alone (using ITP).

In the absence of inhibition the cooperative interactions with respect to Fru-6-*P* remained present (Fig. 2B). This was confirmed by calculations of the Hill coefficients: values of 2.2 were calculated which were independent on the concentrations of Mg · ITP^{2-} in the range 0.04—1.98 mM Mg · ITP^{2-} (Fig. 3). When Mg · ATP^{2-} was used the Hill values were slightly higher (n = 3.0). Under our experimental conditions, again this value was not dependent on the concentrations of Mg · ATP^{2-}. Thus, both the absence (Fig. 3B) and the degree (Fig. 3A) of allosteric inhibition by Mg · ATP^{2-} had no effect on the cooperativity towards Fru-6-*P*. In all experiments the n value with respect to Fru-6-*P* tended to be somewhat lower with Mg · ITP^{2-} than with Mg · ATP^{2-}. This may indicate that binding of Mg · ITP^{2-} and of Mg · ATP^{2-} have a different effect on the cooperativity towards Fru-6-*P*.

The involvement of the inhibitor site for Mg · ATP^{2-}, however, greatly effected the affinity for Fru-6-*P* (Table I). Increasing concentrations of the phosphate donors raised the apparent K_m values for Fru-6-*P*. At about 0.5 mM phosphate donor the catalytic site for the nucleotide phosphates appeared to

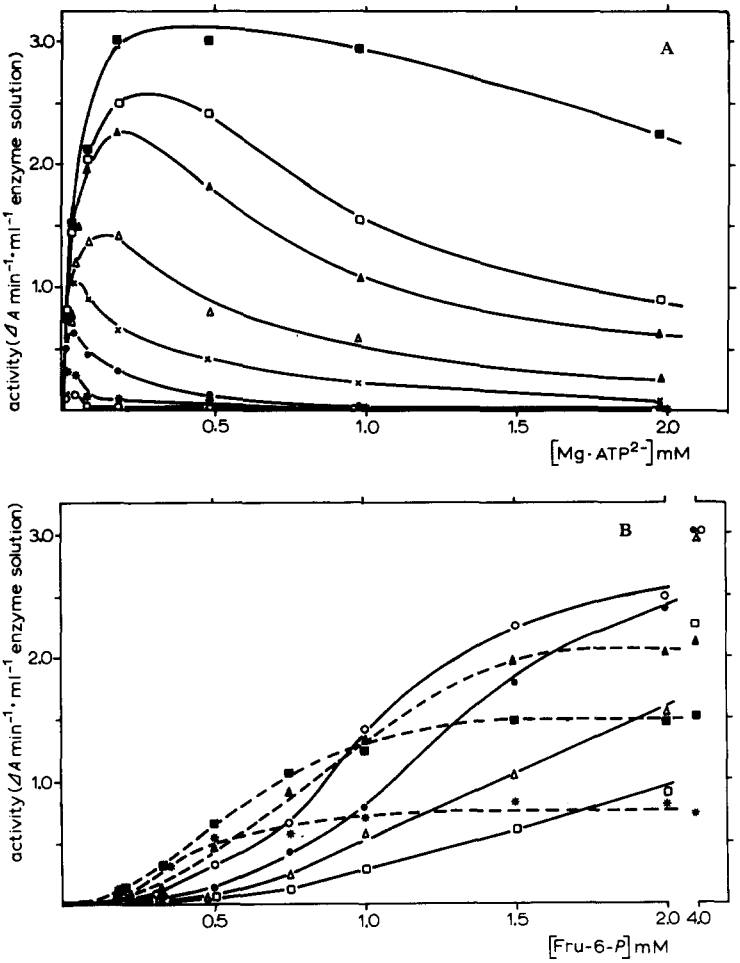

Fig. 1. Phosphofructokinase activity (expressed as ΔA min^{-1} · ml^{-1} enzyme solution) versus [Mg ATP^{2-}] at various [Fru-6-*P*]: 0.2 mM, (○———○); 0.33 mM, (★———★); 0.5 mM, (●———●); 0.75 mM, (X———X); 1.0 mM, (△———△); 1.5 mM, (▲———▲); 2.0 mM, (□———□) and 4.0 mM, (■———■), shown in Fig. 1A and the activity versus [Fru-6-*P*] at various [Mg · ATP^{2-}]: 0.01 mM, (★- - - - -★); 0.04 mM, (■- - - - -■); 0.08 mM, (▲- - - - -▲); 0.18 mM, (○———○); 0.48 mM, (●———●); 0.98 mM, (△———△) and 1.98 mM, (□———□) shown in Fig. 1B, as tested at pH 8.1. Data represent 1 out of 5 similar experiments.

be saturated and no further effect on the affinity for Fru-6-*P* was measured when Mg · ITP^{2-} was used. In contrast, the involvement of the inhibitory site became apparent at higher Mg · ATP^{2-} levels leading to pronounced influences on apparent K_m for Fru-6-*P* values.

