AMINO ACID SEQUENCE OF THE N-TERMINAL 158 RESIDUES OF RABBIT MUSCLE ALDOLASE

M. SAJGÓ and Gy. HAJÓS

Enzymology Department, Institute of Biochemistry, Hungarian Academy of Sciences, H-1502 Budapest, P.O. Box 7, Hungary

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1. Introduction

The amino acid sequence of two out of the four cyanogen bromide fragments of rabbit muscle aldolase which represent the medium part of the polypeptide chain has already been described [1–3]. As for the N-terminal cyanogen bromide fragment (CBl) only the sequence of some smaller segments were known, that of the N-terminal tryptic peptide [4, 5] and the sequence or composition of the cysteine-containing peptides [6–9].

In this paper the sequence analysis of the N-terminal cyanogen bromide fragment containing 158 amino acid residues is presented.

2. Materials and methods

Rabbit muscle aldolase, recrystallized 3 times, was prepared according to Taylor et al. [10]. Trypsin (Calbiochem, B grade) was free of chymotrypsin, as tested with *N*-benzoyl-L-tyrosine-ethylester as substrate.

Carboxymethylation of aldolase and the isolation of the N-terminal cyanogen bromide fragment (CBl) was carried out as described earlier [1].

Digestion with trypsin and chymotrypsin (Calbiochem, A grade) was performed in 0.1 M ammonium bicarbonate, pH 8.2, for 2 hr at 37°C. Enzyme–substrate ratio 1:50, by weight. Digestion was arrested by diisopropyl-fluorophosphate. The digests were gel-filtered on a 2 × 180 cm Sephadex G-25 (fine) column with 0.1 M ammonium bicarbonate as eluent. The fractions were purified by paper electrophoresis and paper chromatography in the buffers and solvent systems previously applied [6].

Digestion with plasmin was performed by a freshly prepared mixture of plasminogen (HUMÁN, Budapest) and streptokinase (Seheringwerke A.G.). The ratio of plasminogen to streptokinase was 20:1, by weight; the ratio of plasminogen to the substrate was 1:20, by weight. The pH was adjusted to 8 with solid ammonium bicarbonate, then after 2 hr incubation at 37°C the digestion was stopped by heat denaturation. The plasmic digest was gel-filtered as mentioned above. The breakthrough fraction was further purified on a 1.5 × 25 cm DEAE-cellulose (DE-52, Whatman) column equilibrated with Tris buffer, pH 8.3, containing 0.01 M Cl^- and 8 M urea. Elution was carried out with a linear Cl^- gradient by the continuous mixing of 100 ml of equilibrating buffer and 100 ml of the equilibrating buffer containing 0.1 M NaCl. The salt content of the pooled fractions was removed by gel-filtration on a 3 × 80 cm Sephadex G-25 (coarse) column eluted with 0.1 M ammonium bicarbonate.

N-terminal sequences were determined by the dansyl–Edman method, cf. [11], the dansyl amino acids were identified by thin-layer electrophoresis [12] or by chromatography on polyamide thin-layer sheets, cf. [11]. C-terminal sequence were determined by a micro method [13] using ion-exchange resin-coated chromatoplates (Ionex-25 SA, Macherey and Nagel, Düren) for the identification of the amino acids released [14].

Amide groups were assigned by examining the change in the electrophoretic mobility of peptides after removing the residue Asx or Glx by Edman degradation.

Amino acid analysis was carried out according to the single column procedure of Dévényi [15] on a Beckman-Unichrom analyzer.

Radioactivity was measured by liquid scintillation in a Packard Tricarb scintillation spectrometer, type 2420.

3. Results and discussion

The sequence analysis of fragment CB1 was based on the analysis of the tryptic peptides separated by gel-filtration (fig. 1) and further purified by paper electrophoresis and paper chromatography.

To establish the alignment of tryptic peptides, apart from the usual chymotryptic digestion we also prepared large fragments by plasmic digestion as proposed by Gráf [16]. Plasmin is tailored to split the Lys–X and Arg–X bonds like trypsin but under the conditions applied not all of these peptide bonds are cleaved. This results in the formation of large fragments comprising two or even more tryptic peptides.

