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A STUDY OF PHOSPHOGLYCERATE KINASE IN HUMAN ERYTHROCYTES

II. KINETIC PROPERTIES

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Summary

Kinetic studies on phosphoglycerate kinase (EC 2.7.2.3) were performed in the forward reaction leading from 1,3-diphosphoglycerate to 3-phosphoglycerate. Substrate activation was observed at fixed levels of ADP or Mg^{2+} and varying concentrations of 1,3-diphosphoglycerate. A biphasic curve was obtained in both linear and double reciprocal plots demonstrating two K_m values (K_{m_1} $1.9 \cdot 10^{-6}$ and K_{m_2} $9.8 \cdot 10^{-6}$ M). Michaelis—Menten-type kinetics were observed in both the linear and double reciprocal plots at fixed levels of 1,3-diphosphoglycerate or ADP and varying concentrations of Mg^{2+}. Apparent Michaelis-Menten kinetics were observed in linear plots when conditions of fixed concentrations of 1,3-diphosphoglycerate or Mg^{2+} were maintained with varying concentrations of ADP. However, the double-reciprocal plots demonstrated biphasic curves with two K_m values (K_{m_1} $1.7 \cdot 10^{-5}$ and K_{m_2} $1.0 \cdot 10^{-4}$ M). Apparent negative cooperativity was observed with respect to 1,3-diphosphoglycerate and ADP.

Phosphoglycerate kinase activity was found to be inhibited by AMP and 2,3-diphosphoglycerate. Substrate activation by 1,3-diphosphoglycerate was maintained in the presence of AMP or 2,3-diphosphoglycerate but at a reduced level of enzyme activity. AMP was found to inhibit enzyme activity non-competitively with respect to 1,3-diphosphoglycerate, ADP and Mg^{2+}. 2,3-Diphosphoglycerate inhibits phosphoglycerate kinase activity with respect to 1,3-diphosphoglycerate, ADP and Mg^{2+}. 2,3-Diphosphoglycerate inhibits phosphoglycerate kinase activity non-competitively with respect of 1,3-diphosphoglycerate.

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Abbreviation: PGM, phosphoglyceromutase (EC 2.7.5.3).

Introduction

Phosphoglycerate kinase (EC 2.7.2.3) has been isolated in crystalline form from human red cells by several workers [1—3]. Kinetic studies on yeast and muscle phosphoglycerate kinase have been published in detail by previous workers [4,5]. The enzyme assay in their investigations were done in the backward and indirect forward direction.

The present investigations were undertaken to elucidate the characteristics of phosphoglycerate kinase in human erythrocytes and the factors which effect its activity. A highly sensitive fluorimetric assay procedure was developed [6] to study the kinetics in the forward reaction 1,3-diphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP.

Materials and Methods

The enzyme phosphoglycerate kinase was purified from human erythrocytes as described earlier [2,6]. The assay of the enzyme phosphoglycerate kinase was carried out fluorimetrically in the forward direction leading from 1,3-diphosphoglycerate to 3-phosphoglycerate [6]. The reaction mixture was composed of 34 mM Tris buffer, pH 8.5, 5 mM phosphate buffer, pH 7.3, 6 mM cysteine · HCl, pH 7.0, 0.4 mM ADP, 14 μM NADH, 1 mM $MgCl_2$, 10 μM 1,3-diphosphoglycerate, 4.2 μg PGM *, 40 μg enolase, 17 μg pyruvate kinase, 6 μg lactate dehydrogenase and 0.87 ng phosphoglycerate kinase per ml of assay mixture, The change in fluorescence was recorded at 37° C. Enzyme activity was expressed on the basis of change in fluorescence (i.e. ΔR/min) throughout the kinetic studies.

Experimental results

The effect of ADP or Mg^{2+} on phosphoglycerate kinase activity (fixed [ADP] or [Mg^{2+}], variable 1,3-diphosphoglycerate). Substrate activation of phosphoglycerate kinase was observed with fixed ADP or Mg^{2+} and varying concentrations of 1,3-diphosphoglycerate. Biphasic curves (Figs. 1a + 1b and 2a + 2b, respectively) were obtained in the linear and double reciprocal plots demonstrating two K_m values. At optimal concentrations of Mg^{2+} and ADP the K_m values calculated for 1,3-diphosphoglycerate from double reciprocal plots were K_{m_1} $1.9 \cdot 10^{-6}$ M and K_{m_2} $9.8 \cdot 10^{-6}$ M respectively. When these results were plotted using the Hill equation [7] the interaction coefficient was 0.8 (example shown in Fig. 1c is for optimal Mg^{2+} and ADP concentrations). A Hill coefficient less than 1 has been proposed as characteristic for enzymes exhibiting negative cooperativity [8]. Apparent negative cooperativity persisted at suboptimal to optimal (0.025—0.4 mM) concentrations of ADP with the Hill coefficient ranging from 0.6 to 0.8. A Hill coefficient of 0.6 was calculated (the example shown in Fig. 2c is for optimal Mg^{2+} and ADP concentrations). Apparent negative cooperativity was observed at fixed suboptimal (0.125 mM) to optimal (1.0 mM) concentrations of Mg^{2+} and varying concentrations of 1,3-diphosphoglycerate, with Hill coefficients at these levels ranging from 0.6 to 0.7.

* See footnote p. 89.

