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ACTIVATION AND INHIBITION OF THE PHOSPHOGLYCERATE KINASE REACTION BY ATP^{4-}

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Summary

A kinetic study for understanding how the phosphoglycerate kinase (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) reaction discriminates between $MgATP^{2-}$ and ATP^{4-} is presented. The results show that in contrast to $MgATP^{2-}$, ATP^{4-} is competitive with 3-phospho-D-glycerate. ATP^{4-} binds to the free enzyme as an inhibitor. When binding to the enzyme-$MgATP^{2-}$-(3-phospho-D-glycerate) complex, ATP^{4-} acts as an activator.

Phosphoglycerate kinase (EC 2.7.2.3) catalyses the reversible transfer of a phosphoryl group from $MgATP^{2-}$ to 3-*P*-glycerate. The substrates are capable of binding to the enzyme independently, and a rapid equilibrium random mechanism fits the available data [1—5]. At elevated concentrations either substrate causes activation probably by binding to a second site in the protein molecule [1]. The ability of the enzyme molecule to bind two molecules of either substrate has been shown by equilibrium studies [6,7]. $MgATP^{2-}$ is the true substrate in the catalytic reaction [8]. After it became evident [2] that $MgATP^{2-}$ and ADP^{3-} preferentially bind to different sites, the obvious question was: Does the metal ion steer the nucleotide to a specific site in the enzyme?

Phosphoglycerate kinase was prepared from baker's yeast by a method described earlier [9] and the main electrophoretic component 2 was used. The reagents for determining the activity were from Sigma Chemical Co.

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Only analytical grade reagents were used and all solutions were made with glass-distilled water. Contaminating metal ions were removed as described earlier [8].

The activity of phosphoglycerate kinase was measured spectrophotometrically [10] and expressed as the initial velocity, $v = (dA_{366}/dt)_{t=0}$ in min^{-1} (cf. ref. 8). The assay mixture contained 50 mM Tris·HCl buffer (pH 7.80 at 25°C) and 0.5 mM NADH in addition to the reagents mentioned in the text. About 0.2 U of phosphoglycerate kinase and 60 U of glyceraldehydephosphate dehydrogenase (EC 1.2.1.12) were used per ml of the assay mixture.

For estimation of the concentrations of ATP^{4-}, $MgATP^{2-}$ and Mg^{2+}, the following dissociation constants were used: $MgATP^{2-}$, 0.08 mM (cf. ref. 2) and 3-*P*-glycerate-Mg^{2+}, 10 mM [11].

Effects of ATP^{4-} on the phosphoglycerate kinase activity is illustrated in Fig. 1. ATP^{4-} up to about 1 mM appears as an activator. At higher concentrations inhibition becomes more and more predominant. A considerable change in ionic strength occurs with the increase in ATP^{4-} concentration. As seen in Fig. 1 its possible effect on the activity appears negligible.

Figs. 2 and 3 show that ATP^{4-} is a competitive inhibitor of both the substrates. The secondary plots derived from these figures have a parabolic form (inset plots), indicating that there are at least two inhibitor molecules binding simultaneously to the enzyme. Assuming that the ATP^{4-} molecules are bound in sequence to two different sites in the free enzyme, the rate of the inhibited reaction can be described as:

$$\frac{1}{v} = \frac{1}{V} \left\{ 1 + \frac{K_A}{[A]} + \frac{K_B}{[B]} + \left(1 + \frac{[I]}{K_i} + \frac{[I]^2}{K_i K_i'}\right) \frac{K_A K_B}{[A][B]} \right\} \qquad (1)$$

