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REGULATION OF HUMAN ERYTHROCYTE HEXOKINASE

THE INFLUENCE OF GLYCOLYTIC INTERMEDIATES AND INORGANIC PHOSPHATE

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Summary

Human erythrocyte hexokinase (ATP : D-hexose 6-phosphotransferase, EC 2.7.1.1) was inhibited competitively with respect to $MgATP^{2-}$ by glucose-6-*P* (K_i = 10.8 μM) and fructose-6-*P* (K_i = 160 μM). Low concentrations of inorganic phosphate were competitive with respect to glucose-6-*P* and fructose-6-*P*, although higher concentrations of P_i were not able to overcome completely the inhibition by the hexose phosphates. The results are consistent with a model in which hexokinase exists in equilibrium either as free or phosphate-associated enzyme, the latter having a reduced but still substantial affinity for hexose phosphate. An alternative explanation could be found in the presence of two different enzymes, one with a high affinity for glucose-6-*P* being sensitive to regulation by P_i, one with a lower affinity for glucose-6-*P* being insensitive to P_i. A similar but less pronounced effect of P_i, was found on the inhibition by 2,3-diphosphoglycerate (K_i = 4.0 mM). P_i in the absence of inhibitor was also a competitive inhibitor with respect to $MgATP^{2-}$ (K_i = 20 mM).

Furthermore a competitive inhibition with respect to $MgATP^{2-}$ was found by fructose 1,6-diphosphate (K_i = 4.3 mM), glycerate-3-*P* (K_i = 3.8 mM), glycerate-2-*P* (K_i = 12.5 mM), $MgADP^-$ (K_i = 1.0 mM) and MgAMP (K_i = 1.7 mM).

Introduction

Erythrocyte glycolysis is known to be regulated by the enzymes hexokinase (ATP : D-hexose 6-phosphotransferase, EC 2.7.1.1) and phosphofructokinase (ATP : D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) [1,2]. Of these two enzymes hexokinase has a much higher 'control strength' as was shown by mathematical models [2]. An explanation of this result was found in

the low activity of hexokinase relative to the other enzymes of glycolysis, which suggests a rate-limiting role for this enzyme. Furthermore hexokinase activity is markedly suppressed by intracellular concentrations of glucose-6-*P* [2,4—7]. The level of glucose-6-*P* is controlled by the enzymes hexokinase and phosphofructokinase, which enables the latter enzyme to participate in the regulation of the flux.

The importance of the glucose-6-*P* level as a control point in glucolysis was already pointed out in 1964 by Rose and O'Connel [3].

Because of the regulatory role of hexokinase in erythrocyte glycolysis, it is of interest to study the influence of various effectors on human erythrocyte hexokinase. These studies have been hampered by the presence of contaminating enzymes in the hexokinase preparation. Therefore laborious and inaccurate methods have been used, which may be the reason for contradictory reports in literature. Most of these contradictions concern the role of phosphate in the inhibition by glucose-6-*P*. For human erythrocyte hexokinase P_i has been found to relieve partly [4] or even completely [5,6] the inhibition of glucose-6-*P*, while Gerber et al. [7] found no influence of P_i at all. Phosphate on its own was reported to have no influence on the enzyme activity [4—7]. There is no doubt about the inhibitory action of 2,3-diphosphoglycerate on the enzyme [8,9]. Although the inhibition constants are in the range of the intracellular concentrations of 2,3-diphosphoglycerate, it remains still unclear whether 2,3-diphosphoglycerate in vivo is important as a regulator of hexokinase, because 2,3-diphosphoglycerate is partly bound to hemoglobin dependent on the oxygenation state of hemoglobin [10]. No inhibition of human erythrocyte hexokinase was reported by fructose-6-*P*, fructose 1,6-diphosphate [4,7], glycerate-3-*P*, and phospho*enol*pyruvate [7].

Recently we published the preparation of a highly purified human erythrocyte hexokinase [11], which enabled the study of the influence of various effectors on the enzyme making use of simple coupled enzyme systems.

The present paper shows the effects of various glycolytic intermediates and phosphate on highly purified hexokinase, which are markedly different from those described in literature.

Materials and Methods

Materials. Glucose 6-phosphate (disodium salt), fructose 6-phosphate (disodium salt), fructose 1,6-diphosphate (trisodium salt), dihydroxyacetone phosphate-dimethylketal (DHAP, dicyclohexylammonium salt), DL-glyceraldehyde-3-phosphate-diethylacetal (GAP, monobarium salt), 2,3-diphosphoglycerate (pentacyclohexylammonium salt), 3-phosphoglycerate, trisodium salt, grade II), 2-phosphoglycerate (trisodium salt), phospho*enol*pyruvate (tricyclohexylammonium salt) pyruvate (monosodium salt), D (—)-lactate (monolithium salt), adenosine 5′-triphosphate (ATP, disodium salt), adenosine 5′-diphosphate (ADP, disodium salt) and adenosine 5′-monophosphate (AMP, disodium salt) were obtained from Boehringer Mannheim, as were the enzymes used for the measurement of hexokinase activity. NaH_2PO_4 (P_i) and all other reagents used were of analytical grade of purity.