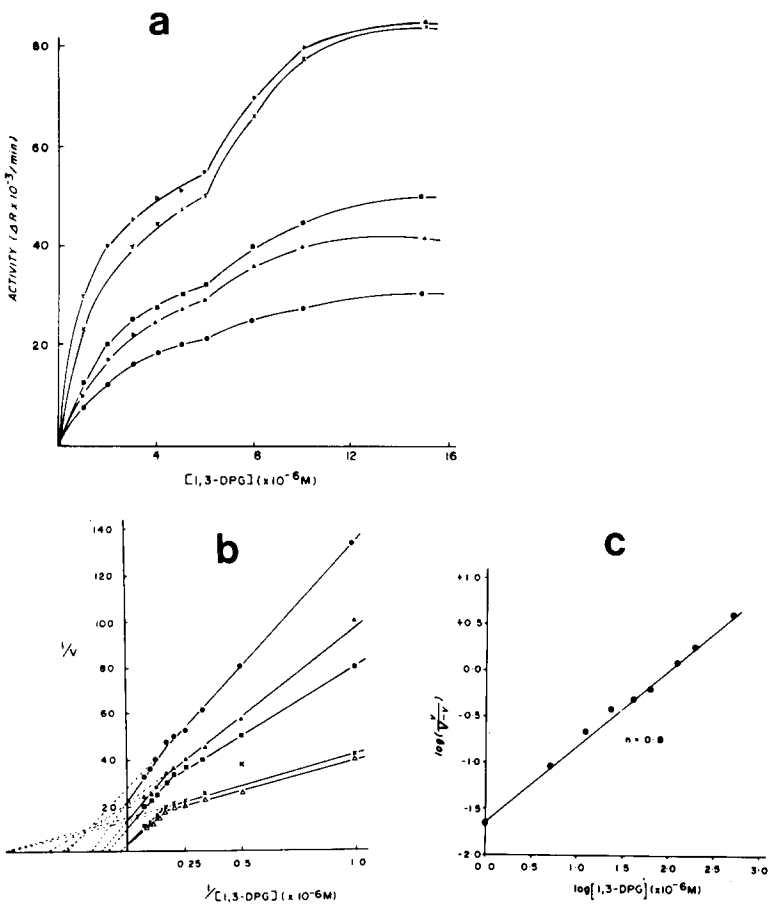

Fig. 1. The effect of ADP and 1,3-diphosphoglycerate (DPG) on phosphoglycerate kinase activity (fixed [ADP], variable [1,3-diphosphoglycerate]). [ADP]: ●———●, $0.025 \cdot 10^{-3}$ M; ▲———▲, $0.05 \cdot 10^{-3}$ M; ■———■, $0.10 \cdot 10^{-3}$ M; X———X, $0.40 \cdot 10^{-3}$ M; △———△, $0.80 \cdot 10^{-3}$ M. (a) Linear plot. (b) Double reciprocal plot. (c) Hill plot. Conditions as described in the text.

The effect of 1,3-diphosphoglycerate and ADP or Mg^{2+} on phosphoglycerate kinase activity (fixed [1,3-diphosphoglycerate], variable [ADP] or [Mg^{2+}]). Michaelis-Menten kinetics were observed in both linear and double reciprocal plots with fixed levels of 1,3-diphosphoglycerate and varying concentrations of Mg^{2+} (Figs. 3a and 3b, respectively). A Hill coefficient of 1.0 was calculated (the example shown in Fig. 3c is for optimal ADP and saturating 1,3-diphosphoglycerate concentrations).

A Michaelis-Menten-type curve was obtained in the linear plots at fixed levels of 1,3-diphosphoglycerate and varying concentrations of ADP, but the double reciprocal plots described biphasic curves (Figs. 4a and 4b, respectively). The interaction coefficient was 1.0 at higher levels of ADP concentrations for each fixed level of 1,3-diphosphoglycerate (Fig. 4c). At subsaturating concentrations of ADP apparent negative cooperativity persisted at fixed levels of 1,3-diphosphoglycerate with the Hill coefficient ranging between 0.6 and 0.75.

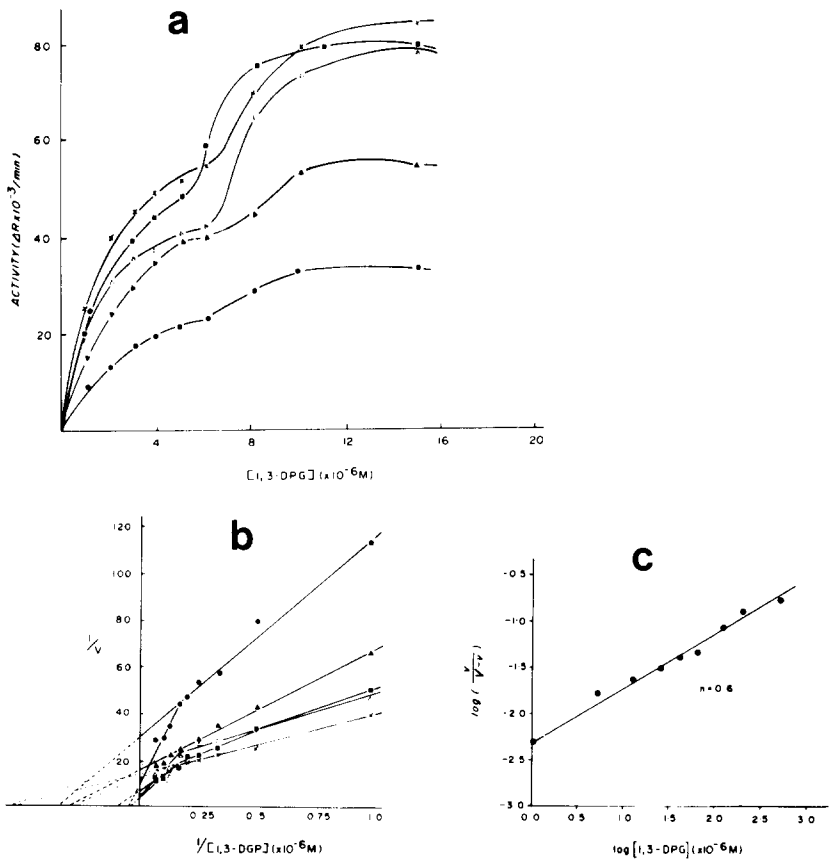

Fig. 2. The effect of Mg^{2+} and 1,3-diphosphoglycerate (DPG) on phosphoglycerate kinase activity (fixed [Mg^{2+}], variable [1,3-diphosphoglycerate]). [Mg^{2+}]: ●———●, $0.125 \cdot 10^{-3}$ M; ▲———▲, $0.25 \cdot 10^{-3}$ M; X———X, $1.0 \cdot 10^{-3}$ M; ■———■, $2.5 \cdot 10^{-3}$ M; △———△, $5.0 \cdot 10^{-3}$ M. (a) Linear plot. (b) Double reciprocal plot. (c) Hill Plot. Conditions as described in the text.

The effect of Mg^{2+} and ADP on phosphoglycerate kinase activity (fixed [Mg^{2+}], variable [ADP]). Apparent Michaelis-Menten kinetics were observed in the linear plots with fixed concentrations of Mg^{2+} and varying concentrations of ADP (Fig. 5a). However, the double reciprocal plots for ADP described biphasic curves with two K_m values (K_{m_1} $1.7 \cdot 10^{-5}$ and K_{m_2} $1.0 \cdot 10^{-4}$ M, respectively, Fig. 5b). The Hill plot also described a biphasic curve demonstrating two values for the interaction coefficient (n): 1.0 and 0.6 for the saturating and subsaturating levels of ADP concentrations, respectively (the example shown in Fig. 5c is for optimal Mg^{2+} and saturating 1,3-diphosphoglycerate concentrations).

