PEPTIDES ISOLATED FROM TRYPTIC AND CHYMOTRYPTIC DIGEST OF FRAGMENT CB6(Pro) OF HUMAN PLASMA ALBUMIN

L.MORÁVEK, V.KOSTKA, M.A.SABER* and B.MELOUN

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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As a part of sequential studies on human plasma albumin, peptides were isolated from tryptic and chymotryptic digest of cyanogen bromide fragment CB6(Pro) which is derived from the region between methionine residues No V and VI of the polypeptide chain of this protein. The data obtained were arranged into eight sequential regions: Pro-Cys-Ala-Glu-Asp-Tyr(Ser,Val,Val,Leu)-Leu-Asn-Gln-Leu-Cys-Val-Leu-Glu-His-Lys-Thr-Pro-Val-Ser-Asp-Arg-Val-Thr-Lys-Cys-Cys-Thr--Glu-Ser-Leu-Val-Asn-Arg; Arg-Arg-Pro-Cys-Phe-Ser-Ala-Leu-Glu-Val-Asp-Glu-Thr-Tyr-Val--Pro-Lys-Glu-Phe-Asn-Ala-Glu-Thr-Phe; Thr-Phe-His-Ala-Asp-Ile-Cys-Thr-Leu-Ser-Glu-Lys--Glu-Arg; Gln-Ile-Lys-Lys-Gln-Thr-Ala-Leu-Val-Glu-Leu-Val-Lys; His(Lys,Pro)Lys; (Ala,Thr) Lys; Lys-Glu-Gln-Leu-Lys; Lys-Ala-Val-Hse.

In studies on the amino-acid sequence of human plasma albumin, which have been carried out in this Laboratory, the protein was subjected to cyanogen bromide cleavage with subsequent interruption of its disulfide bonds[1]; the seven fragments thus formed were isolated[2,3]. These fragments cover the entire polypeptide chain of the protein and have been subjected to sequential investigation. On the plasma basis of the knowledge of amino-acid sequences around methionine residues of human albumin, the order of these fragments in the chain was determined[4,5]. A rational nomenclature of the fragments was adopted which designates their order in the polypeptide chain: CB1(Asp)-CB2(Ala)-CB3(Cys)-CB4(Pro)-CB5(Phe)-CB6(Pro)-CB7(Asp). As yet, complete amino-acid sequences of fragments[6-9] CB7(Asp), CB4(Pro), CB2(Ala), and CB1(Asp) have been determined.

As a part of sequential investigation of fragment CB6(Pro), peptides from the tryptic and chymotryptic digest of the fragment were isolated and characterized in this study.

EXPERIMENTAL

Material. Human plasma albumin was a product of the Institute for Sera and Vaccines, Prague. Cyanogen bromide fragment CB6(Pro) was prepared as described earlier[2,3]. TPCK-trypsin and

* National Research Centre, Cairo, Arab Republic of Egypt.

chymotrypsin were from Worthington Biochemical Corporation, Freehold, N. J., U.S.A. Soy-bean trypsin inhibitor was a B-grade product of Calbiochem, San Diego, California, U.S.A. Sephadex G-75 and G-25, and SE-Sephadex were products of Pharmacia, Uppsala, Sweden. All chemicals used were of analytical purity grade.

Methods. Electrophoresis at pH 5·6 (ref.[10]) and at pH 1·9 (ref.[11]), as well as paper chromatography in the system n-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 12, by vol.) were carried out on Whatman No 3 paper. Peptide maps of samples were obtained by using electrophoresis at pH 5·6 in the first direction and chromatography in the system given above in the second direction. Amino-acid analyses were done on 20 h and 70 h hydrolysates of peptides by the method of Spackman and coworkers[12]. Sequential analysis of peptides was effected by the phenylthiohydantoin technique in combination with chromatographic identification of PTH-amino acids on silica gel thin layers[13,14]. The net charge of the peptides was used to judge the presence of amides in several instances. Some peptides were ascribed their C-terminal amino acids with regard to the specificity of tryptic or chymotryptic cleavage.

