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Glucose-6-phosphate isomerase

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Glucose-6-phosphate isomerase (EC 5.3.1.9) is a dimeric enzyme of molecular mass 132 000 which catalyses the interconversion of D-glucose-6-phosphate and D-fructose-6-phosphate. The crystal structure of the enzyme from pig muscle has been determined at a nominal resolution of 2.6 Å. The structure is of the α/β type. Each subunit consists of two domains and the active site is in both the domain interface and the subunit interface (P. J. Shaw & H. Muirhead (1976), *FEBS Lett.* **65**, 50–55). Each subunit contains 13 methionine residues so that cyanogen bromide cleavage will produce 14 fragments, most of which have been identified and at least partly purified. Sequence information is given for about one-third of the molecule from 5 cyanogen bromide fragments. One of the sequences includes a modified lysine residue. Modification of this residue leads to a parallel loss of enzymatic activity. A tentative fit of two of the peptides to the electron density map has been made. It seems possible that glucose-6-phosphate isomerase, triose phosphate isomerase and pyruvate kinase all contain a histidine and a glutamate residue at the active site.

Introduction

Glucose-6-phosphate isomerase (or phosphoglucose isomerase (PGI)) plays a central role in the metabolism of phosphorylated sugars, and phosphoglucose isomerase deficiency has an effect on the metabolism of red blood cells, giving rise to haemolytic anaemia (Paglia & Valentine 1974). The enzyme is an aldose–ketose isomerase, which catalyses the reversible interconversion of D-glucopyranose-6-phosphate and D-fructofuranose-6-phosphate by promoting the intramolecular transfer of a proton between C-1 and C-2. The properties of the enzyme have been reviewed by Noltmann (1972) and Rose (1975).

PGI has been isolated from a wide variety of sources. It is present in large excess compared with the rates of metabolic flux and has long been considered to be a bifunctional enzyme of glycolysis and gluconeogenesis so that tissue-specific enzymes have not evolved. However, it has been shown that tissue-specific isoenzymes do occur in catfish and conger (Mo *et al.* 1975). PGI is present in multiple forms in pig muscle and the relative distributions of three isoenzymes vary depending upon the animal species from which the enzyme is isolated. In addition, both genetic and nongenetic factors have been postulated to be responsible for the polymorphism of glucose phosphate isomerases from numerous origins with no clear evolutionary or taxonomic patterns as to when either or both kinds are to be expected. For the pig muscle PGI the multiplicity is now attributed to genetic causes (Gee & Noltmann, unpublished experiments). It has also been established unequivocally that the enzyme species being subjected to X-ray diffraction analysis represents isoenzyme III (Gee *et al.* 1980), and sequence

work currently under way is performed on that same enzyme species. PGI is specific for a single substrate–product pair. The enzyme is composed of two identical subunits each of molecular mass 66000. The dimer contains two identical active sites and shows no interaction between them.

Proposals for mechanism of action

PGI has no requirement for cofactors or other bound molecules or ions, it is a highly efficient enzyme with an equilibrium constant close to unity, and the high rates of proton transfer imply that solvent is not involved in the mechanism. The turnover number is about

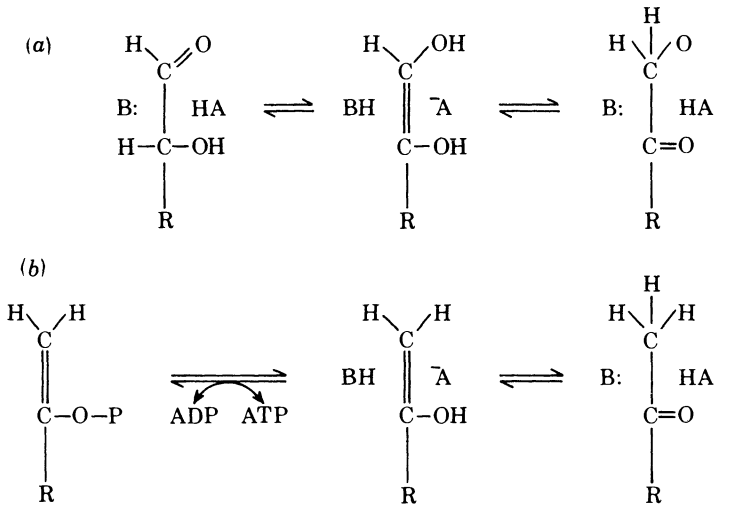


Figure 1. Postulated mechanism via a *cis*-enediol intermediate for (*a*) phosphoglucose isomerase and triose phosphate isomerase, and (*b*) pyruvate kinase. See Rose (1975).

