AMINO ACID SEQUENCE OF AN ACTIVE SITE FRAGMENT FROM HUMAN DIPHOSPHOGLYCERATE MUTASE

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1. Introduction

Diphosphoglycerate mutase (DPGM, bisphosphoglycerate synthase, EC 2.7.5.4) controls the concentration of 2,3-diphosphoglycerate (2,3-DPG) in erythrocytes [1–4]. The discovery that 2,3-DPG profoundly influences the affinity of haemoglobin for oxygen [5,6] has stimulated much interest in this system. It is now apparent that DPGM catalyses three reactions: the conversion of 1,3-diphosphoglycerate to 2,3-DPG; the dephosphorylation of 2,3-DPG; and the interconversion of 3- and 2-phosphoglycerates [2–4].

These three reactions are also catalysed by the glycolytic enzyme, monophosphoglycerate mutase (MPGM, EC 2.7.5.3), although at different relative rates [2–4]. The two enzymes have other features in common including subunit size [2,7], tryptic peptide elution profiles [8], and a phosphorylated histidine intermediate [2,9]. A correlation of the amino acid sequence of yeast MPGM with its 2.8 Å resolution electron density map shows two histidines (His 8 and His 179) at the active site [10]. The sequence of a tryptic phosphohistidine containing peptide from horse DPGM homologous with residues 8–15 of yeast MPGM has been determined [9].

We report here the isolation and amino acid sequence determination of a 32 residue fragment from the N-terminus of human DPGM. A comparison with the yeast MPGM sequence shows them to be highly homologous, and implies a structurally very similar active site, and a common evolutionary origin.

2. Materials and methods

2.1. *Purification and characterization of human DPGM*

DPGM was prepared from outdated human erythrocytes by the method in [2] except that the final purification step involving affinity chromatography on Blue Sepharose CL-6B (Pharmacia) was altered. It was found that elution with 3-phosphoglycerate (Sigma, grade 1) instead of 2,3-DPG gave a better separation of DPGM from MPGM, which copurify until this step.

The DPGM was characterized by enzymic assay, SDS–polyacrylamide gel electrophoresis, and amino acid analysis, and was shown to be of adequate purity.

2.2. *Separation of CNBr fragments*

Purified DPGM (195 mg) was dialysed, freeze-dried and dissolved in 2 ml 70% (v/v) formic acid. A 50-fold molar excess of CNBr (Eastman) over methionine residues was added, and digestion was for 24 h at room temperature (15–20°C). The CNBr fragments were separated on Sephadex G-75 eluted with 50% (v/v) formic acid.

2.3. *Sub-digestion of CNBr fragments*

Fragments (~400 nmol) dissolved in 1% (w/v) ammonium bicarbonate were digested by 200 μg trypsin or chymotrypsin (Worthington) for 18 h at 37°C. The sub-peptides were purified by high-voltage paper electrophoresis.

2.4. *Analytical methods*

A Beckman 890C sequencer was used to determine the sequence of fragment CN4. The phenylthiohydantoin (PTH) amino acids were identified by reversed-phase high-pressure liquid chromatography using Waters Associates equipment. PTH amino acids were hydrolysed in evacuated tubes with 25 μl hydriodic acid (BDH Chemicals) at 130°C for 20 h, and amino acid analysis done to confirm the PTH identification. The sequence was completed and verified by isolating sub-peptides, and determining

their sequence by the manual dansyl-Edman procedure [11] if necessary. Peptides were hydrolysed in evacuated tubes with 0.5 ml 50% (v/v) Aristar HCl at 110°C for 20 h. Quantitative amino acid analysis was done using a Locarte amino acid analyser with a single column eluted by a 3.5 h sequence of 4 stepwise buffer changes. Tryptophan was detected after reaction with Ehrlich reagent [12].

3. Results and discussion

3.1. *Isolation of CNBr fragments*

Human DPGM contains 4 methionine residues (table 1), and the separation of the CNBr fragments is shown in fig.1. Fragments CN4 and CN5 elute together, and were separated by high-voltage paper electrophoresis. Their amino acid compositions, electrophoretic mobilities and N-termini are given in table 1. It is apparent from a correlation of the amino acid composition and electrophoretic mobility [13] of CN5 (and also sub-peptide CN5TB2) that it has a blocked N-terminus, and therefore is derived from the N-terminus of DPGM.

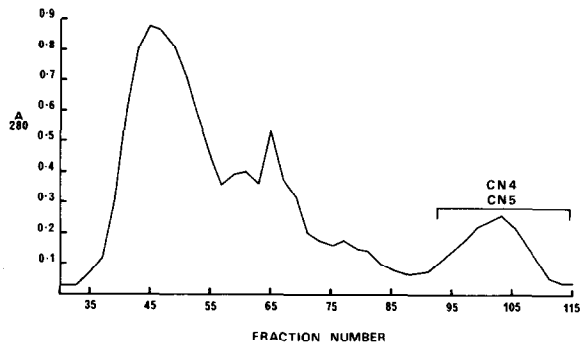

Fig.1. Gel filtration of CNBr fragments on Sephadex G-75. The CNBr digest was applied directly to a 2.5 × 1350 cm column that had been equilibrated with 50% (v/v) formic acid. The flow rate was 6 ml/h, and fractions of 2.5 ml were collected. Fractions containing CN4 and CN5 were pooled as indicated.

