AMINO ACID SEQUENCE OF A FRAGMENT OF RABBIT MUSCLE ALDOLASE

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Cleavage of rabbit muscle aldolase by cyanogen bromide results in the formation of four fragments of different size [1]. Study of the primary structure of the enzyme has been based on the examination of these fragments [1, 2].

Because of the insolubility and associated problems due to the relatively large size of the fragments Lai [1], as well as Anderson et al. [3], used pyridine–acetic acid buffer as eluant for the Sephadex G75 column in the separation procedure.

By combining cyanogen bromide cleavage and the reversible modification of amino groups we could successfully overcome these difficulties without impairing resolution [2]. Aldolase, carboxymethylated with ^{14}C-bromoacetate in the presence of 8 M urea, was treated with cyanogen bromide as described by Lai [1]. The mixture of the fragments was acylated with maleic anhydride [4] or citraconyl anhydride [5] in the presence of 8 M urea, at pH 8.5. At the end of the reaction, the solution of the acylated fragments was directly applied to a Sephadex G-75 (fine) column. Elution was performed with 0.1 M ammonium bicarbonate (fig. 1).

The alignment of the four fragments separated by gel chromatography was first described by Lai [1] as CB1–CB3–CB4–CB2 and we confirmed his results by determining the *N*- and *C*-terminal sequences of the unblocked fragments.

We further analysed the peptides of the tryptic and chymotryptic digest of acylated and deacylated CB1, CB3 and CB4, as well as the chymotryptic, peptic and partial acid hydrolysate of the fragments obtained from the tryptic digest of the unblocked cyanogen bromide fragments. The *N*-terminal se-

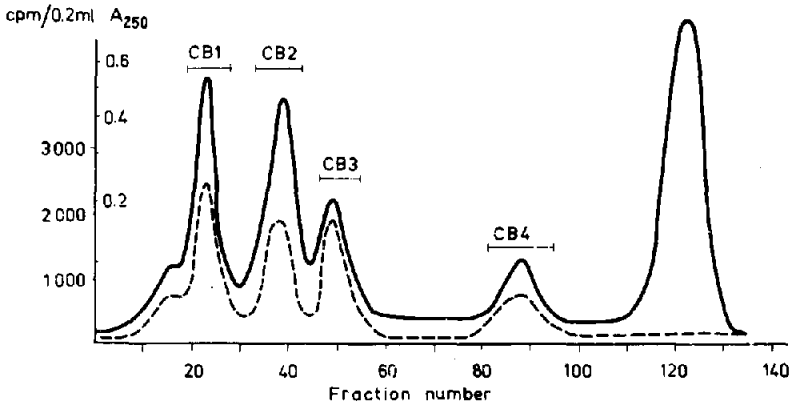

Fig. 1. Separation of the acylated cyanogen bromide fragments of aldolase. Sephadex G-75 (fine) column (3.5 X 180 cm) equilibrated and eluted with 0.1 M ammonium bicarbonate, pH 8.2. Elution velocity 34 ml/hr, fraction volume 8.5 ml. Breakthrough volume 340 ml. Fractions from tube 100 did not contain peptides or other ninhydrin positive substances. The fifth peak represents the salt fraction (urea, citraconic acid etc.). —— A_{250}; – – – – radioactivity.

quences were determined by the dansyl-Edman method [6] and dansyl amino acids were identified by thin layer electrophoresis [7].

From these studies the following partial sequence could be deduced:

145 150
Cmc–Ala–Gln–Tyr–Lys–Lys–Asp–Gly–Ala–Asp–
CB1

155 160
–Phe–Ala–Lys–Arg–Met–Glx–Asx–Ala–Asx–Val–

165 170
–Leu–Ala–Arg–Tyr–Ala–Ser–Ile–Gly–Val–/Cmc_1
CB3

181 185
$Asx_1Glx_3Pro_1Ile_1Leu_1$/–Pro–Glx–Ile–Leu–Pro–Gly–
CB3

192 195
–/$His_1Asx_3Val_1$/–Lys–Arg–Cmc–Gln–Tyr–Val–Thr–
CB3

200 205
–Glu–Lys–Val–Leu–Ala–Ala–Val–Lys–Ala–Leu–

210 215
Ser–Asn–His–His–Ile–Tyr–Leu–Gln–Gly–Thr–Leu–
CB3

220 225
–Leu–Lys–Pro–Asn–Met–Val–Thr–Pro–Gly–His–
CB3

230 235
–Ala–Cmc–Thr–Gly–Lys–Tyr–Ser–His–Glu–Gln–
CB4 CB4

240
–Ile–Ala–Met

From the tryptic digest of the acylated CB1 we obtained peptide 144–158* which contained the cysteinyl residue at position 144. This peptide had homoserine as *C*-terminal residue; it was thus the *C*-terminal section of fragment CB1. Sequence studies on the *N*-terminal tryptic peptide of CB1, which contains 11 amino acids, are described elsewhere [8].

The tryptic digest of the maleylated fragment CB3 contained peptides 159–166, 167–193 and 194–224, the alignment of which was deduced from the common *N*-terminal sequence of CB3 and peptide 159–166, the obvious *C*-terminal position of peptide 207–224 and the analysis of overlapping chymotryptic peptides. Peptide 207–224 has already been characterized by Lai et al. [9] when they located the substrate-binding ϵ-amino group of Lys-221.

The above sequence of CB4 (225–242) is in good agreement with the result of Lai and Chen [10]. The only discrepancy is at position 238 where we found Glu instead of Gln.

The preliminary sequence described here accounts for 99 amino acids which are located in the middle region of the linear structure. Four out of seven cysteinyl residues of the protein are included in this region of the molecule. One of the four cysteinyl residues, Cys-231, has been found to be most reactive towards bromoacetate but its alkylation did not impair enzymic activity [11–13]. In the sequential vicinity of this Cys residue, the substrate-binding Lys-221 is located. We assume that the side chains of these residues are on the surface of the molecule.

The charge distribution shows certain anomalies in this region: from Lys-192 to Met-242 there are only 2 acidic but 10 basic side chains. This inequality may contribute to the formation of the microenvironment of the active site.

* The numbering of residues is based on the assumption that fragment CB1 consists of 158 amino acid residues [1]. These figures may slightly change as sequence work proceeds.

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