A ternary complex in the reaction mechanism of phosphofructokinase?

The following rate equation for a two substrate reaction delivers an overall picture of the reaction catalyzed by human platelet phosphofructokinase:

$$v = V \cdot \left\{ 1 + \frac{K_m \text{ Fru-6-}P}{[\text{Fru-6-}P]^n} + \frac{K_m \text{ Mg} \cdot \text{ATP}^{2-}}{[\text{Mg} \cdot \text{ATP}^{2-}]} + \frac{K_m \text{ Fru-6-}P\text{-}E\text{-Mg} \cdot \text{ATP}^{2-}}{[\text{Fru-6-}P]^n [\text{Mg} \cdot \text{ATP}^{2-}]} \right\}^{-1} \quad (1)$$

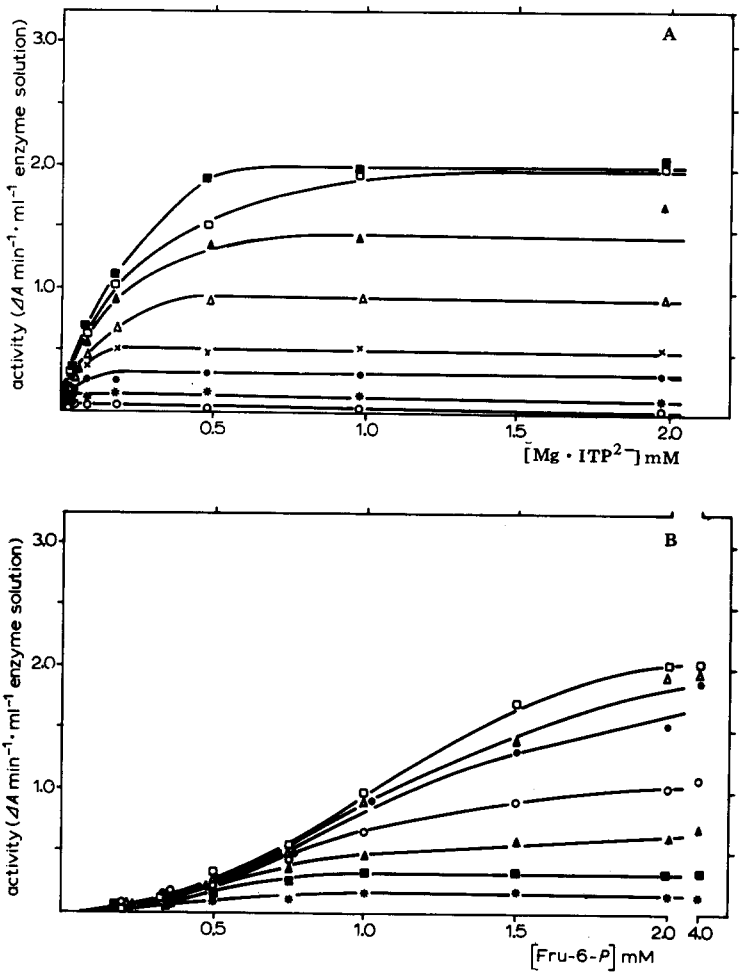

Fig. 2. Phosphofructokinase activity (expressed as ΔA $min^{-1} \cdot ml^{-1}$ enzyme solution) versus [Mg · ITP^{2-}] at various [Fru-6-*P*] shown in A and the activity versus [Fru-6-*P*] at various [Mg · ITP^{2-}] (B) as tested on the same enzyme preparation used in Fig. 1 at pH 8.1. Symbols for [Fru-6-*P*] and [phosphate donor] as in Fig. 1. Data represent one out of five similar experiments.

