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Sequence and structure of yeast phosphoglycerate kinase

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The structure of yeast phosphoglycerate kinase has been determined with data obtained from amino acid sequence, nucleotide sequence, and X-ray crystallographic studies. The substrate binding sites, as deduced from electron density maps, are compatible with known substrate specificity and the stereochemical requirements for the enzymic reaction. A carboxyl-imidazole interaction appears to be involved in controlling the transition between the open and closed forms of the enzyme.

Key words: crystal/kinase/sequence/structure/substrates

Introduction

Phosphoglycerate kinase (EC 2.7.2.3) is one of two enzymes required for ATP generation in glycolysis. All phosphoglycerate kinases, whether glycolytic gluconeogenic, or 'photosynthetic' (carbon fixation) are monomers with mol. wts. close to 45 000 (Scopes, 1973). The glycolytic, reaction catalysed by phosphoglycerate kinase (PGK) is that of the transfer of a phosphoryl group from the acyl phosphate of 1,3-diphosphoglycerate (1,3-DPG) to ADP thus forming ATP and 3-phosphoglycerate (3-PGA).

X-ray studies of the yeast (Bryant *et al.*, 1974) and horse-muscle (Blake and Evans, 1974) enzymes, have shown that they are structurally homologous; the principal structural feature being the occurrence of two widely separated domains of almost equal size. This bilobal feature of the enzyme together with the inferred binding position for 3-PGA has led to the suggestion (Banks *et al.*, 1979) that the domains move towards each other in order to complete the fully developed active site. Evidence obtained from n.m.r. (Tanswell *et al.*, 1976), low angle X-ray scattering (Pickover *et al.*, 1979), and sedimentation experiments (Roustan *et al.*, 1980) is consistent with the molecule undergoing a relatively large conformational change during catalysis.

High resolution X-ray studies of the yeast (Bryant *et al.*, 1978) and horse-muscle (Blake and Rice, 1981) enzymes have been reported but a detailed description of the structure of the yeast enzyme has had to await the determination of its amino acid sequence. The complete amino acid sequence reported in this publication is 65% identical with that of the horse enzyme (Banks *et al.*, 1979). The crystallographic results also reported here confirm that the enzyme's conformation has been highly conserved during its evolution. Indeed, the only significant difference in the results obtained from structural studies of the yeast and horse enzymes concerns the binding of substrates. In this paper we present evidence for the location of the 3-PGA and ATP binding sites and show how this information correlates with the reported biochemical, physical, and mechanistic properties of the enzyme.

[1]Dr Wendell worked on this project from its beginnings in 1969 until his untimely death in 1978. His Bristol colleagues would like to dedicate this paper to his memory.

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Experimental Methods

PGK was prepared from dried baker's yeast (*Saccharomyces cerevisiae*) by methods described by Scopes (1971). Cyanogen bromide cleavage of the pure enzyme yields four unique non-overlapping peptides. The longest peptide (173 residues) has an acylated serine residue and forms the major part of the amino-terminal domain. The amino acid sequence of this portion of the molecule has been deduced from the nucleotide sequence of the isolated gene (Dobson *et al.*, 1982). The carboxy-terminal portion of the molecule (residues 174 – 415) has been sequenced by manual and automated Edman degradation of the three smaller cyanogen bromide peptides and fragments derived from them by digestion with proteolytic enzymes (Perkins, 1980). All but residues 228 – 281 of the earlier primary structure results have been confirmed by an extension of the nucleotide sequence work. The complete sequence is shown in Table I. The mol. wt. (44 513) and amino acid composition derived from the sequence are close to previously published values (Larsson-Raznikiewicz, 1970). Details of the primary structure determination and its correlation with amino acid sequence data from other species will be published elsewhere.

Yeast PGK crystallizes from solutions (pH 7) containing ~2.5 M ammonium sulphate, in the space group C2 with one molecule in the asymmetric unit (Watson *et al.*, 1971). The low (6 Å) (Wendell *et al.*, 1972) and medium (3.5 Å) (Bryant *et al.*, 1974) resolution electron density maps were derived using conventional isomorphous replacement techniques employing two single site heavy atom derivatives. The phasing power of the gold ($KAuCl_4$) derivative does not continue much beyond 4 Å and it has been necessary to extend the resolution of the structure determination using the mercury derivative [$Hg(CH_3COO)_2$] alone. Six crystals were used to re-collect the medium resolution diffractometer data for the parent and for each derivative (Bryant, 1974). Only two crystals each of the parent and mercury derivative were needed to extend the data to 2.5 Å resolution using rotation camera techniques (Arndt *et al.*, 1973). The mean error in phase angle, expressed in terms of its cosine (figure of merit), is 0.87 for the reflections (strongest 60%) out to 3.5 Å resolution and 0.59 for those (80% of total set) between 3.5 and 2.5 Å resolution. The details of the X-ray work, including a description of the special steps that were taken to include the most accurate anomalous dispersion information in the phase calculations, will be reported elsewhere.

