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The complete amino acid sequence of human erythrocyte diphosphoglycerate mutase

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The complete amino acid sequence of human erythrocyte diphosphoglycerate mutase, comprising 239 residues, was determined. The sequence was deduced from the four cyanogen bromide fragments, and from the peptides derived from these fragments after digestion with a number of proteolytic enzymes. Comparison of this sequence with that of the yeast glycolytic enzyme, phosphoglycerate mutase, shows that these enzymes are 47% identical. Most, but not all, of the residues implicated as being important for the activity of the glycolytic mutase are conserved in the erythrocyte diphosphoglycerate mutase.

Key words: bisphosphoglycerate/2,3-diphosphoglycerate/mutase/sequence/synthase

Introduction

Diphosphoglycerate mutase (DPGAM, bisphosphoglycerate synthase, EC 2.7.5.4) controls the concentration of 2,3-diphosphoglycerate (2,3-DPG) in erythrocytes (Rapoport and Luebering, 1950; see also reviews by Chiba and Sasaki, 1978; Rose, 1980). The discovery that 2,3-DPG profoundly influences the affinity of haemoglobin for oxygen (Chanutin and Curnish, 1967; Benesch *et al.*, 1968) has stimulated much interest in this system. There are three key reactions involved:

1. The synthesis of 2,3-DPG or synthase reaction

$$\text{1,3-DPG} + \text{3-PGA} \longrightarrow \text{2,3-DPG} + \text{3-PGA}.$$

2. The degradation of 2,3-DGP or phosphatase reaction

$$\text{2,3-DPG} \longrightarrow \text{PGA} + \text{Pi}.$$

This reaction is stimulated by 2-phosphoglycollate (Rose and Liebowitz, 1970; Sasaki *et al.*, 1971).

3. The glycolytic mutase reaction

$$\text{3-PGA} \underset{\text{2,3-DPG}}{\rightleftharpoons} \text{2-PGA}$$

(abbreviations: 1,3-DPG, 1,3-diphosphoglycerate; 3-PGA, 3-phosphoglycerate; PGA, phosphoglycerate; 2-PGA, 2-phosphoglycerate). It was originally expected that these three reactions were catalysed by three different enzymes. It is now known that two enzymes are involved, and, quite surprisingly, that both these enzymes catalyse all three reactions, although at substantially different relative rates.

Work in three laboratories to purify and characterise DPGAM established that both the synthase and phosphatase activities were always associated (Rose and Whalen, 1973; Sasaki *et al.*, 1975; Kappel and Hass, 1976). It was also shown that highly purified DPGAM had an inherent glycolytic mutase activity but that this was relatively small (~1/10 the other activities). The purified enzyme is a dimer with identical subunits of mol. wt. 30 000 (Rose and Whalen, 1976).

The other enzyme that catalyses the same three reactions involving 2,3-DPG is the glycolytic enzyme, monophosphoglycerate mutase (MPGAM, EC 2.7.5.3). In the case of MPGAM, the mutase activity is very much greater than the synthase and phosphatase activities [$10^3 - 10^5$ times (Rodwell *et al.*, 1957)]. Yeast MPGAM has been extensively studied and its detailed structure is known from amino acid sequence (Fothergill and Harkins, 1982) and high-resolution crystallographic (Winn *et al.*, 1981) studies. The yeast enzyme is a tetramer with identical subunits of mol. wt. 26 882.

We report here the determination of the complete amino acid sequence of DPGAM. A comparison with the yeast MPGAM sequence shows these enzymes to be highly homologous, and implies a structurally similar active site, and a common evolutionary origin.

Results

Purification and characteriation of human DPGAM

Several schemes for the purification of DPGAM have been published, and we have found the affinity elution method described by Rose and Dube (1976) to be significantly less cumbersome than the others, and to give a good yield of enzyme (~25 mg from 1 litre packed, outdated erythrocytes). DPGAM and MPGAM are apparently chemically very similar, and co-purify through most chromatographic procedures other than affinity elution from Blue Sepharose. In our hands, 3-PGA was more successful in achieving the elution of DPGAM free from MPGAM (Figure 1), than was the 2,3-DGP used by Rose and Dube.

It was obviously necessary to be certain that the purified DPGAM was free from MPGAM. We relied on SDS-polyacrylamide gel electrophoresis (DPGAM has a slightly greater mobility), on relative synthase/mutase activities (MPGAM has very little synthase activity), on heat stability (DPGAM remains active after heating at 60°C for 5 min, whereas MPGAM does not) and on amino acid composition (MPGAM has a much higher alanine content). The amino acid composition of the purified DPGAM is compared with that of yeast MPGAM in Table I.

CNBr fragments

Cleavage of DPGAM at the three methionine residues yielded the expected four fragments. The two smaller fragments CN3 and CN4, were isolated by gel filtration on Sephadex G-75 in 50% formic acid (Figure 2a). The larger fragments and partially cleaved material were not adequately separated by this column, as judged by SDS-polyacrylamide gel electrophoresis, and a further purification step was required. Gel filtration on Sephadex G-75 in 0.2 M pyridine/acetate, pH 3.0 gave a good separation of fragments CN1 and CN2 (Figure 2b). Sephadex swells to a much greater extent in pyridine/acetate than in 50% formic acid, and therefore is suitable for fractionation in a larger mol. wt. range.

