SEQUENTIAL ANALYSIS OF FRAGMENT CB5(Phe) OF HUMAN PLASMA ALBUMIN. N-TERMINAL SEQUENCE AND TRYPTIC PEPTIDES

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As a part of the sequential studies on human plasma albumin, the amino-acid sequence was studied of fragment CB5(Phe) representing the region of the polypeptide chain of this protein between methionine residues No IV and V. Edman degradation of the fragment and an investigation of peptides resulting from tryptic cleavage of the oxidized fragment permitted its partial amino acid-sequence to be derived as follows: Phe-Leu-Tyr-Glu-Tyr-Ala-Arg-Arg-His-Pro-Asp-Tyr--Ser-Val-Val-Leu-Leu-Leu-Arg-Leu-Ala-Lys-Thr-Tyr-Glu-Thr-Thr-Leu-Glu-Lys-Cys-Cys-Ala--Ala-His-Asp-Pro-Tyr-Glu-Cys-Ala-Ala-Lys/Val-Phe-Asp-Glu-Phe(Lys,Pro_2,Leu_2,Val,Glx_3, Asx,Ile)Lys/Gln(Asx,Cys,Phe,Glx_4,Leu_2,Gly,Tyr)Lys/Phe-Gln-Asn-Ala-Leu-Leu-Val-Arg/Tyr--Thr-Lys/Lys-Val-Pro-Gln-Val-Ser-Thr-Pro-Thr-Leu-Val-Glu-Val-Ser-Arg/Asn-Leu-Gly-Lys/ /Val-Gly-Ser-Lys-Cys-Cys-Lys-His-Pro-Glu-Ala-Lys/Arg-Hse.

In earlier studies[1,2] from this Laboratory, the isolation was described of seven fragments formed by cyanogen bromide cleavage of human plasma albumin with subsequent interruption of the disulfide bonds by oxidation[3]. These fragments cover the entire polypeptide chain of the protein. Their order has been determined[4,5] in a study on the amino-acid sequences around the six methionine residues of human plasma albumin. This order was used as a basis of a new rational nomenclature of the fragments which replaced the earlier working symbols[5]. The complete amino-acid sequences of four fragments[6-9], *i.e.* CB7(Asp), CB4(Pro), CB2(Ala), and CB1(Asp) and a partial amino-acid sequence of another fragment, CB6(Pro) (ref.[10]) were determined in our earlier studies.

This paper reports on the N-terminal analysis of fragment CB5(Phe) and on the investigation of peptides resulting from its tryptic digestion.

EXPERIMENTAL

Material. Human plasma albumin was a product of the Institute for Sera and Vaccines, Prague. Cyanogen bromide fragment CB5(Phe) was prepared as described elsewhere[1,2]. TPCK-Trypsin and chymotrypsin were from Worthington Biochemical Corporation, Freehold, N.J., U.S.A. Sephadex G-75, Sephadex G-25 fine, and QAE-Sephadex A-25 were products of Pharmacia, Uppsala, Sweden. All the chemicals used were of analytical purity grade.

Methods. The techniques of paper electrophoreses[11,12] and chromatography, the amino-acid analysis of peptides[13], and the preparation of peptide maps were described in one of the preceding papers[10]. The sequential analysis of tryptic peptides was effected by Edman degradation[14] and the phenylthiohydantoins of amino acids were identified by chromatography on a thin layer of silica gel[14,15]. Some peptides were ascribed their C-terminal amino acids with respect to the known specificity of trypsin.

Preparation and fractionation of tryptic digest of fragment CB5(Phe). The substrate (1 272 mg) was dissolved in 100 ml of water and the solution was made alkaline by 0·1M ammonium carbonate; phenol red was used as an indicator. TPCK-Trypsin (12·5 mg) was added to the solution which was subsequently incubated 2 h at 37°C. The same quantity of trypsin was added at the end of this period and the incubation was continued for 2 more hours. The digest was cooled down to room temperature and applied onto a 200 . 4·4 cm column of Sephadex G-25, equilibrated in 0·01M ammonium carbonate. The column was eluted by the same solution and 20 ml fractions were collected at 10 min intervals. The course of the gel filtration was monitored by paper electrophoresis (at pH 5·6) of aliquots (0·5%) of the fractions. According to the results of the paper electrophoretic analysis, the effluent was pooled into 7 fractions. Individual peptides were isolated from the latter by paper electrophoresis and chromatography.

Chymotryptic digestion of peptide T9. The peptide (1 μmol) was dissolved in 200 μl of water made alkaline by 1% solution of ammonium carbonate; phenol red was used as an indicator. Chymotrypsin was added (1 : 100, molar ratio) and the peptide was digested 2 h at 37°C. The addition of chymotrypsin was repeated afterwards and the digestion was allowed to proceed for 2 more hours. The chymotryptic fragments of peptide T9 were isolated by paper electrophoresis (at pH 5·6) and by chromatography.