The electron density map calculated using the merged phase data is of high quality allowing a clear and unambiguous tracing of the polypeptide chain. Correlation of the

Table I. Primary structure of yeast phosphoglycerate kinase determined by a combination of amino acid and nucleotide sequencing techniques

												///	///	///	///	+++	+++	A	+++
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ser	Leu	Ser	Ser	Lys	Leu	Ser	Val	Gln	Asp	Leu	Asp	Leu	Lys	Asp	Lys	Arg	Val	Phe	Ile
+++								///	///	///	///					***	***	***	***
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Arg	Val	Asp	Phe	Asn	Val	Pro	Leu	Asp	Gly	Lys	Lys	Ile	Thr	Ser	Asn	Gln	Arg	Ile	Val
***	***	***	I	***	***	***	***	***	***	***						+++	B	+++	
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Ala	Ala	Leu	Pro	Thr	Ile	Lys	Tyr	Val	Leu	Glu	His	His	Pro	Arg	Tyr	Val	Val	Leu	Ala
								///	///	///	///					***	***	***	***
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Ser	His	Leu	Gly	Arg	Pro	Asn	Gly	Glu	Arg	Asn	Glu	Lys	Tyr	Ser	Leu	Ala	Pro	Val	Ala
***	II	***	***	***	***	***				+++	C	+++	+++						***
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Lys	Glu	Leu	Gln	Ser	Leu	Leu	Gly	Lys	Asp	Val	Thr	Phe	Leu	Asn	Asp	Cys	Val	Gly	Pro
***	***	***	III	***	***	***	***					+++	D	+++	+++				
101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
Glu	Val	Glu	Ala	Ala	Val	Lys	Ala	Ser	Ala	Pro	Gly	Ser	Val	Ile	Leu	Leu	Glu	Asn	Leu
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140
Arg	Tyr	His	Ile	Glu	Glu	Glu	Gly	Ser	Arg	Lys	Val	Asp	Gly	Gln	Lys	Val	Lys	Ala	Ser
	***	***	***	***	IV	***	***	***	***	***	///	///	///			+++	E	+++	
141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160
Lys	Glu	Asp	Val	Gln	Lys	Phe	Arg	His	Glu	Leu	Ser	Ser	Leu	Ala	Asp	Val	Tyr	Ile	Asn
									///	///	///	///							
161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
Asp	Ala	Phe	Gly	Thr	Ala	His	Arg	Ala	His	Ser	Ser	Met	Val	Gly	Phe	Asp	Leu	Pro	Gln
+++	F	+++		***	***	***	***	***	***	***	V	***	***	***	***	***	***	***	
181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200
Arg	Ala	Ala	Gly	Phe	Leu	Leu	Glu	Lys	Glu	Leu	Lys	Tyr	Phe	Gly	Lys	Ala	Leu	Glu	Asn
				+++	+++	G	+++	+++					///	///	///	***	***	***	***
201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220
Pro	Thr	Arg	Pro	Phe	Leu	Ala	Ile	Leu	Gly	Gly	Ala	Lys	Val	Ala	Asp	Lys	Ile	Gln	Leu
***	VI	***	***	***	***					+++	+++	H	+++	+++		***	***	VII	*
221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240
Ile	Asp	Asn	Leu	Leu	Asp	Lys	Val	Asp	Ser	Ile	Ile	Ile	Gly	Gly	Gly	Met	Ala	Phe	Thr
***	***	***		***	***	***	VIII	***	***	***						***	***	***	***
241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260
Phe	Lys	Lys	Val	Leu	Glu	Asn	Thr	Glu	Ile	Gly	Asp	Ser	Ile	Phe	Asp	Lys	Ala	Gly	Ala
***	***	***	IX	***	***	***	***	***	***	***	***				+++	I	+++	+++	
261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280
Glu	Ile	Val	Pro	Lys	Leu	Met	Glu	Lys	Ala	Lys	Ala	Lys	Gly	Val	Glu	Val	Val	Leu	Pro
	+-+	+-+	J	+-+	+-+								+-+	+-+	K	+-+	+-+		
281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300
Val	Asp	Phe	Ile	Ile	Ala	Asp	Ala	Phe	Ser	Ala	Ser	Ala	Asn	Thr	Lys	Thr	Val	Thr	Asp
		///	///	///	///	///	///	///						***	***	***	***	X	*
301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320
Lys	Glu	Gly	Ile	Pro	Ala	Gly	Trp	Gln	Gly	Leu	Asp	Asn	Gly	Pro	Glu	Ser	Arg	Lys	Leu
***	***	***	***	***						+++	L	+++						///	///
321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
Phe	Ala	Ala	Thr	Val	Ala	Lys	Ala	Lys	Thr	Ile	Val	Trp	Asn	Gly	Pro	Pro	Gly	Val	Phe
///	///	///	///	///			***	***	***	***	***	***	XI	***	***	***	***	***	///
341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360
Glu	Phe	Glu	Lys	Phe	Ala	Ala	Gly	Thr	Lys	Ala	Leu	Leu	Asp	Glu	Val	Val	Lys	Ser	Ser
///	///				+++	M	+++				***	***	***	***	XII	***	***	***	***
361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380
Ala	Ala	Gly	Asn	Thr	Val	Ile	Ile	Gly	Gly	Gly	Asp	Thr	Ala	Thr	Val	Ala	Lys	Lys	Tyr
	///	///	///	///		+++	N	+++				***	***	***	***	XIII	***	***	***
381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400
Gly	Val	Thr	Asp	Lys	Ile	Ser	His	Val	Ser	Thr	Gly	Gly	Gly	Ala	Ser	Leu	Glu	Leu	Leu
***					***	***	XIV	***	***										
401	402	403	404	405	406	407	408	409	410	411	412	413	414	415					
Glu	Gly	Lys	Glu	Leu	Pro	Gly	Val	Ala	Phe	Leu	Ser	Glu	Lys	Lys					