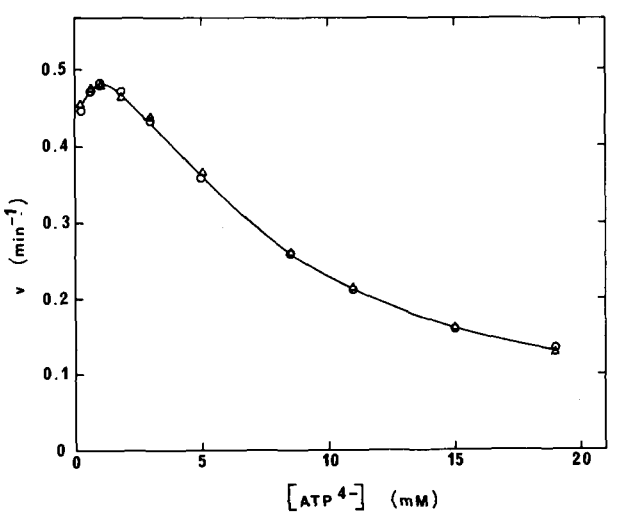

Fig. 1. Overall effects of ATP^{4-} on the phosphoglycerate kinase activity. The experiment was performed at a constant KCl concentration of 0.25 M (△). A successive increase in ionic strength (0.19 M as a maximum increase) occurred with increasing ATP^{4-} concentrations. The KCl concentration was varied (○) to counteract the corresponding ionic strength effect. The 3-*P*-glycerate concentration was 2 mM and the $MgATP^{2-}$ concentration 1 mM.

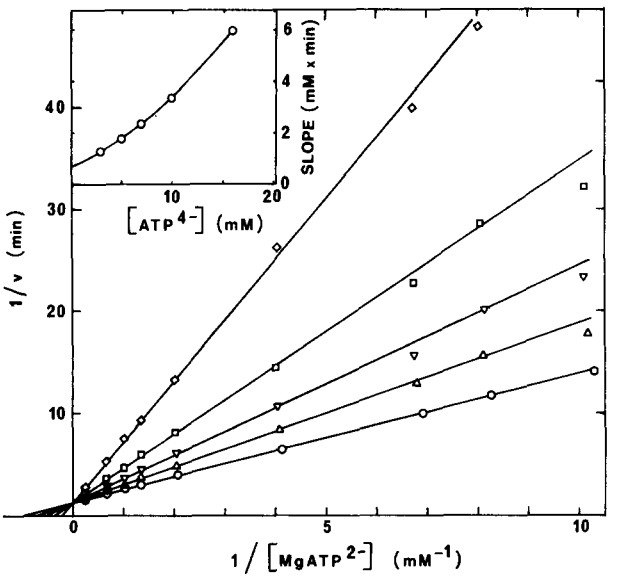

Fig. 2. Effects of ATP^{4-} on the phosphoglycerate kinase activity at varying $MgATP^{2-}$ concentrations. The ATP^{4-} concentrations were ○, 3 mM; △, 5 mM; ▽, 7 mM; □, 10 mM; ◇, 16 mM. The 3-*P*-glycerate concentration was 2 mM. The inset figure shows the slope of the Lineweaver-Burk plots as a function of the ATP^{4-} concentration. The points represent the slopes experimentally obtained and the solid line the equation of the slope estimated from Eqn. 1, with the inhibitor constants determined (cf. the text).

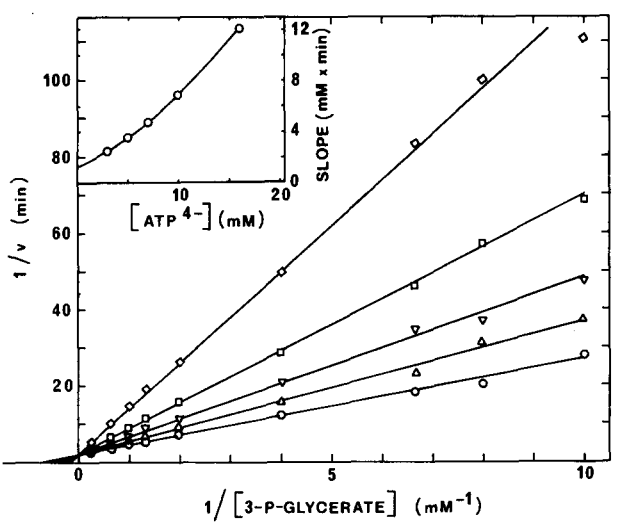

Fig. 3. Effects of ATP^{4-} on the activity of phosphoglycerate kinase at varying concentrations of free 3-*P*-glycerate. The ATP^{4-} concentrations and symbols are as in Fig. 2. The $MgATP^{2-}$ concentration was 1 mM. The inset figure shows the slope of the Lineweaver-Burk plots as a function of ATP^{4-}. The points represent the slopes experimentally obtained and the solid line the equation of the slope estimated from Eqn. 1, with the inhibitor constants determined (cf. the text).