Preparation and fractionation of tryptic digest of fragment CB6(Pro). The substrate (1532 mg) was dissolved in 100 ml of 0·1% ammonium carbonate. TPCK-trypsin (15 mg) was added and the mixture was kept at 37°C for 2 h. Another 15 mg portion of TPCK-trypsin was added afterwards and incubation was continued for 2 more hours. The digest was subsequently cooled down to room temperature and fractionated by gel filtration. The digest was applied onto a column of Sephadex G-25 fine (200 . 4·5 cm) equilibrated in 0·1M ammonium carbonate. The column was eluted by the latter solution at a rate of 20 ml/20 min (1 fraction). Aliquots (0·5%) of each fraction were evaluated by paper chromatography and the effluent was pooled into ten fractions. From the latter, individual peptides were isolated by paper chromatography and electrophoresis.

Preparation and fractionation of chymotryptic digest of fragment CB6(Pro). The substrate (500 mg) was dissolved in 35 ml of water and the solution was made alkaline with respect to phenol red by 0·1M ammonium carbonate. Chymotrypsin (5 mg) and soy-bean trypsin inhibitor (0·1 mg) were added and the solution was incubated at 37°C for 2 h. The same quantity of chymotrypsin and inhibitor was added afterwards and incubation was continued for two more hours.

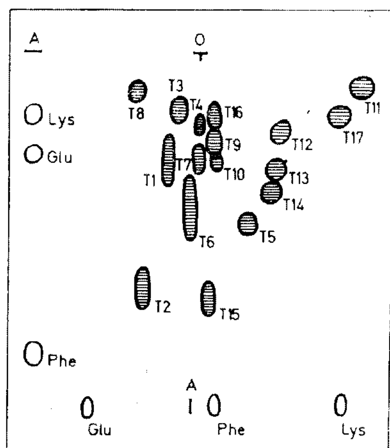

FIG. 1

Peptide Map of Tryptic Digest (1·6 mg) of Oxidized Fragment CB6(Pro)

First direction (horizontally) high-voltage electrophoresis, pH 5·6, anodes to the left. Second direction (vertically) descending chromatography. See text for details. O origin, A reference mixture of amino acids (Lys,Glu, Phe).

The digest was fractionated by gel filtration. The solution was cooled down to room temperature and applied onto a column of Sephadex G-25 fine (2·8 . 150 cm) equilibrated in 0·01M ammonium carbonate. The column was eluted by the same solution. Fractions of 20 ml/30 min were collected. Aliquots (1%) of each fraction were evaluated by paper chromatography and the effluent was pooled into six fractions. Individual peptides were isolated by paper chromatography and electrophoresis.

Tryptic cleavage of peptide C2. The peptide (0·8 μmol), dissolved in 200 μl of water made alkaline with respect to phenol red by 1% ammonium carbonate, was digested with trypsin at a molar ratio 1 : 100, 2 h at 37°C. The same addition of trypsin was made afterwards. Incubation was terminated at the end of a total period of 4 h. Tryptic peptides were isolated by electrophoresis at pH 5·6 and by paper chromatography.

RESULTS AND DISCUSSION

The amino-acid composition of peptides isolated from both digests of fragment CB6(Pro) is given in Table I. Since oxidation was used to interrupt disulfide bonds

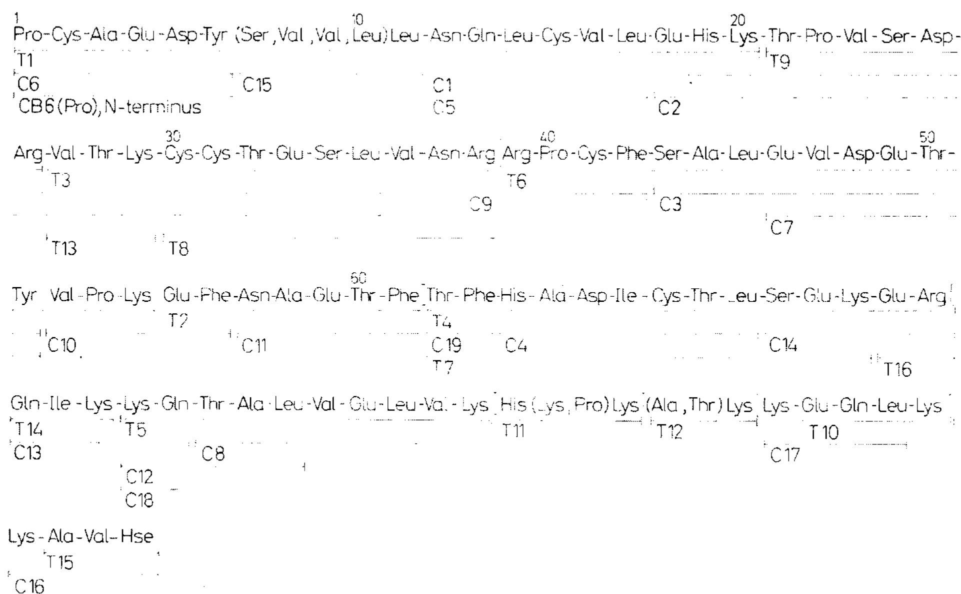

FIG. 2

Alignment of Tryptic and Chymotryptic Peptides of Fragment CB6(Pro)