The residues forming helices (***), parallel (+ + +) and anti-parallel (+ − +) beta sheets and beta turns (///) are overscored with distinguishing markers and labeled in accordance with Figure 1. Residues underlined are the same in the horse (Banks *et al.*, 1979) and human (Huang *et al.*, 1980) PGK sequences.

side chain information, implicit in the map, with the amino acid sequence data shows excellent agreement over the entire molecule. Tertiary structure information has been recorded from a skeletal model (scale 2 cm/Å) built using a Richard's box (Richards, 1968) and the complete, regularised, coordinate data set deposited with the Brookhaven Protein Data Bank.

Molecular Structure

The protein folds into a bilobal structure in which two domains of similar size are connected by a narrow waist region through which the chain passes twice as is shown in Figure 1. Each domain consists of a core of six parallel strands of β-pleated sheet surrounded by helices which are connected with the β-sheet strands by β turns and sections of irregular structure. The connections between sheet strands in each domain are different (CDBAEF and IHGLMN). The polypeptide chain forms most of one domain before traversing the waist region to form the whole of the second domain. The 12 carboxyl-terminal residues cross the waist region to complete the amino-terminal domain.

Readers wishing to compare the primary and secondary structures of the yeast and horse enzymes will find that the paper published by Banks *et al.* (1979) describing the horse enzyme contains information similar to that presented in Table I and Figure 1 of this paper. Apart from a problem of hydrogen bond formation for a third strand of anti-parallel sheet (residues 310–312), the absence of two short helical units (between residues 165 and 178) and a very pronounced turn (145°) between helices VII and VIII (one long helix in the horse enzyme) there appear to be few significant differences at the secondary structure level.

Several parts of the general tertiary structure are worthy of special note. The residues from 210 to 212 (212 is alanine), 234 to 236, 369 to 371, 392 to 394 are consecutive glycine residues. These rather unusual, side chain-free regions of the polypeptide chain are conserved in known PGK sequences and presumably have special mechanistic significance (see below). Banks *et al.* (1979) have suggested that an Ala-Gly-Gly sequence might be important for domain movement in the horse enzyme. The equivalent residues (182–184) in the yeast enzyme are Ala-Ala-Gly. The same authors have also discussed the probable mechanistic significance of the numerous basic residues facing the adenosine phosphate binding position in the horse structure. Because of its special interest, we show the equivalent portion of the yeast structure in Figure 2. The grouping of the side chains of histidines 62 and 170 and of arginine 21 is very similar to that found at the active site of yeast phosphoglycerate mutase (Winn *et al.*, 1981). Although both enzymes bind 3-PGA the reactions catalysed are very different. The mutase forms a phosphoryl intermediate (Rose, 1980) whereas the kinase does not (Johnson *et al.*, 1976).

Crystal Soaking Experiments

Difference Fourier maps calculated using data collected from crystals soaked in Mg-ADP and Mg-ATP (10 mM) revealed the positions for the adenine and phosphate binding sites but showed little density between these two locations suggesting that the ribose ring was not correctly located (Bryant *et al.*, 1978). The Mg-ATP showed little extra density