N-*Terminal analysis of fragment* CB5(Phe). The fragment (3·5 mg, c. 0·3 μmol) was subjected to Edman degradation[14] carried out in Beckman Model 890C Sequencer (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif., U.S.A.). The degradation was allowed to proceed according to the fast protein-quadrol program. The phenylthiohydantoins obtained by the conversion

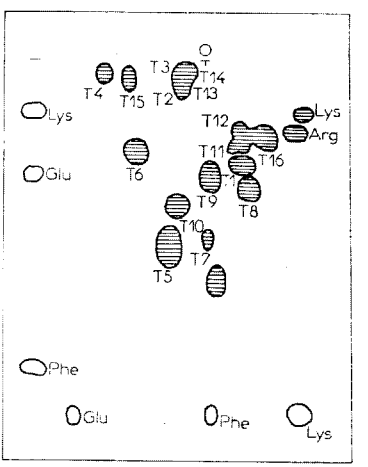

FIG. 1

Peptide Map of Tryptic Digest of Fragment CB5(Phe)

First direction (horizontally) high-voltage electrophoresis, pH 5·6, anode to the left. Second direction (vertically) descending chromatography. The details were described elsewhere[10]. o origin; Lys,Glu,Phe reference mixture of amino acids. The peptide without the symbol was not isolated in this study.

of the thiazolinones were identified by gas chromatography in Beckman Model GC-65 Gas Chromatograph as such or after their sililation[16]. In several instances the phenylthiohydantoins were identified by thin-layer chromatography on silica gel as described above.

RESULTS AND DISCUSSION

The tryptic cleavage of fragment CB5(Phe) afforded peptides whose map is shown in Fig. 1. We were able to isolate 16 peptides from the digest, together with free lysine and arginine. The amino-acid composition of the peptides isolated is given

TABLE I

Amino-Acid Analyses of Peptides Isolated

The values are not corrected; half-cystine residues were determined as cysteic acid. The analyses were done on 20 h hydrolysates of peptides. None of the peptides analyzed contained methionine or tryptophan.

Designation of peptide	Lys	His	Arg	Cys	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	Hse[a]
T1	1·0										1·0			1·0			
T2	2·0	1·0		2·7	1·0	2·5		2·7	1·0		3·7			1·0	1·7		
T3	1·6	0·7		2·8	1·2	2·0		3·1	1·2		3·8			0·9	0·9		
T4	1·0	1·0		3·0	1·3			1·3	1·0		3·8				0·8		
T5	2·0				2·2			3·8	2·2			2·2	1·1	2·1		2·0	
T6	1·1			1·1	1·1			5·0		1·0				2·0	0·8	1·0	
T7			0·8		1·1			1·1			1·1	1·0		2·1		1·0	
T8	1·0					1·0									0·8		
T9	0·9		1·0			2·0	1·9	2·0	2·0			4·2		1·1			
T10			1·1			1·8	1·7	1·9	2·1			3·9		1·0			
T11	0·9				0·9					1·2				1·0			
T12	1·2						1·0			1·0		1·0					
T13	1·8			2·1			1·0			1·2		1·2					
T14	1·9	1·1		2·0				1·0	0·9		1·0						
T15	1·0			1·9													
T16			1·0														1·0
T9—C1	1·1							1·0	0·9			0·9					
T9—C2						1·9	1·1		0·9			1·3					
T9—C3			1·0				1·0	1·0				2·1		1·0			
T9—C4	0·9					1·9	1·2	1·2	2·2			2·2					

[a] Homoserine.

in Table I; the peptides are marked by symbol T and a serial number, chymotryptic fragments of these peptides are marked by symbol C. Selected tryptic peptides were submitted to stepwise degradation carried out manually. The characteristics of all peptides investigated in this study are given in Table II.

We were able to perform 40 degradation steps with fragment CB5(Phe) in the automatic sequenator and thus to determine their order (Fig. 2). A comparison of the results of Edman degradation of fragment CB5(Phe) with the results of the analyses of tryptic peptides permits us to assign certain peptides to defined sites of the molecule of the fragment. Peptides T1, T2, T3, and T4 fall into the 43-residue N-terminal region of the fragment. Peptide T16 containing homoserine is derived from the C-terminus of the fragment. The positions of the remaining tryptic peptides in the polypeptide chain of the fragment are interchangeable. The tryptic peptides isolated by us do not account for the entire chain: we were not able to isolate all