A symbolizes $MgATP^{2-}$ and B 3-*P*-glycerate. K_A and K_B are the corresponding Michaelis constants, 0.5 mM and 0.62 mM, respectively [1]. ATP^{4-} is represented by I and its inhibitor constants by K_i and K_i'. The slope of both curves in the insets of Figs. 2 and 3 was plotted as a function of the ATP^{4-} concentration. The two straight lines obtained were then used to calculate the inhibitor constants. K_i and K_i' equal to 0.92 mM and 16 mM, respectively, fit the data of Fig. 2. The corresponding values from the results in Fig. 3 are 1.1 mM and 17 mM. In the calculations no consideration has been taken to the activation observed at low ATP^{4-} concentrations.

Multiple binding sites for ATP^{4-} in the enzyme are required to explain the phenomena of activation and inhibition presented in Fig. 1 as well as the parabolic secondary plots shown in Figs. 2 and 3. Either substrate is able to nullify the inhibition. Thus ATP^{4-} competes with the substrates for the free form of the enzyme. $LnATP^{-}$ has earlier been shown to behave very much as ATP^{4-}, when related to the $MgATP^{2-}$ kinetics [7].

It now appears possible to discriminate between different alternatives [6] of binding two ATP^{4-} molecules to the enzyme. X-ray crystallographic results [12] show that it is improbable that the binding sites are equivalent. The absence of a non-competitive contribution in the inhibition patterns would rule out the possibility that the binding sites are independent. It seems very likely that binding in sequence is the correct alternative. These conclusions agree with the earlier suggestions on 3-*P*-glycerate binding to the enzyme [6].

The inhibitor constants estimated from data presented in Figs. 2 and 3 were compared with the dissociation constants obtained from equilibrium studies [6], assuming ATP^{4-} binding in a compulsory order. K_i agrees nicely with the corresponding dissociation constant. K_i' appears to be twice as large, a phenomenon to be explained by the activation that has not been considered in Eqn. 1.

Our results suggest that ATP^{4-} binding as an activator occurs only when the catalytic site is occupied by $MgATP^{2-}$ and/or 3-*P*-glycerate. ATP^{4-} binding as an inhibitor occurs only to the free form of the enzyme. Neither $MgATP^{2-}$ nor 3-*P*-glycerate was present in the system used in the studies on binding of ATP^{4-} by equilibrium dialysis [6].

Simultaneous binding of ATP^{4-} and 3-*P*-glycerate to the active centre appears to be impossible, supporting the earlier suggestion that $MgATP^{2-}$ is the true form of the nucleotide substrate [8]. Thus, complex formation with the divalent metal ion prior to binding to the active centre is necessary in case ATP is needed to form a catalytically functional enzyme-substrate complex.

Some interaction appears to occur between the catalytic centre and an additional nucleotide binding site, offering possibilities of regulating the catalytic reaction. Work in progress will further discuss that phenomenon.

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References

1 Larsson-Raźnikiewicz, M. (1967) Biochim. Biophys. Acta 132, 33—40
2 Larsson-Raźnikiewicz, M. and Arvidsson, L. (1971) Eur. J. Biochem. 22, 506—512
3 Roustan, C., Brevet, A., Pradel, L.-A. and van Thoai, N. (1973) Eur. J. Biochem. 37, 248—255
4 Janson, C.A. and Cleland, W.W. (1974) J. Biol. Chem. 249, 2567—2571
5 Larsson-Raźnikiewicz, M. and Schierbeck, B. (1974) Biochem. Biophys. Res. Commun. 57, 627—634
6 Larsson-Raźnikiewicz, M. (1973) Arch. Biochem. Biophys. 158, 754—762
7 Tanswell, P., Westhead, E.W. and Williams, R.J.P. (1974) FEBS Lett. 48, 60—63
8 Larsson-Raźnikiewicz, M. (1964) Biochim. Biophys. Acta 85, 60—68
9 Arvidsson, L., Schierbeck, B. and Larsson-Raźnikiewicz, M. (1976) Acta Chem. Scand. B30, 228—234
10 Bücher, Th. (1947) Biochim. Biophys. Acta 1, 292—314
11 Larsson-Raźnikiewicz, M. (1972) Eur. J. Biochem. 30, 579—583
12 Bryant, T.N., Watson, H.C. and Wendell, P.L. (1974) Nature 247, 14—17