BINDING OF MnADP$^-$ TO PHOSPHOGLYCERATE KINASE

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1. Introduction

Phosphoglycerate kinase (EC 2.7.2.3) catalyzes the reaction: MADP$^-$ + 1,3-diphosphoglycerate ⇌ MATP^{2-} + 3-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$^-$ 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_s, 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$^-$

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

Of other nucleotides tested $MnIDP^-$ gave similar enhancements to MnADP, viz. ϵ_t, 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 MndADP 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

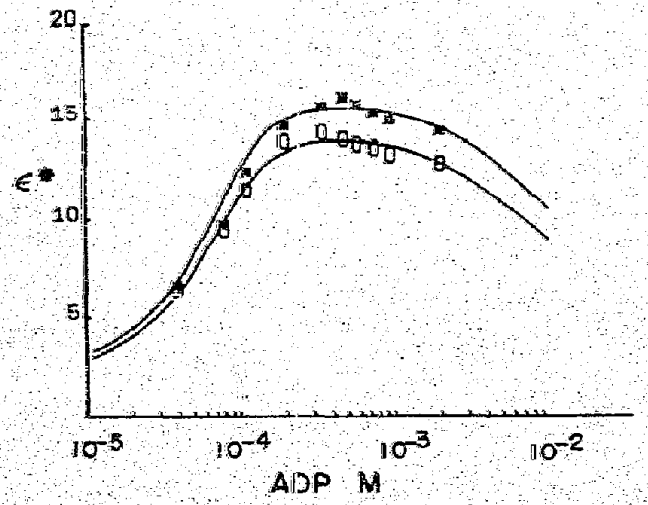

Fig. 1. PRR titration data for phosphoglycerate kinase and $MnCl_2$ (0.1 mM) with ADP in 50 mM HEPES–KOH, pH 8.0. Upper curve, phosphoglycerate kinase = 0.317 mM; lower curve, phosphoglycerate kinase = 0.231 mM. Temperature, 25°C. Solid curves drawn with K_D = 1 mM, ϵ_b = 4.3, K_1 = 0.03 mM, ϵ_a = 1.7,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

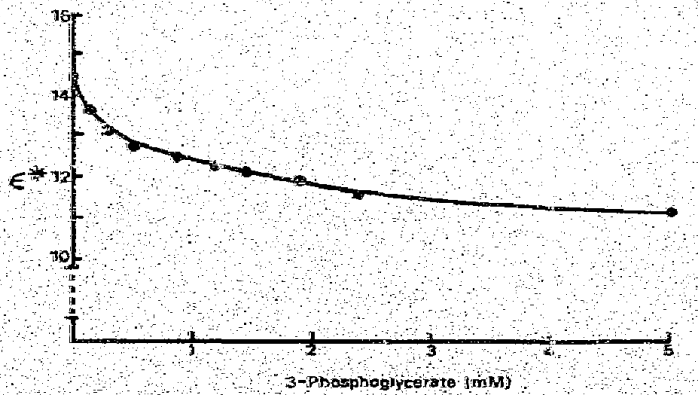

Fig. 2. PRR titration of MnADP-phosphoglycerate kinase with 3-phosphoglycerate in 50 mM HEPES–KOH, pH 8.0; ADP, 0.5 mM; $MnCl_2$, 0.1 mM; enzyme, 0.162 mM. Temperature, 25°C.

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 $E\diagdown^{M}_{S}$ 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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