

BINDING OF $MnADP^{3-}$ TO PHOSPHOGLYCERATE KINASE

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1. Introduction

Phosphoglycerate kinase (EC 2.7.2.3) catalyzes the reaction: $MADP^{3-} + 1,3\text{-diphosphoglycerate} \rightleftharpoons MATP^{2-} + 3\text{-phosphoglycerate}$.

The properties of the enzyme isolated from several sources and the enzyme's reaction kinetics have been reviewed recently by Scopes [1]. Investigations have dealt substantially with the kinetic activation of the enzyme by metal ion cofactors [2-5].

Preliminary X-ray crystallographic studies have been reported on the enzyme from yeast [6] and horse muscle [7]. Recently a 3.5 Å resolution electron density map of the yeast enzyme was calculated [8]. By soaking crystals in solutions containing magnesium ions and ADP, the position of a unique $MgADP$ (or $MnADP$) binding site on one lobe of the enzyme was located, though no definite conclusions about the mode of binding of the metal ion were reached [8].

In this communication, we present evidence from equilibrium binding measurements by NMR relaxation methods that the metal ion and ADP^{3-} when present individually interact weakly with the yeast enzyme whereas the $MADP^{3-}$ complex is very strongly bound to the enzyme.

2. Experimental procedures

Phosphoglycerate kinase was prepared from yeast as described by Scopes [9]. Measurements of the PRR[†] of water were made at 30 MHz in 50 mM HEPES-KOH, pH 8.0, at 25°C and titrations carried out as described previously [10]. Nucleotides were obtained from Sigma. The relationship between ϵ^* , the observed enhancement of the PRR and ϵ_a , ϵ_b , and ϵ_t , the characteristic enhancements for the MS, EM and EMS complexes have been described [11, 12]. Enhancement data were analyzed as described by Reed et al. [12]. Values obtained from computer analysis were ϵ_t and the constants, K_2 and K_3 , for the dissociation of MS and S from EMS and ES complexes, respectively. The values obtained previously for ϵ_a (1.7) and the dissociation constant of $MnADP^{3-}$

[†] The Abbreviations used are: PRR, proton relaxation rate; XDP, xanthosine-5'-diphosphate; % S.D., percentage standard deviation; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

(0.03 mM) were used for the nucleoside diphosphates [12, 13].

3. Results

Addition of Mn^{2+} to phosphoglycerate kinase in 50 mM HEPES-KOH, pH 8.0, produced a moderate degree of enhancement of the PRR. Analysis of two titrations, with $MnCl_2$ fixed at 0.1 mM and varying enzyme concentration gave a best fit for a one site model with ϵ_b , 4.3 and K_d , the dissociation constant for the EM complex, in the range 0.5–1.0 mM.

PRR titrations, at two concentrations of phosphoglycerate kinase with constant $MnCl_2$ (0.1 mM), and with varying ADP are shown in fig. 1. The peaks of the curves are much broader than observed with, for example, creatine kinase [12] or adenylate kinase [14], consistent with relatively weak competition by ADP^{3-} for the $MnADP$ -site [12]. This was confirmed by computer analysis which yielded values of 85 μM and 3.2 mM for K_2 and K_s , respectively, with ϵ_1 , 21.0 (% S.D. = 3.74) for a K_d value of 1 mM. Lowering the value of K_d to 0.5 mM did not alter either

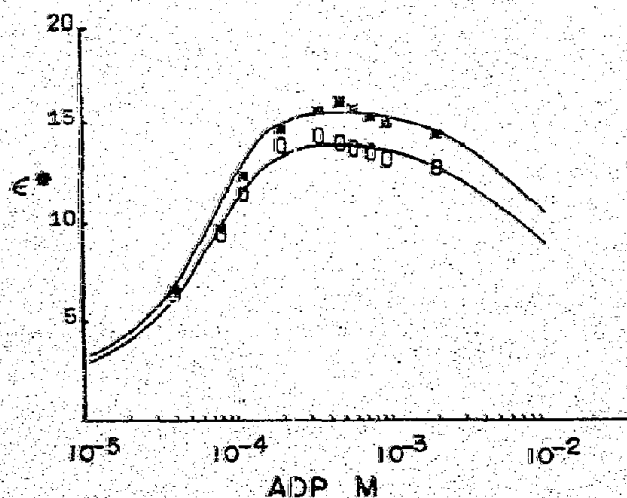


Fig. 1. PRR titration data for phosphoglycerate kinase and $MnCl_2$ (0.1 mM) with ADP in 50 mM HEPES-KOH, pH 8.0. Upper curve, phosphoglycerate kinase = 0.317 mM; lower curve, phosphoglycerate kinase = 0.231 mM. Temperature, 25°C. Solid curves drawn with $K_D = 1$ mM, $\epsilon_b = 4.3$, $K_1 = 0.03$ mM, $\epsilon_a = 1.7$, $K_2 = 85$ μM , $\epsilon_1 = 21.0$, $K_s = 3.2$ mM. Values of K_2 , K_s and ϵ_1 taken from minimum percentage of S.D. (3.7) in regression analysis.

the fit or the constants appreciably. A second set of titrations gave similar values; viz. ϵ_1 , 23; K_2 , 110 μM ; K_s , 5 mM.