The effect of ADP and Mg^{2+} on phosphoglycerate kinase activity (fixed [ADP], variable [Mg^{2+}]). Michaelis-Menten kinetics were obtained in both linear and double reciprocal plots with fixed levels of ADP and varying concentrations of Mg^{2+} (Figs. 6a and 6b). The K_m for Mg^{2+} was calculated at 0.5 mM. The interaction coefficient was calculated to be 1.0 (the example shown in Fig. 6c is for the optimal ADP and saturating 1,3-diphosphoglycerate concentra-

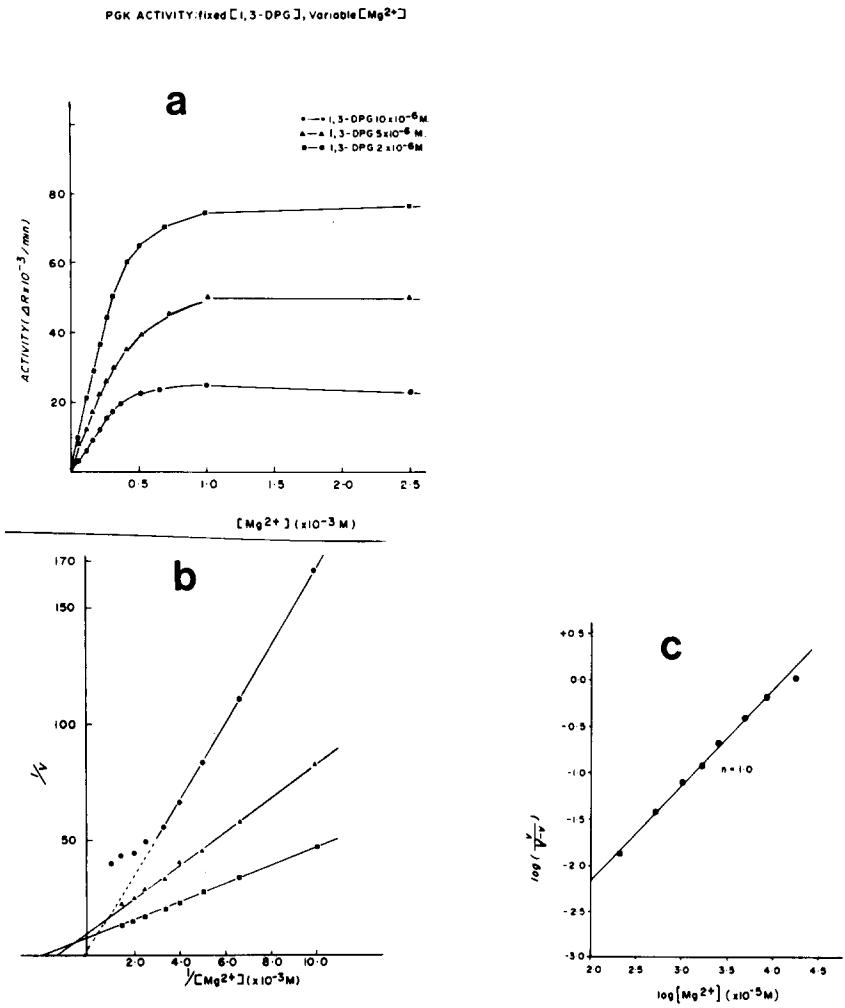

Fig. 3. The effect of 1,3-diphosphoglycerate (DPG) and Mg^{2+} on phosphoglycerate kinase activity (fixed [1,3-diphosphoglycerate], variable [Mg^{2+}]). [1,3-Diphosphoglycerate]: ●———●, $2.0 \cdot 10^{-6}$ M; ▲———▲, $5.0 \cdot 10^{-6}$ M; ■———■, $10.0 \cdot 10^{-6}$ M. (a) Linear plot. (b) Double reciprocal plot. (c) Hill plot. Conditions as described in the text.

tions) which is characteristic for Michaelis-Menten-type kinetics.

Effects of AMP on phosphoglycerate kinase activity. AMP was found to have an inhibitory effect on phosphoglycerate kinase activity (Fig. 7). Enzyme activity was inhibited to a maximum of 54% with added AMP ($10 \cdot 10^{-3}$ M).

The effect of AMP and 1,3-diphosphoglycerate on phosphoglycerate kinase activity (fixed [AMP], variable [1,3-diphosphoglycerate]). Substrate activation persisted at lower levels of AMP but at higher concentrations of this inhibitor ($5 \cdot 10^{-3}$ M) the curve approached the hyperbolic at a decreased level of activity (Fig. 8a). The V at these fixed levels of AMP was lower when compared with the control due to the inhibition of phosphoglycerate kinase activity. The inhibition by AMP was of a non-competitive type with respect to 1,3-diphos-

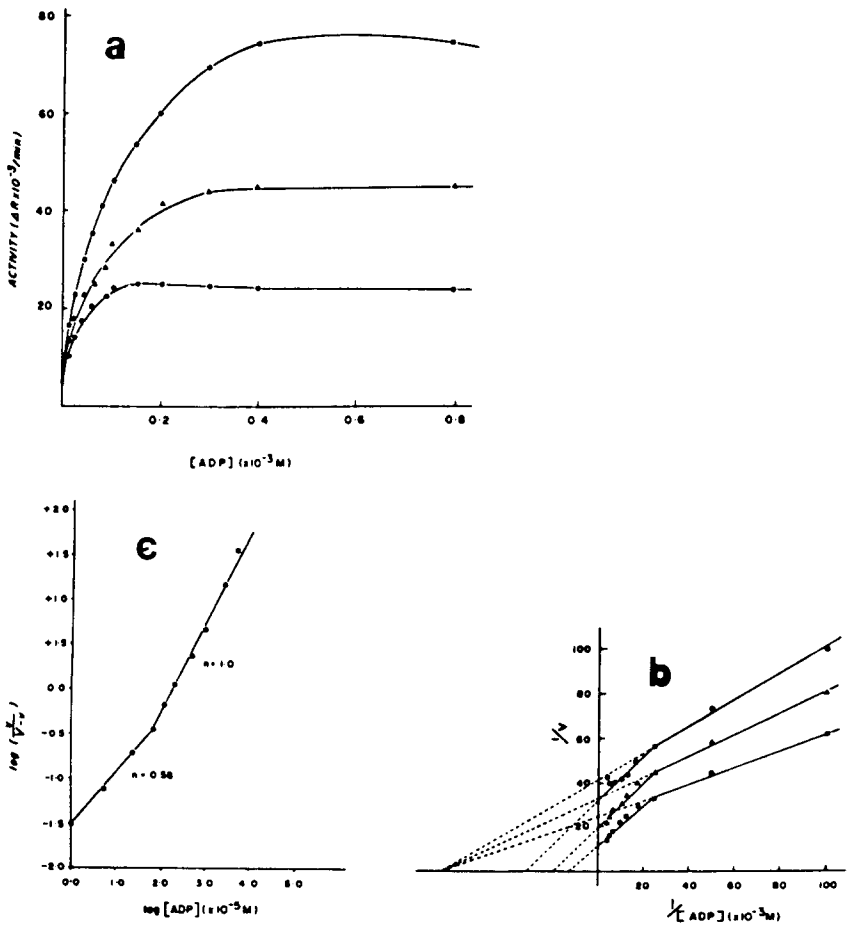

Fig. 4. The effect of 1,3-diphosphoglycerate and ADP on phosphoglycerate kinase activity (fixed [1,3-diphosphoglycerate], variable [ADP]). [1,3-Diphosphoglycerate]: ●———●, $2.0 \cdot 10^{-6}$ M; ▲———▲, $5.0 \cdot 10^{-6}$ M; ■———■, $10.0 \cdot 10^{-6}$ M. (a) Linear plot. (b) Double reciprocal plot. (c) Hill plot. Conditions as described in the text.