1000 catalytic events per second. The evidence for the mechanism for the base-catalysed interconversion of aldoses and ketoses via a *cis*-enediolate anion has been reviewed by Rose (1975) (figure 1). Functional catalytic groups would be required to open the ring, to polarize the carbonyl group present in the open chain form of the substrates, and to transfer the proton between C-1 and C-2. A general base at the active centre of the enzyme is thought to transfer stereospecifically a carbon-bound proton of the substrate between C-1 and C-2. Because the isomerization is catalysed by a protein-bound base, proton abstraction and addition must occur on the same side of the enediol intermediate and only a *cis*-enediol will give the correct products. The base involved has no exchangeable protons and hence could be either a carboxylate or the non-protonated nitrogen of an imidazole. It seems likely that an electrophilic group (AH) would be present to polarize the carbonyl group and to stabilize the enolate ion. Ring opening requires an acid group on the opposite side of the enol plane from the proton-transferring base.

Kinetic data suggest that two ionizable groups in the enzyme with average pK values of 6.75 and 9.3 are involved in catalysis. Dyson & Noltmann (1968) have proposed a mechanism involving a protonated lysine and a non-protonated imidazole in which the enzyme first catalyses the ring-opening step and then the isomerization via the enediolate intermediate.

O'Connell & Rose (1973) have shown that the epoxide 1,2-anhydro-D-mannitol-6-phosphate inactivates PGI and becomes covalently bound in an ester linkage to a glutamate residue. In yeast PGI the sequence around this labelled glutamate is either Asp-Ser-Glu or Ser-Glu-Asp. Neither of these sequences occur in the peptides of known sequence from pig muscle PGI (tables 4 and 5). O'Connell & Rose suggest that this glutamate is the base that participates in the isomerization via the *cis*-enediolate. Chirgwin & Noltmann (1975) suggest that the acidic residue that polarizes the substrate carbonyl and thus enhances enediolate formation could be an arginine side chain.

PGI is inactivated by the chemical modification of lysine residues. The dimer binds two equivalents of pyridoxal-5′-phosphate (Bruch *et al.* 1973). Thus the dimer possesses two catalytic sites and the modification of one specific lysine residue per active site destroys catalytic activity.

The enzyme substrates exist as a mixture of cyclic configurations, α and β pyranoses and α and β furanoses, equilibrated via acyclic configurations (aldehydes and ketones respectively). PGI can use and produce both the α and β anomeric forms; however, the β-anomers react at a slower rate. It has been suggested (Schray *et al.* 1973; Plessor *et al.* 1979) that both anomers of each substrate react at the same enzyme site but that rotation must take place about a C–C single bond for the β-anomers to arrive at the same enzyme-bound intermediate as the α-anomers. After proton abstraction, the planar enediol cannot rotate. This may play an important role in coupling enzymes with different anomeric specificity for substrate.

X-ray analysis at 3.5 Å resolution

Low-resolution X-ray analysis of PGI (Muirhead & Shaw 1974; Shaw & Muirhead 1977) confirmed that the dimer of molecular mass 132000 consists of two crystallographically identical subunits. At 3.5 Å resolution it was possible to trace the course of a considerable portion of the polypeptide chain, although there were several ambiguous regions. A total of 514 amino acid residues were included in each subunit, which would correspond to a molecular mass for the dimer of about 113000. Each subunit comprises a large domain and a small domain. The large domain contains a six-stranded parallel β-sheet surrounded by α-helices. The small domain contains a smaller, less regular, β-structure surrounded by helices and an irregularly folded chain. The stable core structure is consistent with the high stability of muscle PGI, which requires rigorous conditions for complete denaturation. The active site was located by binding the open-chain competitive inhibitor 6-phosphogluconate and the enediolate analogue 5-phosphoarabinonate (Chirgwin & Noltmann 1975). Both inhibitors gave similar results, showing that each active site lies both in the domain interface and in the subunit interface. Thus each active site is composed of part of each subunit. The two binding sites are well separated by rigid secondary structure. Significant conformational changes take place when the inhibitors bind.

The phosphate group, which is essential for binding, is tightly bound to the enzyme. The binding site could be either a loop in the main chain or two long positively charged side chains. There is density that could represent a bound sulphate ion in the electron density map. The mode of binding of 5-phosphoarabinonate suggests that glucose-6-phosphate would bind as the chair conformer with substituents C-2 to C-5 equatorial (the C1) conformer. A large side chain is close to the ring oxygen and a second side chain is close to the C-1 and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