Enzyme purification. Human erythrocyte hexokinase was purified as

reported earlier [11]. The preparations had specific activities of 30—50 units/mg of protein. The enzyme solution contained 0.05 M Tris · HCl (pH 8.0), 0.5 M NaCl and 0.003 M mercaptoethanol.

Enzyme assays. Except for the experiments concerning the glucose-6-*P* inhibition of hexokinase, the enzyme activity was determined spectrophotometrically at 37°C in a system coupled with glucose-6-phosphate dehydrogenase.

The assay mixture (system I) contained in a final volume of 3 ml: 33 mM Tris · HCl (pH 7.7), 0.33 mM $NADP^+$, 0.15 I.U. glucose-6-phosphate dehydrogenase, 10 mM glucose (unless otherwise indicated) and ATP at the concentrations as indicated in the text. $MgCl_2$ was added in the concentrations necessary to maintain an excess of 5 mM Mg^{2+} over ATP (unless otherwise indicated). In the studies concerning the inhibition by glucose-6-*P* the initial rate of ADP formation was measured at 37°C in a coupled enzyme system with pyruvate kinase and lactate dehydrogenase. In addition to the concentrations of ATP, glucose and $MgCl_2$ (see system I) the assay mixture (system II) contained in a final volume of 3 ml: 0.33 mM Tris · HCl (pH 7.7), 3.0 mM phospho*enol*pyruvate, 1.0 mM NADH, 3 I.U. pyruvate kinase and 3 I.U. lactate dehydrogenase. The reaction was started by the addition of 0.03 unit of hexokinase.

Initial rate measurements were performed by following the reduction of $NADP^+$ (system I), respectively, the oxidation of NADH (system II) at 340 nm with a Perkin-Elmer spectrophotometer Model 124. One unit of enzyme activity is defined as the amount of enzyme which catalyzes the formation of 1 μmol of glucose 6-phosphate and 1 μmol of ADP per min.

Statistical analysis of the results. Double reciprocal plots were statistically treated as described earlier [12]. The inhibition constants derived from secondary plots of slope vs. inhibitor concentration are calculated according to the method of the least squares with weighting factors proportional to 1/variance of the slope.

Results

Inhibition by glucose-6-P and fructose-6-P

The double reciprocal plots in Fig. 1 show that human erythrocyte hexokinase is inhibited by glucose-6-*P* competitively with respect to $MgATP^{2-}$. The replot of the slopes of the Lineweaver-Burk plots (inset Fig. 1) are linear with added glucose-6-*P* up to a concentration of about 40 μM and a K_i (glucose-6-*P*) = 10.8 μM can be calculated. A non-competitive inhibition was found with respect to glucose (results not shown). Furthermore the same inhibition constant was found when the experiments were carried out at pH 7.0 instead of pH 7.7, indicating that there is probably no influence of pH.

Hexokinase from various sources including human erythrocyte hexokinase is reported [4,7] to be not inhibited by fructose-6-*P*. However, we found a competitive inhibition by fructose-6-*P* with respect to $MgATP^{2-}$ (Fig. 2). From the secondary plot of slope vs. concentration of fructose-6-*P* (inset Fig. 2) an inhibition constant of K_i (fructose-6-*P*) = 0.16 mM can be calculated. This inhibition by fructose-6-*P* cannot be caused by a contamination of glucose-6-*P* in the fructose-6-*P*, because assay mixture I is used in which possible glucose-6-

