

The effect of substrates on the inter-domain interactions of the hinge-bending enzyme 3-phosphoglycerate kinase

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A hinge-bending mechanism has been proposed for phosphoglycerate kinase, in which the two domains bend about the connecting 'waist' region. In partially denaturing concentrations of guanidinium chloride the substrate 3-phosphoglycerate stabilises one domain against denaturation and destabilises the other. The reduction of mutual stabilisation of the two domains on binding substrate indicates a freeing of the hinge to allow the protein to take up other states rather than a directive mechanism. The stabilisation of both domains at higher concentrations of ATP at which the enzyme is inhibited supports this mechanism.

Phosphoglycerate kinase Enzyme mechanism Conformational stability Hinge-bending Denaturation

1. INTRODUCTION

Phosphoglycerate kinase (PGK) is a monomeric, two-domain enzyme which catalyses the transfer of a phosphate group between ATP and 3-phosphoglycerate (PGA). It has been shown that while ATP and ADP bind to the same site on the C-terminal domain, the most plausible binding site for PGA is close to the N-terminal domain, approx. 1 nm away from the other site [1,2]. This distance is too great for phosphoryl transfer to occur, so a hinge-bending mechanism, which would bring the two substrates close together, has been proposed [2]. Such a mechanism is supported by several observations. Binding of substrate has been reported to reduce the radius of gyration [3] and to increase the sedimentation coefficient [4]; the binding of PGA causes crystals of PGK to disintegrate [5] and brings about a conformational change in solution [6].

Hinge-bending must involve a rearrangement of the inter-domain contacts. The model proposed by Blake and Rice [7] involves rotation of helices 7

and 14 (V and XIII in yeast PGK [1]) about the normal through their contact area. Watson et al. [1] propose the critical involvement of the His 338-Glu 190 interaction.

The binding of substrates to PGK can bring about changes in the inter-domain contacts in one of two ways. It may act in a permissive manner by destabilising the normal, non-bent interactions and, by lowering the free energy barrier between the two states, allow the enzyme to take up preferentially the bent conformation. Alternatively, the energy of binding of the substrates could be used to stabilise the enzyme directly in the bent conformation.

The results presented here for the yeast enzyme show that the binding of substrates brings about a reduction in the mutual stabilisation between the domains and hence in destabilisation of the inter-domain interactions.

2. MATERIALS AND METHODS

Aristar grade guanidinium chloride (GdmCl) was from BDH Chemicals, Poole, England. Other reagents were of analar grade.

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Yeast PGK was isolated as described in [8] and its concentration estimated assuming $A_1^{1\text{ mg/ml}} = 0.495$ at 280 nm. Bound PGA was removed from PGK by incubation with NADH, ATP and GAPDH [9].

Circular dichroism (CD) measurements were made using a Jobin-Yvon Dichrographe III, with temperature-controlled cell holder. Readings were converted to units of mean residue ellipticity using the relationship: $[\theta] = 3299M_0\Delta A/c \cdot l$ deg $\cdot$ cm² $\cdot$ dmol⁻¹; where ΔA = difference in absorbance between left- and right-handed circularly polarised radiation, M_0 = mean residue M_r value, c = protein concentration (g/dm³) and l = path length (cm).

In order to observe the effect of substrates on its stability, PGK was dissolved (0.1 mg $\cdot$ ml⁻¹) in 10 mM Tris/100 mM NaCl/1 mM EDTA/0.001% 2-mercaptoethanol adjusted to pH 8.0 with HCl and containing a concentration of GdmCl such that the protein was partly unfolded as determined by CD [8]. 200 mM substrate was prepared in the same buffer at the same concentration of GdmCl. Aliquots of the substrate solution were added to 300 or 500 μ l volumes of the PGK solution. The samples were allowed to stand overnight before measurement because of the slow kinetics involved in equilibration.

Values of ellipticity were corrected for dilution and for the contribution of the substrate.

3. RESULTS AND DISCUSSION

Yeast PGK unfolds in GdmCl in a single cooperative transition with complete thermodynamic reversibility [8,10,11]. Detailed analysis shows that the two structural domains [1] unfold and refold independently, the C-terminal domain unfolding at a slightly higher concentration [8] (cf. similar behaviour for horse PGK [12]).

In the absence of substrate and in 0.7 M GdmCl yeast PGK exhibits 77% of the ellipticity at 222 nm of the native enzyme. On addition of PGA the ellipticity is steadily reduced until at between 1 and 2 mM PGA it equals 50% of the native ellipticity (fig.1). In apparent contradiction to this, in 0.8 M GdmCl, where the protein exhibits 30% of the native ellipticity, the addition of PGA brings about an increase in ellipticity, again to approx. 50% of the native value.

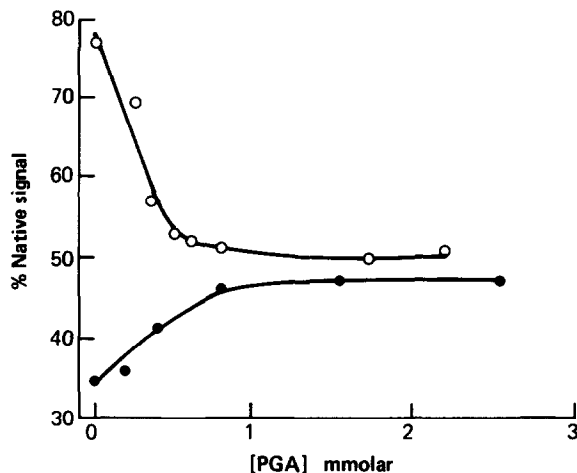


Fig.1. The effect of 3 PGA on the ellipticity of partially unfolded PGK. PGK was dissolved at 0.1 mg $\cdot$ ml⁻¹ in 10 mM Tris, 100 mM NaCl, 1 mM EDTA, 0.001% 2-mercaptoethanol adjusted to pH 8.0 with HCl. The buffer contained GdmCl at the stated concentration. PGA at 200 mM in the same solvent was added to the protein solution and the ellipticity measured at 222 nm at 20°C as in section 2. (●) 0.8 M GdmCl, (○) 0.64 M GdmCl.

