AMINO-ACID SEQUENCE OF HOMOSERINE CHAIN VI-Pro FROM C-TERMINAL REGION OF HUMAN PLASMA ALBUMIN

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The N-terminal amino-acid sequence of the oxidized homoserine chain VI-Pro of human plasma albumin was determined by a protein sequenator. Four peptides, which account for the whole chain, were isolated from the tryptic digest by a combination of paper electrophoresis and paper chromatography. The amino-acid sequences of these peptides were determined by Edman degradation. The primary structure of the chain was derived from a comparison with the amino-acid compositions of overlapping peptides isolated from the chymotryptic digest.

As we have shown earlier[1,2], human plasma albumin is hydrolyzed by cyanogen bromide at all six methionine residues to three fragments in which the chains arisen from the hydrolysis are linked together by disulfide bonds. After oxidation of the first fragment by performic acid we were able to isolate two homoserine chains, designated I-Asp (88 residues) and II-Ala (36 residues). The second fragment consists of one single homoserine chain, III-Cys (162 residues). The third fragment gives four homoserine chains after oxidation, namely IV-Phe (95 residues), V-Pro (110 residues), VI-Pro (31 residues), and chain VII-Asp (37 residues), which does not contain homoserine. The chains are designated according to their N-terminal amino acid, the Roman numerals indicate the probable sequence of the chains in the molecule of human plasma albumin[2]. The present paper reports on the determination of the amino-acid sequence of homoserine chain VI-Pro by a combination of classical techniques of sequential analysis with stepwise degradation in a protein sequenator.

EXPERIMENTAL

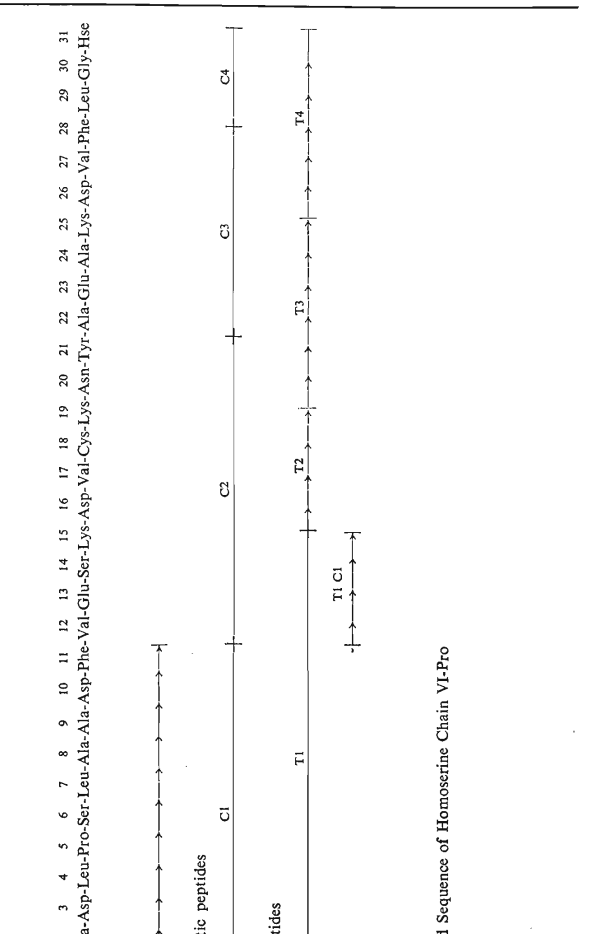
Chymotrypsin and trypsin were prepared by the procedure of Northrop and Kunitz[3]; trypsin was treated with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone[4]. Human plasma albumin was a product of Imuna, Šarišské Michaľany. Homoserine chain VI-Pro was obtained[2] from the cyanogen bromide hydrolysate of human plasma albumin. The enzymatic digestion of the homoserine chain (2 μmol) by chymotrypsin or trypsin was allowed to proceed 4 h at 37°C and a molar enzyme to substrate ratio of 1 : 50. The enzyme was added to 1% solution of the chain in 0·05M-NH_4HCO_3. The digest was lyophilized. The fractionation of the enzymatic digests was effected by electrophoretic[5,6] (pH 1·9 and 5·6) and chromatographic[7] methods. For sequential analysis of smaller peptides the Dansyl technique was employed[8]. The qualitative determination of DNS-amino acids was carried out on polyamide[9] thin layers by the technique introduced by

TABLE I

Amino-Acid Composition of Homoserine Chain VI-Pro and of its Enzymatic Fragments

Peptides isolated from chymotryptic digest (C 1—C 4), peptides isolated from tryptic digest (T 1—T 4). The number of amino-acid residues is given; amide groups are not included.