Tryptic peptides are marked "T", chymotryptic peptides "C". The peptides are symbolized by horizontal bars; full lines are used for peptides sequenced completely, dashed lines for unsequenced peptides. Vertical bars separate individual sequential regions. The link between arginine residues No 38 and 39 is tentative. The N-terminal amino-acid sequence of fragment CB6(Pro), Pro-Cys-Ala-Glu-Asp-Tyr, as well as its C-terminal sequence, Ala-Val-Hse, have been determined in a preceding study[3].

TABLE I

Amino-Acid Analyses of Peptides Isolated

The values are not corrected; half-cystine was determined as cysteic acid. The analyses were carried out with 20 h hydrolysates of peptides. None of the peptides contained glycine, methionine, or tryptophan.

Designation of peptide	Lys	His	Arg	Cys	Asp	Thr	Ser	Glu	Pro	Ala	Val	Ile	Leu	Tyr	Phe	Hse[a]
T1	1·0	0·8		1·9	2·0		0·9	2·7	0·9	1·0	3·1[b]		3·6[b]	0·6		
T2					1·1	1·0		2·0		1·0					1·8	
T3	1·1		0·9	2·1	1·2	1·8	1·1	1·2			1·8		1·0			
T4	1·2	0·8	1·1	1·0	1·0	2·0	1·1	2·1		1·1		1·1	1·2		1·0	
T5	1·5					1·1		1·9		1·0	1·8		1·8			
T6	1·0		0·9	1·1	1·1	1·0	1·0	2·0	1·9	1·0	1·8		1·0	0·8	1·0	
T7	1·0	0·9		1·1	1·1	1·9	1·0	1·3		1·2		0·9	1·1		1·1	
T8			1·0	1·8	1·0	1·0	0·9	1·0			1·0		1·0			
T9			1·0		1·0	0·9	0·9		0·9		0·9					
T10	1·0							1·9					1·0			
T11	2·0	0·9							1·1							
T12	1·0					0·8				0·8						
T13	1·0					0·9					0·9					
T14	1·0							0·8				0·8				
T15										0·8	0·9					1·0
T16			1·0					1·1								
T17	1·0															

C1	1·8	0·7	1·0	3·1	1·6	3·1	2·0	2·6	1·0		3·1		2·5			
C2	1·8	0·6	0·8	2·0	1·1	2·8	1·8	2·0	1·3		1·9		0·9			
C3					1·1	0·7	0·7	1·9		1·0	1·1		1·1	0·6		
C4		0·5		0·9	1·0	0·9				0·9		0·8	1·0			
C5				1·1	1·0			1·1			1·0		2·0			
C6				1·0	1·0			1·0	1·1	1·0				0·7		
C7					1·1	1·0		2·0			1·0			0·6		
C8						0·5		1·1		0·9	1·0		2·0			
C9			1·8	0·8					1·1						1·0	
C10	1·0							1·0	1·1		1·0				1·0	
C11					0·9	1·0		1·1			1·0				1·0	
C12	0·9					1·0		1·2			1·0		0·9			
C13	2·0							2·1				1·0				
C14	1·0		1·0				0·9	1·9								
C15							0·8				1·8		2·2			
C16	1·1									1·0	1·0					0·8
C17	1·0							2·0					1·0			
C18	1·0							1·0								
C19						0·7									1·0	
C2—T1	1·1		1·1		1·0	0·9	1·0	1·0	0·9		0·9					
C2—T2	1·0					1·0					1·0					
C2—T3				2·0		1·0	1·0	1·0					1·0			
C2—T4			1·0		1·1	0·9	1·0		1·0		1·0					

[a] Hse stands for homoserine, [b] value after 70 h hydrolysis.