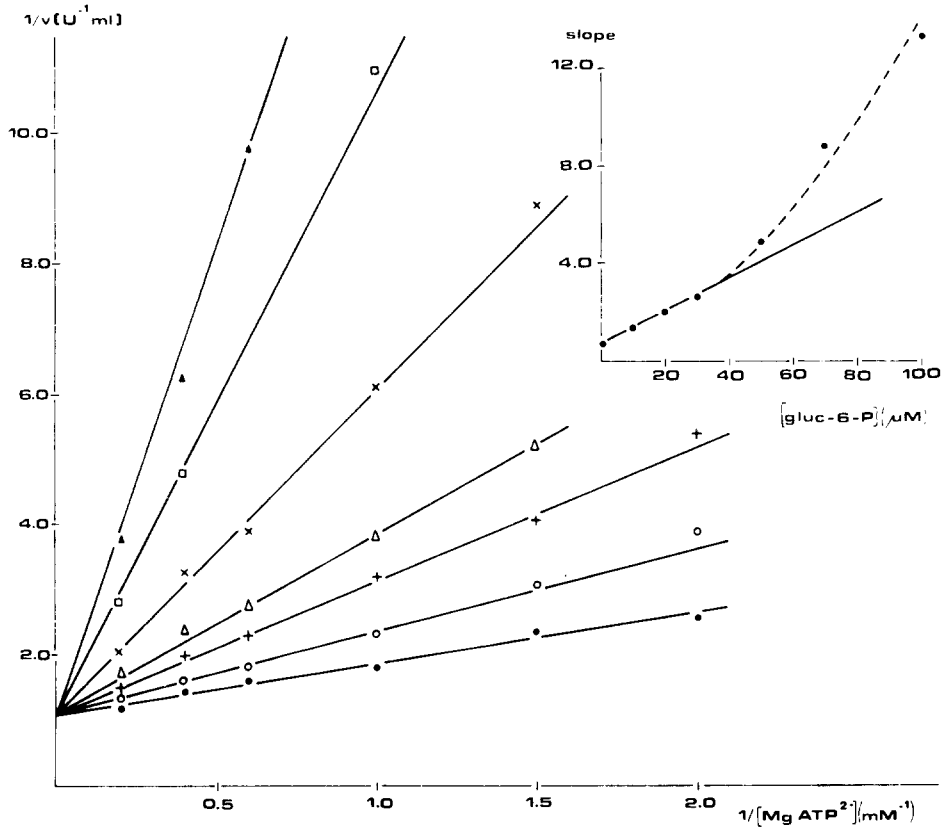

Fig. 1. Lineweaver-Burk plot of hexokinase activity vs. [$MgATP^{2-}$] at concentrations of glucose-6-*P* of 0 μM (●), 10 μM (○), 20 μM (+), 30 μM (△), 50 μM (X), 70 μM (□) and 100 μM (▲). Further conditions as described in Materials and Methods. Inset: Secondary plot of slope vs. added [glucose-6-*P*].

P contamination is converted to gluconate-6-*P* prior to initiation of the hexokinase reaction.

Influence of P_i on the inhibition by glucose-6-P and fructose-6-P

Previous reports on hexokinase from various sources [4—6,13,14] including human erythrocyte hexokinase [4—6] have demonstrated the competing effect of P_i on the inhibition by glucose-6-*P* while P_i in the absence of glucose-6-*P* had no effect either on the *V* or on the K_m of $MgATP^{2-}$.

In contrast Gerber et al. [7] found no influence of P_i at all either in the presence or in the absence of glucose-6-*P* for human erythrocyte hexokinase.

In our experiments P_i was found to overcome at least partly the inhibition by glucose-6-*P*. This influence of P_i is competitive with respect to glucose-6-*P* (Fig. 3). The same influence of P_i on the inhibition by fructose-6-*P* was found (Fig. 4).

Kosow et al. [6] interpreted the influence of P_i on the inhibition of human erythrocye hexokinase by glucose-6-*P* using modified Michaelis-Menten equations. They concluded that P_i could completely relieve the glucose-6-*P* inhibi-

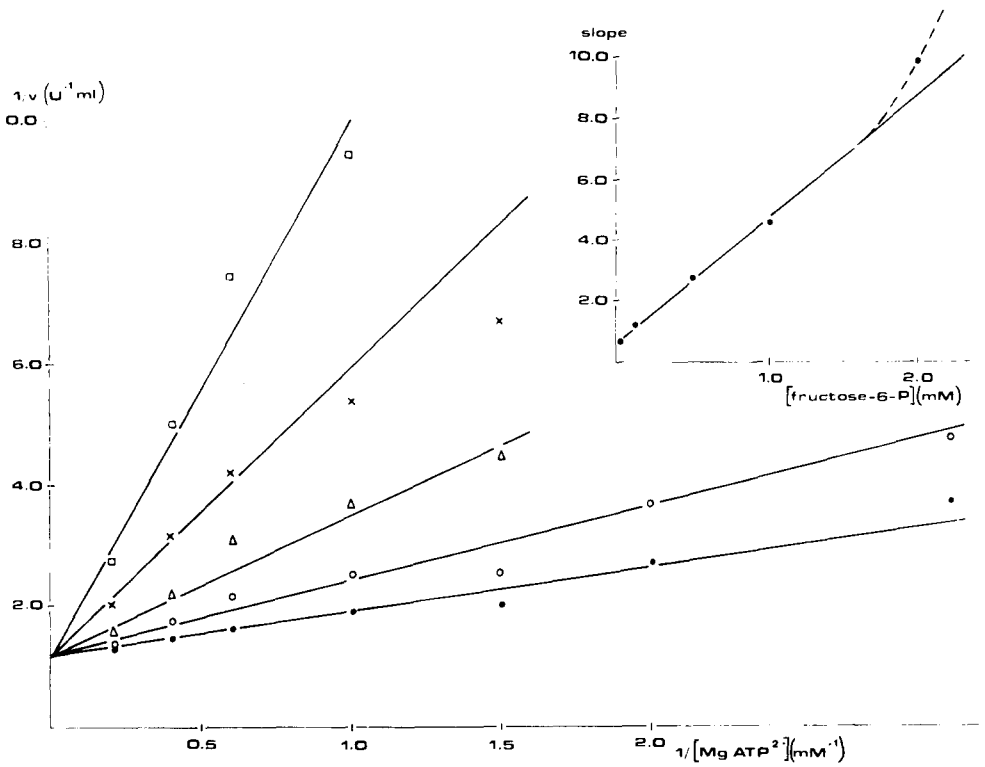

Fig. 2. Lineweaver-Burk plot of hexokinase activity vs. [$MgATP^{2-}$] at concentrations of fructose-6-*P* of 0.0 mM (•), 0.1 mM (○), 0.5 mM (△), 1.0 mM (X) and 2.0 mM (□). Further conditions as described in Materials and Methods. Inset: secondary plot of slope vs. [fructose-6-*P*].