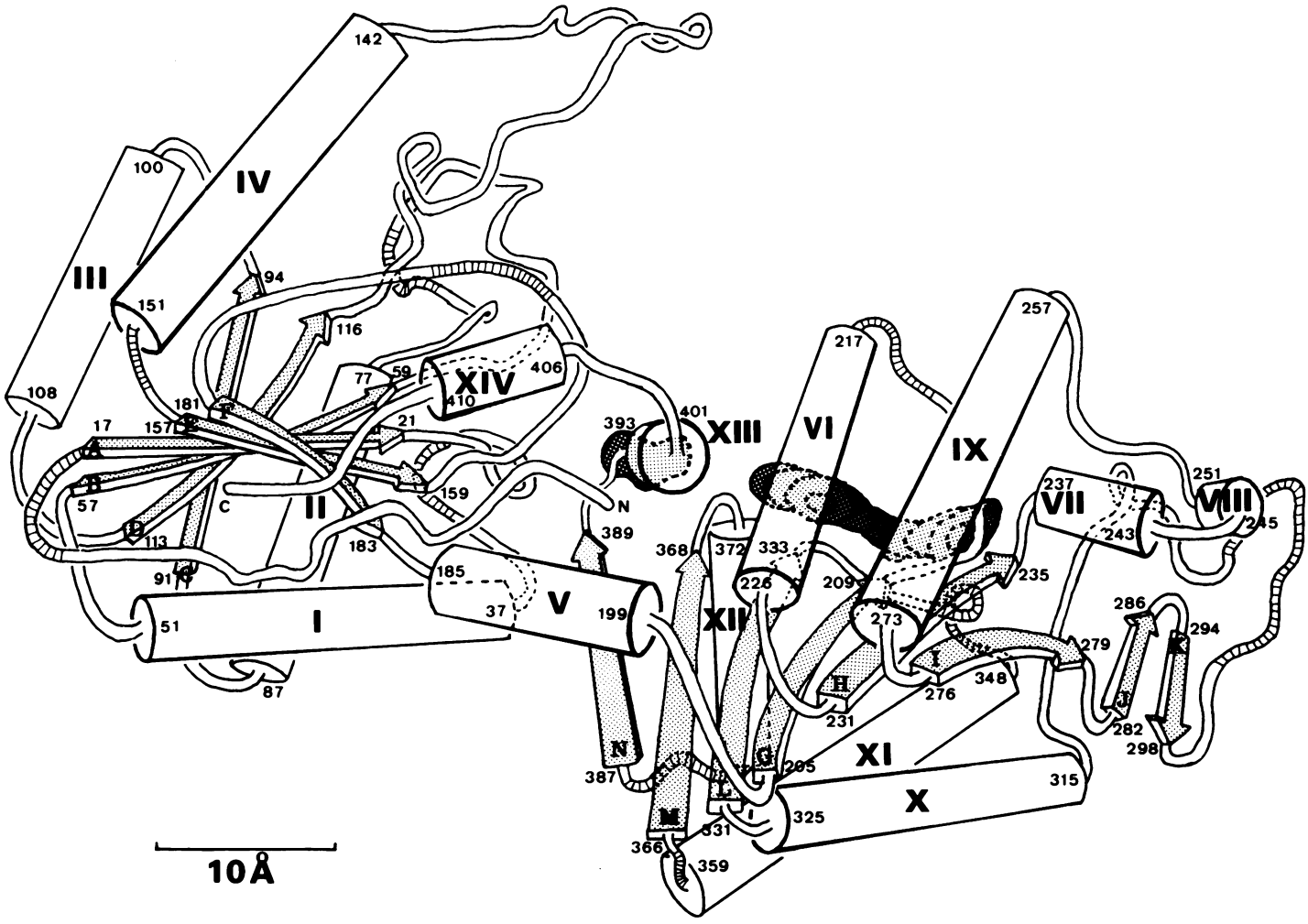

Fig. 1. A schematic drawing of the structure of the open form of the yeast PGK molecule. The helices are denoted by cylinders and the sheet strands by arrows. The view point of the drawing is from the solvent looking towards the substrate binding region through the protein matrix. The binding position for Mg-ATP is shown behind helices VI and IX. The 3-PGA binding site is behind helix XIII.

in the phosphate peak when compared with the equivalent Mg-ADP result suggesting that, under the conditions used, the terminal phosphoryl group had not taken up a definite position. Difference maps, calculated to locate the metal ion, showed more density in the phosphate peak when manganese was used in experiments with ATP and ADP (Bryant, 1974).

Direct comparison of peaks in difference electron density maps with those found in the native Fourier map suggested that, in all the soaking experiments, no more than 20% occupancy had been achieved. Increasing the nucleoside phosphate concentration in the soaking experiments did not appear to improve the overall site occupancy or the density associated with the ribose and terminal phosphoryl groups. Concentrations of the nucleoside phosphates much above 20 mM resulted in either a loss of crystallinity or disintegration of the soaking crystal. Crystals could not be grown when either Mg-ATP or Mg-ADP (up to 10 mM) was present.

Solution studies designed to examine the effects of anions on enzyme activity (Scopes, 1978) have suggested that a sulphate ion binds in the active site of yeast PGK. Difference Fourier maps calculated using data collected from crystals grown in ammonium sulphate and then transferred to vials containing ammonium selenate showed one large peak almost coincident with the phosphate peak observed in the ATP difference maps (Bryant *et al.*, 1978). Adenosine phosphate binding experiments were therefore carried out at different hydrogen ion concentrations in an effort to find the conditions where the inhibitory sulphate ion would have least effect on the binding of the phosphate moiety. A difference Fourier map, calculated using data collected from crystals soaked in mother liquor at pH 9 containing Mn-adenylyl β,γ-imidodiphosphate (Mn-AMP-P-N-P) at 10 mM and 3-PGA at 200 mM concentrations, showed continuous density for the whole of the nucleotide substrate including the ribose ring and γ-phosphate (see Figure 3). Presumably, the stronger phosphoryl binding at the higher pH allows the γ-phosphate to take up its correct position at the amino end of helix XII thus enabling the ribose group to assume the location where it makes hydrogen bonds through its hydroxyls. Although 3-PGA had been added to the soaking solution, and the precaution taken of using the imido-phosphate analogue of ATP to prohibit the formation of 1,3-DPG, no electron density attributable to 3-PGA was observed in the difference Fourier map.