The amino acid compositions of the CNBr fragments are given in Table I, and it can be seen that the sum of the

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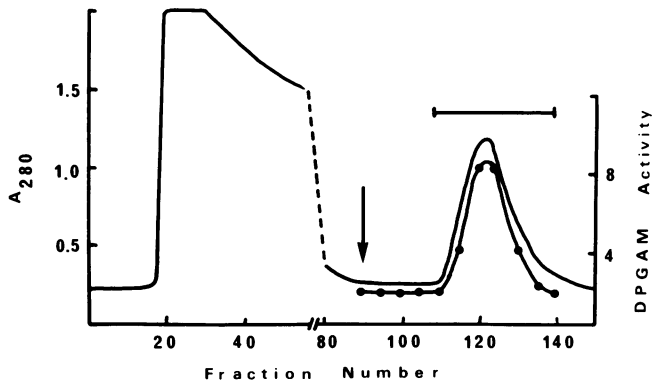


Fig. 1. Affinity elution of DPGAM from Blue Sepharose. DPGAM was partially purified from 4 litres packed out-dated erythrocytes by chromatography on DEAE-cellulose and by $(NH_4)_2SO_4$ fractionation (40–60% saturation). The precipitate was desalted on a Sephadex G-25 column (5 x 75 cm) into 5 mM triethanolamine buffer, pH 7.5 and loaded onto a column (2.4 cm^2 x 54 cm) of Blue Sepharose-CL6B. The column was eluted at a flow rate of 30 ml/h with the same buffer until the absorbance returned to a low value. The arrow indicates the start of a linear gradient of 3-PGA (200 ml buffer + 200 ml buffer with 3 mM 3-PGA). DPGAM activity (—●——●—) is in units/ml, and the fraction size is 9 ml. Fractions containing DPGAM were pooled as indicated.

fragments agrees well with the composition of whole DPGAM. Fragment CN1 did not contain homoserine, and was therefore the C-terminal fragment. The order of the other fragments was established by the sequences of peptides from a chymotrypsin digest of whole DPGAM.

The sequence of DPGAM

A sample of DPGAM was subjected to automatic Edman degradation in the sequencer, but no phenylthiohydantoin (PTH) amino acid was detected, indicating that the enzyme has a blocked N terminus. This was confirmed by sequence analysis of the pool containing fragments CN3 and CN4. Initially it was not realised that this pool contained more than one fragment, since automatic Edman degradation gave a single sequence corresponding to residues 6–30 (Figure 3). However, purification of peptides from a chymotrypsin digest of this pool gave two peptides CN3/4TB2 and CN3/4TB3 which were not present within CN3, and which indicated the presence of the five-residue CNBr peptide, CN4. The electrophoretic mobility of CN3/4TB2 (Table II) and the sequence of CN3/4TB3 and C176C confirmed this. Liquid-phase sequencing of CN3 allowed 30 of the 32 residues to be identified. The initial yield was 15 nmol, and the repetitive yield was 91% by regression analysis. Manual

Table I. Amino acid compositions of DPGAM and its CNBr fragments

Amino acid	MPGAM	DPGAM	Sum of CNBr fragments	CN1	CN2	CN3 + 4
CM-Cys	(0)	1.2 (1)	1.2 (1)	0 (0)	1.2 (1)	0 (0)
Asp	(15)	27.7 (11)	25.6 (11)	14.7 (8)	7.0 (2)	3.9 (1)
Asn	(12)	(15)	(15)	(7)	(5)	(3)
Thr	(8)	5.3 (5)	5.5 (5)	2.5 (2)	2.2 (2)	0.8 (1)
Ser	(15)	15.1 (15)	14.5 (15)	8.2 (8)	3.7 (4)	2.6 (3)
Hse	(0)	0 (0)	(3)+ (3)	0 (0)	+ (1)	(2)+(2)
Glu	(16)	38.5 (27)	39.0 (27)	23.5 (17)	11.3 (7)	4.2 (3)
Gln	(9)	(12)	(12)	(7)	(4)	(1)
Pro	(15)	12.0 (12)	11.6 (12)	9.8 (10)	1.8 (2)	0 (0)
Gly	(16)	16.5 (17)	16.7 (17)	8.4 (9)	5.1 (5)	3.2 (3)
Ala	(21)	12.5 (12)	12.1 (12)	7.0 (7)	4.0 (4)	1.1 (1)
Val	(17)	13.3 (13)	13.0 (13)	7.0 (7)	4.9 (5)	1.1 (1)
Met	(1)	2.9 (3)	0 (0)	0 (0)	0 (0)	0 (0)
Ile	(11)	12.3 (12)	11.4 (12)	8.8 (9)	2.1 (2)	0.5 (1)
Leu	(27)	27.2 (28)	26.9 (28)	15.6 (16)	8.8 (9)	2.5 (3)
Tyr	(8)	7.0 (7)	6.8 (7)	6.0 (6)	0.8 (1)	0 (0)
Phe	(6)	5.5 (6)	5.7 (6)	1.8 (2)	2.9 (3)	1.0 (1)
His	(4)	8.1 (8)	8.2 (8)	6.2 (6)	1.2 (1)	0.8 (1)
Lys	(23)	12.6 (12)	11.4 (12)	5.7 (6)	2.8 (3)	2.9 (3)
Arg	(12)	17.0 (17)	16.5 (17)	9.7 (10)	4.9 (5)	1.9 (2)
Trp	(5)	6.0 (6)	5.8 (6)	2.0 (2)	2.1 (2)	1.7 (2)
Total	(241)	240.7 (239)	234.9 (239)	136.9 (139)	67.8 (68)	30.2 (32)
N terminus	Pro	Blocked		Ala	Glu	Leu + blocked

