AMINO ACID SEQUENCE OF 37 RESIDUES AT THE CARBOXYL–TERMINAL END OF HUMAN PLASMA ALBUMIN

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1. Introduction

Plasma albumin plays in the organism the important role of a transport protein. Its amino acid sequence has been studied systematically only during the past few years. Fragments of bovine [1, 2] and porcine [2] albumin have been investigated in more detail. The amino acid sequence of the 24-residue N-terminal region of human albumin has been determined [3]. The peptides from the neighborhood of the only SH-group [4] and the only tryptophan residue of human plasma albumin [5] have been isolated. The C-terminal end [6] of its molecule has been characterized by the sequence (Leu, Val, Ala_{3-4})Gly – Leu.

All fragments resulting from specific cleavage of human plasma albumin by cyanogen bromide have been isolated recently [7–10]. Six of these peptide chains containing 88, 36, 162, 95, 110, and 31 amino acid residues have C-terminal homoserine. One chain composed of 37 amino acids does not contain homoserine [9, 10]. As yet, the amino acid sequence of the 31-residue chain has been determined [9, 11]. The remaining chains have been characterized by sequential analysis of five N- and C-terminal amino acid residues of each chain. The aim of this study is to report on sequential studies on the C-terminal region of human plasma albumin represented by a 37-residue chain not containing homoserine.

2. Material and methods

The C-terminal chain has been isolated as described before [10] from human plasma albumin (Imuna, Sarisske Michalany, Czechoslovakia). The albumin was hydrolyzed by cyanogen bromide in 70% formic acid for 22 hr at 5°. For first fractionation a Sephadex G-75 column equilibrated with 0.03 M ammonium formate at pH 2.86 was used. The material emerging as the first peak was oxidized by performic acid, lyophilized, and rechromatographed on the same column. The C-terminal chain, contained in the last elution peak, was fractionated further on QAE-Sephadex A-25 in 0.01 M sodium acetate, pH 5.0. A linear elution gradient of 0 to 1.0 M NaCl was used. The material contained in the main peak was desalted on Sephadex G-25 in 0.02 M NH_4OH. It was designated VII-Asp, i.e. by a symbol of its N-terminal amino acid.

The enzymatic digests of the chains were fractionated on Whatman No. 3 paper by electrophoresis at pH 1.9 and 5.6 [12, 13], and by chromatography in systems S1, n-butanol – pyridine – acetic acid – water (15:10:3:12) or S3, methyl ethyl ketone – tert-butanol – water (2:2:1). The methods of amino acid analysis and sequential analysis by dansyl-Edman degradation have been described earlier [8].

The chymotryptic digest of chain VII-Asp was prepared with 2.3 μmole of material. It was dissolved in 4 ml of 0.05 M NH_4HCO_3 and digested with the enzyme at a molar ratio of 1:50 for 16 hr at 23°. The digest was lyophilized. A sample of 0.12 μmole was used for the peptide map (fig. 1). The main portion of the digest was prefractionated on a 1.6 × 50 cm column of Biogel P2, equilibrated with 0.02 M NH_4OH. A flow rate of 6 ml per hr was maintained and the absorbance of the effluent at 260 nm was measured. Only two fractions were obtained. They were fractionated further by chromatography in system S1 and by

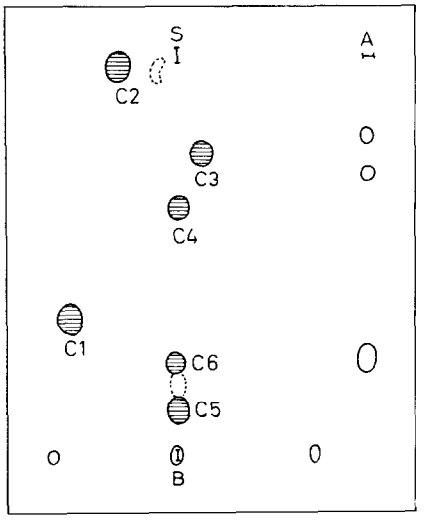

Fig. 1. Peptide map of chymotrypsin digest of chain VII-Asp. 1st direction (horizontally), electrophoresis at pH 5.6, anode to the left, 2nd direction (vertically), descending chromatography in the system n-butanol–pyridine–acetic acid–water (10:15:3:12). S, origin. Reference mixture of amino acids (A and B), Lys, Glu, Leu. Peptides C1 through C6.

