AMINO ACID SEQUENCE HOMOLOGY IN THE ACTIVE SITE OF RABBIT, BEEF, WHALE AND CALAMARY MUSCLE ALDOLASES

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1. Introduction

Useful information can often be obtained from comparison of selected regions of the same protein isolated from a variety of species for the understanding of the genetic and functional relationships.

It has been shown that an impressive homology exists in amino acid sequence around the substrate-binding lysine residue of fructose diphosphate aldolase (E.C.4.1.2.13) isolated from the muscle of distantly related sources such as mammals [1], amphibia [2], shell-fish [3], birds [4] and fish [5,6].

The present investigation was undertaken to extend this study to a molluscan muscle aldolase from which kinetic and molecular properties have already been reported [7]. In addition, we present the sequence of the active centre peptides of beef and whale aldolase.

2. Materials and methods

Aldolase from calamary muscle (*Loligo vulgaris*) beef and whale muscle was isolated by ammonium sulfate fractionation and substrate affinity chromatography according to the procedure of Penhoet et al. [8]. A specific extinction coefficient $E^{1\%}_{280} = 9.1$ was assumed for calamary aldolase. Labelling of the substrate binding site with [^{14}C]DHAP was carried out as described for rabbit muscle aldolase [1]. Carboxymethylation of the [^{14}C]glycerophosphate aldolase was performed in 8 M urea at pH 8.5 using iodoacetic acid. The protein was digested with 5% trypsin (w/w) in 0.5% NH_4HCO_3, pH 8.5, for 4 hr at 37°C. Tryptic peptides were fractionated by gel filtration on Sephadex G-25 (100 cm × 2.5 cm) in 0.5% NH_4HCO_3, pH 7.7. Radioactive fractions were recovered by freeze-drying. Further purification of the active site peptide was performed by high voltage electrophoresis on Whatman 3 MM paper in 0.2 M pyridine-acetate buffer, pH 4.62.

Amino acid analyses were carried out with a Locarte automatic amino acid analyser (Locarte company, London UK). N-terminal amino acid determination was carried out using the dansyl technique of Gray and Hartley [10]. C-terminal digestion was carried out on 0.1 mole of peptide with a mixture of CPA-CPB** (20:1, substrate: enzyme, w/w) in 0.2 M *N*-ethylmorpholine acetate, pH 8.5, at 37°C.

Peptide sequencing was performed automatically with a protein sequencer Beckman 890 B using the DMAA–propanol–water system Niall [11]. Prior to sequence determination, the peptide was treated according the method of Braunitzer, to render it more hydrophilic [12]. PTH derivatives were identified by

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** Carboxypeptidases A and B.

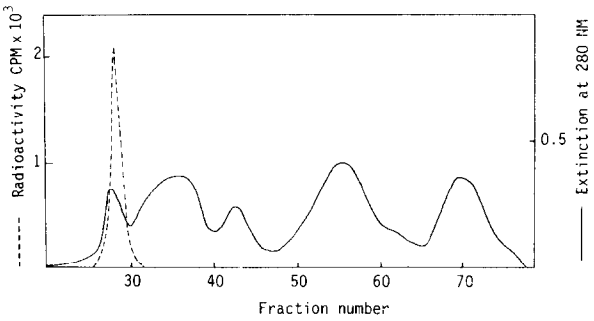

Fig.1. Fractionation on Sephadex G-25 of 500 mg of tryptic peptides of molluscan muscle [^{14}C]glycerophosphate aldolase. The column is equilibrated in NH_4HCO_3 0.005 M, pH 7.7. The flow-rate is 50 ml/hr. Fractions containing 8 ml of the efluent were collected.

gas chromatography [13] and amino acid analysis after back hydrolysis [14] was carried out with Durrum high pressure amino acid analyser (Durrum Instruments, Palo Alto, Calif. USA).

3. Results

The elution profile on Sephadex G-25 of the tryptic peptides of labelled calamary muscle aldolase is shown in fig.1. About 90% of the radioactivity is eluted in the exclusion volume. The active site peptide was further purified by high voltage electrophoresis on paper with a recovery of 50%. All three active site peptides were shown to be homogeneous by N-terminal analysis and autoradiography. The N-terminal amino acid was in all cases alanine. Table 1 gives the amino acid composition of the three peptides. Fig.2 shows the amino acid sequences of the active site peptides as determined by the automatic Edman degradation procedure. For calamary muscle aldolase the first 22 residues were determined without any ambiguity, except positions 5 and 6 which are probably occupied