The results are expressed as mol of residue per mol of DPGAM subunit or per mol of fragment, and the values for serine and threonine are corrected by extrapolation to zero time. Other values are averages for the three different times of hydrolysis. The values in parentheses are those found in the sequence. The composition of yeast MPGAM is from Fothergill and Harkins (1982).

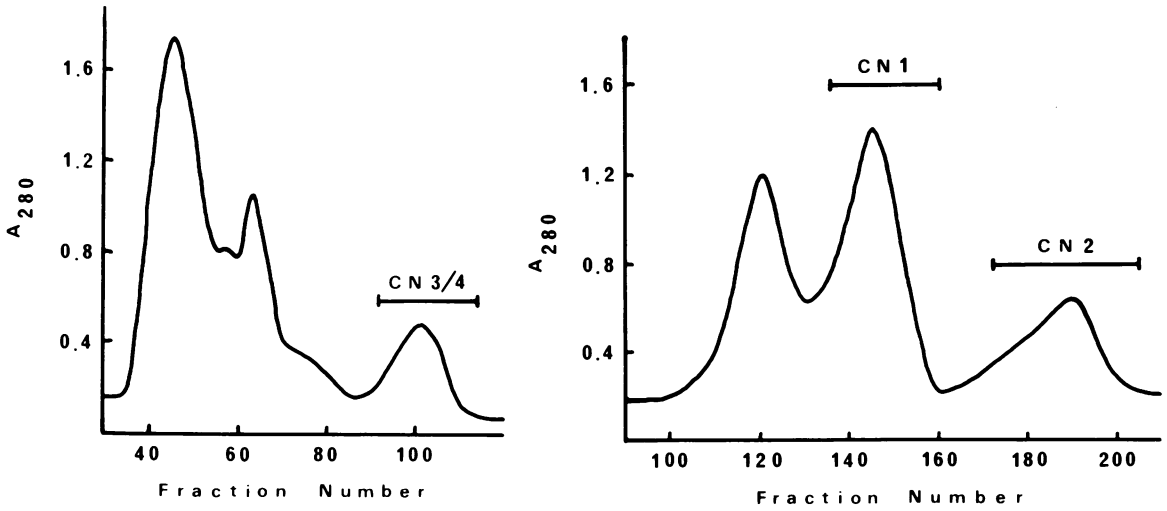


Fig. 2. Gel filtration of CNBr fragments of DPGAM. (**a**) DPGAM (105 mg) was dissolved in 5 ml of 70% (v/v) formic acid, and 24 mg of CNBr was added. The mixture was left for 24 h at room temperature, and then loaded onto a column (2.5 cm x 1350 cm) of Sephadex G-75 and eluted with 50% (v/v) formic acid. The flow rate was 6 ml/h. Fractions containing fragments CN3 and CN4 were pooled as indicated. (**b**) Fractions containing partially cleaved DPGAM, CN1 and CN2 were pooled together, freeze dried and further fractionated on a column (2.4 cm x 1800 cm) of Sephadex G-75 eluted with 0.2 M pyridine/30% (v/v) acetic acid buffer, pH 3.0. The flow rate was 10 ml/h. Fractions with CN1 and CN2 were pooled as indicated.

sequencing of peptides from trypsin and chymotrypsin digests of CN3/4, and from a chymotrypsin digest of whole DPGAM established the complete sequence of fragments CN3 and CN4 (Figure 3). The amide assignments were made by direct identification of the amino acid PTH derivatives.

The sequence of fragment CN2, comprising 68 residues, was determined by automatic N-terminal sequence analysis, and by manual sequencing of peptides derived by digestion with trypsin, chymotrypsin and staphylococcal proteinase. The automatic sequencing penetrated 66 residues. The initial yield was 30 nmol and the repetitive yield was 93% by regression analysis. Tryptic and staphylococcal proteinase peptides from CN2, and chymotryptic peptides from whole DPGAM contributed to the sequence determination. The amide assignments were made by direct identification of the amino acid PTH derivatives except for Gln-99. This residue was deduced to be an amide since the pH 6.5 electrophoretic mobility of peptide C164.6 showed that it contained only one acidic residue, which was located at position 98 by PTH identification (see Figure 3 and Table II). The N terminus of fragment CN2 corresponds to residue 33 of DPGAM.