tion and that glucose-6-*P* does not interact with red cell hexokinase to which P_i is associated. This conclusion could not be extended to hexokinase type I from Sarcoma 37 cells, since P_i could not reverse all of the inhibition observed at higher glucose-6-*P* levels. In contrast to these authors, we found in our experiments a competitive inhibition by P_i competitive with respect to $MgATP^{2-}$ in the absence of glucose-6-*P* or fructose-6-*P* with an inhibition constant of about 20 mM (results not shown).

The equations given by Kosow et al. [6] can be extended with this phosphate inhibition. The rate equation for the hexokinase reaction, assuming a rapid equilibrium random mechanism, in the presence of hexose phosphate, P_i and saturating concentrations of glucose, can then be described as:

$$V_{AIP_i} = \frac{V}{1 + \frac{K_A}{[A]}\left(1 + \frac{[I]}{K_i f(P_i)} + \frac{[P_i]}{K_{P_i}}\right)}$$

in which A stands for $MgATP^{2-}$ and I for either glucose-6-*P* or fructose-6-*P*.

The following definitions have now to be made: Rate equation in presence

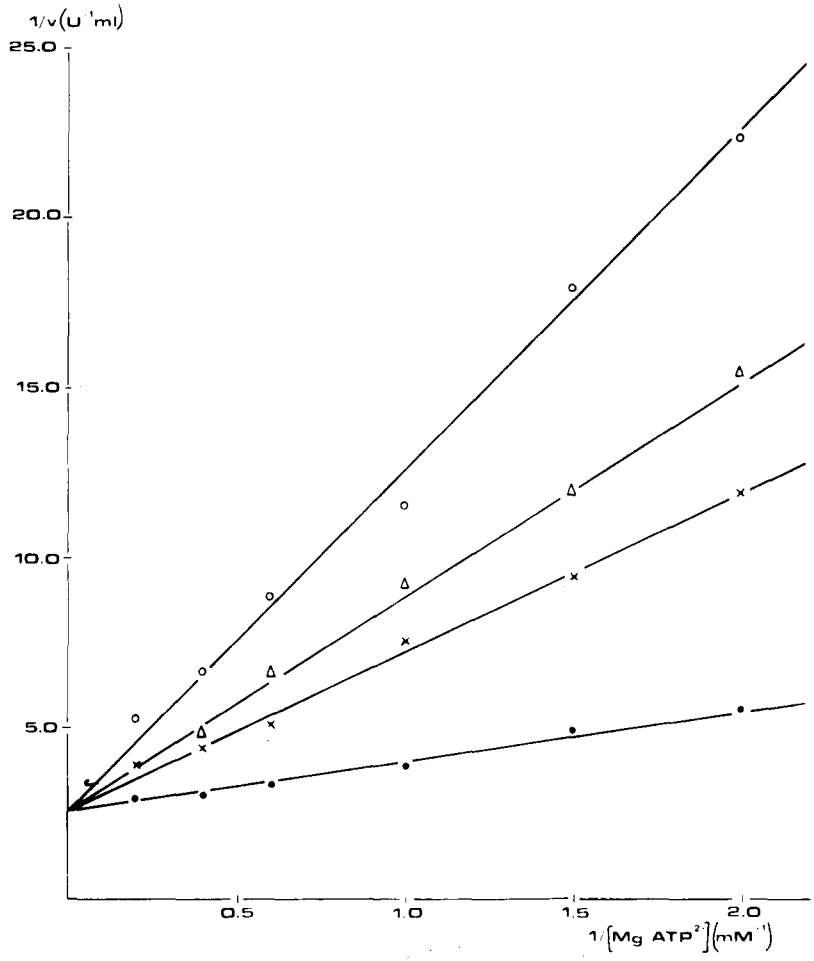

Fig. 3. Influence of P_i on the inhibition of hexokinase by glucose-6-*P*. The reciprocal of hexokinase activity is plotted vs. the reciprocal of [$MgATP^{2-}$] in the presence (○, □, X) and in the absence (●) of 50 μM glucose-6-*P*. The concentrations of added phosphate are 0.0 mM (●,○), 1.0 mM (△) and 5.0 mM (X), The concentration of glucose is kept constant at 0.16 mM. Further conditions as described in Materials and Methods.

