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ACTIVATION AND INHIBITION OF PHOSPHOGLYCERATE KINASE BY SULPHATE ION

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Summary

A kinetic study of the effects of SO_4^{2-} on the activity of phosphoglycerate kinase (ATP : 3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) is presented. SO_4^{2-} behaves both as an activator and inhibitor of the reaction. Activation does not appear to affect binding of one or the other of the two substrates to the catalytic centre. As an inhibitor SO_4^{2-} competes with both the substrates. Thus, each substrate can constrict SO_4^{2-} from the inhibitor binding site, probably the catalytic centre. Under these conditions activation becomes more and more evident. There appear to exist at least two SO_4^{2-} binding sites, one which earlier has been defined as an anion binding site, and a second, being the catalytic centre. The former seems to have a higher affinity for SO_4^{2-} than the latter.

Introduction

Anions behave both as activators and inhibitors of the phosphoglycerate kinase (ATP : 3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) reaction [1,2]. The substrates also behave as activators [3]. Anion binding to the enzyme has been presented by Wrobel and Stinson [4] and Scopes [5]. There appears to be at least one specific anion binding site. Multiple sites binding the substrates are also evident [5—7]. An anion binding site outside the catalytic centre has been proposed as responsible for the activation [8,9]. A different opinion has recently been put forward by Scopes, who suggested that the anion binding site for activation and inhibition is the same, located in the active centre [5]. A clarifying kinetic analysis appeared necessary. The kinetic patterns may be very complex. As the kinetics were shown to be of rapid equilib-

rium random type with the two substrates $MgATP^{2-}$ and 3-phosphoglycerate independently binding to the enzyme, explicit kinetic patterns can be expected using selected conditions.

Materials and Methods

Phosphoglycerate kinase was prepared from baker's yeast and the main electrophoretic component B [10] was used. Glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.2) from rabbit muscle was purchased from Boehringer Mannheim GmbH. Before use the crystalline enzyme was centrifuged (27 000 × g, 20 min) and dissolved in 50 mM Tris-HCl (pH 7.8) with or without 100 mM NaCl, depending on the experimental conditions. The barium salt of 3-phosphoglycerate, disodium salts of equine muscle ATP and yeast NADH were all purchased from Sigma Chemical Co. 3-phosphoglycerate was liberated from its barium salt with Na_2SO_4 and neutralized by NaOH. The concentration was determined as described earlier [11]. A dissociation constant of 80 μM was used in the calculation of the $MgATP^{2-}$ concentrations (cf. Ref. 12). Only analytical grade reagents were used and all solutions were made with double distilled water. Dithizone was used to remove contaminating metal ions.

The activity of phosphoglycerate kinase was determined by the conventional spectrophotometric method of Bücher [13] under the conditions described earlier [12]. The initial velocity was expressed as $v = (dA_{366}/dt)_{t=0}$ (in min^{-1}). About 0.2 μg phosphoglycerate kinase was used per ml of the substrate. The experiments were performed in 50 mM Tris-HCl (pH 7.8, 25°C) with or without 100 mM NaCl, the optimal salt concentration [1].

Results and Discussion

Overall activation or inhibition by SO_4^{2-} of the phosphoglycerate kinase reaction was studied in the absence or presence of 100 mM NaCl (optimal concentration [1]) and two different concentrations of Mg^{2+}, 2 and 10 mM. The results presented in Fig. 1 show that the lower Mg^{2+} concentration is preferable in the experiments of activation and inhibition. Although the optimal rate appears independent of the NaCl concentration activation is more pronounced in the absence, and inhibition in the presence of NaCl. The experiments presented in Fig. 2 show that activation by SO_4^{2-} affects neither the K_m value for $MgATP^{2-}$ nor 3-phosphoglycerate. For a two substrate reaction following a rapid equilibrium random mechanism these results predict that the sulphate binding site is outside the catalytic centre. The kinetic patterns for the SO_4^{2-} inhibition in Fig. 3 show that the SO_4^{2-} is a strictly competitive inhibitor of both the substrates. Although this type of inhibition does not prove that the inhibitor binds to the catalytic centre this possibility is quite clear. It also appears reasonable that SO_4^{2-} as an activator and an inhibitor binds to different sites.