Fragment CN1, comprising 139 residues, was significantly larger than the other CNBr fragments, and the sequence determination relied significantly on information from tryptic, chymotryptic (from whole DPGAM) and staphylococcal proteinase peptides. Cleavage of CN1 with *o*-iodosobenzoic acid led to the isolation of peptide CN1W1 corresponding to residues 162–239. Automatic N-terminal sequence analysis of CN1 yielded the sequence of the first 43 residues. The initial yield was 30 nmol and the repetitive yield was 95% by regression analysis. The relatively poor penetration was due to significant loss of yield at Gln-108 and at Gln-131. The Pro-Pro-Pro- sequence at residues 120–122, however, did not lead to a drop in yield. The amide assignments were made either by direct identification of the PTH amino acids, or by peptide mobility (see Table II) and/or by staphyococcal proteinase specificity. The N terminus of fragment CN1 corresponds to residue 101 of DPGAM.

The complete amino acid sequence of DPGAM comprises 239 residues. The subunit relative molecular mass calculated from the sequence is 27533; this value is close to that estimated by physical methods.

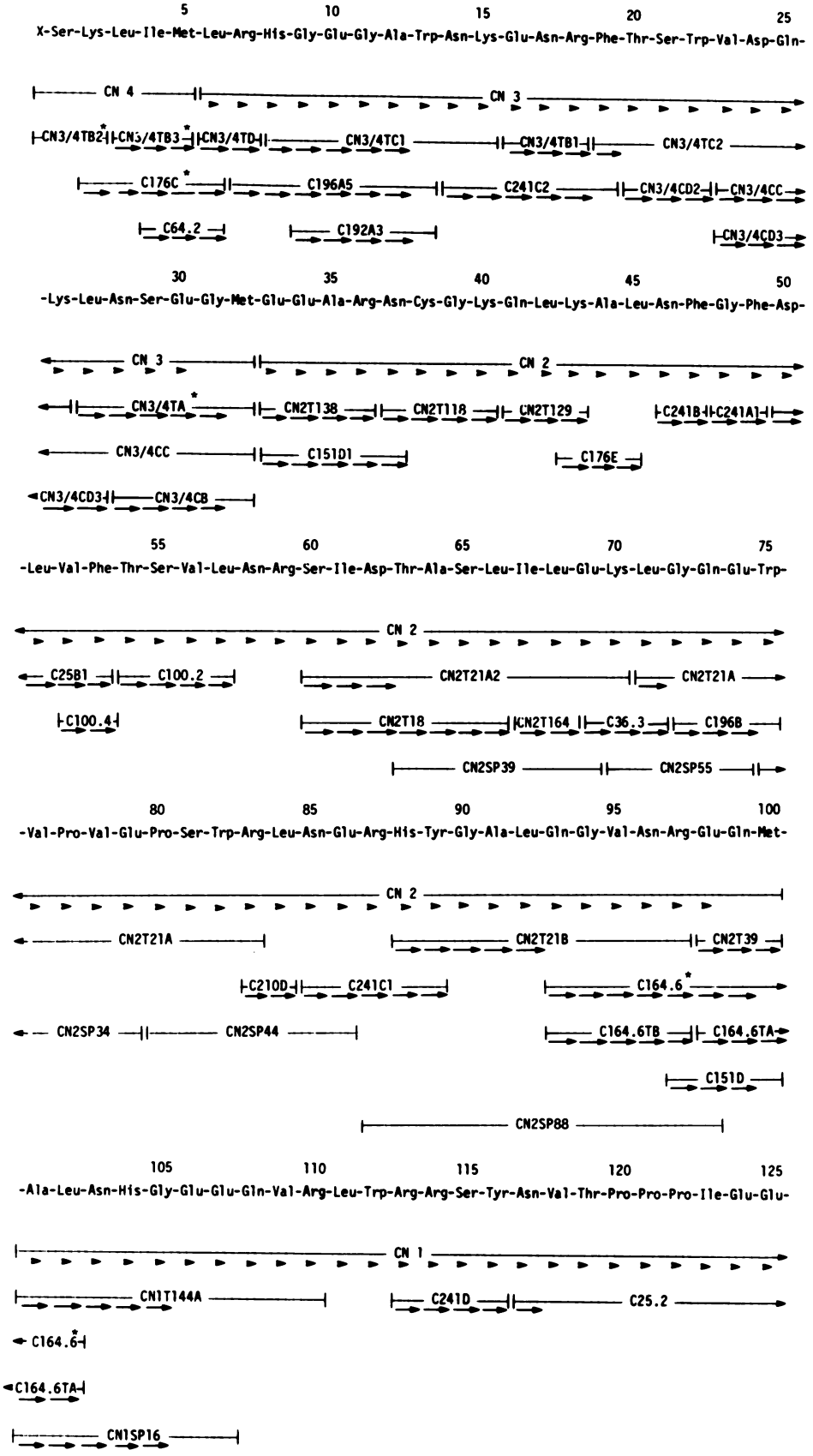
Discussion

We have been investigating the structure and activity of the glycolytic enzyme MPGAM for several years (e.g., Fothergill and Harkins, 1982; Winn *et al.*, 1981). Two lines of evidence suggested that the erythrocyte enzyme that catalyses the synthesis of 2,3-DPG, DPGAM, might be structurally similar to the glycolytic mutase. (i) Both enzymes catalyse the same three reactions (see Introduction), albeit at very different relative rates (reviewed by Rose, 1980). (ii) The subunit relative molecular mass of the two enzymes is similar [27 000 for DPGAM and 28 500 for MPGAM (Hass *et al.*, 1976)]. In addition, the amino acid compositions, although by no means identical, show striking similarities. We therefore decided to test the hypothesis that the two enzymes were structurally related by doing amino acid sequence studies.