of $MgATP^{2-}$ (A) and in absence of inhibitor and P_i is defined as:

$$V_A = \frac{V}{1 + \frac{K_A}{[A]}}$$

Rate equation in presence of $MgATP^{2-}$ (A) and hexose phosphate (I) and in absence of P_i is defined as:

$$V_{AI} = \frac{V}{1 + \frac{K_A}{[A]}\left(1 + \frac{[I]}{K_i}\right)}$$

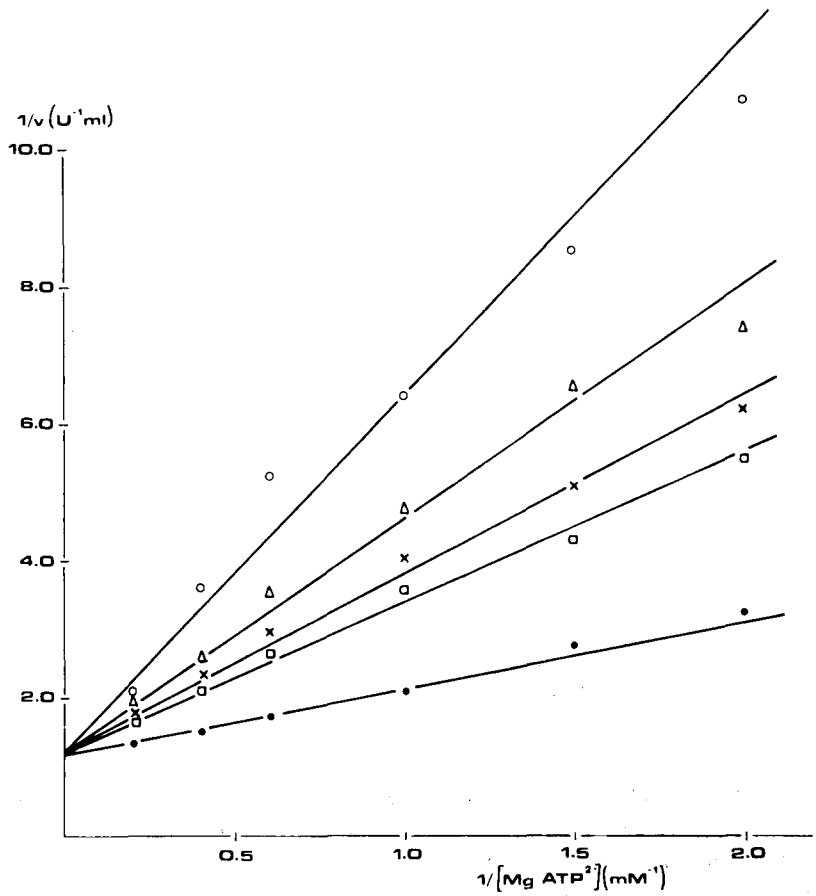

Fig. 4. Influence of P_i on the inhibition of hexokinase by fructose-6-*P*. The reciprocal of hexokinase activity is plotted vs. the reciprocal of $[MgATP^{2-}]$ in the presence (○,△,X,□) and in the absence (●) of 0.8 mM fructose-6-*P*. The concentrations of added phosphate are 0.0 mM (●,○), 0.3 mM (△), 1.0 mM (X) and 5.0 mM (□). Further conditions as described in Materials and Methods.

Rate equation in presence of $MgATP^{2-}$ (A) and P_i and in absence of inhibitor:

$$V_{AP_i} = \frac{V}{1 + \frac{K_A}{[A]}\left(1 + \frac{[P_i]}{K_{P_i}}\right)}$$

Starting from these definitions it can be derived that

$$f(P_i) = \frac{V_{AIP_i} \cdot V_{AP_i}(V_{AI} - V_A)}{V_{AI}(V_{AIP_i} \cdot V_A - V_A \cdot V_{AP_i})}$$

If P_i acts competitively with respect to glucose-6-*P*, the function $f(P_i)$ should be $1 + [P_i]/K$ and therefore be linear. However, a curved function is found both for glucose-6-*P* and fructose-6-*P* (Fig. 5), reflecting the inability of P_i to overcome completely the inhibition by these hexose phosphates. The affinity for