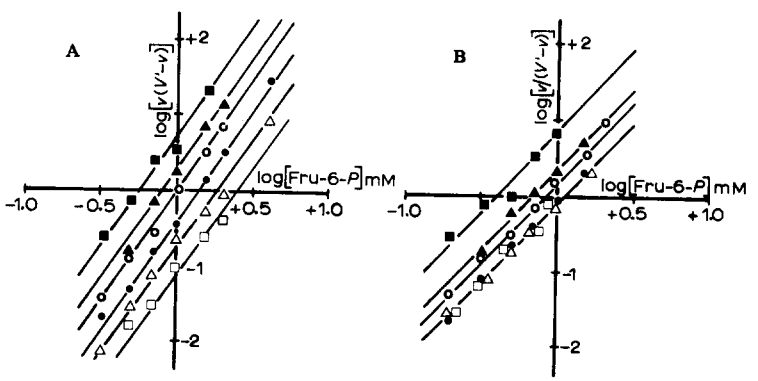

Fig. 3. Hill plots for Fru-6-*P* at various [Mg · ATP^{2-}] (Fig. 3A) and Mg · ITP^{2-} (Fig. 3B). Data calculated from Figs 1B and 2B [Phosphate donor] and symbols as in Fig. 1.

TABLE I

MICHAELIS-MENTEN CONSTANTS FOR Fru-6-*P* WITH AND WITHOUT INHIBITION BY Mg · ATP^{2-}

The apparent K_m values for Fru-6-*P* in the presence of Mg · ATP^{2-} or Mg · ITP^{2-} as the phosphate donor were calculated from Fig. 3A and Fig. 3B respectively by extrapolation to log $v/(V'-v) = 0$, where V' stands for apparent V.

Phosphate donor (mM)	Apparent K_m for Fru-6-*P* (mM)	
	Inhibition (Mg · ATP^{2-})	No inhibition (Mg · ITP^{2-})
0.04	0.58	0.40
0.08	0.81	0.68
0.18	1.00	0.83
0.48	1.41	1.12
0.98	1.82	1.12
1.98	2.51	1.12

where E and n stand for phosphofructokinase and the Hill coefficient, respectively. If the term K_m Fru-6-*P*-*E*-Mg · ATP^{2-} is equal to zero, Eqn 1 will represent a Ping Pong mechanism. If this term is unequal to zero a ternary complex is involved in the reaction [18,20—23]. For calculations of this factor the data from Figs 1 and 2 were replotted in Lineweaver-Burk plots for Fru-6-*P* (Fig. 4) and Mg · ITP^{2-} (not shown in a figure). From Fig. 4 apparent V_{max} values at various Mg · ATP^{2-} (Fig. 4A) and Mg · ITP^{2-} (Fig. 4B) levels at infinite concentrations of Fru-6-*P* were derived by extrapolation to $[\text{Fru-6-}P]^{-1} = 0$.