Another way of getting large fragments of the N-terminal portion of the molecule, which facilitated establishment of overlaps, was the limited tryptic digestion of PMB-treated aldolase. Under the experimental conditions applied a limited proteolytic process leads to the formation of a 50% active aldolase, denoted as aldolase-T [17, 18]. During the course of this limited digestion two large peptides could be isolated which contained 27 and 41 amino acid residues from the N-terminal end of the protein [18]. These peptides, called Nt-27 and Nt-41, contained peptides T1b, T2a and T3 (Nt-27) and T1b, T2a, T3 and T4a (Nt-41). The amino acid sequence of peptide T1a has previously been described [4, 5]. However, later we confirmed the result of Lai et al. [5] who – in contrast to our earlier results [4] – reported an additional proline at position 9.

In the course of the sequence analysis of fragment CB1 we also located a cysteinyl residue (Cys-84) which had not been previously found. Earlier only two tryptic peptides containing carboxymethyl cysteine could be isolated and identified in fragment CB1, peptides T16 and T13a [6, 8, 9]. Later we isolated from the tryptic digest of fragment CB1 an additional, carboxymethyl cysteine-containing peptide, T9a, which did not contain lysine or arginine [7]. The 'real'

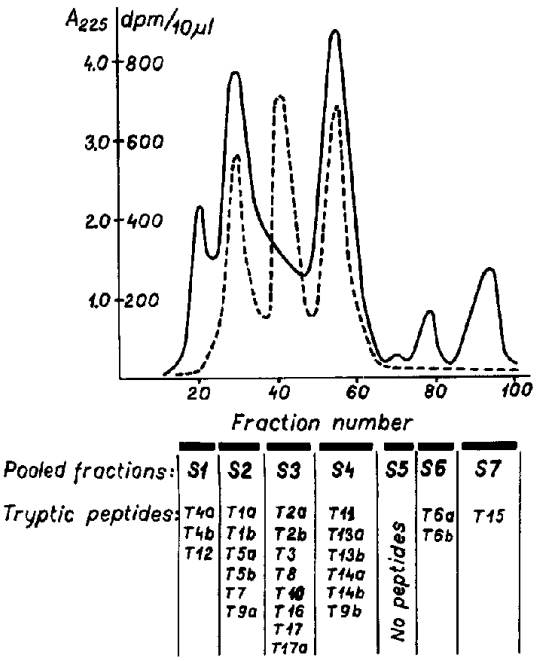

Fig. 1. Fractionation of the tryptic digest of fragment CB1 on a Sephadex G-25 (fine) column. Column size: 2 × 180 cm, flow rate: 31 ml/hr. Elution was carried out with 0.1 M ammonium bicarbonate, pH 8.2, containing 1% butanol. Void volume 160 ml, fraction volume 7.8 ml. S1, S2 etc. mean the pooled fractions, under the marks of the fractions the marks of the tryptic peptides are indicated. (————) A_{225}, (- - - - - - - -) radioactivity.

tryptic peptide of this region, T9, could only be obtained if we applied a short tryptic hydrolysis – 30 min instead of 2 hr. Since trypsin was free of chymotryptic activity in the esterase test, we ascribe the splitting of the Phe-91–His-92 peptide bond to the inherent chymotryptic activity of trypsin. Similar chymotryptic-like splitting was also found between residues 156–157, indeed, in the tryptic digest peptides T17 and T17a were found nearly in the same amount.

Anderson et al. [19] isolated from beef muscle aldolase a tryptic peptide which differs from T2a by a serine–cysteine replacement. This cysteine is reactive beef aldolase towards alkylation, consequently, it has to be on the surface of the molecule, exposed to the solvent medium. Since T2a is at the N-terminal region of the molecule, by analogy we may speculate whether this N-terminal region is on the surface of the native protein.