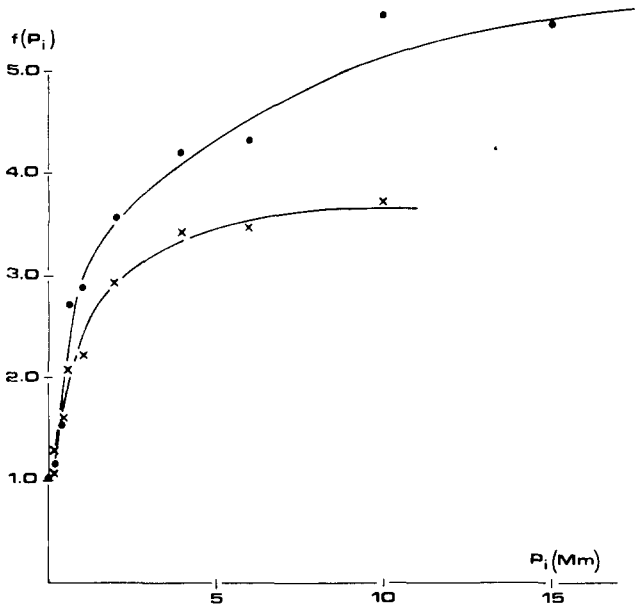

Fig. 5. Influence of P_i on the inhibition of hexokinase by 50 μM glucose-6-*P* (•) and by 0.8 mM fructose-6-*P* (×). $f(P_i)$ is determined as defined in the text. The concentration of $MgATP^{2-}$ was kept constant at 0.5 mM. Further conditions as described in Materials and Methods.

the hexose phosphate is maximally decreased by a factor of about 6 in the case of glucose-6-*P* and by a factor of about 3.5 in the case of fructose-6-*P*, when the concentration of phosphate is increased.

These findings are contrary to the reports of Kosow et al. on the P_i effect on the red cell enzyme, but might be in accordance with their results on hexokinase type I from Sarcoma 37 cells.

Inhibition by 2,3-diphosphoglycerate

In agreement with other reports on the inhibition of human erythrocyte hexokinase by 2,3-diphosphoglycerate, we found a competitive inhibition with respect to $MgATP^{2-}$ (results not shown). An inhibition constant of 4.0 mM was calculated.

We also could detect an influence of P_i on the inhibition by 2,3-diphosphoglycerate, although this influence was very small compared to the effect of P_i on the inhibition by glucose-6-*P* and fructose-6-*P*. This can be seen from Fig. 6 in which the degree of inhibition by glucose-6-*P*, fructose-6-*P* and 2,3-diphosphoglycerate is plotted vs. the added P_i. Again the inability of P_i to overcome the inhibition by glucose-6-*P*, fructose-6-*P* or 2,3-diphosphoglycerate completely, is shown.

Inhibition by other glycolytic intermediates

The results concerning a possible inhibitory action of other glycolytic intermediates are given in Table I. Furthermore the results of the inhibition by intermediates already mentioned and of some intermediates of the hexose

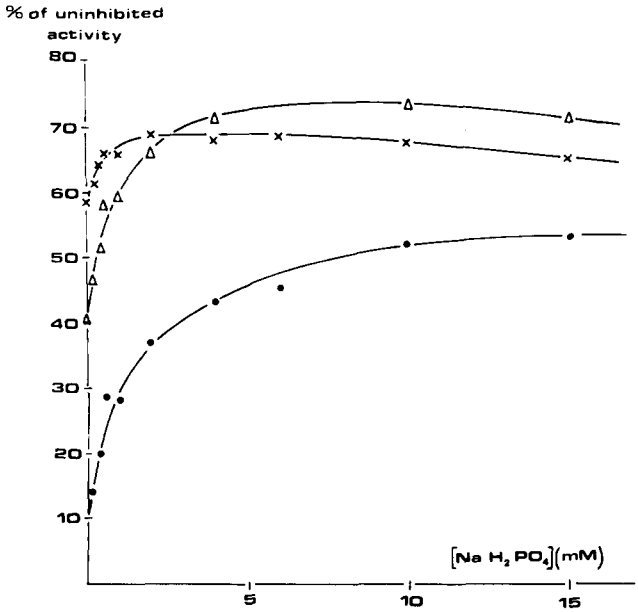

Fig. 6. Influence of P_i on the inhibition of hexokinase by 50 μM Glu-6-*P* (•), 0.8 mM Fru-6-*P* (△) and 10 mM 2,3-diphosphoglycerate (X). The percentage of inhibition is plotted vs. the concentration of added phosphate. The concentration of $MgATP^{2-}$ in these experiments was kept constant at 0.5 mM. The excess of Mg^{2+} over the sum of ATP and 2,3-diphosphoglycerate was kept at 5.0 mM. Further conditions as described in Materials and Methods.

TABLE I

INHIBITION CONSTANTS AND INTRACELLULAR CONCENTRATIONS * OF GLYCOLYTIC INTERMEDIATES AND SOME OTHER COMPOUNDS

	Inhibition constant K_i ± deviation (s) ** (μM)	Intracellular concentration * (mean ±S.D.) (μM)
Glucose 6-phosphate	10.8 ± 0.6	39.7 ± 10.7
Fructose 6-phosphate	160 ± 15	13.0 ± 2.9
Fructose 1,6-diphosphate	4 300 ± 800	2.7 ± 0.9
Dihydroxyacetone phosphate	no inhibition	13.4 ± 4.0
Glyceraldehydephosphate	no inhibition	not detectable
1,3-Diphosphoglycerate	not measured	not detectable
2,3-Diphosphoglycerate	4 000 ± 200	3700 ± 100
3-Phosphoglycerate	3 800 ± 300	64.1 ± 7.3
2-Phosphoglycerate	12 500 ± 2800	10.4 ± 3.6
Phospho*enol*pyruvate	>10 000	17.4 ± 3.1
Pyruvate	no inhibition	53.3 ± 21.5
Lactate	no inhibition	932 ± 211
MgADP	1 000 ± 200	190 ± 30
MgAMP	700 ± 200	18 ± 3
P_i	20 400 ± 2100	1000
GSH	no inhibition	2000 ± 300
GSSG	no inhibition	3.7 ± 1.4
Gluconate-6-phosphate	no inhibition	

* These values are derived from Beutler [15] assuming a normal intracellular concentration of 300 g hemoglobin/l.