phoglycerate (with optimal concentrations of Mg^{2+} and ADP). The interaction coefficient calculated from the Hill plot was 0.75 (the example shown in Fig. 8c for the test with $0.25 \cdot 10^{-3}$ M added AMP). Negative cooperativity persisted up to a level of 2.0 mM AMP with an interaction coefficient calculated at 0.90. However, at higher concentrations of AMP (5.0 mM) cooperativity was abolished with a Hill coefficient of 1.0. The interaction coefficient of the control (without added AMP) was 0.6.

The effect of AMP and ADP on phosphoglycerate kinase activity (fixed [AMP], variable [ADP]). Apparent Michaelis-Menten curves were obtained in linear plots with fixed levels of AMP and varying concentrations of ADP (Fig. 9a). However, the double reciprocal plot described biphasic curves with two K_m values (Fig. 9b). A non-competitive type of inhibition was observed by AMP with respect to ADP. A biphasic curve was obtained on the Hill plot with two interaction coefficients, 0.6 and 1.0, respectively (the example shown in

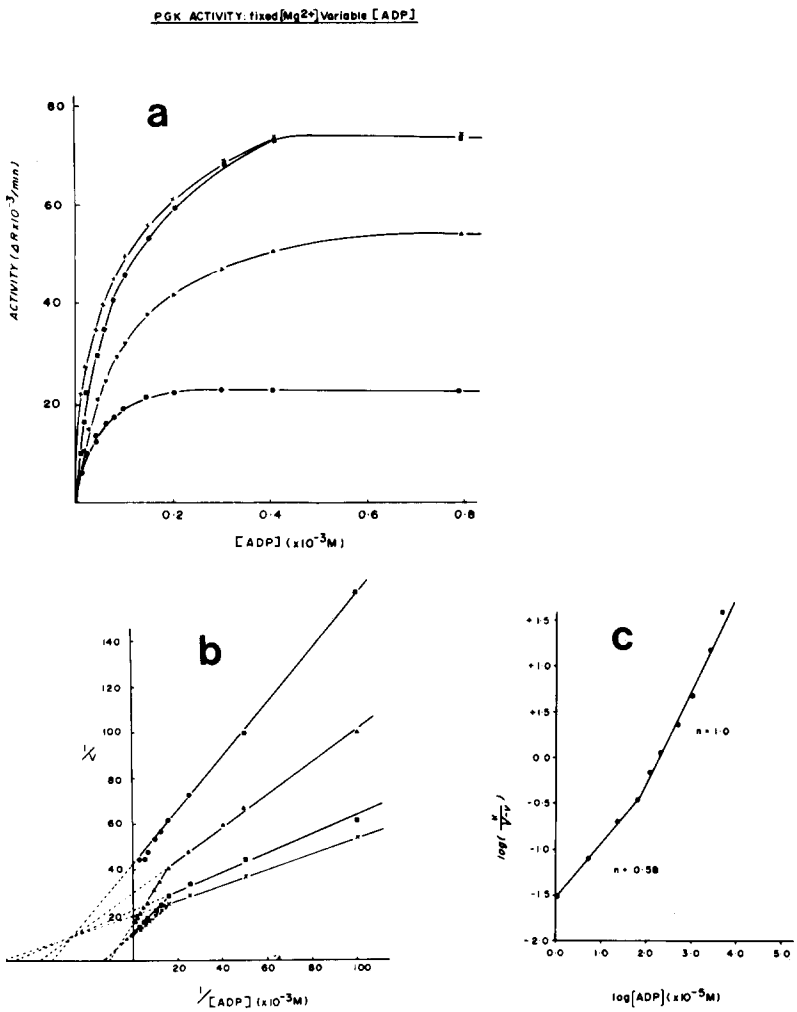

Fig. 5. The effect of Mg^{2+} and ADP on phosphoglycerate kinase (PGK) activity (fixed [Mg^{2+}], variable [ADP]). [Mg^{2+}]: ●——●, $0.125 \cdot 10^{-3}$ M; ▲——▲, $0.25 \cdot 10^{-3}$ M; ■——■, $1.0 \cdot 10^{-3}$ M; X——X, $2.5 \cdot 10^{-3}$ M. (a) Linear plot. (b) Double reciprocal plot. (c) Hill plot. Conditions as described in the text.

Fig. 9c is for the optimal Mg^{2+} and saturating 1,3-diphosphoglycerate concentration in the presence of $5.0 \cdot 10^{-3}$ M AMP). The interaction coefficient values for the enzyme treated with AMP was similar to the control without added AMP.

The effect of AMP and Mg^{2+} on phosphoglycerate kinase activity (fixed [AMP], variable [Mg^{2+}]). Inhibition of a non-competitive type with respect to Mg^{2+} was observed at various levels of AMP (Figs. 10a and 10b). The interaction coefficient was 1.0 at all fixed levels of AMP and varying concentrations of Mg^{2+} (the example in Fig. 10c is for the optimal ADP and saturating concentration of 1,3-diphosphoglycerate in the presence of 1.0 mM added AMP). Similarly, the interaction coefficient of the control (without added AMP) was 1.0.

The effect of 2,3-diphosphoglycerate on phosphoglycerate kinase activity.