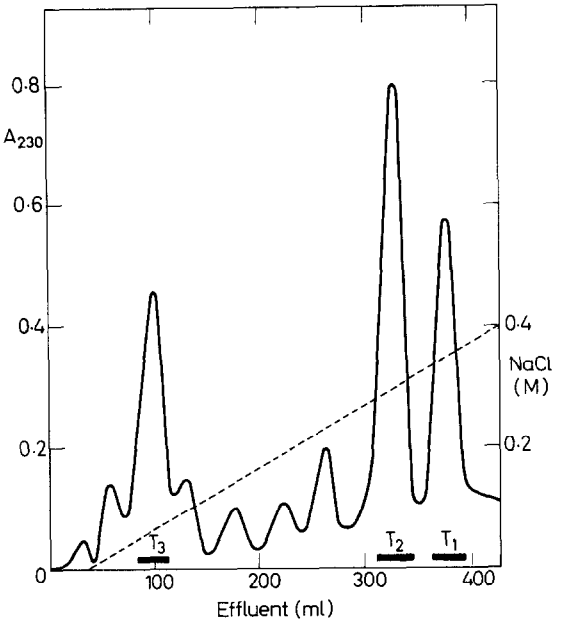

Fig. 2. Elution profile of tryptic digest of chain VII-Asp on QAE-Sephadex A-25. Column 1.6 × 19 cm, eluted by 0.02 M Tris-HCl buffer, pH 6.5. Fractions 5.2 ml per 7 min. After the emergence of 30 ml of buffer, a linear gradient of 0 to 0.4 M NaCl in 400 ml of 0.02 M Tris-HCl buffer, pH 6.5, was applied.

electrophoresis at pH 5.6. Peptides C1 to C6 were isolated. Peptide C2 was digested further with trypsin under conditions identical to those used for chymotryptic cleavage. The digest was fractionated electrophoretically (pH 5.6) and peptides C2T1, C2T2, and C2T3 were isolated.

The tryptic digestion of chain VII-Asp was carried out with 10 μmole of the material. It was dissolved in 4 ml of 0.05M NH_4HCO_3 and hydrolyzed at a molar ratio of 1:100 for 1 hr at 37°. An equal portion of trypsin was added afterwards and the digestion was continued for 3 hr. The digest was lyophilized and then dissolved in 2 ml of 0.02 M Tris-HCl buffer. This solution was adjusted to pH 8 by ammonium hydroxide and fractionated on a Sephadex A-25 column (see fig. 2). Peptides T1, T2, and T3 were obtained. Peptide T1 was subsequently digested with chymotrypsin. The digest was resolved electrophoretically at pH 5.6. Peptides T1C1 to T1C2 were obtained.

Peptide T3 (2 μmole) was dissolved in 1 ml of 0.1 M NH_4HCO_3, containing 0.001 M $CaCl_2$, and digested with thermolysin (Calbiochem, B-grade) at a molar ratio of 1:50 for 6 hr at 37°. The digest was lyophilized and fractionated electrophoretically at pH 1.9. Peptide T3R3 was isolated from the zone showing the same mobility as serine. The main zone, showing the mobility of tyrosine, was fractionated in system S1 and afforded peptide T3R2. The second zone of a R_f-value higher than that of leucine was fractionated in system S2 and gave peptides T3R1 and T3R4.

3. Results and discussion

The amino acid composition of the C-terminal region (VII-Asp) of human plasma albumin is the following: Lys_5, Asp_4, Thr, Ser, Glu_5, Gly_2, Ala_8, Cys_3, Val_2, Leu_3, Phe_3. The derived amino acid sequence of the fragment is shown in fig. 3. The N-terminal amino acid sequence of five residues was determined by Edman degradation of the intact chain as Asp – Asp – Phe – Ala – Ala –. Peptides C1 through C6, isolated from the chymotryptic digest account for