** The standard deviation of the inhibition constants are calculated as described in Materials and Methods.

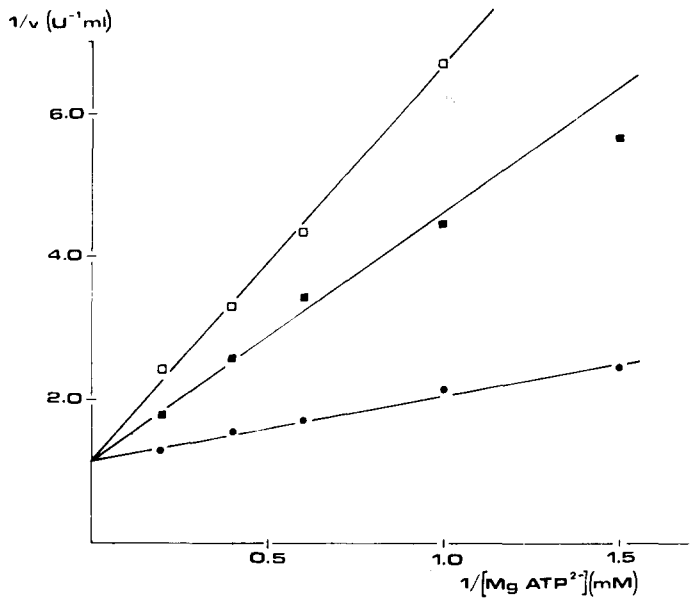

Fig. 7. Lineweaver-Burk plot of hexokinase activity vs. [$MgATP^{2-}$]. Additions: none (●), 5 mM MgADP (□) and 5 mM MgAMP (■). An excess of 5.0 mM Mg^{2+} was maintained over the sum of ATP and ADP or AMP. Further conditions as described in Materials and Methods.

monophosphate shunt are summarized. The inhibition constants are calculated from Lineweaver-Burk plots of enzyme activity vs. $MgATP^{2-}$ in the presence and in the absence of 5 mM of inhibitor. These plots are statistically treated as described in Materials and Methods. Besides the intermediates already mentioned Fru-1,6-P_2, 3-phosphoglycerate, 2-phosphoglycerate, MgADP and MgAMP appeared to be competitive inhibitors with respect to $MgATP^{2-}$ (see Table I). Of these only MgADP and MgAMP might have physiological importance, because their K_i is not too high compared to the intracellular concentrations [15]. The inhibition by MgADP and MgAMP was strictly competitive with respect to $MgATP^{2-}$ (Fig. 7), although other authors [14] report mixed inhibition patterns for hexokinase obtained from other mammalian sources.

The inhibition by Fru-1,6-P_2 and 2-phosphoglycerate might have been caused by contamination of respectively, Fru-6-P and 2,3-diphosphoglycerate. However, since there seemed to be no physiological importance, this possibility was not further investigated.

1,3-Diphosphoglycerate was not assayed because this compound is not commercially available. However, because its concentration in the red cell is very low [1], 1,3-diphosphoglycerate is not likely to be directly important for the regulation of hexokinase.

Discussion

From binding studies on hexokinase type I from bovine brain Ellison et al. [13] suggested a model which implies that there is an equilibrium between a free and a phosphate-associated enzyme. The kinetic parameters of the two