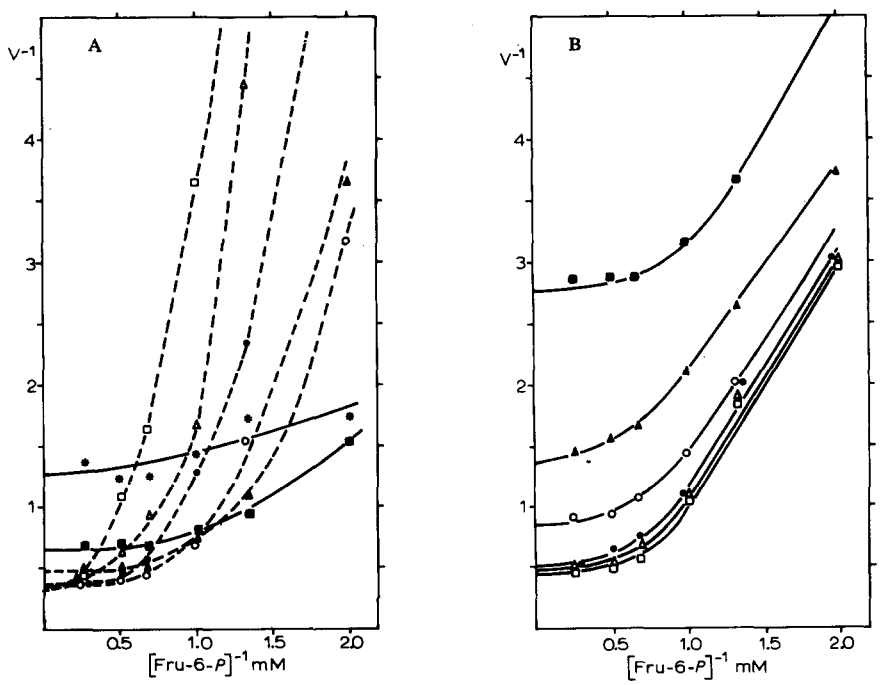

Fig. 4. Lineweaver Burk plots of phosphofructokinase activity (expressed as ΔA min^{-1} · ml^{-1} enzyme solution) versus [Fru-6-*P*] at various [Mg · ATP^{2-}]. (Fig. 4A) and [Mg · ITP^{2-}] (Fig. 4B). Values were calculated from Figs 1B and 2B. Symbols as in Fig. 1.

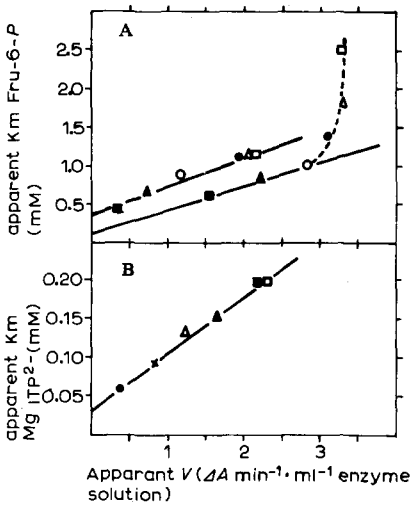

Fig. 5. Plots of apparent V values ($V_{app.}$ Fru-6-P in Eqn 2; expressed as ΔA min^{-1} · ml^{-1} enzyme solution) at corresponding apparent K_m data for Fru-6-P (mM) (K_m app. Fru-6-P in Eqn 2) using Mg · ATP^{2-} (lower line) and Mg · ITP^{2-} (upper line) as the second substrate (Fig. 5A). Similar plot for Mg · ITP^{2-} using Fru-6-P as the second substrate shown in Fig. 5B. Values calculated from Figs 1, 2 and 4. Points corresponding to inhibition by Mg · ATP^{2-} were excluded from the calculations. (Regression lines with correlation coefficient (r): Fig. 5A. $y = 0.323\ x + 0.086$; $r = 1.00$ for Mg · ATP^{2-} and $y = 0.377\ x + 0.343$; $r = 0.98$ for Mg · ITP^{2-}. Fig. 5B. $y = 0.076\ x + 0.029$; $r = 1.00$). Symbols defined in Fig. 1.

These values were plotted against the respective apparent K_m values for Fru-6-P (Fig. 5A). The lines in Fig. 5A represent the graphic notation of Eqn 2, which can be derived from Eqn 1 by converting the latter to the form $y = a + bx$ in which y = apparent K_m Fru-6-P and x = apparent V at [Fru-6-P] = ∞ (V_{app} Fru-6-P)

$$K_{m\,app}\,\text{Fru-6-}P = \frac{K_m\,\text{Fru-6-}P\text{-}E\text{-Mg} \cdot ATP^{2-}}{K_m\,\text{Mg} \cdot ATP^{2-}} + \frac{K_m\,\text{Fru-6-}P \cdot K_m\,\text{Mg} \cdot ATP^{2-} - K_m\,\text{Fru-6-}P\text{-}E\text{-Mg} \cdot ATP^{2-}}{K_m\,\text{Mg} \cdot ATP^{2-} \cdot V} \cdot V_{app}\,\text{Fru-6-}P \quad (2)$$

Assuming that Fru-6-P is the first substrate the lines of Fig. 5A do not pass through the origin, whether Mg · ATP^{2-} or Mg · ITP^{2-} is the other substrate (regression analysis: $p < 0.01$ using Mg · ITP^{2-} as phosphate donor). Therefore, the term K_m Fru-6-P-E-Mg · NTP^{2-}/K_m Mg · NTP^{2-} is unequal to zero. If Mg · ITP^{2-} is the first substrate Fig. 5B is valid. This line crosses the ordinate at a significant distance from the origin (regression analysis $p < 0.01$). The strong inhibition by Mg · ATP^{2-} hampers similar calculations for this substrate. However, if this inhibition was suppressed by the addition of sulphate [7] or cyclic 3′, 5′ AMP [30] results similar to those of Mg · ITP^{2-} were obtained. Furthermore, in the presence of sulphate at pH 8.1 the platelet enzyme shows strongly reduced cooperativity towards Fru-6-P ($n = 1.2$) [7]. Under these conditions a ternary complex can also be calculated, which means

that sigmoidal kinetics do not disturb these calculations. This is not surprising since in the calculations of the complex the velocity data are extrapolated to infinite Fru-6-*P* concentration, i.e. to conditions in which $n = 1.0$. Whatever the first substrate may be these data strongly suggest the involvement of a ternary complex in the reaction mechanism.