The two structural domains possess similar secondary structure patterns so that the value of 50% native ellipticity corresponds to approx. 50% of the native secondary structure. Since the reversible unfolding of the enzyme is highly cooperative [8] and since the effect of added PGA clearly levels off above 1 mM, the 50% of native ellipticity is considered to correspond to a state with one domain intact and the other unfolded.

These observations can be reconciled by the model shown in fig.2. On the left-hand side are shown the major states involved in the reversible unfolding of PGK by GdmCl [8], the two intermediate states being transient. On the addition of PGA, the equilibria of the partially unfolded protein are shifted from the native and from the fully unfolded state to a state with 50% of the native conformation, i.e. to that intermediate state which is capable of binding PGA. Thus one domain is stabilised by PGA and the other destabilised. The former would be expected to possess the substrate-binding site and the latter must be the result of reduced mutual stabilisation of the two domains consequent on binding substrate. Thus the 'waist'

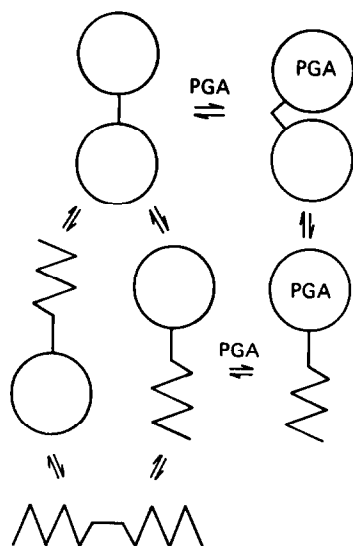


Fig.2. Unfolding and refolding equilibria of PGK: the effect of substrate. Folded domains of PGK are represented by circles, unfolded domains by zig-zag lines. The hinge region is bent (active) or straight (inactive).

region inter-domain contacts are being destabilised on binding PGA.

The consequences of adding ATP to partially unfolded PGK are shown in fig.3. In 0.7 M GdmCl, where the protein is 30% unfolded, an initial fall in ellipticity is again observed, followed by an increase to nearly 100% of the native ellipticity by 0.6 mM ATP. The initial reduction in folded structure by ATP is interpreted in the same way as for PGA, in that the binding of ATP to one domain reduces the mutual stabilisation of the domain by destabilising the waist region interactions.

It has been shown that PGK has two binding sites with different affinities for ATP [13] but it is not known on which domain the second site resides. If it is on the N terminal domain – the high-affinity site is on the C-terminal domain – binding of ATP at higher concentrations would be expected to stabilise both domains. Alternatively, if the second were on the C-terminal domain, binding to this site would have to result in a stabilisation of the waist region interactions with increased mutual domain stabilisation.

These results lead to the conclusion that there is

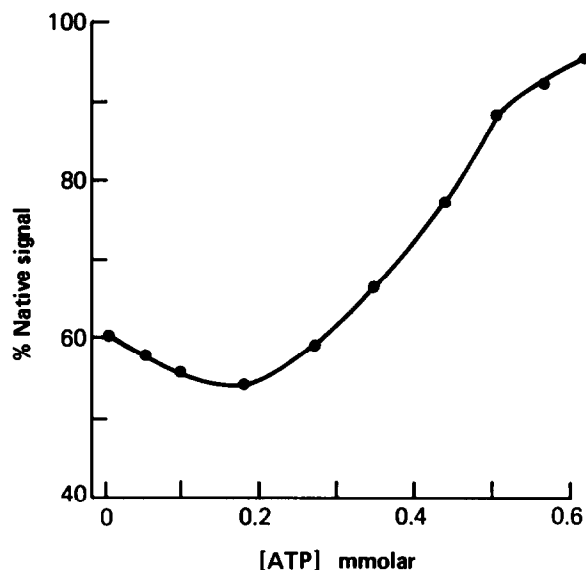


Fig.3. The effect of ATP on the ellipticity of partially unfolded PGK. PGK was dissolved at 0.1 mg·ml⁻¹ in the same buffer as in fig.1 containing 0.7 M GdmCl. ATP was dissolved in the same solvent. Values of ellipticity were read at 222 nm at 20°C.

mutual stabilisation of the two domains and that binding of either PGA or ATP destabilises the waist region between the domains of PGK. This supports a hinge-bending mechanism in which the enzyme is set free by bound substrates to explore other conformations, of which the closed or bent conformation, in which the substrates are sufficiently close to one another to allow phosphate transfer, is the most stable. Loss of products of reaction will allow the enzyme to regain its open conformation in which it is most accessible to fresh substrate.

The fact that higher concentrations of ATP which stabilise the enzyme against GdmCl denaturation also inhibit enzyme activity [14] further strengthens the proposal that the catalytic activity of PGK depends on substrate-induced destabilisation of the waist region to allow hinge binding. The effect of substrates in the hinge-bending mechanism is therefore 'permissive' rather than 'directive', consistent with the proposal of Watson et al. [1] who suggest that the binding of substrates induced a weakening of the His 338-Glu 190 interaction.

ACKNOWLEDGEMENT

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