It was known that the catalytic mechanism of MPGAM involved a phosphohistidine intermediate (Rose, 1970, 1971). In addition it was known from high resolution X-ray crystallographic studies of yeast MPGAM that two histidine residues (His-8 and His-179) were present at the active site (Winn *et al.*, 1977). We directed our initial efforts toward the isolation of histidine-containing peptides from DPGAM, and reported the sequence of residues 1–32 (Haggarty and Fothergill, 1980). Meanwhile, Han and Rose (1979) independently isolated phosphohistidine-containing tryptic peptides from yeast MPGAM and horse DPGAM corresponding to residues 8–15. Taken together, these results showed that one of the histidines at the active site (His-8) was the one that was phosphorylated, and also demonstrated that human DPGAM and yeast MPGAM were homologous (16 of the 32 residues compared were identical). The determination of the complete sequence of human DPGAM now permits a more extensive comparision to be made (see Figure 4).

Overall, human DPGAM and yeast MPGAM are 47%

identical (114 of 241 residues). This clearly indicates that the two enzymes have a common evolutionary origin, especially when the evolutionary divergence of *Homo sapiens* and *Saccharomyces cerevisiae* is taken into consideration. The two sequences are not quite as similar, however, as would be expected for a single glycolytic enzyme. For example, phosphoglycerate kinases from *H. sapiens* and *S. cerevisiae* are 64% identical (Perkins *et al.*, 1983), and fructose-1,6-bisphosphate