Discussion

Phosphofructokinase isolated from different sources generally shows allosteric inhibition at high Mg · ATP^{2-} levels and cooperativity towards Fru-6-*P*. The way in which these properties are related, however, is not general. In one group of phosphofructokinases both properties are completely independent and each may be influenced separately by various effectors. Examples of this class are phosphofructokinase from yeast [13,14] *Bacillus licheniformis* [15] or human erythrocytes [16]. In contrast, the other group of phosphofructokinases shows strong interdependence of these phenomena. The enzyme from pig spleen [10], rat thymocytes [11] and rabbit muscle [12] is characterized by a complete loss of cooperativity towards Fru-6-*P* when Mg · ATP^{2-} inhibition is absent and furthermore the degree of cooperativity corresponds to the degree of inhibition by Mg · ATP^{2-} (For a recent review see ref. 31).

The data presented in this paper show that the human platelet enzyme behaves in the former way i.e. the cooperativity towards Fru-6-*P* remains present in the absence of Mg · ATP^{2-} inhibition and this cooperativity is not affected by various degrees of inhibition. Between 0.04 mM and 1.98 mM Mg · ATP^{2-} the Hill coefficient remained constant ($n = 3.0$). A similar observation has been made by Kopperschläger on yeast phosphofructokinase [32]. Unlike this enzyme which gave Hill values of 1.0 when ITP was present in stead of ATP, the platelet enzyme still exhibits cooperativity towards Fru-6-*P* when Mg · ITP^{2-} is the phosphate donor ($n = 2.2$).

Recently, Lee et al. demonstrated that photo-oxidized phosphofructokinase from human red blood cells looses its cooperativity towards Fru-6-*P* but still shows the inhibition pattern of ATP [33]. They concluded that cooperativity towards Fru-6-*P* and inhibition by ATP are independent phenomena and this is well in accordance with the findings presented here for the platelet enzyme.

From the data for Mg · ITP^{2-} a second conclusion may be drawn. In the range of Mg · ITP^{2-} concentrations used this substrate did not effect the Hill coefficient for Fru-6-*P*. This indicates that the degree of saturation of the catalytic site for the phosphate donor does not influence the cooperativity towards Fru-6-*P*. In this respect the platelet enzyme resembles phosphofructokinase from *Escherichia coli*. Here ATP only functions as a substrate and does not inhibit enzyme activity. The various degrees of saturation with ATP had no effect on the cooperativity of this enzyme towards Fru-6-*P* [34].

Investigations on the reaction mechanism of phosphofructokinase catalysis have been performed using isotope exchange techniques, various substrate analogs [19] or calculations of kinetic data at varying levels of both substrates [18,20—23]. Using the latter method the involvement of a ternary complex in the reaction mechanism of the platelet enzyme can be calculated although the

reaction mechanism itself remains obscure. In this respect, our findings disagree with those of Layzer et al. [17], who concluded from parallel lines in the reciprocal plots that phosphofructokinase from human red blood cells follows a Ping Pong mechanism. However, the demonstration that the reciprocal plots produce apparent parallel lines is not conclusive evidence for a Ping Pong mechanism [31]. In contrast to Layzer, Staal et al. [18] calculated a ternary complex for the same enzyme using the methods described here for the platelet enzyme. Such a complex was found also in rabbit muscle phosphofructokinase catalysis; in fact the presently available information suggests that a ternary complex is involved in the reaction mechanism of most types of phosphofructokinases [31]. The potent activities of the free constituents of the Mg · ATP^{2-} complex already mentioned in the introduction may interfere with formation of the ternary complex thus leading to a more complicated reaction mechanism.

The physiological meaning of the results reported here is until now uncertain. The independence of cooperative interactions towards Fru-6-*P* and allosteric inhibition by Mg · ATP^{2-} may perhaps provide an extra dimension to regulation phenomena in human platelet glycolysis. But since various ligands similarly effect both properties the netto result might be of relatively minor importance [7,8,30].