TABLE II
Amino-Acid Sequences of Peptides Isolated

Designation of peptide	Sequence of peptide
T1	Leu-Ala-Lys
T2	Thr-Tyr-Glu-Thr-Thr-Leu-Glu-Lys-Cys-Cys-Ala-Ala-His-Asp-Pro-Tyr(Glx, Cys,Ala,Ala)Lys
T3	(Glx,Thr,Thr,Leu,Glx,Lys,Cys,Cys,Ala,Ala,His,Asx,Pro,Tyr,Glx,Cys,Ala, Ala)Lys
T4	(Cys,Ala,Ala,His,Asx,Pro,Tyr,Glx,Cys,Ala,Ala)Lys
T5	Val-Phe-Asp-Glu-Phe(Lys,Pro_2,Leu_2,Val,Glx_3,Asx,Ile)Lys
T6	Gln(Asx,Cys,Phe,Glx_4,Leu_2,Gly,Tyr)Lys
T7	Phe-Gln-Asn-Ala-Leu-Leu-Val-Arg
T8	Tyr-Thr-Lys
T9	Lys-Val-Pro-Gln-Val-Ser-Thr-Pro-Thr-Leu-Val-Glu-Val-Ser-Arg
T10	(Val,Pro,Glx,Val,Ser,Thr,Pro,Thr,Leu,Val,Glx,Val,Ser)Arg
T11	Asn-Leu-Gly-Lys
T12	(Val,Gly,Ser)Lys
T13	Val-Gly-Ser-Lys-Cys-Cys-Lys
T14	Cys-Cys-Lys-His-Pro-Glu-Ala-Lys
T15	Cys-Cys-Lys
T16	Arg-Hse
T9—C1	(Lys,Val,Pro,Gln)
T9—C2	(Val,Ser,Thr,Pro,Thr)
T9—C3	(Leu,Val,Glu,Val,Ser,Arg)
T9—C4	(Lys,Val,Pro,Gln,Val,Ser,Thr,Pro,Thr)

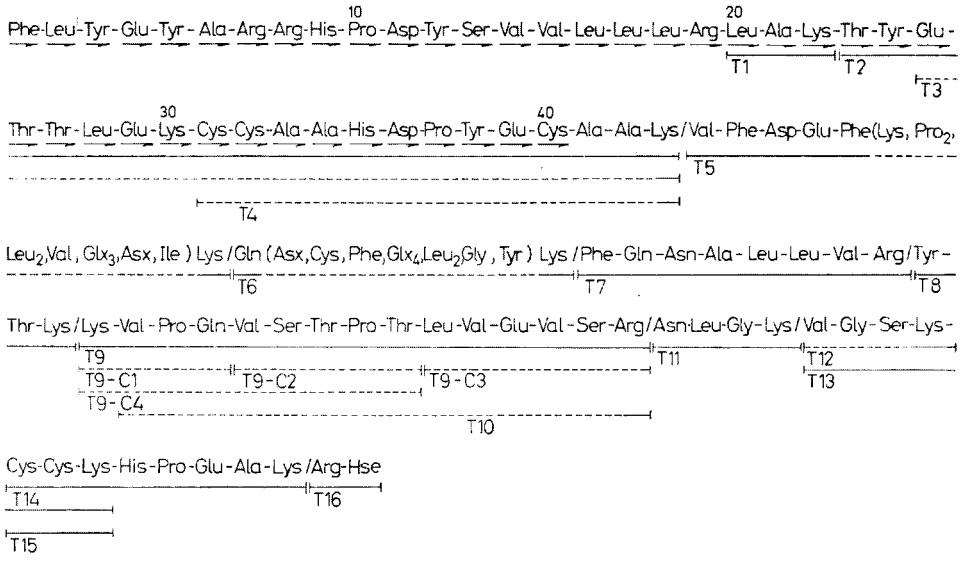

FIG. 2

Edman Degradation of Fragment CB5(Phe) and Amino-Acid Sequences of Peptides Resulting from its Tryptic Digestion

The peptides are symbolized by horizontal bars (full line-completely determined sequence, dashed line-sequence undetermined) and letters T for tryptic peptides and C for chymotryptic peptides. The results of Edman degradation of fragment CB5(Phe) are shown by arrows (→). The first 43 amino-acid residues from the N-terminus bear serial numbers. Sequenced regions whose positions are interchangeable within the fragment are separated by a slanting line (/).

the peptides derived from the N-terminus (residues 1–20) of the chain. The data presented here permit us to define unambiguously the sequence of residues 1 to 43 in the molecule from the N-terminus and to present the remaining part of the fragment in the form of 8 interchangeable regions. The complete amino-acid sequence of fragment CB5(Phe) will be determined after the necessary overlaps of the tryptic peptides analyzed here have been obtained.

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