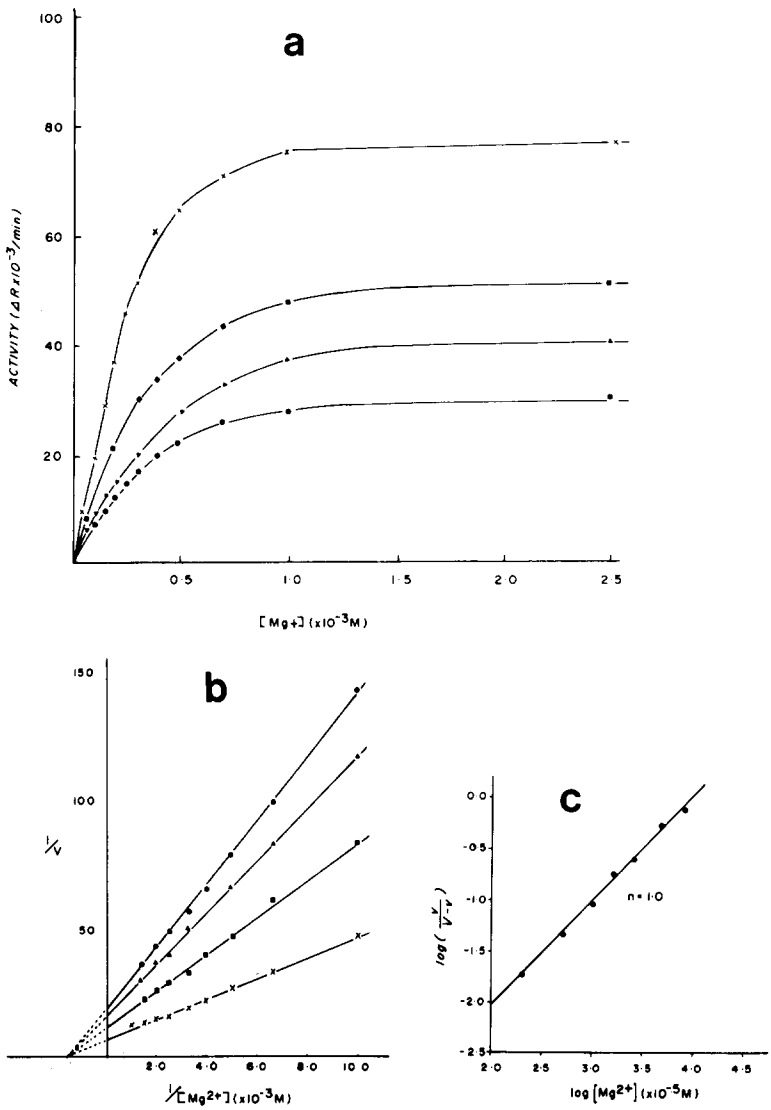

Fig. 6. The effect of ADP and Mg^{2+} on phosphoglycerate kinase activity (fixed [ADP], variable [Mg^{2+}]). [ADP]: ●———●, $0.025 \cdot 10^{-3}$ M; ▲———▲, $0.05 \cdot 10^{-3}$ M; ■———■, $0.10 \cdot 10^{-3}$ M; X———X, $0.40 \cdot 10^{-3}$ M. (a) Linear plot. (b) Double reciprocal plot. (c) Hill plot. Conditions as described in the text.

2,3-Diphosphoglycerate had an inhibitory effect on phosphoglycerate kinase activity (Fig. 11). Enzyme activity was inhibited to a maximum of 57% with added 2,3-diphosphoglycerate ($10 \cdot 10^{-3}$ M).

Although enzyme inhibition was observed in the presence of 2,3-diphosphoglycerate, substrate activation persisted in the presence of this inhibitor. At higher concentrations of 2,3-diphosphoglycerate ($5.0 \cdot 10^{-3}$ M) the curve approached the hyperbolic (Fig. 12a). A non-competitive type of inhibition by 2,3-diphosphoglycerate with respect to 1,3-diphosphoglycerate was observed (Fig. 12b). The interaction coefficient was 0.85 (the example shown in Fig. 12c

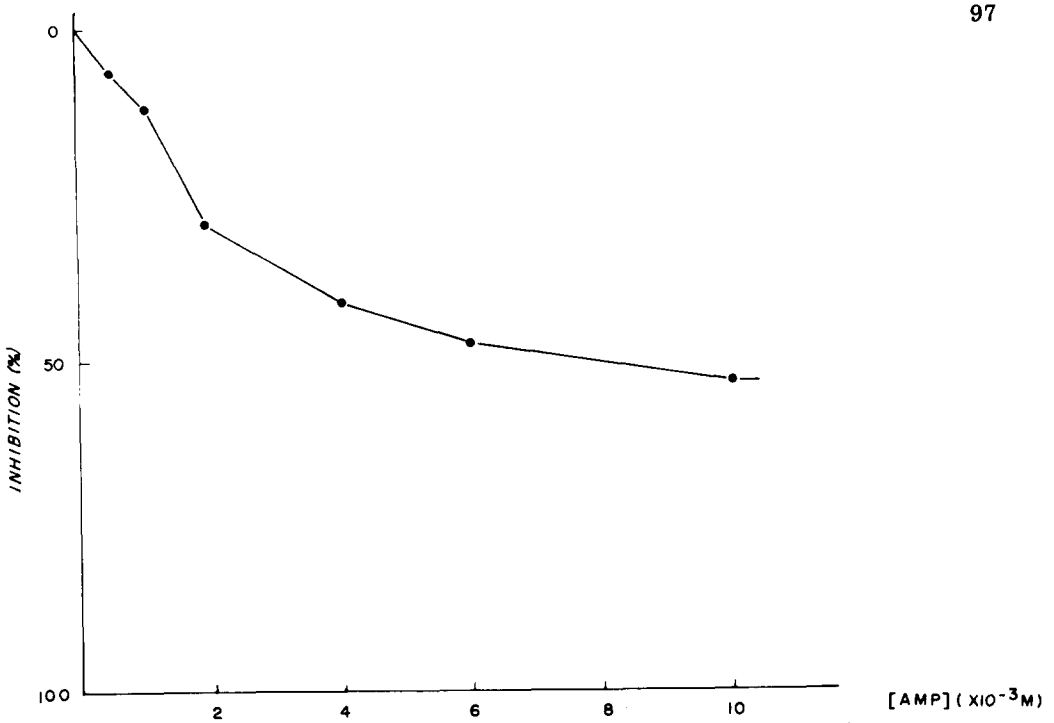

Fig. 7. Effect of AMP on phosphoglycerate kinase activity. Conditions as described in the text.