As was pointed out by Scopes [2] the anion effects are related to the concentrations of the substrate. Therefore it appeared reasonable to study how optimal SO_4^{2-} concentrations affect the substrate kinetics. The results are presented in Fig. 4. It is evident that the anion behaves as an inhibitor at $MgATP^{2-}$ and 3-phosphoglycerate concentrations below 0.5—1 mM, but as an activator at

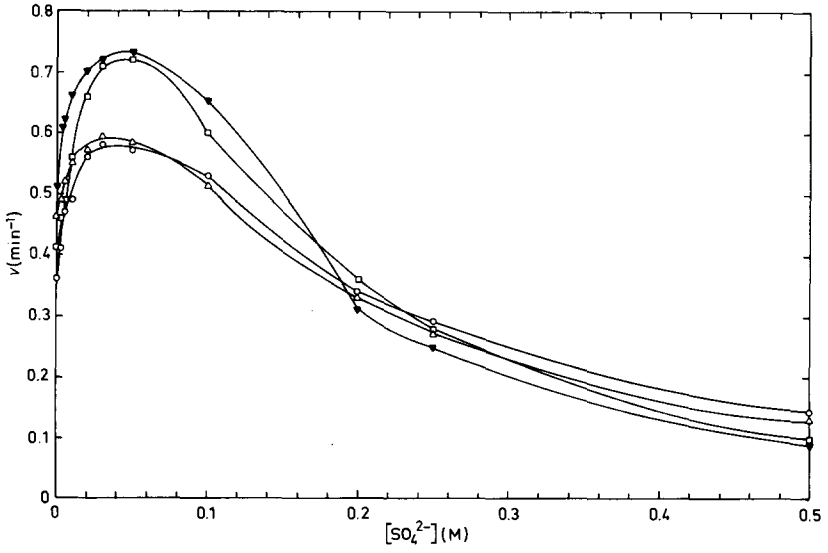

Fig. 1. The overall effects of SO_4^{2-} on the phosphoglycerate kinase activity. The experiment was performed at NaCl concentrations of 0 or 0.1 M. The total Mg^{2+} was 2 or 10 mM. △———△, 10 mM Mg^{2+} and 0.1 M NaCl; ○———○, 10 mM Mg^{2+} no NaCl; ▲———▲, 2 mM Mg^{2+} and 0.1 M NaCl; □———□, 2 mM Mg^{2+} no NaCl. The assay mixture contained 2 mM ATP/2 mM 3-phosphoglycerate and 0.5 mM NADH.

higher substrate concentrations. These results fit very nicely with those presented in Figs. 1 and 2. SO_4^{2-} under the conditions of Fig. 4 appears to bind to two different sites. At substrate concentrations in the range around the K_m values SO_4^{2-} inhibits. With increasing concentration of the respective substrate the active centre becomes saturated and the inhibitor is constricted from the

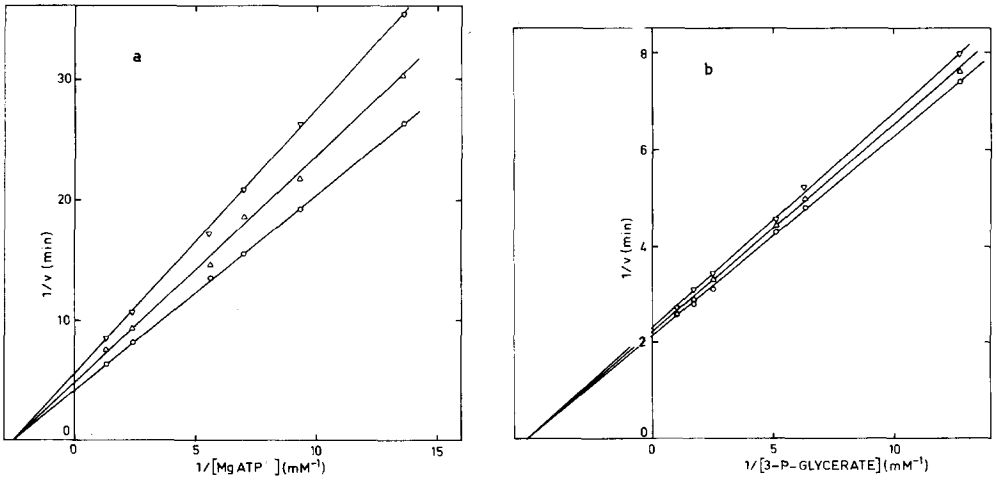

Fig. 2. The activation by SO_4^{2-} of the kinetics of (a) $MgATP^{2-}$ with ▽———▽, 0 mM; △———△, 1 mM; ○———○, 2 mM SO_4^{2-}. The 3-phosphoglycerate concentration was 2 mM, and NADH 0.5 mM. (b) 3-phosphoglycerate with ▽———▽, 0 mM; △———△, 1 mM; ○———○, 2 mM SO_4^{2-}. The total Mg^{2+} and ATP was 2 mM, NADH was 0.5 mM.