The ATP Binding Site

The model of yeast PGK shows that the adenine group binds in a marked depression in the enzyme surface. The adenine nitrogen (N6) forms a hydrogen bond with the main chain carbonyl group of residue 311 and sits on top of an

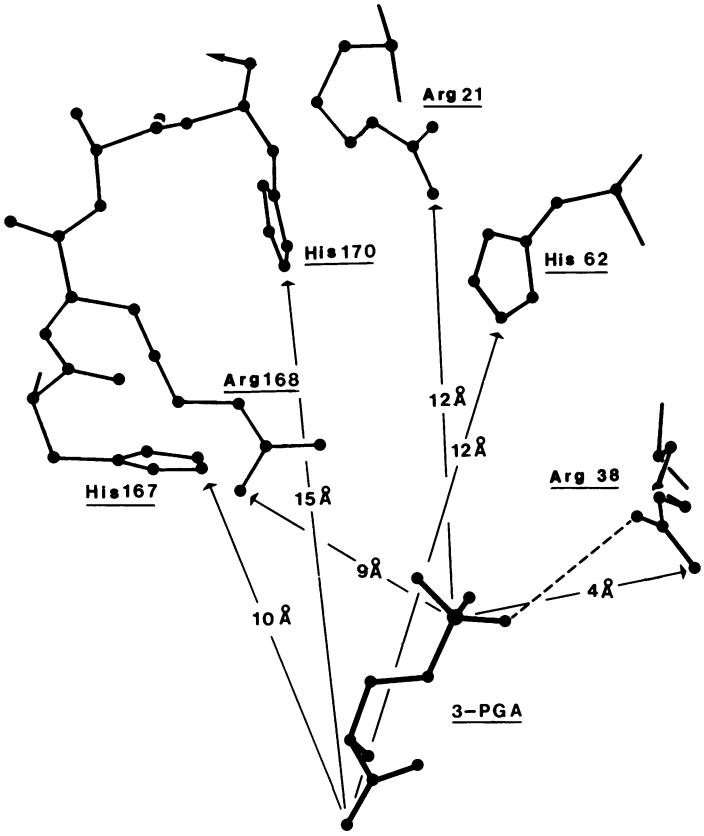

Fig. 2. A computer drawing of the basic residues which form much of the surface of the amino-terminal domain as seen from the adenosine phosphate binding site. Distances from the triose substrate (3-PGA) relate to the open form of the enzyme.

obligatory glycine residue (338). Glycine 211 and the aliphatic side chains of leucine 311 and valine 339 define the boundaries of the adenine binding hollow. The ribose ring lies in a shallow depression between a main chain peptide unit (338) and a proline side chain (336) with the 2′ hydroxyl forming a hydrogen bond with the glutamate side chain 341 and the 3′ hydroxyl making a similar bond with the side chain of aspartate 372. The α and β phosphates lie along the main chain and above an obligatory glycine (335). The γ-phosphate crosses from the main chain to the amino end of helix XII where it makes hydrogen bonds with the main chain nitrogen atoms of residue 372 (see Figure 4b) and probably 371. With the phosphoryl groups positioned in this way there remains considerable density in the difference map for the metal ion. The interpretation of the 'phosphate peak' that gives the best overall fit places the manganese atom 3 Å from the γ-phosphate and 5 Å from both the α- and β-phosphates. In this position the metal ion is linked with the hydroxyl of the ribose by hydrogen bonds through the carboxyl group of aspartate 372. Neighbouring residues appear to restrict the movement of this carboxyl group suggesting that it must also interact with the metal ion when Mg-ADP binds to the enzyme (see Tanswell *et al.*, 1976).

When the phosphoryl transfer occurs following domain movement it seems probable that one of the arginine side chains found protruding towards the adenosine phosphate binding site from the amino domain (see Figure 2) is better placed than the metal ion to stabilize the charge on the developing transition state. Presumably the metal ion moves towards the α- and β-phosphates in parallel with the formation of 1,3-DPG thus reducing the probable charge stabilization roles of the lysines 213 and 217 (see Figure 3).