3.2. *Amino acid sequence of an active site fragment*

The amino acid sequences of fragments CN4 and CN5 are given in fig.2. The liquid-phase sequencer analysis was done on the pooled fractions indicated in fig.1, and therefore contained both fragments. The

Table 1

Peptide data

Name	CN4	CN5	TA	TB1	TB2	TB3	TC1	TD4	TD5	ChB	ChC	ChE	ChD3	ChD8
$m_{6.5}$	0	0	-0.34	0	0	0	+0.10	+0.55	+0.55	-0.41	-0.27	0	0	0
Asx	3.9	-	1.1	-	-	1.0	1.8	-	-	0.9	1.8	-	-	1.2
Thr	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ser	2.0	0.9	0.9	-	1.0	-	0.9	-	-	0.9	1.2	-	1.0	-
Glx	4.2	-	1.1	-	-	0.9	2.1	-	-	1.1	1.8	-	-	1.2
Pro	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gly	3.2	-	1.0	-	-	-	2.1	-	-	1.2	1.1	-	-	-
Ala	1.1	-	-	-	-	-	1.0	-	-	-	-	-	-	-
Cys	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Val	1.1	-	-	-	-	-	0.9	-	-	-	1.0	-	-	1.0
Ile	-	1.1	-	1.0	-	-	-	-	-	-	-	1.0	-	-
Leu	2.0	1.0	0.8	0.9	-	-	-	-	1.0	-	1.1	1.1	-	1.0
Tyr	-	-	-	-	-	-	0.8	-	-	-	-	-	0.9	-
Phe	1.0	-	-	-	-	-	0.8	-	-	-	-	-	-	-
His	0.8	-	-	-	-	-	0.6	-	-	-	-	-	-	-
Lys	2.9	0.9	-	-	1.0	-	1.8	1.0	-	-	0.9	1.0	-	0.8
Arg	1.9	-	-	-	-	1.0	-	-	1.0	-	-	-	-	-
Hse	+	+	+	+	-	-	-	-	-	+	+	+	-	-
Trp	+	-	-	-	-	-	+	-	-	-	-	-	-	-
Total	27	5	6	3	2	3	7 8	1	2	5	10	4	2	5
N-terminus	Leu	X-Ser	Leu	Leu	X-Ser	Glx	Phe His	Lys	Leu	Asn	Val	Lys	Ser	Val

Experimental details are given in the text. Composition values are molar ratios, and are uncorrected for destruction or partial hydrolysis. (-) means insignificant amount (≤0.3). Electrophoretic mobility is expressed relative to the mobility of aspartic acid, using valine as a neutral marker

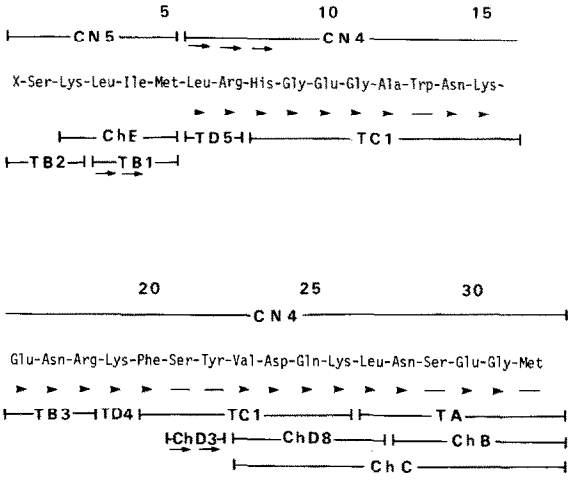

Fig.2. Amino acid sequence of an active site fragment. Residues identified from the sequencer (▶) or by the manual dansyl-Edman procedure (→) are indicated. Degradation cycles for which no residue was identified are shown by (—). Peptide data are given in table 1. The assignment of residue 13 as Trp is based on the compositions of peptides TC1, ChD3 and ChD8. Peptides TC1 contained tryptophan as shown by positive reaction with Ehrlich reagent, whereas peptides ChD3 and ChD8 did not.

Table 2

Comparison of active-site peptides

	5	10	15	20	25	30
Horse DPGM [9]		H	G	Q	G	A W N K
Human DPGM	X-S	K	L	I M L	R H G E G A W N K	E N R K F S Y V D Q K L N S E G M
Yeast MPGM [14]	P	K	L	V L	V R H G E S	Q W N E K N L F T G W V D V K L S A K G Q
Rabbit MPGM [15]					V R H G E S	T W

Residues that are identical in two or more sequences are boxed in. The active-site histidine is starred (✱). The one letter code for the amino acids is that recommended by IUPAC-IUB [16]

presence of fragment CN5 was not a problem since it has a blocked N-terminus and was not degraded. The sequence was completed and verified by purifying and partially sequencing tryptic and chymotryptic peptides as shown in fig.1 and table 1.

It is apparent from a comparison of the sequences in table 2 that fragments CN4 and CN5 of DPGM correspond to the N-terminal, active-site region of yeast MPGM. The phosphohistidine peptide isolated from horse DPGM [9] is virtually identical to residues 8–15 of the human enzyme with only a single amide difference.

The sequence homology between the human DPGM and yeast MPGM would suggest they have structurally similar active sites and would be consistent with their ability to catalyse the same reactions. The sequence similarity would also be consistent with the DPGM having evolved from the ubiquitous glycolytic enzyme, MPGM.

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