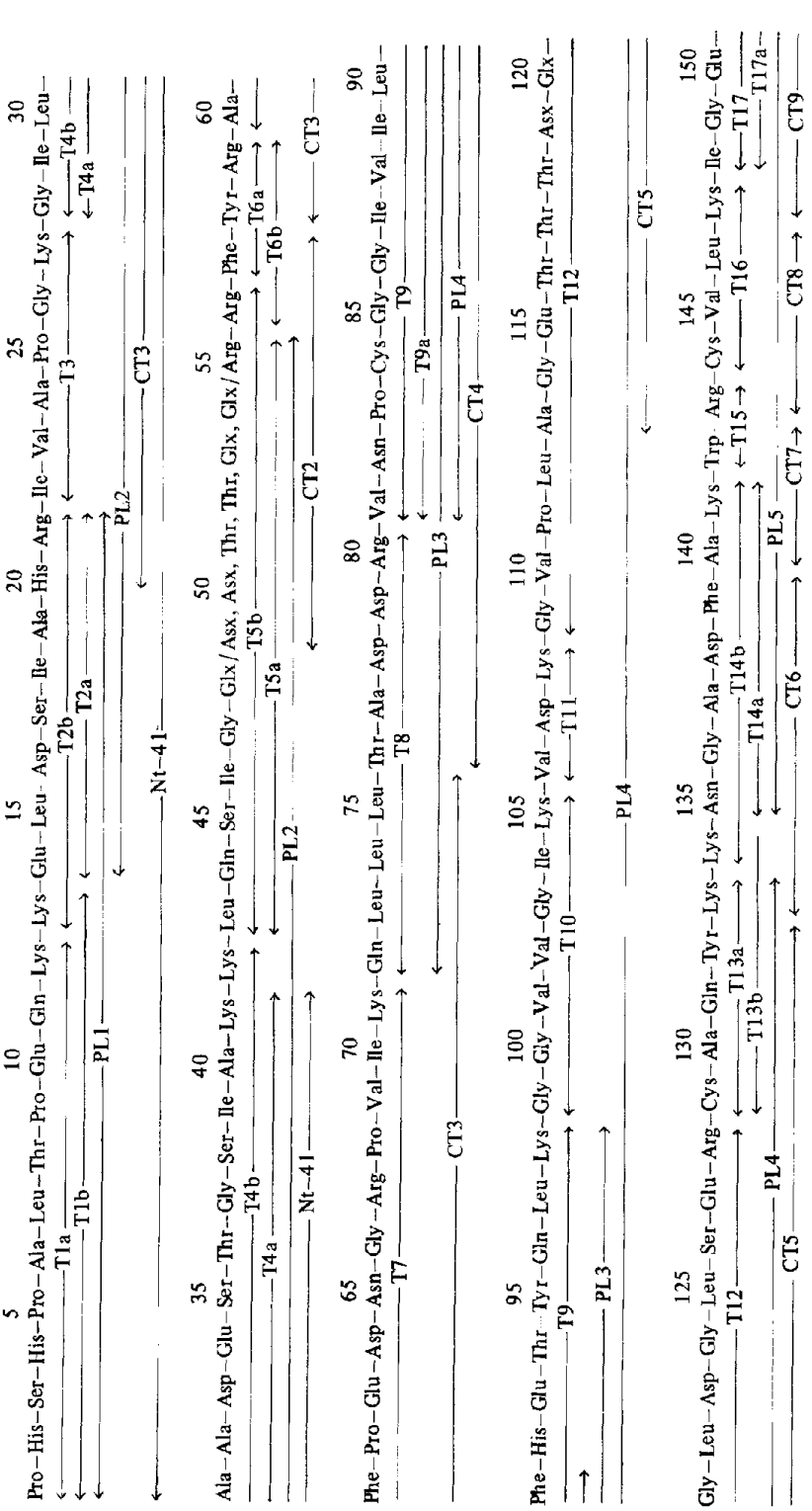

Fig. 2. Amino acid sequence of fragment CBl. T: tryptic, CT: chymotryptic, PL: plasmic peptides. In the figure all tryptic peptides are marked, whereas from the plasmic and chymotryptic peptides only the informative ones are indicated.

The results presented did not reveal further differences between the primary structure of subunits from that reported earlier. We namely found a serine–histidine and a glutamine–glutamic acid replacement at positions 3 and 11, respectively [11]. However, the fractional amount of the peptides showing these differences varied from one aldolase preparation to the other.

Together with the data already published on the structure of fragments CB3 and CB4 [1–3] the tentative primary structure of two-thirds of the molecule is already known.

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