# AMINO ACID SEQUENCES OF ACTIVE-SITE HISTIDINE PEPTIDES FROM RABBIT MUSCLE PHOSPHOGLYCERATE MUTASE

Neill W. HAGGARTY and Linda A. FOTHERGILL
*Department of Biochemistry, Marischal College, University of Aberdeen, Aberdeen, AB9 1AS, Scotland*

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## 1. Introduction

Monophosphoglycerate mutase (MPGM) catalyses the interconversion of 3-phosphoglycerate and 2-phosphoglycerate, in a reaction mechanism involving a phosphohistidine intermediate [1,2]. A correlation of the 2.8 Å resolution electron-density map from X-ray crystallographic studies of *yeast* MPGM with its amino acid sequence shows 2 histidines at the active site [3]. Structural studies of *muscle* MGPM are much less complete, although the enzyme has been used extensively in kinetic work, e.g. [4,5]. The muscle and yeast enzymes have been shown to differ in several respects. The muscle enzyme is a dimer with identical subunits of mol. wt 28 000 [6], whereas the yeast enzyme is isolated as a tetramer composed of 4 identical subunits of mol. wt 27 000 [7]. The activity of the muscle enzyme is sensitive to modification of cysteinyl and arginyl residues [8,9], in contrast to the yeast enzyme that has no thiol [10], and is only moderately sensitive to butanedione [11]. In addition, a phosphohistidine peptide isolated from chicken breast muscle MGPM has been sequenced [12], and shows no homology with any of the 4 histidine sequences of the yeast enzyme (see table 2).

We report here the purification and determination of the amino acid sequences of histidine-containing peptides from rabbit muscle MPGM to obtain an estimate of the extent of structural similarity at the active sites of the muscle and yeast enzymes.

## 2. Materials and methods

### 2.1. *Chymotrypsin digestion of rabbit muscle MPGM*

Rabbit muscle MPGM (Sigma) was characterised by SDS–polyacrylamide gel electrophoresis and amino acid analysis, and shown to be of adequate purity. The enzyme (105 mg) was dialysed, freeze-dried and dissolved in 1% (w/v) ammonium bicarbonate. Chymotrypsin (Worthington) was added in two 1 mg amounts, and digestion was for 24 h at 37°C.

### 2.2. *Isolation of histidine-containing peptides*

The chymotryptic peptides were separated by ion-exchange chromatography on Technicon P resin using a gradient of acetic acid and pyridine, and detected by ninhydrin reagent [10]. After elution, the resin was washed with two 25 ml samples of ammonia solution (spec. grav. 0.88): pyridine (1:1, v/v). Each peak was tested for the presence of histidine by Pauly reagent [13] and amino acid analysis. Peptides were further purified by high-voltage paper electrophoresis at pH 6.5 and/or pH 2 [14].

### 2.3. *Sub-digestion of peptides*

Peptide (~200 nmol) dissolved in 1% (w/v) ammonium bicarbonate was digested by 100 μg subtilisin (Nova Industr. A/S) for 1 h at 37°C. The sub-peptides were purified by high-voltage paper electrophoresis.

### 2.4. *Analytical methods*

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.

Peptides were hydrolysed in evacuated tubes with 0.5 ml of 50% (v/v) Aristar HCl at 110°C for 20 h. Tryptophan was detected after reaction with Ehrlich reagent [15]. Sequences were determined by the manual dansyl-Edman procedure [10].



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## 3. Results and discussion

### 3.1. *Isolation of histidine peptides*

The ion-exchange separation of the chymotryptic peptides is shown in fig.1, and peaks containing histidine are indicated. An additional histidine-containing peptide was found in the ammonia–pyridine wash. The amino acid compositions and electrophoretic mobilities of peptides RM1 and RM2 and their subtilisin subpeptides are given in table 1.

### 3.2. *Amino acid sequence of active site peptides*

The amino acid compositions of peptides RM1 and RM2 suggested these peptides corresponded to the 2 yeast active site peptides (see table 2), and their amino acid sequences were determined (fig.2). It is apparent from a comparison of the sequences in table 2 that the yeast and rabbit muscle active-site peptides are virtually identical, with only a single

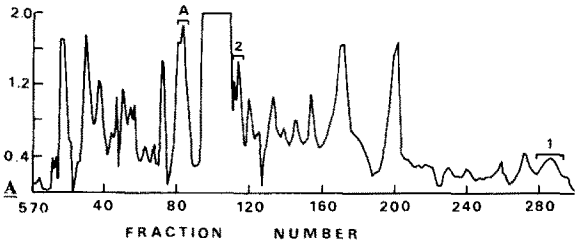


Fig.1. Ion-exchange chromatography of chymotryptic peptides on Technicon P resin. Freeze-dried peptides were dissolved in 2 ml 0.2 M pyridine and adjusted to pH 2–3 with 98% formic acid. The sample was applied to a 0.6 × 150 cm column that had been equilibrated with 0.2 M pyridine acetate (pH 3.1). Elution was at 40°C at 48 ml/h flow rate with a gradient of pyridine and acetic acid using a 9-chambered Technicon Autograd [10]. The effluent (~10%) was mixed with ninhydrin reagent for the detection of peptides, and the remainder was collected in 2.5 ml fractions. The bracketed fractions A, 1 and 2 contained histidine.