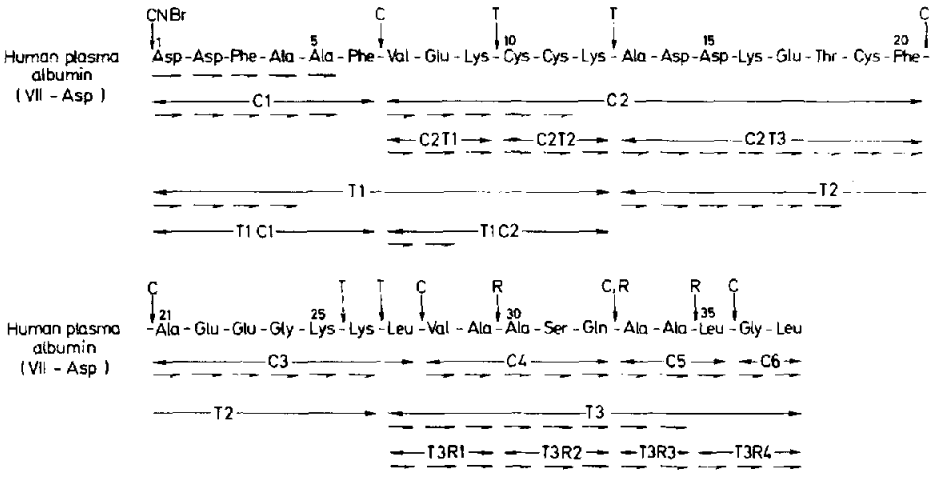

Fig. 3. Amino acid sequence of C-terminal region (VII-Asp) of human plasma albumin. ↓ CNBr, ↓ C, ↓ T, and ↓ R denote sites of cleavage by cyanogen bromide, chymotrypsin, trypsin, or thermolysin, respectively. → Symbolizes the sequence determined by the dansyl-Edman degradation method.

the whole chain. Peptide C1: Asp – Asp – Phe – Ala – Ala – Phe, is identical with the N-terminal region of the chain. Peptide C2: Val – Glu – Lys – Cys – Cys (Lys_2, Asp_2, Thr, Glu, Ala, Cys, Phe), contains all three cysteic acid residues and the only threonine residue of the chain. Peptide C2 was digested with trypsin and the complete amino acid sequence of the resulting peptides was determined as follows. C2T1: Val – Glu – Lys, C2T2: Cys – Cys – Lys, C2T3: Ala –Asp – Asp – Lys – Glu – Thr – Cys – Phe. Peptide C3: Ala – Glu – Glu – Gly – Lys – Lys – Leu, contains one of the two glycine residues present in the chain and one leucine residue. Peptide C4: Val – Ala – Ala – Ser – Gln, contains the only serine residue of the chain. A characteristic feature of peptides C5: Ala – Ala – Leu and C6: Gly – Leu, is their content of leucine.

Peptide T1: Asp – Asp – Phe – Ala (Lys_2, Glu, Ala, Cys_2, Val, Phe), can be assigned to the N-terminus of the chain with respect to the sequence of the first four amino acids. Chymotryptic digestion of the peptide afforded peptide T1C1: (Asp_2, Ala_2, Phe_2) and peptide T1C2: Val – Glu (Lys_2, Cys_2). The presence of glutamic acid in this peptide was determined by Edman degradation. The sequence of peptide T2: Ala – Asp – Asp – Lys – Glu – Thr (Lys_2, Glu_2, Gly, Ala, Cys, Phe), was elucidated by the same method. This peptide represents the middle region of chain VII-Asp and overlaps chymotryptic peptide C2T3. Peptide T3: Leu – Val – Ala – Ala – Ser – Gln – Ala – Ala (Leu_2, Gly) contains all three leucine residues of chain VII-Asp and completes its C-terminal region. The complete amino acid sequence of peptide T3 follows from the sequence of its thermolysin fragments T3R1: Leu – Val – Ala, T3R2: Ser – Gln – Ala, T3R3: Ala – Ala, and T3R4: Leu – Gly – Leu. The amino acid sequence of peptide T3 is in agreement with the sequence of the overlapping chymotryp-

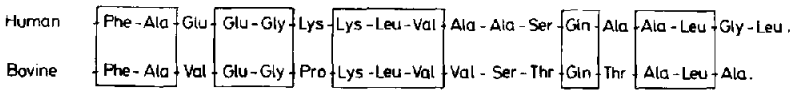

Fig. 4. Comparison of amino acid sequence of C-terminal region of human and bovine plasma albumin.

tic peptides C4, C5, and C6.

No microheterogeneity in the primary structure of human plasma albumin was observed during the sequential studies on chain VII-Asp. Fig. 4 shows the degree of homology between a part of the 37-residue C-terminal region of human plasma albumin reported here and the corresponding part of bovine plasma albumin described by other authors [2].

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