C-2 hydroxyls. This could be the glutamate residue identified by Rose (O'Connell & Rose 1973). These two side chains plus the groups involved in phosphate binding are all in the same subunit. Three more side chains in the other subunit are brought into position close to the transition state analogue by means of the conformational change in the enzyme. Two of these align with the C-3 and C-4 hydroxyls. It is known that C-3 and C-4 of the substrate are involved in hydrogen bonding to the enzyme and that the C-3 hydroxyl maintains a rigid conformation throughout the catalytic cycle (Bessell & Thomas 1973). The third side chain is on the correct side of the substrate to act as the base that transfers the proton between C-1 and C-2. Using this information, Shaw & Muirhead (1976) proposed a possible mechanism in which the carboxylate, together with the electrophilic group near the ring oxygen, promotes the ring-opening step, the base on the other subunit transfers the proton via the *cis*-enediolate intermediate, and the ring-closure step is essentially the reverse of the ring-opening step. This mechanism implies that the phosphate group and the hydroxyl groups on C-3 and C-4 are involved in binding and that two bases (a carboxylate plus another) and an electrophilic group are necessary for catalysis. The mechanism has been presented above as a sequential mechanism. However, since the overall rate is fast it could be considered as a concerted one. The binding of the substrate and the resulting conformational change in the enzyme would shield the carboxylate group from water and enhance the effect of its negative charge. A charge relay system similar to that found in the proteolytic enzymes could exist (Blow *et al.* 1969). In this tentative mechanism three catalytic groups are involved and the active site has the correct stereochemistry to satisfy the known specificity of PGI.

The proposal that the active site of PGI is created by the association of subunits is consistent with the results of experiments on matrix-bound PGI from rabbit muscle (Bruch *et al.* 1976). Dimeric PGI can be immobilized by reaction with Sepharose 4B activated with cyanogen bromide. Its catalytic parameters are not affected. Immobilized monomers will bind substrate but possess no catalytic activity. However, these subunits will react with soluble subunits to give catalytically active dimers. If the soluble subunits are inactivated with pyridoxal-5′-phosphate they will hybridize with and induce catalytic activity in matrix-bound monomers, but only half the sites are active. These experiments suggest that the binding of the dimeric enzyme to the matrix occurs through one subunit only, that the dimer is necessary for catalytic activity, and that the two active sites per dimer are independent.

X-ray analysis at 2.6 Å resolution

X-ray diffraction patterns were recorded photographically with an Arndt–Wonacott rotation camera (Arndt *et al.* 1973). Data were collected for native PGI and a mercurial derivative (Muirhead & Shaw 1974). Phases were determined from seven data sets by using the method of single isomorphous replacement with anomalous scattering. The value of E/f for the data sets varied between 0.40 and 0.52 and the average figure of merit varied between 0.64 and 0.77 (Muirhead *et al.* 1967). Data from the previous 3.5 Å structure determination were included in the calculation of the final electron density map by merging phases (Stuart *et al.* 1979). The average observed deviation from the mean for multiply measured phases was 45.9°. A total of 15146 reflexions was included and this is about 87% of the total number of independent reflexions in the 2.6 Å sphere.

The resulting electron density map was plotted in sections perpendicular to the crystallo-

graphic z axis. In many areas the map showed considerably more detail than the 3.5 Å map. In particular, many side chains were visible in more detail (figure 2). Since the high-resolution phases are determined from only one isomorphous derivative, the noise level is high and there are some problems in following the course of the polypeptide chain. However, it seems clear that the connectivity of the chain, at least in the small domain, is not correct in the model built from the 3.5 Å map. Extra density has appeared in one or two regions that will account for some of the missing residues in the 3.5 Å model.

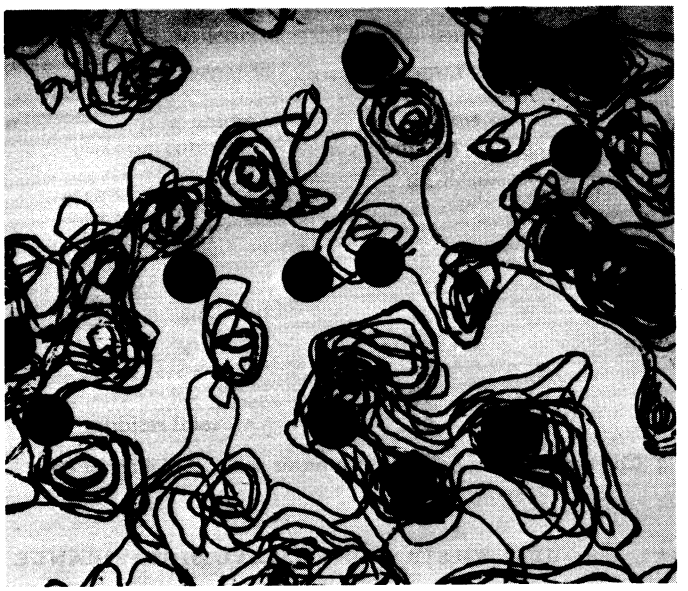


FIGURE 2. Part of the 2.6 Å electron density map of phosphoglucose isomerase. The line of three markers in the centre represents the substrate binding site. The other markers label C_α positions. The side chain just below the right-side of the active site is that postulated to be the base involved in transferring the proton between C-1 and C-2 of the substrate (B in figure 1).