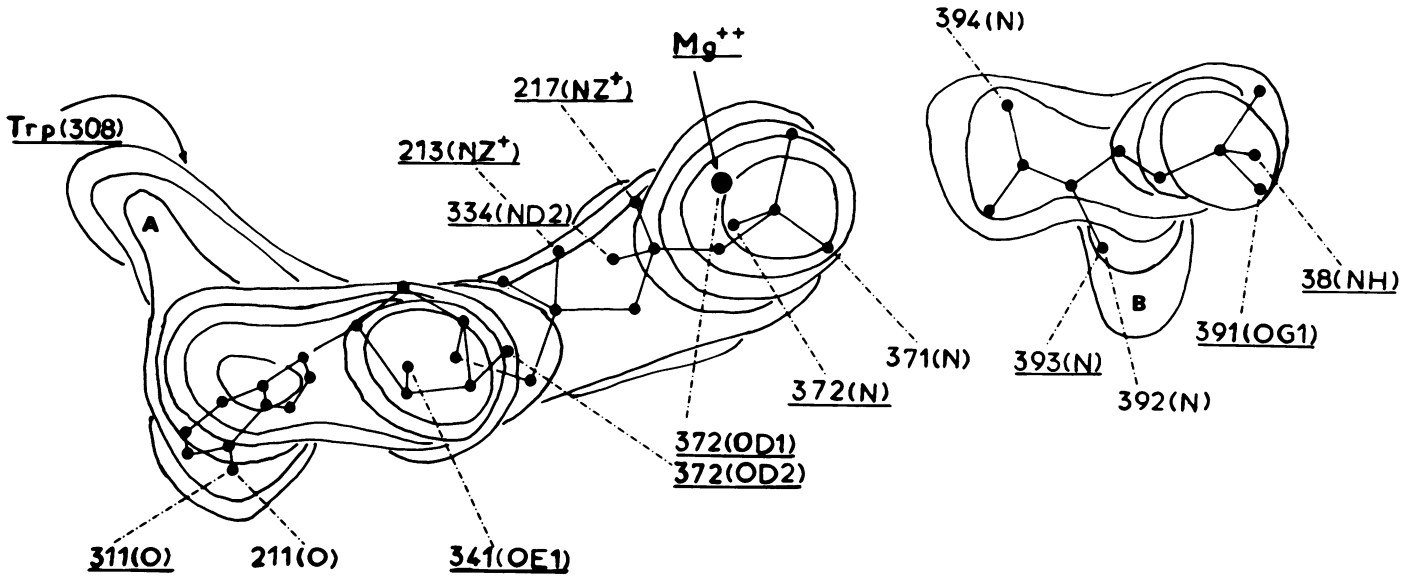

Fig. 3. The electron density associated with the Mg-ATP and 3-PGA binding sites with appropriate models superimposed. Only the lowest contour levels are shown. Density peaks associated with the β-phosphoryl group and parts of the ribose are masked by contour levels nearer the point of view which is from the solvent. Contours for the Mg-ATP site are taken from a 4 Å resolution difference Fourier map calculated using Mn-AMP-P-N-P and those for 3-PGA from the equivalent sections of the original Fourier map (for explanation see text). The electron density bulge marked A is associated with the indole ring of tryptophan 308 which moves into a position adjacent to the adenine ring when ATP is bound to the enzyme. The bulge in the 3-PGA density, marked B, is associated with a helical portion of the peptide chain which helps align the substrate and makes a hydrogen bond with the 2-hydroxyl. As built into the density, the ribose is in the 2′ endo conformation and the glycocidic and exocyclic linkages are anti ($\chi \sim 100°$) and trans, gauche ($\psi \sim 180°$) respectively. Possible interactions between substrates and protein are indicated by broken lines. The residue numbers and atom names follow the usual conventions and correspond to those deposited with the Data Bank.

3-PGA Binding

Experiments with yeast PGK crystals soaked in mother liquor at pH 5, 7, and 9 containing 3-PGA at varying concentrations up to 200 mM failed to reveal any evidence of triose binding (Bryant *et al.*, 1978). Crystals which grew in the presence of 200 mM 3-PGA were identical to crystals grown under the same conditions but without 3-PGA. From these experiments, it was concluded that the triose substrate could not bind either because its site was not fully developed in the open form of the structure, or that its binding site was already occupied by 3-PGA or a molecule which could not be displaced by high concentrations of 3-PGA. The fact that crystals could be grown in the presence of high concentrations of 3-PGA but not Mg-ATP appeared to suggest that the nucleoside phosphate alone caused the conformational change between the open and closed forms of the enzyme. This seemed improbable since it appeared more logical to assume that the enzyme would be able to form a solvent-inaccessible site if the molecule closed after binding both substrates. The second possibility, that the 3-PGA site was already occupied in native crystals of PGK, seemed more plausible. In this situation only those molecules with no bound triose (see section dealing with adenosine phosphate soaking experiments) would take up Mg-ATP without inducing strain in the crystal lattice. The only problem with this argument appeared to be the source of a 3-PGA-like molecule in our enzyme preparations.