forms are similar except in their ability to bind glucose-6-*P*. It was suggested that the dissociation constant for glucose-6-*P* is relatively very high for hexokinase to which P_i is bound. This model was in agreement with the results of the kinetic studies by Kosow et al. [6] on hexokinase type I from human erythrocytes and with a model proposed by Wilson [16] for rat brain hexokinase. However, our results concerning the influence of P_i on the inhibition by glucose-6-*P* as well by fructose-6-*P* do not agree with this model, because P_i was shown to be not completely competitive with respect to glucose-6-*P*. So if a phosphate-associated enzyme exists, glucose-6-*P* is indeed capable to interact with this form of the enzyme. Therefore our observations can only be made consistent with the model proposed by Ellison et al. [13] by assuming that the phosphate-associated form of the enzyme has a reduced but still substantial affinity for glucose-6-*P* or fructose-6-*P*. This assumption is favoured by the observations that $f(P_i)$ (Fig. 5) reaches a maximum at higher concentrations of P_i. The observation that P_i at low concentrations has only little influence on the enzyme in the absence of glucose-6-*P*, while it strongly effects enzyme activity in the presence of the inhibitor, indicates that at least two distinct sites must exist, one catalytic site for the binding of $MgATP^{2-}$, one effector site for the binding of P_i. If our assumption is true that a phosphate-associated enzyme with a reduced affinity for glucose-6-*P* exists, it seems reasonable that glucose-6-*P* binds at a site which overlaps both the catalytic site and the P_i site. The inhibitory action of higher concentrations of phosphate in the absence of glucose-6-*P* can be explained by a low affinity binding of P_i at the catalytic site. An alternative explanation of our results is the presence of two different enzymes, one with a high affinity for glucose-6-*P* and fructose-6-*P* being sensitive to regulation by P_i, one with a lower affinity for hexose phosphate being insensitive to P_i; the K_m ($MgATP^{2-}$) being the same for both enzymes. The former may operate according to the model proposed by Ellison et al. [13]. In favour of this explanation are the secondary plots of slope vs. concentration of inhibitor in Figs. 1 and 2, which are deviating from linearity in the higher concentration range of inhibitor. This can be expected when two enzymes with different affinity for the inhibitor are present.

As in the red cell hexokinase operates in conditions of saturating glucose concentrations, while the affinity constant for the $MgATP^{2-}$ complex (K_m = 0.57—1.0 mM, ref. 11) lay well in the range of the intracellular concentration, it is obvious that all factors which can effect the concentration of $MgATP^{2-}$ or act competitively with respect to $MgATP^{2-}$ are potentially regulators of the enzyme.

Comparing the inhibition constant for glucose-6-*P* with the intracellular concentration (Table I) one can see that hexokinase in vivo operates markedly inhibited and is therefore strongly regulated by the glucose-6-*P* level. At phosphate exerts its most pronounced influence on this inhibition at concentrations that are physiologically important, there is a regulator role of phosphate too.

It remains still unclear whether the inhibition by 2,3-diphosphoglycerate in vivo is important for the hexokinase activity. Although the inhibition constant (K_i = 4.0 mM) is well in the range of the physiological concentration, it is still unknown which part of it is bound to hemoglobin and thus not available to affect enzyme activity. In a study on the influence of 2,3-diphosphoglycerate

on red cell glycolysis Duhm [9] reported that only a part of the inhibition (about 50%) observed in 'high-diphosphoglycerate' cells (five times normal amount) must be attributed to an inhibition by 2,3-diphosphoglycerate of glycolytic enzymes. This inhibition can be attributed for the greater part to the enzyme hexokinase [8,9].

Investigation of Table I shows that there may also be some physiological importance in the inhibition by MgADP and to a much lesser degree for MgAMP, suggesting a role of the adenylate energy charge in the regulation of hexokinase. The inhibition observed by fructose-6-*P*, fructose 1,6-diphosphate, glycerate-3-*P*, glycerate-2-*P* and phospho*enol*pyruvate seems to have no physiological importance at all.

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References

1 Minakami, S. and Yoshikawa, H. (1966) J. Biochem. 59, 139—144
2 Rapoport, T., Heinrich, R., Jacobasch, G. and Rapoport, S. (1974) Eur. J. Biochem. 42, 107—120
3 Rose, I.A. and O'Connell, E. (1964) J. Biol. Chem. 239, 12—17
4 de Verdier, C.H. and Garby, L. (1965) Scand. J. Haematol. 2, 305—317
5 Rose, I.A., Warms, J.V.B. and O'Connell, E.L. (1964) Biochem. Biophys. Res. Commun. 15, 33—37
6 Kosow, D.P., Oski, F.A., Warms, J.V.B. and Rose, I.A. (1973) Arch. Biochem. Biophys. 157, 114—124
7 Gerber, G., Preissler, H., Heinrich, R. and Rapoport, S.M. (1974) Eur. J. Biochem. 45, 39—52
8 Ponce, J., Roth, S. and Harkness, D.R. (1971) Biochim. Biophys. Acta 250, 63—74
9 Duhm, J. (1975) Biochim. Biophys. Acta 385, 68—80
10 Gerber, G., Berger, H., Jänig, G.R. and Rapoport, S.M. (1973) Eur. J. Biochem. 38, 563—571
11 Rijksen, G. and Staal, G.E.J. (1976) Biochim. Biophys. Acta 445, 330—341
12 Rijksen, G. and Staal, G.E.J. (1976) Biochim. Biophys. Acta 452, 382—391
13 Ellison, W.R., Lueck, J.D. and Fromm, H.J. (1975) J. Biol. Chem. 250, 1864—1871
14 Purich, D.L. and Fromm, H.J. (1971) J. Biol. Chem. 246, 3456—3463
15 Beutler, E. (1975) in Red cell metabolism: a manual of biochemical methods, 2nd edn., pp. 106—133, Grune and Stratton, New York
16 Wilson, J.E. (1973) Arch. Biochem. Biophys. 159, 543—549