PHYSICAL AND CHEMICAL PROPERTIES

Extensive comparative studies have shown that pig muscle PGI, in terms of gross molecular structure, is very similar to the enzyme from rabbit skeletal muscle, detailed information about which had been reported earlier (Pon *et al.* 1970). Data for the pig muscle enzyme are summarized in table 1. Its native molecular mass is 132 000 and its sedimentation coefficient is 7.1 *S*. Like its counterpart from rabbit, the pig muscle enzyme is composed of two subunits of equal molecular mass (66 000).

The total amino acid composition of the pig muscle enzyme, shown in table 2, is similar, but not identical, to that of the rabbit muscle isomerase. Of interest to the topic of this paper is the arginine content of 25 residues per subunit, so that tryptic hydrolysis, after carbamylation to block the lysine residues, will yield 26 peptides; this has been confirmed by peptide mapping (Gee *et al.* 1980). A subunit contains 13 methionine residues, so that cyanogen bromide cleavage will produce 14 fragments, most of which have now been identified and at least partly purified (see below). The most characteristic distinction between the rabbit and pig muscle PGIs is their cysteine content, which is six residues per subunit for the former and three per subunit for the latter.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 1. Physical properties of pig muscle phosphoglucose isomerase isoenzyme III

parameter	method	value
$s^{\circ}_{20,w}$	velocity sedimentation, pH = 7.1	7.25×10^{-13} s
M_{eq}	equilibrium sedimentation (native enzyme)	132000 g mol^{-1}
$M_{subunit}$	equilibrium sedimentation in 6 M guanidine HCl (enzyme subunit)	67000 g mol^{-1}
$M_{subunit,\ theoretical}$ $(= \frac{1}{2}M_{eq})$		66000 g mol^{-1}

Table 2. Amino acid composition of pig muscle phosphoglucose isomerase isoenzyme III

amino acid residue	residues per subunit	amino acid residue	residues per subunit
Lys	39	Val	30
His	22	Met	13
Arg	25	Ile	33
Asp/Asn	58	Leu	56
Thr	37	Tyr	12
Ser	37	Phe	33
Glu/Gln	68	Trp	11
Pro	25	CySH	3
Gly	43	—	—
Ala	40	total residues	585†

† Corresponds to a calculated subunit molecular mass of 65929.

Sulphydryl groups: their structural significance and their usefulness in the preparation of heavy atom derivatives

As referred to in the preceding section, pig muscle PGI contains only three sulphydryl groups per subunit (and no disulphide bonds). Furthermore, two of these can be modified with sulphydryl reagents such as *p*-mercuribenzoate (figure 3) without loss of enzyme activity. The third requires the presence of a denaturing agent (sodium dodecyl sulphate) before it will react with mercurial. However, sodium dodecyl sulphate at 5 g/l will cause the enzyme to lose its catalytic activity; these conditions are known to cause both dissociation of the native enzyme into its subunits and unfolding of the polypeptide chains. Since two –SH groups can be labelled with mercurial by soaking enzyme crystals in a medium containing the reagent (Muirhead & Shaw 1974), it might be concluded that the two sulphydryl groups that can be modified without loss of enzyme activity are the same that are labelled in preparing heavy atom derivatives. Also, after being derivatized, these two –SH groups can be precisely located in a three-dimensional model of the enzyme, and the knowledge of their location will assist in placing the corresponding sulphydryl peptides in the linear sequence.

Locating the third –SH group will be of significance because it appears to be related to the conformational integrity of the enzyme. Preliminary data from sulphydryl titrations at very low sodium dodecyl sulphate concentrations (less than 0.4 g/l) suggest that it is possible to find conditions under which the third sulphydryl group will react with mercurial without a parallel loss of activity (R. J. Scott, R. H. Palmieri and E. A. Noltmann, unpublished experiments). The structural implications of this finding are the subject of current physical, chemical and kinetic studies.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

General sequence strategy

Early attempts at sequencing PGI from pig skeletal muscle met with difficulties because of the comparatively large size of its subunit polypeptide chain and because the enzyme maintains a large portion of its tertiary structure in β-pleated sheet arrangements (Shaw & Muirhead 1977) with the ensuing technical problems of obtaining soluble peptides suitable for sequencing.