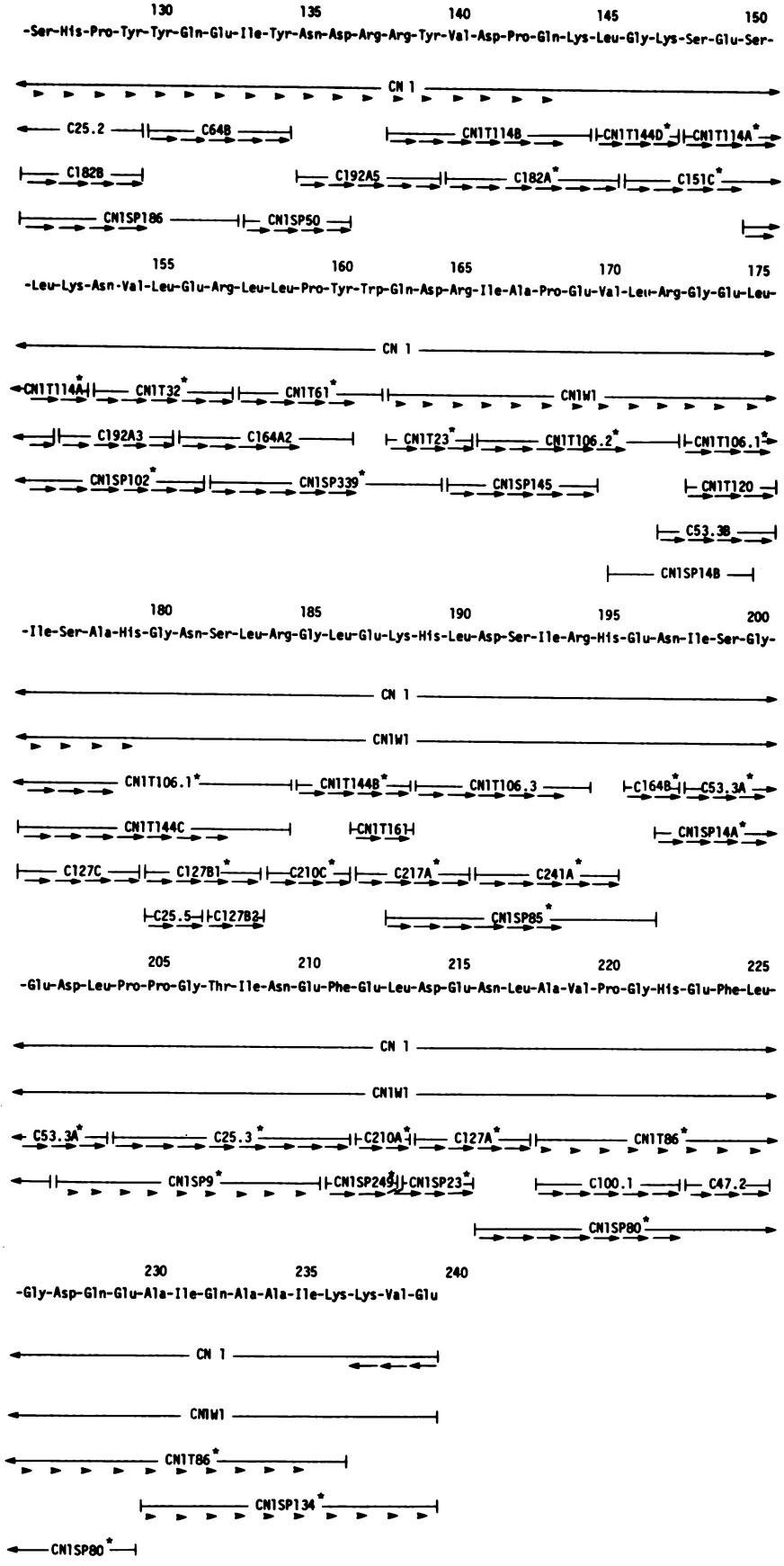


Fig. 3. Amino acid sequence of DPGAM. Residues were identified by automatic Edman degradation (►), by the manual dansyl-Edman procedure (-►), or by carboxypeptidase digestion (◄-). The amino acid compositions and peptide mobilities of those peptides indicated by * are given in Table II. Peptide nomenclature and details of the methods are given in the text.

adolases from *H. sapiens* and *Drosophila melanogaster* are 80% identical (Freemont *et al.*, 1983). [Glycolytic enzymes are probably among the most highly conserved enzymes known, and are changing at a lower rate than, for example, cytochrome c (Dayhoff, 1972).]

The high degree of homology between the sequences of DPGAM and yeast MPGAM strongly suggests that the two enzymes have similar tertiary structures. In particular, it is probable that the active sites are very similar, although it is likely that there are some differences consistent with the different catalytic properties of the two enzymes. A prominent feature of the active site of yeast MPGAM (Winn *et al.*, 1981) is the presence of two histidine side chains (residues 8 and 179) separated by the guanidinium group of an arginine (residue 7). Another arginine side chain (residue 59) is in a suitable position to interact with the carboxyl group of the substrate. All four of these residues are conserved in DPGAM. A notable difference between the two enzymes involves residue 11. In MPGAM residue 11 is a serine that is in a suitable position to form a hydrogen bond with a substrate phosphoryl group. This residue is replaced by glycine in DPGAM.

Another region of MPGAM implicated as essential for activity involves the carboxy-terminal 8–12 residues (Sasaki *et al.*, 1966; Fothergill and Harkins, 1982). These amino acids apparently constitute a flexible 'tail' since they do not contribute to the electron-density map (Winn *et al.*, 1981). The loss of the carboxyl 'tail' by proteolysis is associated with complete loss of mutase activity. The phosphatase activity is retained, although it is no longer stimulated by 2-phosphoglycollate. Model building studies show that the cluster of small side chains of residues 230–237 confers great flexibility on this region and also show that it is possible for the 'tail' to adopt a conformation that could modulate access to the active site. In addition, the side chains of the two lysine residues (239 and 240) could approach the active site, and might be important for retaining the negatively charged substrate molecules on the enzyme during catalysis. Interestingly, this region is rather different in DPGAM, although some features are conserved. The 'tail' is shorter and significantly less flexible but the two consecutive lysine residues are retained.

It seems likely from these comparisons that MPGAM and DPGAM have essentially the same active sites, with the two histidine residues playing the same roles. The differences in catalytic behaviour probably result from differences in ligand binding. These possibilities are currently being investigated by detailed model building studies.

Materials and methods

Purification of human DPGAM

The purification procedure was based on that of Rose and Dube (1976), and involved DEAE-cellulose chromatography to remove haemoglobin, $(NH_4)_2SO_4$ precipitation and affinity elution with 3-PGA from Blue Sepharose-CL6B (Figure 1). DPGAM synthase activity was measured by an aldolase/glyceraldehyde-3-phosphate dehydrogenase coupled assay modified from Rapoport and Luebering (1952). It was found necessary to include

Table II. Amino acid compositions, electrophoretic mobilities and N-terminal residues of peptides from proteolytic digests of DPGAM and its CNBr fragments