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References

1 Holmsen, H., Day, H.J. and Setkowsky, C.A. (1972) Biochem. J. 129, 67—82
2 Mills, D.C.B. (1973) Nat. New Biol. 243, 220—222
3 Warshaw, A.L., Laster, L. and Shulman, N.R. (1966) J. Clin. Invest. 45, 1923—1934
4 De Vreker, G.G. and De Vreker, R.A. (1965) Rev. Belge Pathol. 31, 79—85
5 Detwiler, T.C. (1969) Biochim. Biophys. Acta 177, 161—163
6 Detwiler, T.C. (1970) Biochim. Biophys. Acta 197, 117—126
7 Akkerman, J.W.N., Gorter, G., Sixma, J.J. and Staal, G.E.J. (1974) Biochim. Biophys. Acta 370, 102—112
8 Akkerman, J.W.N., Gorter, G., Sixma, J.J. and Staal, G.E.J. (1974) Int. J. Biochem 5, 853, 857
9 Akkerman, J.W.N., Gorter, G., Sixma, J.J. and Staal, G.E.J. (1974) Biochim. Biophys. Acta 370, 113—119
10 Hickman, P.E. and Weideman, M.J. (1973) FEBS Lett. 38, 1—3
11 Ohyama, H. and Yamada, T. (1973) Biochim. Biophys. Acta 302, 261—266
12 Kemp, R.G. (1969) Biochemistry 8, 4490—4496
13 Afting, E.G. and Ruppert, D. (1973) Arch. Biochem. Biophys. 156, 720—729
14 Atkinson, D.E., Hathaway, J.A. and Smith, E.C. (1965) Biochem. Biophys. Res. Comm. 18, 1—5
15 Marschke, C.K. and Bernlohr, R.W. (1973) Arch. Biochem. Biophys. 156, 1—16
16 Mandereau, J. and Boivin, P. (1973) Biochimie 55, 1341—1351
17 Layzer, R.B., Rowland, L.P. and Bank, W.J. (1969) J. Biol. Chem. 244, 3823—3831

18 Staal, G.E.J., Koster, J.F., Bänziger, C.J.M. and Van Milligen-Boersma, L. (1972) Biochim. Biophys. Acta 276, 113—123
19 Bar-tana, J. and Cleland, W.W. (1974) J. Biol. Chem. 249, 1263—1270
20 Slater, E.C. (1953) Disc. Faraday Soc. 20, 231—240
21 Koster, J.F. and Veeger, C. (1968) Biochim. Biophys. Acta 151, 11—19
22 Staal, G.E.J. and Veeger, C. (1969) Biochim. Biophys. Acta 185, 49—62
23 Staal, G.E.J., Koster, J.F., Kamp, H., Van Milligen-Boersma, L. and Veeger, C. (1971) Biochim. Biophys. Acta 227, 86—96
24 Taquikhan, M.M. and Martell, A.E. (1962) J. Phys. Chem. 66, 10—15
25 O'Sullivan, W.J. and Perrin, D.D. (1964) Biochemistry 3, 18—26
26 Purich, P.L. and Fromm, H.J. (1972) Biochem. J. 130, 63—69
27 McGilvery, R.W. (1965) Biochemistry 4, 1924—1930
28 Morrison, J.F. and Heyde, E. (1972) Annu. Rev. Biochem. 41, 29—54
29 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
30 Akkerman, J.W.N., Gorter, G., Corbey, H.M.A., Staal, G.E.J. and Sixma, J.J. (1973) in Erythrocytes, Thrombocytes and Leukocytes (Gerlach, E., Moser, K., Deutsch, E. and Wilmanns, W. eds) p. 342—344, Thieme, Stuttgart
31 Bloxham, D.P. and Lardy, H.A. (1973) in The Enzymes VIII-A (Boyer, P.D., ed.), p. 240—278, Academic Press, New York
32 Kopperschläger, G., Freyer, R., Diezel, W. and Hoffmann, E. (1968) FEBS Lett. 1, 137—141
33 Lee, L.M.Y., Krupka, R.M. and Cook, R.A. (1973) Biochemistry 12, 3503—3508
34 Blangy, D., Buc, H. and Monod, J. (1968) J. Mol. Biol. 31, 13—35