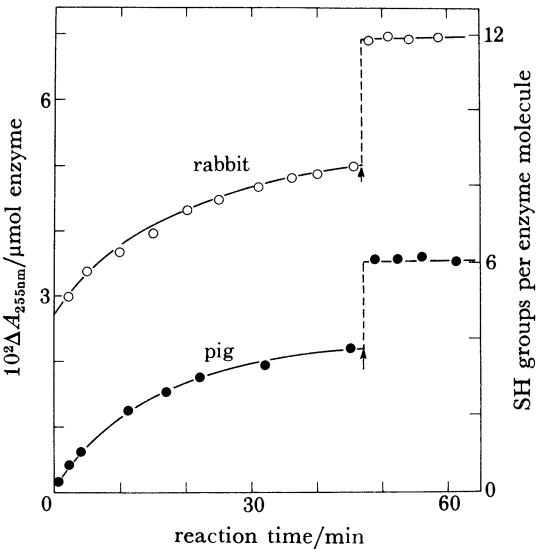
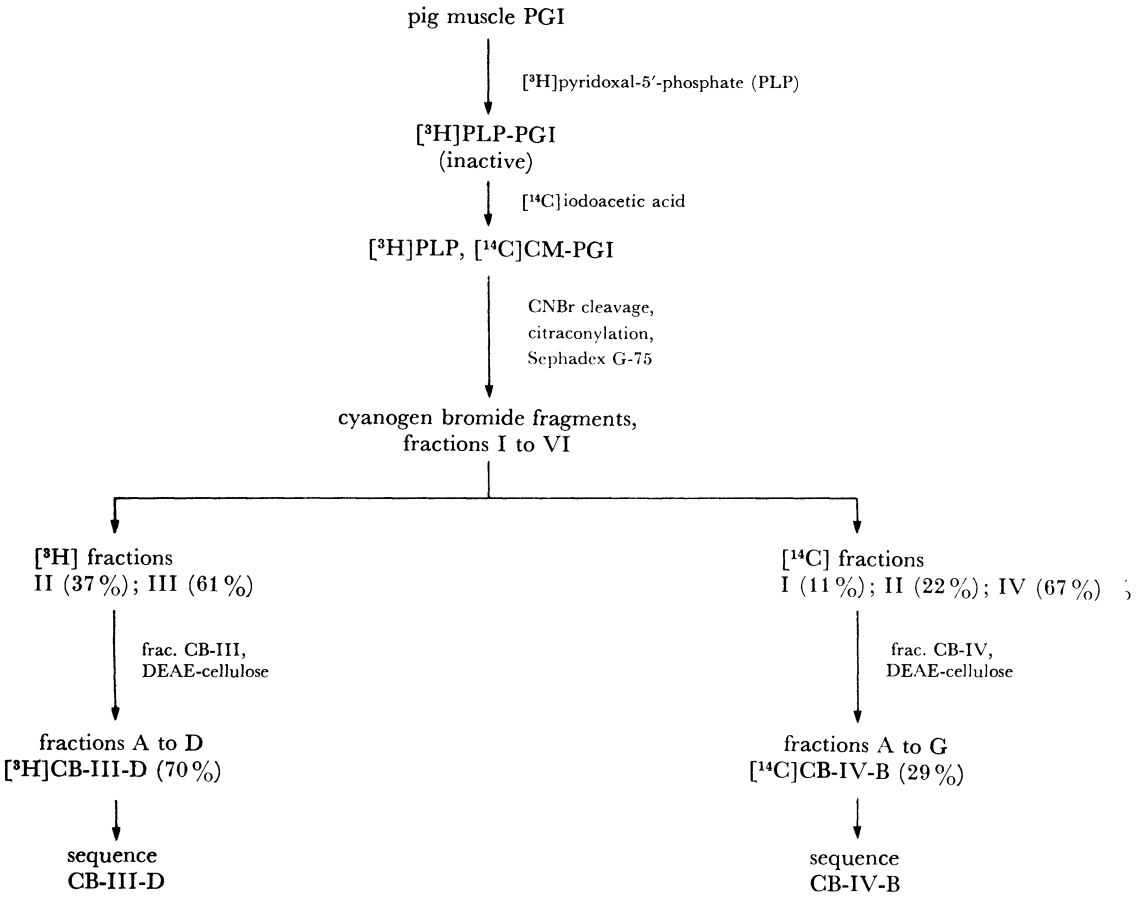


Figure 3. Reaction of rabbit and pig muscle phosphoglucose isomerases with *p*-mercuribenzoate. Sulphydryl groups were measured by ultraviolet absorption at 255 nm from reaction with *p*-mercuribenzoate in 50 mM sodium phosphate buffer (pH 7.1). No loss in enzyme activity was observed before the addition of sodium dodecyl sulphate (0.5%) as indicated by the arrows. Data for the rabbit muscle enzyme are taken from Chatterjee & Noltmann (1967).

These problems have been overcome by citraconylation of the peptide fractions and/or performing isolations and purifications by gel filtration and ion exchange chromatography in the presence of 8 M urea. The routine scheme for obtaining peptides suitable for sequence analysis now followed is shown in table 3. It involves cyanogen bromide cleavage either directly of the denatured protein or after labelling with suitable radioactive markers, automated sequencing by the Edman procedure and/or further tryptic digestion and sequencing by either automated or manual methods. A typical chromatogram of the initial separation of the cyanogen bromide peptides is shown in figure 4. The nomenclature for identifying peptides is first to list the cleavage method (CB), then to use Roman numerals for the fractions obtained by the first separation technique, and to follow this by letters to identify the individual peptides purified in subsequent chromatographic procedures. In addition to the cyanogen bromide approach, direct thermolysin cleavage of the total protein has been used successfully in the isolation of small, radioactivity labelled peptides. Also, limited proteolytic digestion of the native enzyme is being pursued to obtain large peptide fragments.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 3. TYPICAL PURIFICATION SCHEME FOR CYANOGEN BROMIDE PEPTIDES FROM PIG MUSCLE PHOSPHOGLUCOSE ISOMERASE ISOENZYME III