Peptide	CN3/4 TB2	CN3/4 TB3	C176C	CN3/4 TA	C164.6	C182A	CN1 T144D	C151C	CN1 T114A	CN1 SP102	CN1 T32	CN1 SP339	CN1 T61	CN1 T23	CN1 T106.2	CN1 T106.1	C127 B1
$m_{6\cdot5}$	0	0	+0.37	−0.34	0	0	+0.63	0	0	–	0	–	–	0	0	–	0
m_2	–	–	–	–	+0.92	–	–	–	–	–	–	–	–	–	+1.12	–	+0.79
Asx	–	–	–	1.1	1.3	1.1	–	–	–	1.0	1.1	1.0	–	0.9	–	1.0	0.8
Thr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ser	1.0	–	–	0.9	–	–	–	1.8	1.8	0.9	–	–	–	–	–	1.8	0.9
Hse	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Glx	–	–	–	1.1	3.0	0.8	–	0.8	1.2	1.0	0.8	1.0	–	1.1	1.2	1.3	–
Pro	–	–	–	–	–	1.0	–	–	–	–	–	0.9	1.0	–	1.1	–	–
Gly	–	–	–	1.0	1.0	–	1.3	1.0	–	–	–	–	–	–	–	2.0	0.9
Ala	–	–	–	–	1.1	–	–	–	–	–	–	–	–	–	0.9	0.9	–
Val	–	–	–	–	1.0	1.0	–	–	–	0.9	0.8	–	–	–	0.8	–	–
Met	–	–	0.6	–	0.9	–	–	–	–	–	–	–	–	–	–	–	–
Ile	–	1.0	0.9	–	–	–	–	–	–	–	–	–	–	–	0.9	1.3	–
Leu	–	0.9	2.0	0.8	1.0	1.0	1.1	1.0	1.1	1.8	0.9	1.9	1.9	–	1.1	2.0	1.0
Tyr	–	–	–	–	–	–	–	–	–	–	–	0.8	0.9	–	–	–	–
Phe	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
His	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.8	–
Lys	1.0	–	1.1	–	–	0.8	1.0	0.8	1.0	1.0	–	–	–	–	–	–	–
Arg	–	–	–	–	0.9	–	–	–	–	–	1.0	0.9	–	1.0	1.0	1.0	–
Trp	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–
Sum	2	3	5	6	10	6	3	6	5	7	5	8	5	3	7	12	4
N-terminus	Blocked	Leu	Lys	Leu	Glx	Val	Leu	Gly	Ser	Ser	Asx	Arg	Leu	Glx	Ile	Gly	Gly

Table II. continued

Peptide	C210C	CN1 T144B	C217A	CN1 SP85	C241A	C164B	CN1 SP14A	C53.3A	CN1 SP9	C25.3	CN1 SP249	C210A	CN1 SP23	C127A	CN1 SP80	CN1 T86	CN1 SP134
$m_{6\cdot5}$	+0.51	0	+0.36	–	+0.22	−0.66	−0.39	−0.72	–	–	–	−0.66	−1.05	−0.92	–	–	–
m_2	–	–	–	–	–	+1.25	–	+0.71	–	+0.57	–	–	–	–	–	–	–
Asx	–	–	–	1.2	0.9	1.0	1.2	0.8	1.9	1.2	–	–	1.0	1.8	1.8	1.3	–
Thr	–	–	–	–	–	–	–	–	0.8	1.0	–	–	–	–	–	–	–
Ser	–	–	–	0.8	1.1	–	1.1	1.1	–	–	–	–	–	–	–	–	–
Hse	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Glx	–	0.8	1.0	1.0	–	1.0	1.0	0.8	1.0	1.3	1.1	1.0	1.0	1.0	2.9	4.1	1.9
Pro	–	–	–	–	–	–	–	–	1.5	2.1	–	–	–	–	0.9	1.3	–
Gly	1.0	1.2	–	–	–	–	0.9	0.8	0.8	1.1	–	–	–	–	2.3	2.1	–
Ala	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	4.1	3.0
Val	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	1.1	1.1
Met	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ile	–	–	–	0.9	1.0	–	1.2	0.8	1.2	1.1	–	–	–	–	–	2.0	2.0
Leu	1.0	0.9	1.0	1.1	–	–	–	1.0	1.3	–	–	1.0	1.0	1.0	2.1	1.3	–
Tyr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Phe	–	–	–	–	–	–	–	–	–	0.8	1.0	–	–	–	1.0	1.0	–
His	–	–	0.9	1.7	0.9	–	–	–	–	–	–	–	–	–	0.8	1.1	–
Lys	–	1.0	0.9	0.9	–	–	–	–	–	–	–	–	–	–	–	1.0	1.9
Arg	1.0	–	–	1.0	1.1	–	–	–	–	–	–	–	–	–	–	–	–
Trp	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sum	3	4	4	9	5	2	5	6	9	8	2	2	3	4	14	19	10
N terminus	Arg	Gly	Glx	Lys	Asx	Glx	Asx	Ile	Asp	Pro	Phe	Glx	Leu	Asx	Asx	Ala	Ala

Experimental details and peptide nomenclature are given in the text. Values are molar ratios, and are uncorrected for destruction or partial hydrolysis. The symbol – means 'insignificant amount' in the compositions, or 'not done' for electrophoretic mobility.