The crystals used in this study were grown from enzymes prepared using large amounts of lactic acid buffer (Scopes, 1971). Noting that some of this buffer would be metabolised by the gluconeogenic enzymes released into solution by cell lysis, crystals were tested for the presence of 3-PGA on the assumption that, if present originally, 1,3-DPG would be hydrolysed before or during exposure to X-rays. Assay experiments designed to detect 3-PGA at concentrations equivalent to that of the enzyme proved inconclusive but n.m.r. experiments showed that similar enzyme preparations contained significant amounts of organic phosphate (single phosphoryl peak) before, but not after, exhaustive dialysis. This correlates with the finding of Johnson *et al.* (1976) that preparations of yeast PGK can contain contaminating amounts of a triose substrate. Conroy *et al.* (1981) have since shown that active site arginine residues react only slowly with phenylglyoxal unless PGK is first incubated with the enzyme and cofactors which are required to convert 3-PGA to glyceraldehyde-3-phosphate. All these results are most easily interpreted if PGK binds 3-PGA, or a molecule which hydrolyses to 3-PGA (the dissociation constant for 1,3-DPG is <0.1 μM, see Scopes, 1978), at one of the preparative stages and that it remains bound during subsequent procedures.

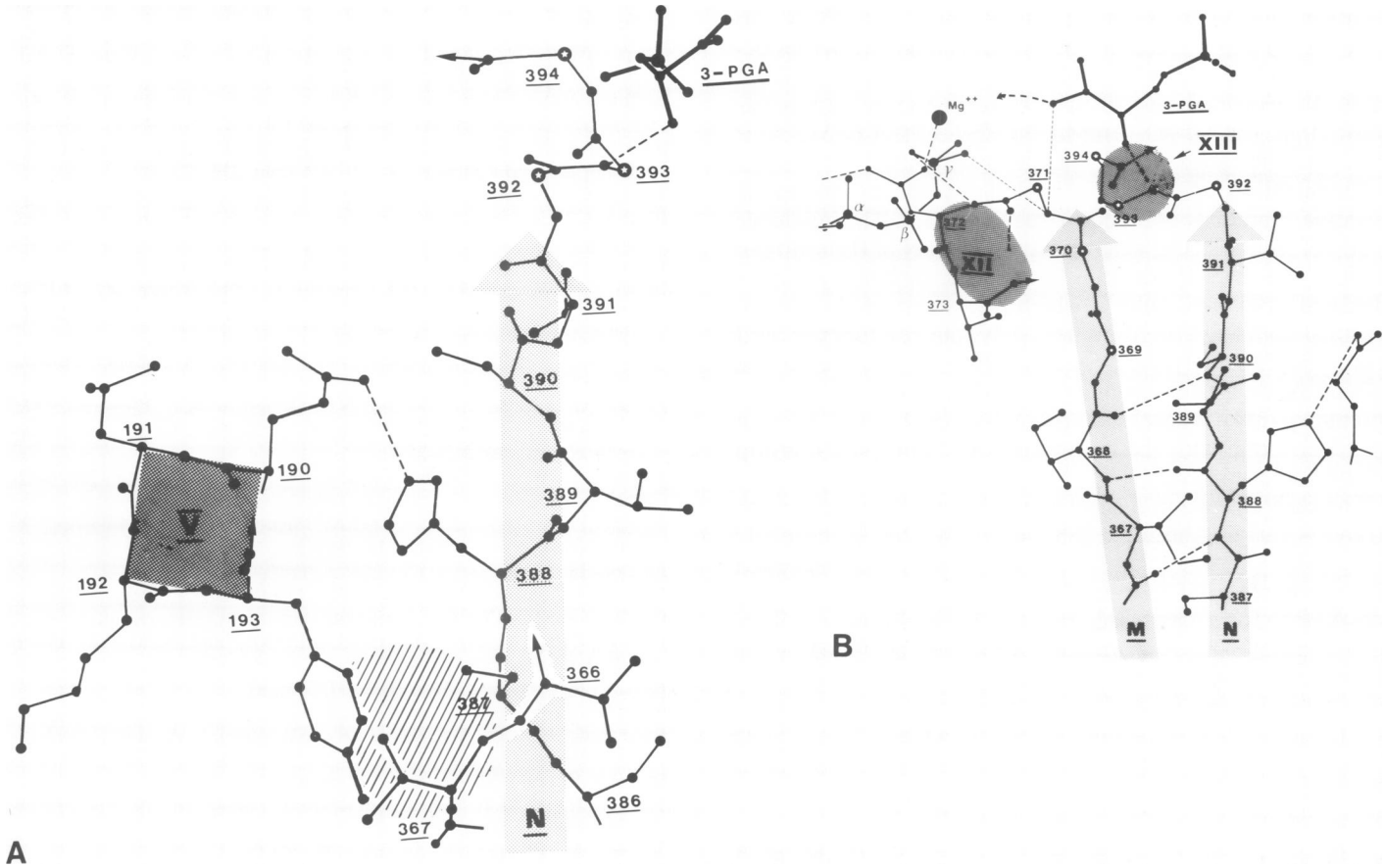

Fig. 4. Computer drawings of structural features associated with the waist region of the molecule. The sterically significant glycine residues are shown with distinguishing atom markers. Hydrogen bonds are represented by bold broken lines. Less prominent broken lines indicate hydrogen bonds which could be formed following minor positional change. The shaded arrows indicate β sheet strands. **(a)** View looking down helix V (shaded) showing the interaction between the carboxyl group of glutamate 190 and the imidazole group of histidine 388. The cross striped region (van der Waals radius) adjacent to tyrosine 193 indicates that there is insufficient room between threonine 367 and serine 387 for a nitrate group bonded to the ϵ carbon of the aromatic side chain. **(b)** The positions of the adenosine phosphates, the metal ion, and 3-PGA are shown relative to strands M and N of the carboxyl domains central β sheet. The histidine/glutamate interaction is included to serve as a reference point for comparing the two parts of this figure. The exposed amino ends of helices XII and XIII are shown shaded to emphasise their role in substrate binding.

With the availability of the yeast PGK sequence we have now been able to look at the high resolution Fourier map for unexplained electron density peaks. The peak shown in Figure 3 is of the correct size and shape for it to represent a 3-PGA molecule. When skeletal models are positioned into the density the 2-hydroxyl forms a hydrogen bond with a main chain nitrogen (393) at the amino end of helix XIII and one of the carboxyl oxygens is 4 Å from, and in a suitable position to react with, the γ-phosphoryl of the ATP molecule (see Figure 4b). The electron density peak, and its interpretation as shown in Figure 3, explains in a very simple way both the absolute requirement for the D-triose and the in-line mode of phosphoryl transfer (Webb and Trentham, 1980). If the orientation of the triose substrate is altered slightly by the movement of the amino-terminal domain then the non-attacking carboxyl oxygen could make a hydrogen bond with a main chain nitrogen (394) located at the amino end of helix XIII. In this position the triose substrate is maximally bonded to the protein and in van der Waals contact with the three glycine residues from residues 392 to 394.