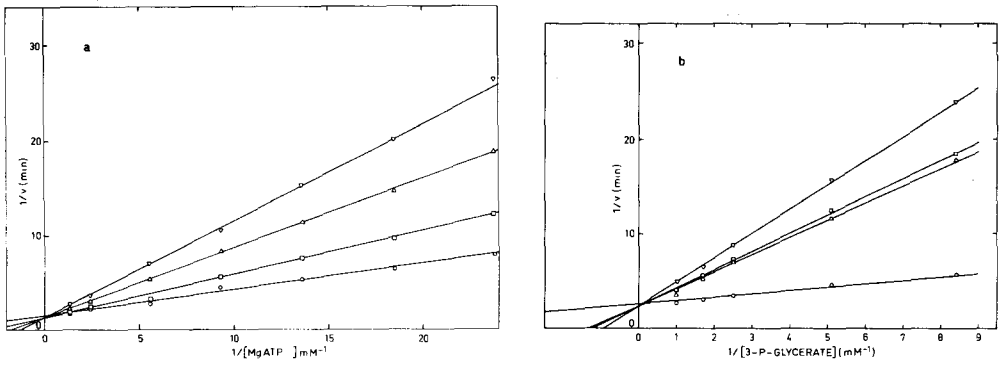

Fig. 3. The inhibition by SO_4^{2-} of the kinetics of (a) $MgATP^{2-}$ with ○———○, 0 mM; □———□, 40 mM; △———△, 75 mM; ▽———▽, 100 mM SO_4^{2-}. (b) 3-phosphoglycerate with ○———○, 0 mM; △———△, 75 mM; □———□, 80 mM; ▽———▽, 100 mM SO_4^{2-}. The total Mg^{2+} and ATP was 2 mM, NADH was 0.5 mM.

corresponding site (cf. Fig. 3). SO_4^{2-} still appears bound to the activator site. Multiple binding sites exist also for the substrates [5—7]; a second centre appears to have a regulatory function [9]. At high concentrations $MgATP^{2-}$ seems to compete with the SO_4^{2-} bound to the activator site. These results agree with those of Wrobel and Stinson [4] providing evidence for a site outside the catalytic centre binding substrates and other anions. Two SO_4^{2-} binding sites have also been suggested from NMR studies [14]. Phosphoglycerate kinase is a monomer having a bilobed structure [15,16]. Recent results, suggesting a hinge bending region [16,17] between the lobes, further support the proposal [9,4]

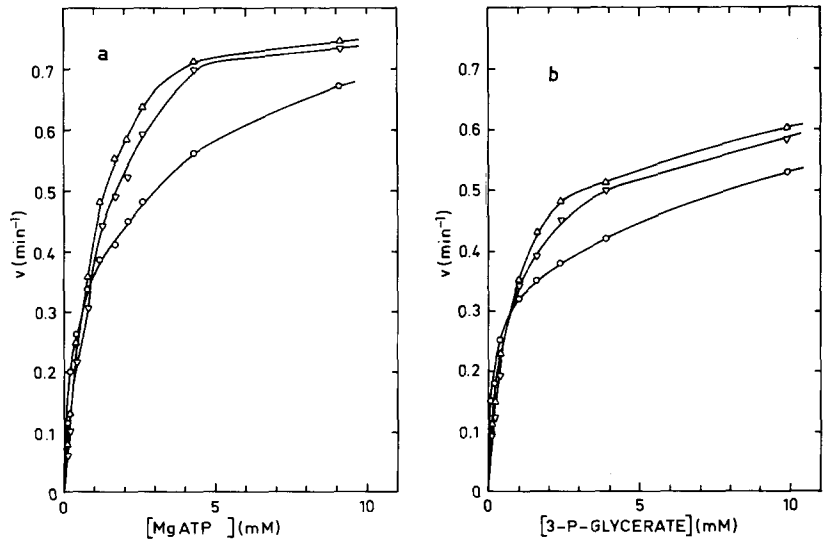

Fig. 4. The effects of SO_4^{2-} on the initial velocity of phosphoglycerate kinase at variable substrate concentrations. (a) $MgATP^{2-}$ with ○———○, 0 mM; △———△, 25 mM; ▽———▽, 40 mM SO_4^{2-}. 3-phosphoglycerate was 2 mM and NADH 0.5 mM. (b) 3-phosphoglycerate with ○———○, 0 mM; △———△, 25 mM; ▽———▽, 40 mM SO_4^{2-}. The total Mg^{2+} and ATP was 2 mM, NADH was 0.5 mM and NaCl was 0.1 M.

that the activator site might well be in a lobe different from the active centre. Earlier observations [1] (cf. also Ref. 18) indicated that anion activation is accompanied by a conformation change of the enzyme.

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