ISOLATION AND SEQUENCE ANALYSIS OF A PYRIDOXAL-5′-PHOSPHATE LABELLED PEPTIDE

Earlier studies had shown that PGI can be labelled stoichometrically with pyridoxal-5′-phosphate, which results in modification of a lysine residue and parallel loss of enzymatic activity (Schnackerz & Noltmann 1971). Enzyme so labelled with [³H]pyridoxal-5′-phosphate, when subjected to cyanogen bromide cleavage and then Sephadex and DEAE cellulose chromatography, was found to retain most of the tritium label in the CB-III-D fraction, which represents a single peptide of approximately 60 residues. Its sequence has been determined directly by manual and automated Edman degradation methods and carboxypeptidase digestions, as well as by additional tryptic digestion and sequencing of the resulting subpeptides, whose separation was accomplished on a Sephadex G-50–G-25 tandem column. The sequence of this peptide is shown in table 4.

SEQUENCE DATA FOR OTHER CYANOGEN BROMIDE PEPTIDES

After cyanogen bromide cleavage and citraconylation of the reaction mixture, isolation and purification of the individual peptides is through size separation on Sephadex, chromatography on ion exchange columns and repeat chromatography after decitraconylation.

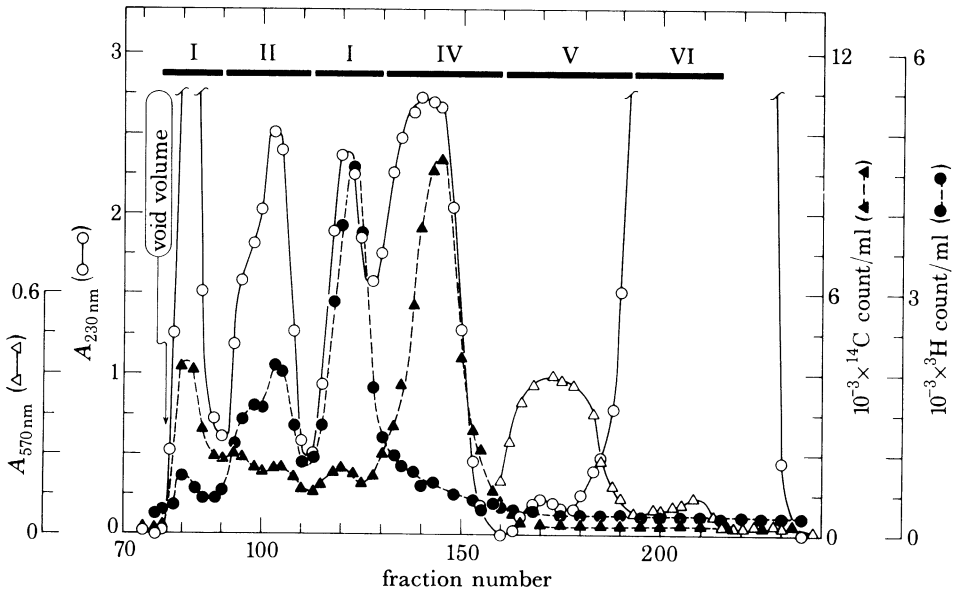
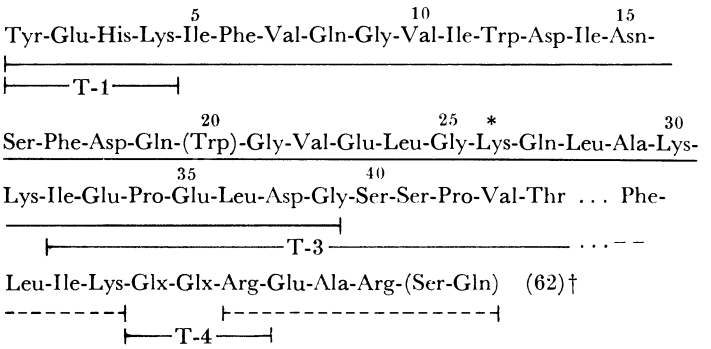


FIGURE 4. Separation of the citraconylated cyanogen bromide peptides of [^{3}H]PLP, [^{14}C]*S*-carboxymethyl pig muscle phosphoglucose isomerase on Sephadex G-75. The cyanogen bromide peptides from the modified enzyme were citraconylated in 50 mM *N*-ethylmorpholine-acetate buffer, pH 8.2. The peptides were then fractionated on a tandem column (2.5 cm × 216 cm and 2.5 cm × 112 cm) of Sephadex G-75 with the use of 50 mM ammonium bicarbonate – 10% isopropanol as eluant buffer. Because of the high ultraviolet absorption of the 'salt' fraction, peptide material in fraction VI was identified by reaction with ninhydrin after alkaline hydrolysis.