Of other nucleotides tested $MnIDP$ gave similar enhancements to $MnADP$, viz. ϵ_1 , 21.4; K_2 , 143 μM ; K_s , 2.8 mM (% S.D. = 4.2). It should be noted that ITP is nearly as effective a substrate as ATP [5]. Significant increases in enhancement were observed for $MnXDP$, $MnGDP$ and $MnATP$. A smaller increase in enhancement was observed with $MnADP$ and $MnUDP$ or $MnCDP$ did not produce a change in enhancement. Details shall be presented elsewhere [15].

Evidence for the formation of a quaternary $MnADP$ -enzyme-3-phosphoglycerate complex was obtained by titration of the ternary complex (ADP, 0.5 mM; $MnCl_2$, 0.1 mM; enzyme, 0.162 mM) with 3-phosphoglycerate at 25°C (fig. 2). The decrease in enhancement appears to be too large to be attributed solely to binding of manganous ions by 3-phosphoglycerate [16]. The calculated change in available $MnADP$ changed from 0.092 to 0.080 mM over the concentration range, zero to 5 mM, of 3-phosphoglycerate used. (A value of 380 M^{-1} was used for the stability constant for Mn -3-phosphoglycerate in these calculations [16]). A plot of the change in enhancement as a function of 3-phosphoglycerate concentration indicated a dissociation constant of approximately 0.5 mM for 3-phosphoglycerate, from the quaternary complex, in reasonable agreement with the kinetically determined value of 0.62 mM [4]. Much smaller

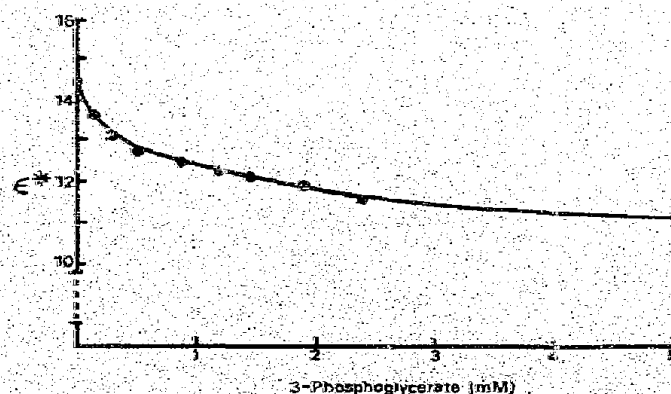


Fig. 2. PRR titration of $MnADP$ -phosphoglycerate kinase with 3-phosphoglycerate in 50 mM HEPES-KOH, pH 8.0; ADP, 0.5 mM; $MnCl_2$, 0.1 mM; enzyme, 0.162 mM. Temperature, 25°C.

changes were observed at 0°C and no significant effect of NO_3^- ion was observed, in contrast to experiments with creatine kinase [17].

4. Discussion

PRR titrations for phosphoglycerate kinase show patterns similar to those observed for metal-nucleotide titrations with the enzymes creatine kinase [12] and adenylate kinase [14]. It is also striking that ϵ_1 for the phosphoglycerate kinase-ADP-Mn complex is virtually identical to that obtained for the MnADP complex with creatine kinase [12]. This observation suggests a similar environment for the Mn(II) in the two ternary complexes. However, in contrast to the situation with creatine kinase where the enzyme's affinity for ADP^{3-} is only two-fold weaker than its affinity for MnADP [12], binding of ADP^{3-} to phosphoglycerate kinase is approximately forty-fold weaker than that of MnADP. The contribution of the metal ion to the stability of the ternary complex could arise from specific metal ion-protein interactions or from partial neutralization of the negative charge of the ADP^{3-} . At present there is insufficient information to differentiate between these two possibilities though the X-ray data on the horse muscle enzyme is consistent with the direct bonding of metal ion to protein in the ternary complex, indicative of a E-S^{M} structure [18]. The considerable specificity of the enzyme towards the nucleoside moiety indicates that protein-nucleoside interactions are important in the overall stability to the ternary complexes [1]. We would like to stress the fact that metal ion-nucleotide binding to phosphoglycerate kinase shows an unusual synergism, the mechanism for which may be revealed in future magnetic resonance and X-ray crystallographic investigations.

It is also noteworthy that the PRR results are suggestive of the formation of a dead-end complex, enzyme-ADP-Mn-3-phosphoglycerate.

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