The Trigger Mechanism

The simplest interpretation of the crystallographic results would suggest that the presence of both Mg-ATP and 3-PGA is required for domain movement. This is in agreement with the conclusions of Pickover *et al.* (1979) and of Roustan *et al.* (1980) who have used low angle X-ray scattering and sedimentation equilibrium measurements, repectively, to show that a large change in the hydrodynamic properties occurs on adding Mg-ATP and 3-PGA to substrate-free enzyme. Moreover, the use of these quite diverse techniques has shown that, for the yeast enzyme, neither 3-PGA or Mg-ATP alone produce a significant change in structure.

Examination of the waist region reveals an interesting histidine/glutamate interaction (see Figure 4a). The imidazole group of histidine 388, extends from strand N of the central β sheet of the carboxyl domain towards the carboxyl group of glutamate 190 from helix V which forms part of the amino-terminal domain. This histidine is only four residues removed from the triple glycine sequence (392 – 394) close to the 3-PGA binding site and only two residues removed from a threonine (391) whose side chain hydroxyl could form a hydrogen bond with the non-transferable phosphoryl group of the triose substrate.

The carboxyl end of β sheet strand M, which interacts with strand N carrying histidine 388, approaches the γ-phosphate site of Mg-ATP (see Figure 4b). It has already been suggested that the nitrogen of residue 371 probably moves on binding Mg-ATP to form a hydrogen bond with the incoming γ-phosphoryl group. This conformational change, facilitated by the triple glycine sequence from residues 369 to 371, would then transmit its effect to the adjacent sheet strand N. It is proposed, therefore, that the cumulative effect on the β sheet strands M and N, when the second substrate binds, weakens the histidine/glutamate interaction thus initiating domain movement. What evidence is there for this proposal?

Bachrach *et al.* (1977) have shown that the nitration of a single tyrosine residue causes almost complete loss of activity. This result is at first sight somewhat surprising since all seven tyrosine residues are remote from the active site region. The published peptide sequence data indicates that the nitrateable tyrosine is located three residues away from glutamate 190 and is in such a position that the additional atoms would interfere with the location of helix V relative to the carboxyl-terminal domain as is shown in Figure 4a. It seems probable, therefore, that the nitration of tyrosine 193 has the effect of precluding the histidine/glutamate interaction thus hindering the movement between the open and closed forms of the enzyme.

A tyrosine residue has also been implicated (Meyer and Westhead, 1976) as being involved in the enzyme activation which occurs at low salt concentrations. It now seems probable that the change in the environment of tyrosine 193 reflects the movement which occurs when ions bind to the exposed δ-nitrogen of histidine 388. Such an ionic interaction would lead to a polarisation of the imidazole ring followed by the domain movement necessary to expose the otherwise buried carboxyl group of glutamate 190. Activation is thus simply explained by a weakening of the switch mechanism and the concomitant formation of a more substrate-responsive enzyme. Experiments are currently underway to test these ideas and to determine the conditions for crystallising the closed form of yeast PGK for further crystallographic studies.

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