Amino acids	VI-Pro	C 1	C 2	C 3	C 4	T 1	T 2	T 3	T 4	T 1 C 1
Lysine	3·0 3	— —	1·90 2	1·00 1	— —	0·91 1	0·84 1	0·87 1	— —	1·07 1
Cysteic acid	1·2 1	— —	1·10 1	— —	— —	— —	1·04 1	— —	— —	— —
Aspartic acid	4·8 5	2·04 2	2·18 2	1·04 1	— —	2·11 2	1·00 1	1·06 1	1·05 1	— —
Serine	1·8 2	0·91 1	1·00 1	— —	— —	2·00 2	— —	— —	— —	1·00 1
Glutamic acid	2·5 2	— —	1·08 1	0·94 1	— —	1·16 1	— —	1·00 1	— —	1·07 1
Proline	1·8 2	1·87 2	— —	— —	— —	1·80 2	— —	— —	— —	— —
Glycine	1·1 1	— —	— —	— —	0·97 1	— —	— —	— —	0·95 1	— —
Alanine	5·1 5	3·00 3	— —	1·98 2	— —	2·90 3	— —	1·94 2	— —	— —
Valine	3·0 3	— —	2·00 2	0·90 1	— —	1·16 1	1·00 1	— —	0·95 1	1·00 1
Leucine	3·1 3	2·02 2	— —	— —	0·97 1	2·00 2	— —	— —	1·00 1	— —
Tyrosine	0·8 1	— —	0·74 1	— —	— —	— —	— —	0·60 1	— —	— —
Phenylalanine	2·0 2	1·00 1	— —	0·83 1	— —	0·97 1	— —	— —	0·84 1	— —
Homoserine	1·0 1	— —	— —	— —	1·00 1	— —	— —	— —	1·00 1	— —
Sum	31	11	10	7	3	15	4	6	6	4



Scheme 1

Amino-Acid Sequence of Homoserine Chain VI-Pro

Hartley[10]. For sequential analysis of the intact homoserine chain (1 μmol), a sequenator built in this Laboratory according to Edman's model[11] was used. The program of the analysis and the technique of identification of amino-acid thiohydantoins have been reported earlier[12]. The presence of amides in the peptides was judged by their mobility on electrophoresis[5]. The amino-acid analyses were performed by the method of Spackman and coworkers[13] on peptides hydrolyzed 20 h at 110°C.

RESULTS AND DISCUSSION

The amino acid composition of homoserine chain VI-Pro and of peptides obtained from its chymotryptic or tryptic digest is given in Table I. As abvious from Scheme 1, homoserine chain VI-Pro contains 31 amino acid residues. Its N-terminal amino-acid sequence (residues 1 through 11) was determined in the sequenator. The remaining information required provided the analysis of the chymotryptic and tryptic digest of the chain. Peptides C 1 through C 4, isolated from the chymotryptic digest, account for the whole sequence of the region studied. Peptide C 1 is identical with the N-terminal region of chain VI-Pro; peptide C 4 contains homoserine and thus represents the C-terminal region. The order of the remaining two peptides, C 2 and C 3, emerged from the analysis of peptides T 1 through T 4, isolated from the tryptic digest of the original chain. Peptide T 1 was digested with chymotrypsin; of the obtained fragments only peptide T 1 C 1 was sequenced. Peptides T 2, T 3, and T 4 were sequenced completely. The two digests described provided information permitting the amino-acid sequence of homoserine peptide VI-Pro to be deduced; the latter involves probably the region between the fifth and the sixth methionine residue in the molecule of human plasma albumin. No structural microheterogeneity was observed in the region studied.

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