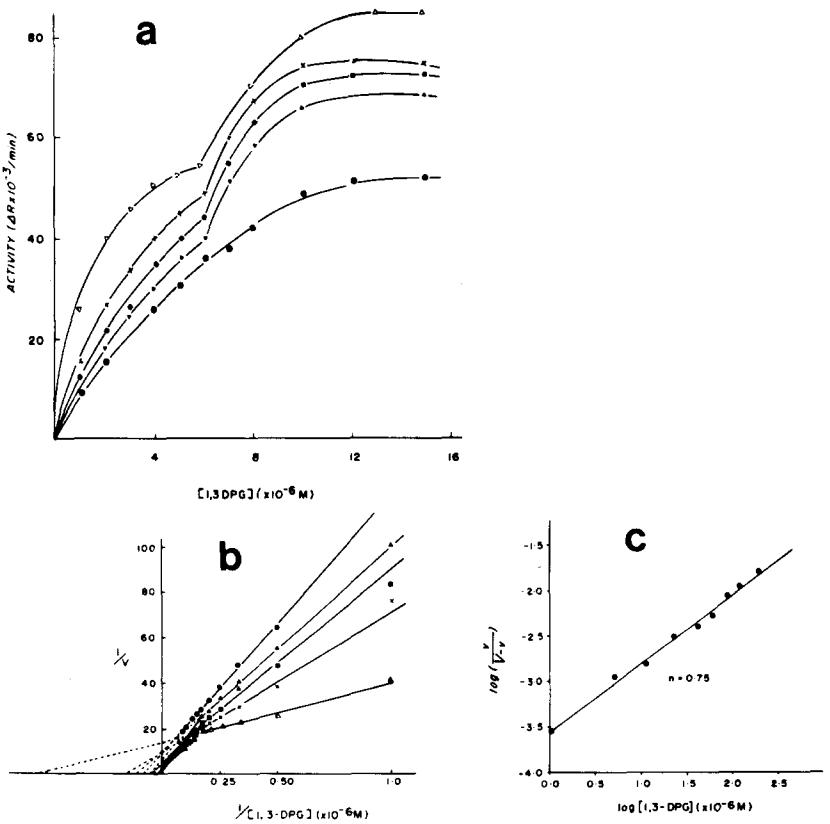

Fig. 8. The effect of AMP and 1,3-diphosphoglycerate (DPG) on phosphoglycerate kinase activity (fixed [AMP], variable [1,3-diphosphoglycerate]). [AMP]: △———△, 0 M; X———X, $0.25 \cdot 10^{-3}$ M; ■———■, $1.0 \cdot 10^{-3}$ M; ▲———▲, $2.0 \cdot 10^{-3}$ M; ●———●, $5.0 \cdot 10^{-3}$ M. (a) Linear plot. (b) Double reciprocal plot.

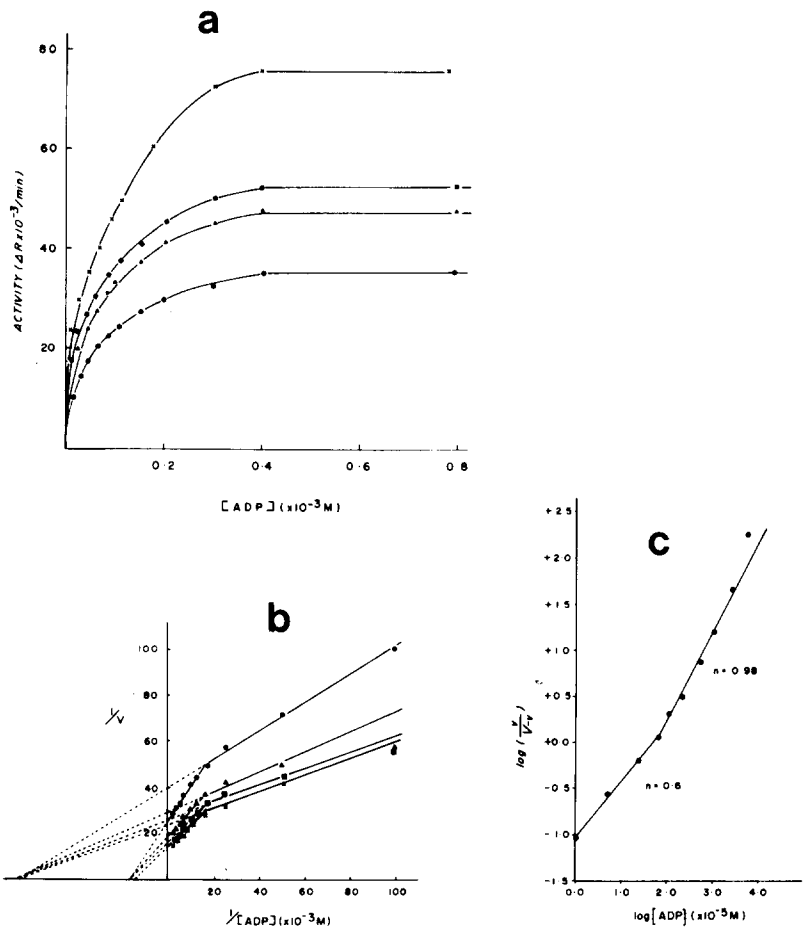

Fig. 9. The effect of AMP and ADP on phosphoglycerate kinase activity (fixed [AMP], variable [ADP]). [AMP]: X———X, 0 M; ■———■, $1.0 \cdot 10^{-3}$M; ▲———▲, $2.0 \cdot 10^{-3}$ M; ●———●, $5.0 \cdot 10^{-3}$ M. (a) Linear plot. (b) Double reciprocal plot. (c) Hill plot. Conditions as described in the text.

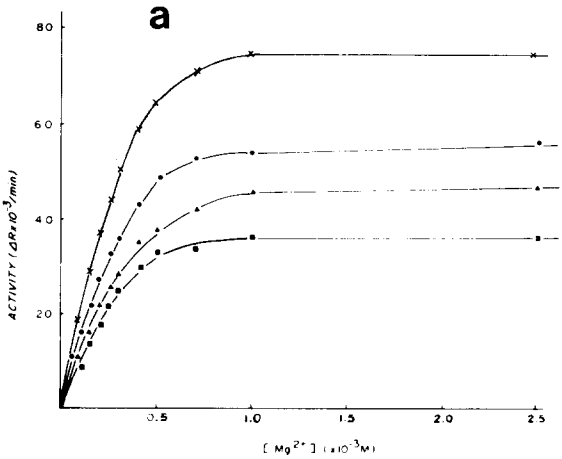

Fig. 10a.

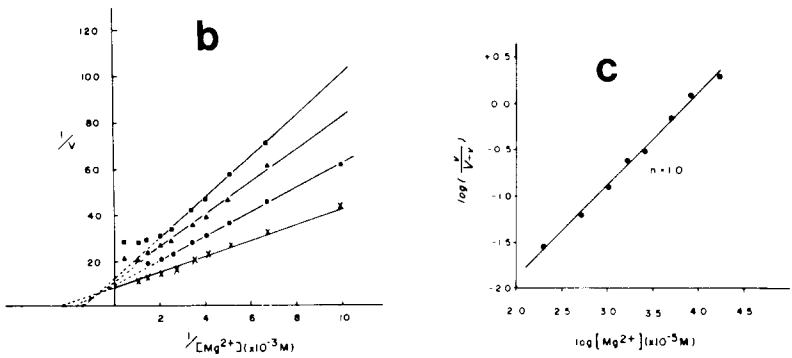

Fig. 10. The effect of AMP and Mg^{2+} on phosphoglycerate kinase activity (fixed [AMP], variable [Mg^{2+}]). [AMP]: X———X, 0 M; ●———●, $1.0 \cdot 10^{-3}$ M; ▲———▲, $2.0 \cdot 10^{-3}$ M; ■———■, $5.0 \cdot 10^{-3}$ M. (a) Linear plot. (b) Double reciprocal plot. (c) Hill plot. Conditions as described in the text.

is for the optimal Mg^{2+} and ADP concentrations in the presence of $5.0 \cdot 10^{-3}$ M 2,3-diphosphoglycerate); the interaction coefficient of the control was 0.6 (without 2,3-diphosphoglycerate). Apparent negative cooperativity persisted at high 2,3-diphosphoglycerate concentrations ($5.0 \cdot 10^{-3}$ M), but to a lesser degree.