TABLE 4. PARTIAL AMINO ACID SEQUENCE OF A [^{3}H]PYRIDOXAL-5′-PHOSPHATE LABELLED CYANOGEN BROMIDE PEPTIDE (CB-III-D) FROM PIG MUSCLE PHOSPHOGLUCOSE ISOMERASE



Notes:

⊢——⊣ Sequence obtained by automated Edman degradation.

⊢T⊣ Sequence obtained by manual/automated Edman methods on tryptic subfragments of the cyanogen bromide peptide.

⊢----⊣ Sequence determined by carboxypeptidase A digestion.

( ) Unassigned residue (X) or tentative residue.

* Position of [^{3}H]pyridoxyllysine.

† Total number of residues estimated from amino acid composition.

Characterization of the peptides occurs by location in the various chromatographic schemes, molecular mass determination, total amino acid analysis and carboxyl terminal and amino terminal analysis. At present, 13 out of the 14 expected cyanogen bromide peptides have been identified, equivalent to about 95 % of the total polypeptide chain. Table 5 provides currently available sequence information on peptides other than that shown in table 4. Note that peptide CB-IV-B contains one of the cysteine residues that carry the ^{14}C label when the sequencing is begun with enzyme labelled with [^{14}C]iodoacetate.

TABLE 5. SUMMARY OF PRELIMINARY SEQUENCE INFORMATION ON SEVERAL CYANOGEN BROMIDE PEPTIDES FROM PIG MUSCLE PHOSPHOGLUCOSE ISOMERASE

CB-I-A

Phe-Glu-Phe-Trp-Asp-Trp-Val-Gly-Gly-Arg-Tyr-Ser-Leu-Trp-Ser-
Ala-Ile-Gly-Leu-Ser-(X)-Ala-Leu-(X)-Val-Gly-Phe-Asp-Asn-Phe-
Glu-Gln-Leu-Leu-(Ser) ... Met (49)†

CB-III-B

Lys-Gly-Lys-Ser-Thr-Glu-Glu-Ala-Arg-Lys-Glu-Leu-Gln-Ala-Ala-
Gly-Lys-Ser-Pro-Glu-Asp-Phe-Glu-Lys-Leu-Leu-Pro-(X)-Lys-Val-
Phe-Glu-Gly-Asn-Arg-Pro-Thr ... Met (72)†

CB-IV-B

Ile-Pro-‡Cys-Asp-Phe-Leu-Ile-Pro-Val-Gln-Thr-Gln-(His)-(Pro)-Ile-
Arg-Lys-Gly-Leu-(His)-(His)-Lys-Ile-(Leu)-Leu-Ala-Asn-Phe-Leu-Ala-
Glx-Thr-Glu ... Glx-Leu-Met (39)†

CB-IV-F

Glu-Ser-Phe-(X)-Lys-Arg-Val-His-(X)-Gly-Glu-Trp-Lys-Gly-Tyr-
(Gly)-Lys-(X)-Ile-(Thr)-(X)-Val-Ile-Asn-Ile- ... Met (34)†

Notes: ‡ Location of [^{14}C]carboxymethyl cysteine. Other symbols as for table 4.

CORRELATION OF AMINO ACID SEQUENCE WITH SECONDARY STRUCTURE

Secondary structures for the two peptides (CB-III-D (table 4) and CB-IV-B (table 5) were predicted by McLachlan, using his computer program (McLachlan 1977). There is a strong probability that in peptide CB-III-D residues 4–12 form a highly hydrophobic strand of β-sheet, while residues 23–32 form an α-helix with a polar and a non-polar surface. The labelled active site lysine is in the centre of this helical region. The best fit for the β-strand is to strand β_1 or possibly to strand β_6 of the large domain (Shaw & Muirhead 1977). Helix A3, which follows β_1, is in both the domain and subunit interface. Peptide CB-IV-B gives a much weaker prediction for a β-strand followed by an α-helix. This peptide contains a

cysteine residue and fits the density for strand β_3 in the large domain. This places the cysteine at one of the reactive sulphydryl groups labelled by mercurials. The other peptides that have been investigated do not contain any sequence predicted to be a strand of β-sheet.