Table 1
Amino acid composition of the active site peptides of rabbit, whale, beef and calamary muscle aldolases

Residue	Rabbit	Calamary	Beef	Whale
Asx	2	2.1	2.1	1.8
Thr	3	1.7	2.6	2.6
Ser	1	0.6	1.3	1.4
Glx	2	2.1	1.3	2.0
Pro	2	1.0	2.1	1.0
Gly	2	3.1	2.3	1.8
Ala	2	2.8	2.5	2.1
Val	1	–	1.2	1.1
Met	1	0.6	0.6	0.8
Ile	1	1.2	1.1	1.1
Leu	4	3.8	3.9	3.7
Tyr	1	–	0.8	0.9
Phe	–	1.2	–	–
His	3	3.2	2.0	2.1
Lys	1	1.2	1.1	1.2
Glys[a]	1	1	1	1
Cm-Cys[b]	1	0.8	0.6	0.8

[a] Glyceryllysine
[b] Carboxymethylcysteine

The results are given in residues of each amino acid/mole of peptide. The data for the rabbit muscle enzyme are from [1].

Rabbit: Ala–Leu–Ser–Asn–His–His–Ile–Tyr–Leu–Glu–Gly–Thr–Leu–Leu–Lys–Pro–Asn–Met–Val–Thr–Pro–Gly–His– (15, 28)
Ala–Cys–Thr–Glu–Lys

Beef: Ala–Leu–Ser–Asn(His–His)Ile–Tyr–Leu–Glu–Gly–Thr–Leu–Leu(Lys)Pro–Gly(Met)Val–Thr( )Gly–( )
Ala( )Thr( )(Lys)

Whale: Ala–Leu–Ser–Asn–His(His)Ile(Tyr)Leu–Glu–Gly–Thr–Leu–Leu(Lys)Pro–Asn(Met)Val–Thr–Pro–Gly–( )
Ala( )Thr–Glu(Lys)

Calamary: Ala–Leu–Ala–Glx(His–His)Ile–Phe–Leu–Glu–Gly–Thr–Leu–Leu–Lys–Pro–Asn–Met–Ala–Thr–Ala–Gly–His
Gly–Cys–Ser–Asx–Lys.

Fig.2. Amino acid sequence of the active site peptide of rabbit, beef, whale and calamary muscle aldolases. The substrate-binding residue is at position 15. The sequence of the rabbit muscle enzyme is from Lai et al [1]. Differences between the calamary sequence and the rabbit sequence are underlined.

by two histidine residues. The assignment of amides was not carried out. The sequence of the last 5 amino acids as determined by carboxypeptidase digestion appeared to be Gly–Cmc–Ser–Asx–Lys. The sequences of the beef and whale peptides were determined although a number of positions were not positively identified. It seems that these two sequences are identical to that of rabbit except for position 17 in beef, where we found a glycine. This is not in good agreement with the amino acid analysis. The subsequent glycine at position 22 was identified without ambiguity.

Residue number 15 is the lysine forming the Schiff-base with the carboxyl group of the substrate.

4. Discussion

The sequence of the beef and whale peptides are probably identical to that of the rabbit. The sequence homology around the active lysine residue in the rabbit and calamary aldolases can be clearly seen in fig.2. Of the 28 amino acids in the peptide at least 19 are identical (assuming that the positions of the 3 histidine residues in the sequence are as indicated, since these residues were not identified unequivocally). The changes are mostly located at the two extremities of the peptide and 5 of them are conservative. Based on the electrophoretic mobility of the calamary peptide it seems likely that at least one amide is substituted by an acid. Thus there are 4 radical replacements: an acid for an amide at either positions 4, 10 or 17; the serine at position 3 and the tyrosine at position 8 in the active site of rabbit aldolase are replaced by alanine and a phenylalanine respectively in calamary aldolase; and there is one replacement, perhaps more critical for the secondary structure, the change of a proline for an alanine at position 21 in the moluscan aldolase.

Such an homology in a 28 amino acid sequence is surprising, since only the lysine at position 15 is implicated in the interaction with the substrate. In rabbit aldolase as in calamary aldolase, it has been shown that other functional residues are situated in the polypeptide chain, far from the substrate binding lysine [15–18]. To explain the active site sequence homology between two muscle aldolases from such phylogenetically distinct sources, we would suggest that this peptide is implicated in some way in the conservation of the three-dimensional structure of the enzymes. Both aldolases are tetrameric enzymes with a subunit molecular weight of approx. 40 000. It is therefore reasonable to suppose that these aldolases do have similar three dimensional structures.

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