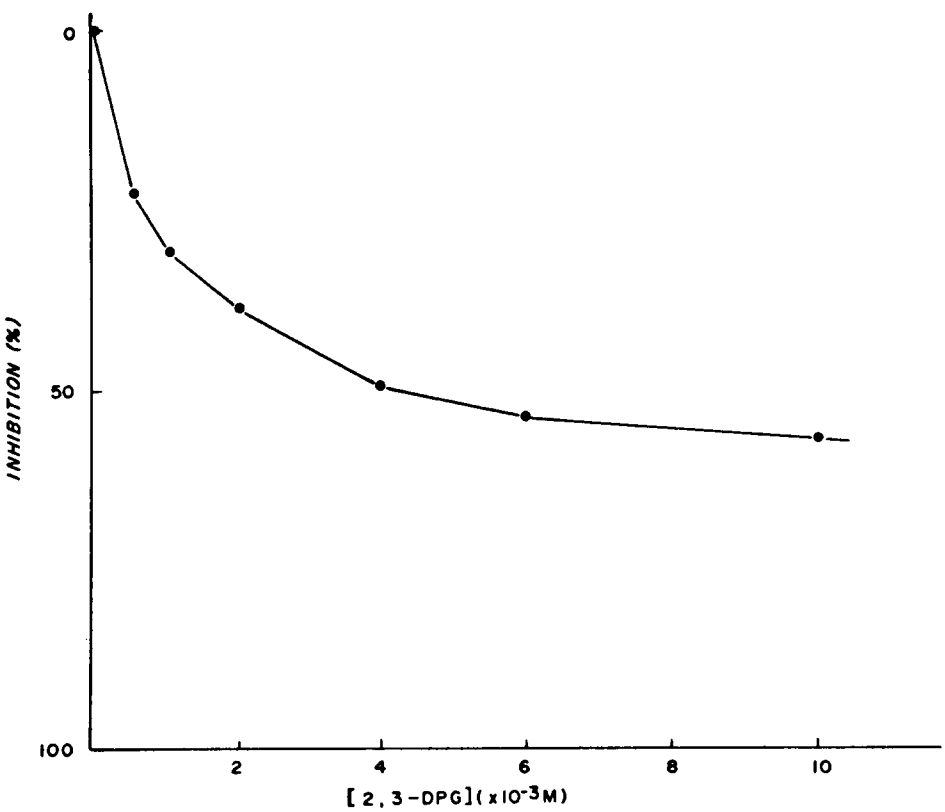

Fig. 11. Effect of 2,3-diphosphoglycerate (DPG) on phosphoglycerate kinase activity. Conditions as described in the text.

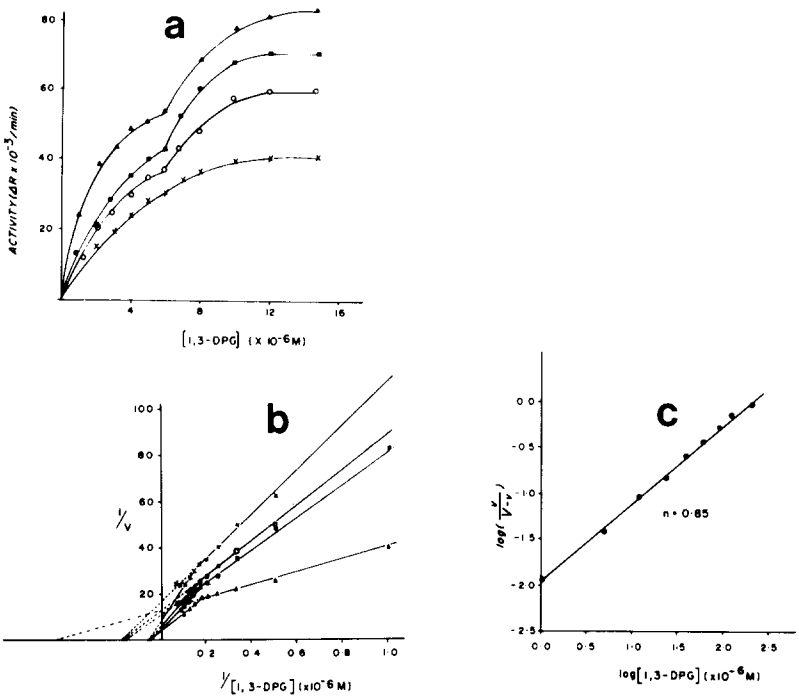

Fig. 12. The effect of 2,3-diphosphoglycerate and 1,3-diphosphoglycerate on phosphoglycerate kinase activity (fixed [2,3-diphosphoglycerate], variable [1,3-diphosphoglycerate]). [2,3-Diphosphoglycerate]: ▲———▲, 0 M; ■———■, $0.50 \cdot 10^{-3}$ M; ○———○, $2.0 \cdot 10^{-3}$ M; X———X, $5.0 \cdot 10^{-3}$ M. (a) Linear plot. (b) Double reciprocal plot. (c) Hill plot. Conditions as described in the text.

Discussion

Substrate activation was observed with respect to 1,3-diphosphoglycerate with fixed levels of ADP or Mg^{2+}. Biphasic curves were obtained on the double reciprocal plot demonstrating two K_m values (Figs. 1 and 2). K_{m_1} ($1.9 \cdot 10^{-6}$ M) for 1,3-diphosphoglycerate obtained in the present work was similar to that reported for phosphoglycerate kinase from yeast [9] and muscle [4] ($1.8 \cdot 10^{-6}$ and $2.2 \cdot 10^{-6}$ M, respectively). The K_m for 1,3-diphosphoglycerate reported from human blood was higher ($3.5 \cdot 10^{-6}$ M) [1]. The K_m for muscle reported by Rao and Oesper [4] was based on activity measurements at three levels of substrate only. The value for yeast was calculated from indirect measurements of the forward reaction [9]. Larsson-Raznikiewicz [10] has reported a noncompetitive type of inhibition at fixed levels of ATP or Mg^{2+} and varying concentrations of 3-phosphoglycerate in the backward reaction in yeast phosphoglycerate kinase. Two K_m values were demonstrated but there was no indication of the appearance of a biphasic curve in the double reciprocal plots.