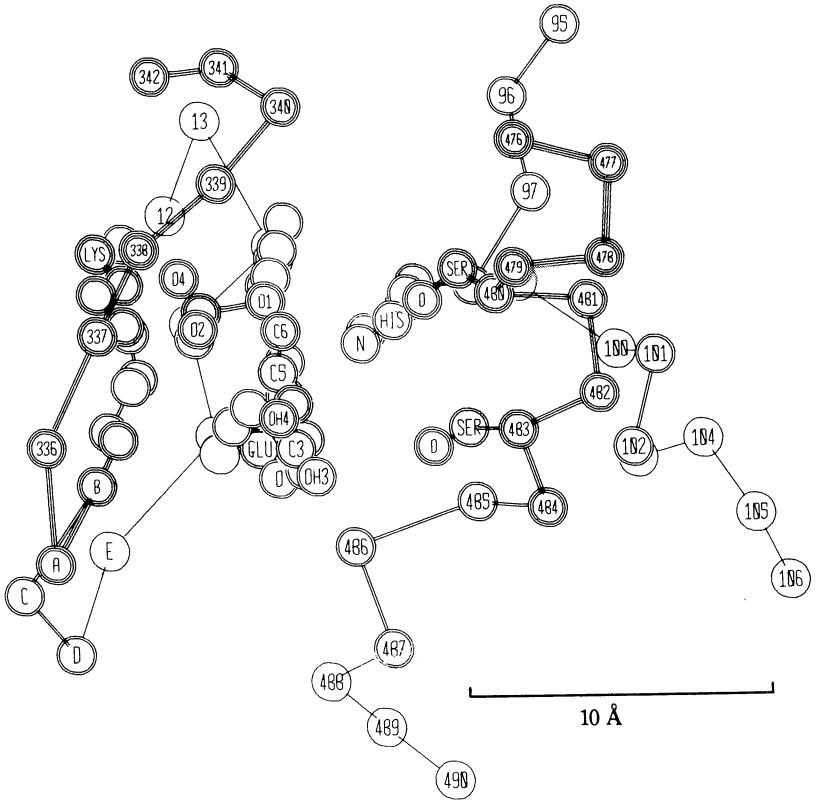


FIGURE 5. Computer drawing of the polypeptide backbone in the vicinity of the active site of pig muscle phosphoglucose isomerase, showing the proposed mode of binding of the substrate glucose-6-phosphate (Shaw & Muirhead 1976). Side chains of residues A and 337 are involved in binding the phosphate group, 480 and 483 react with the C-3 and C-4 hydroxyls, 98 is postulated to be the base B (figures 1 and 2), and 14 and 16 are close to the ring oxygen and the C-1–C-2 bond respectively.

THE ACTIVE SITE

The electron density map at 2.6 Å resolution shows that although the connectivity of the polypeptide chain is not that determined at 3.5 Å resolution, the number of side chains close enough to be involved in binding and catalysis has not been affected. A computer drawing of the polypeptide backbone in the vicinity of the active site with the substrate in position and possible side chains is shown in figure 5. The side chains comprise those involved in binding the phosphate group and five others. The side chain of residue 98 (postulated to be the base) is a flat ring (figure 2) and the side chain of residue 483 (close to substrate C-3) interactions with residue 16 (postulated to be the glutamate labelled by Rose). It is possible that one more side chain could be close enough to interact with the substrate. Thus as well as the phosphate binding site there are five or possibly six groups that could be involved in binding and/or catalysis.

It is interesting to compare the reaction catalysed by PGI with those catalysed by triose phosphate isomerase (TIM) and pyruvate kinase (PK). The reaction catalysed by TIM is

very similar to the isomerization part of the reaction catalysed by PGI. Like PGI, TIM has no requirement for cofactors, metals or activators. TIM is a highly efficient enzyme (Albery & Knowles 1976); PGI is less efficient, perhaps partly because of the requirement for a ring-opening step. For the reaction catalysed by PK, Rose has shown that phosphorylation may be decoupled from the enolization reaction and that the enolization reaction is very similar to that catalysed by PGI and TIM (Rose 1960) (figure 1). The existence of a *cis*-enediol intermediate has been demonstrated for TIM (Rose & Rieder 1959) and PK (Robinson & Rose 1972). In TIM Glu 165, His 95 and Ser 96 are all members of the group of residues implicated in the active site (Phillips *et al.* 1977). The electron density map of PK at 2.6 Å resolution shows that PK could also have a glutamate and a histidine residue, one on each side of the C-1–C-2 bond (Levine *et al.* 1978). In PGI the residues that could be Glu and His are about the same distance apart and are placed on either side of C-1–C-2 (figure 5). Thus in each case the two residues are the same distance apart and on opposite sides of the plane defined by the *cis*-enediol intermediate.

It is feasible that in all three enzymes both a histidine residue and a glutamate residue are present at the active site, one acting as a nucleophilic base and able to maintain the *cis* configuration of the enediolate intermediate, and the other acting as an acidic group to enhance enediolate formation during isomerization. In PGI a third residue is necessary to catalyse ring opening and closing, while in PK bound metal ions and other residues are necessary for phosphoryl transfer.

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FIGURE 2. Part of the 2.6 Å electron density map of phosphoglucose isomerase. The line of three markers in the centre represents the substrate binding site. The other markers label C_α positions. The side chain just below the right-side of the active site is that postulated to be the base involved in transferring the proton between C-1 and C-2 of the substrate (B in figure 1).

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