Table 1
Peptide data

| Name | RM1 | RM1Sa | RM1Sd | RM1Sf | RM2 | RM2Sa | RM2Sb |
|---|---|---|---|---|---|---|---|
| $m_{6.5}$ | +0.23 | −0.38 | 0 | +0.46 | +0.1 | 0 | +0.50 |
| Asx | – | – | – | – | 1.2 | 0.7 | – |
| Thr | 1.0 | 0.7 | 1.0 | – | – | – | – |
| Ser | 0.9 | 1.0 | 1.0 | – | 0.8[a] | 0.5[a] | – |
| Glx | 1.2 | 1.2 | – | 1.0 | – | – | – |
| Pro | – | – | – | – | – | – | – |
| Gly | 1.2 | – | – | 1.4 | 1.3 | 1.0 | – |
| Ala | – | – | – | – | – | – | 2.0 |
| Cys | – | – | – | – | – | – | – |
| Val | 1.0 | – | – | 0.9 | 2.0 | – | – |
| Met | – | – | – | – | – | – | – |
| Ile | – | – | – | – | 1.2 | – | 0.9 |
| Leu | – | – | – | – | – | – | – |
| Tyr | – | – | – | – | – | – | – |
| Phe | – | – | – | – | – | – | – |
| His | 0.9 | – | – | 0.9 | 0.5 | – | 0.9 |
| Lys | – | – | – | – | – | – | – |
| Arg | 1.3 | – | – | 0.9 | – | – | – |
| Trp | + | + | + | – | – | – | – |
| Total | 8 | 4 | 3 | 5 | 6 | 2 | 4 |
| N-terminus | Val | Glx | Ser | n.d. | Ile | Gly | Ile |

[a] The compositions of these peptides contained Ser, although no Dns-Ser was detected at the third position of RM2Sa

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

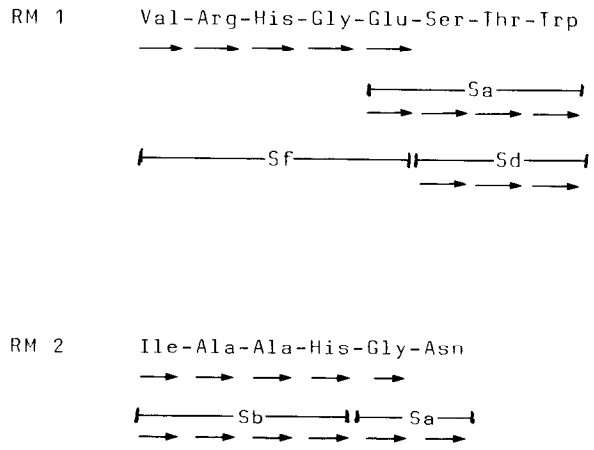


Fig.2. Amino acid sequence of 'active site' histidine-containing peptides. Residues identified by the manual dansyl-Edman procedure are indicated (→). Peptide data is given in table 1.

Table 2
Comparison of histidine-containing peptides

| | |
|---|---|
| RM 1 | Val-Arg-His-Gly-Glu-Ser-Thr-Trp |
| Y 1 (AS)[3] | Val-Arg-His-Gly-Glu-Ser-Gln-Trp |
| RM 2 | Ile-Ala-Ala-His-Gly-Asn |
| Y 2 (AS)[3] | Ile-Ala-Ala-His-Gly-Asn-Ser-Leu |
| Y 3 [10] | Arg-Leu-Asn-Glu-Arg-His-Tyr |
| Y 4 [16] | Val-Lys-His-Leu |
| CM (P-HIS) [12] | Ala-Gly-Gln-Leu-Asp-Asp-Glu-Ser-His-Arg |

*Abbreviations:* RM, rabbit muscle; Y, yeast; CM, chicken muscle; AS, active site; P-HIS, phosphohistidine

conservative replacement. The reason why the chicken muscle phosphohistidine sequence is so dissimilar is not clear.

The structural homology between the yeast and rabbit muscle enzymes active sites would be consistent with their having very similar if not identical reaction mechanisms, and would indicate that kinetic results obtained for the rabbit muscle enzyme are applicable to the yeast enzyme.

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