Substrate activation was not apparent on the linear plot with fixed levels of 1,3-diphosphoglycerate (Fig. 4a) and Mg^{2+} (Fig. 5a) and varying concentration of ADP. However, the double reciprocal plots described biphasic curves. Apparent negative cooperativity was observed at subsaturating levels of ADP at higher levels of ADP it was not operative. This is essentially in agreement with the pro-

posals of Levitzki and Koshland [8] who state that in cases of negative cooperativity the saturation plot looks qualitatively like a Michaelis-Menten curve but the double reciprocal plot is concave downward and the Hill coefficient is less then 1. In comparison to the values obtained in the present study K_{m_1} $1.7 \cdot 10^{-5}$ M, K_{m_2} $1.0 \cdot 10^{-4}$ M, respectively, the K_m values for ADP reported for yeast phosphoglycerate kinase were $2.0 \cdot 10^{-4}$ M [5], $4.0 \cdot 10^{-4}$ M [4] and erythrocyte phosphoglycerate kinase $7.8 \cdot 10^{-4}$ M [1]. The difference may be due to the calculation of K_m values from the indirect estimation of the forward reaction. Furthermore, substrate activation was not observed in these investigations and the calculated K_m is similar to K_{m_2} reported herein.

Michaelis-Menten kinetics were observed with respect to Mg^{2+} at fixed levels of 1,3-diphosphoglycerate or ADP (Figs. 3 and 6, respectively). The K_m for Mg^{2+} ($0.5 \cdot 10^{-3}$ M) in the present study appeared to be somewhat higher than reported in yeast $0.25 \cdot 10^{-3}$—$0.28 \cdot 10^{-3}$ M [4,9], muscle $0.27 \cdot 10^{-3}$ M [4] and human red cells $0.32 \cdot 10^{-3}$ M [1]. These variations may be due to the difference in the assay system used, i.e. the forward reaction compared to the backward reaction.

Larsson-Raznikiewicz [11] reported that free Mg^{2+} and free ATP inhibit phosphoglycerate kinase activity competitively in the backward reaction. In the present study this was not observed in the forward reaction with free Mg^{2+} and free ADP.

The inhibition of phosphoglycerate kinase from human erythrocytes was observed in the presence of AMP and 2,3-diphosphoglycerate. At levels up to 2.0 mM concentration of AMP apparent negative cooperativity was still observed but at a higher level (5.0 mM) cooperativity was abolished (Fig. 8). The Hill coefficients ranged from 0.75 to 1.0, respectively. The interaction coefficient of the control (without added AMP) was 0.6. The increase in the numeric value of the interaction coefficient is indicative of a diminution in cooperativity as the concentration of AMP was increased. The affinity for 1,3-diphosphoglycerate was also decreased in the presence of inhibitor AMP. It may be that the allosteric site has been modified and there is no cooperative interaction at this level of AMP (5.0 mM). Larsson-Raznikiewicz and Arvidsson [12] reported that AMP inhibited yeast phosphoglycerate kinase non-competitively with respect to 3-phosphoglycerate in the backward reaction. A similar type of inhibition was observed with respect to 1,3-diphosphoglycerate in the present study. The effect of AMP on phosphoglycerate kinase activity suggests that AMP and 1,3-diphosphoglycerate have different binding sites on the enzyme.

In relation to ADP and Mg^{2+} the interaction coefficients for the enzyme treated with AMP was similar to that without added AMP (Figs. 9 and 10). This implies that AMP does not affect the binding of ADP and does not induce any conformational change on the enzyme. AMP inhibited the enzyme non-competitively with respect to ADP and Mg^{2+} (Figs. 9b and 10b, respectively). It is therefore reasonable to suggest that AMP, ADP and Mg^{2+} have different binding sites on the enzyme. (A competitive type of inhibition of AMP with respect to $MgATP^{3-}$ was reported by Larsson-Raznikiewicz and Arvidsson [12] in the backward reaction).

2,3-Disphosphoglycerate was found to inhibit the enzyme non-competitively with respect to 1,3-diphosphoglycerate. Apparent negative cooperativity per-

sisted at higher 2,3-diphosphoglycerate concentration ($5.0 \cdot 10^{-3}$ M) but to a lesser degree (Fig. 12a). Working with the backward reaction with partially purified enzyme from human erythrocytes Ponce et al. [13] reported a competitive type of inhibition with respect to 3-phosphoglycerate.

Phosphoglycerate kinase isolated from human erythrocytes in the present study exhibited unique kinetic properties compared to the enzyme from other sources [4,5,10]. Apparent negative cooperativity was observed with respect to 1,3-diphosphoglycerate and ADP. The enzyme exhibited Michaelis-Menten kinetics with respect to Mg^{2+}. Enzyme activity was inhibited by AMP and 2,3-disphosphoglycerate.

The interpretations of the phosphoglycerate kinase kinetics (i.e. bumpy curves) obtained in the present study can be considered on the basis of some of the molecular events suggested by Levitzki and Koshland [8]. The possibility of the presence of isoenzymes of phosphoglycerate kinase with non-identical peptide chains having active sites with different binding constants was ruled out experimentally (by polyacrylamide gel electrophoresis and electrofocusing reported in this study). Other explanations of the presence of non-identical subunits with different catalytic activities and the formation of enzyme aggregates at higher concentrations of substrate can be treated potentially equal. These possibilities have not been tested because of the low yield of the purified enzyme available.

The findings in the present kinetic study of human phosphoglycerate kinase are in conformity with the proposed characteristics of negative cooperativity [8]: the saturation plots look qualitatively like a Michaelis-Menten curve but the double reciprocal plot is concave downward and the Hill coefficient is less than 1.

The recent bilobular model of horse muscle phosphoglycerate kinase presented by Blake and Evans [14] from their X-ray crystallographic studies may afford an explanation of the mechanism of apparent negative cooperativity observed herein. As the two domains are quite apart at a distance of 20 Å from each other they may behave in a similar manner to subunits of the enzyme phosphoglycerate kinase. Although the authors have not been able to produce any clear results with 3-phosphoglycerate binding experiments (nor have they worked with 1,3-diphosphoglycerate) it is reasonable to suggest in the light of our kinetic findings that the enzyme (phosphoglycerate kinase) may have more than one binding site for each substrate. The conformational alteration induced by a ligand on one lobe may effect an unoccupied binding site for the same ligand on the other lobe of the single protein in such a way that its affinity is decreased. A negative homotropic interaction between these two would then be reflected in the type of kinetics observed.

Further investigations, i.e. the effect of urea, sodium dodecyl sulfate and guanidine hydrochloride on the purified phosphoglycerate kinase, the formation of enzyme aggregates and the structure of phosphoglycerate kinase by X-ray crystallography are being carried out.

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