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                    10                  20                  30                  40                  50
DPGAM     X-SKLIMLRHGEGAWNKENRFTSWVDQKLNSEGMEEARNCGKQLKALNFGFD
MPGAM       PKLVLVRHGQSELWNEKNLFTGWVDVKLSAKGQQEAARAGELLKEKGVNVL

                    60                  70                  80                  90                  100
DPGAM     LVFTSVLNRSIDTASLILEKLGQEWVPVEPSWRLNERHYGALQGVNREQM
MPGAM     VDYTSKLSRAIQTANIALEKADRLWIPVNRSWRLNERHYGDLQGKDKAQT

                    110                 120                 130                 140                 150
DPGAM     ALNHGEEQVRLWRRSYNVTPPPIEESHPYYQEIYNDRRYVDPQKLGKSES
MPGAM     LKKFGEEKFNTYRRSFDVPPPPIDASSPFSQKGDERYKYVDPNVLPETES

                    160                 170                 180                 190                 200
DPGAM     LKNVLERLLPYWQDRIAPEVLRGELISAHGNSLRGLEKHLDSIRHENISG
MPGAM     LALVIDRLLPYWQKVIAKLVGKRSMIAAHGNSLRGLVKHLEGISDADIAK

                    210                 220                 230                 240
DPGAM     EDLPPGTINEFELDENLAVPGHQFLGDQEAIQAAIKKVE
MPGAM     LDIPPGTILVFELDENLKPSKPSYYLDPEAAGAAVANQKKG
```

Fig. 4. Comparison of the sequence of human DPGAM with that of yeast MPGAM. The sequence of yeast MPGAM is taken from Fothergill and Harkins (1982). The identical residues are boxed in.

3-PGA (1 mM) in the assay mixture. MPGAM mutase activity was measured by the enolase-coupled assay (Grisolia and Carreras, 1975).

Purification of CNBr- and o-iodosobenzoic acid cleavage fragments

CNBr cleavage of DPGAM was done at room temperature for 24 h with a 100-fold molar excess of CNBr over methionine residues. The resulting fragments were separated on Sephadex G-75 (Figure 2). Cleavage of fragment CN1 at tryptophan residues with *o*-iodosobenzoic acid was for 24 h at room temperature in the dark (Mahoney and Hermodson, 1979). These fragments were separated on Sephadex G-75 eluted with 0.2 M pyridine/30% (v/v) acetic acid, pH 3.0.

Proteolytic digestion and purification of peptides

Whole DPGAM was digested with chymotrypsin, and the CNBr fragments were digested with trypsin, chymotrypsin, staphylococcal proteinase or carboxypeptidase as described previously (Fothergill and Harkins, 1982). Peptide mixtures derived from fragments CN1 and CN2 were fractionated by cation-exchange chromatography on Technicon P resin (Fothergill and Harkins, 1982). Further purification was done, when necessary, by high-voltage electrophoresis at pH 6.5 or 2 (Fothergill and Fothergill, 1970). At pH 6.5, peptide mobility ($m_{6.5}$) was expressed relative to aspartic acid, with valine as a neutral marker. At pH 2, mobility (m_2) was measured relative to valine, with 5-dimethylamino-naphthalene-1-sulphonic acid as a neutral marker. Peptides were detected on paper by staining with cadmium/ninhydrin reagent (Heilmann *et al.*, 1957). Fractionation of peptides derived from fragments CN3 and CN4 was done by high-voltage electrophoresis.

The amide content of a peptide was determined by correlating its electrophoretic mobility at pH 6.5 and its molecular mass (Offord, 1966), or, where appropriate, by identification of the PTH derivatives of the amino acid residues.

Peptide nomenclature

The following abbrevations indicate that the peptide was derived from digestion or cleavage by: C, chymotrypsin; CN, CNBr; SP, staphylococcal proteinase; T, trypsin; W, *o*-iodosobenzoic acid. Numerals that immediately follow these letters refer to the fraction number from the ion-exchange or gel-filtration chromatography separations. The subsequent letters refer to the electrophoretic mobilty at pH 6.5, with 'A' being the most acidic. Numerals refer to electrophoretic mobility at pH 2, with '1' being the most acidic.

Amino acid analysis

Hydrolysis was done in evacuated tubes with 0.5 ml of 5.7 M HCl (Aristar HCl diluted with an equal volume of water) at 110°C for 20, 48 or 96 h. Quantitative analyses were carried out with a Locarte amino acid analyser with a single column. Tryptophan was determined after hydrolysis with 3 M mercaptoethanesulphonic acid (Penke *et al.*, 1974).

Amino acid sequence determination

Amino acid sequences were determined manually by the dansyl-Edman procedure as described previously (Fothergill and Harkins, 1982), or automatically with a Beckman 890C liquid-phase sequencer equipped with the Beckman cold-trap accessory. The details of the sequencer operation and the identification of the amino acid PTH derivatives have been described previously (Smith *et al.* 1982).

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