TABLE II
Amino-Acid Sequences of Peptides Isolated

Designation of peptide	Amino-acid sequence of peptide
T1	(Pro,Cys,Ala,Glx,Asx,Tyr,Ser,Val,Val,Leu,Leu,Asx,Glx,Leu,Cys,Val,Leu,Glx,His)Lys
T2	Glu-Phe-Asn-Ala-Glu-Thr-Phe
T3	(Val,Thr,Lys,Cys,Cys,Thr,Glx,Ser,Leu,Val,Asx,Arg)
T4	(Thr,Phe,His,Ala,Asx,Ile,Cys,Thr,Leu,Ser,Glx,Lys,Glx,Arg)
T5	Lys-Gln-Thr-Ala-Leu-Val-Glu-Leu-Val-Lys
T6	(Arg,Pro,Cys,Phe,Ser,Ala,Leu,Glx,Val,Asx,Glx,Thr,Tyr,Val,Pro,Lys)
T7	Thr-Phe-His-Ala-Asp-Ile-Cys-Thr-Leu-Ser-Glu-Lys
T8	Cys-Cys-Thr-Glu-Ser-Leu-Val-Asn-Arg
T9	Thr-Pro-Val-Ser-Asp Arg
T10	Glu-Gln-Leu-Lys
T11	His(Lys,Pro)Lys
T12	(Ala,Thr)Lys
T13	Val-Thr-Lys
T14	Gln-Ile-Lys
T15	Ala-Val-Hse
T16	Glu-Arg
T17	Lys

C1	(Asx,Glx,Leu,Cys,Val,Leu,Glx,His,Lys,Thr,Pro,Val,Ser,Asx,Arg,Val,Thr,Lys,Cys,Cys,Thr,Glx,Ser)Leu
C2	Glu-His-Lys-Thr(Pro,Val,Ser,Asx)Arg(Val,Thr)Lys(Cys,Cys,Thr,Glu,Ser)Leu
C3	Ser-Ala-Leu-Glu-Val-Asp-Glu-Thr-Tyr
C4	His-Ala-Asp-Ile-Cys-Thr-Leu
C5	Asn-Gln-Leu-Cys-Val-Leu
C6	Pro-Cys-Ala-Glu-Asp-Tyr
C7	Glu-Val-Asp-Glu-Thr-Tyr
C8	(Thr,Ala,Leu,Val,Glu)Leu
C9	Arg-Arg-Pro-Cys-Phe
C10	Val-Pro-Lys-Glu-Phe
C11	(Asx,Ala,Glx,Thr)Phe
C12	Lys-Gln-Thr-Ala-Leu
C13	(Glx,Ile,Lys,Lys,Glx)
C14	Ser-Glu-Lys-Glu-Arg
C15	(Ser,Val,Val,Leu)Leu
C16	(Lys,Ala,Val)Hse
C17	Lys-Glu-Gln-Leu
C18	Lys-Gln
C19	Thr-Phe
C2—T1	(Glx,His,Lys,Thr,Pro,Val,Ser,Asx,Arg)
C2—T2	(Val,Thr)Lys
C2—T3	(Cys,Cys,Thr,Glx,Ser)Leu
C2—T4	(Thr,Pro,Val,Ser,Asp)Arg

in individual cyanogen bromide fragments[3] during their preparation, half-cystine residues are present in the peptides in the form of cysteic acid residues. Peptides from the tryptic digest are marked by symbol "T", chymotryptic peptides by symbol "C". Serial numbers of peptides correspond to the order of their emergence from the Sephadex G-25 column during the fractionation of the digests. A total of 17 peptides were isolated from the tryptic digest and 19 peptides were obtained from the chymotryptic digest. As in preceding cases[6,8,9], the tryptic map is presented here as one of the characteristics of the individual cyanogen bromide fragment. The positions of tryptic peptides obtained in this study are shown in the peptide map in Fig. 1.

The results of the sequential examination of the peptides isolated are given in Table II. Peptides selected from both digests were studied. The partial sequence of peptide C2 was determined from peptides C2–T1 to C2–T4 obtained by tryptic cleavage of peptide C2. The alignment of the peptides into eight sequential regions is shown in Fig. 2. Peptide C6 is identical with the N-terminal sequence of fragment CB6(Pro) reported earlier[3]. Large peptides T1 and C1 provided links for a long continuous sequence extending to arginine No 38. The entire fragment CB6(Pro) contains four arginine residues[3] of which the first one (No 26) is contained in the sequence determined. The next sequentially defined arginine residue in position No 38 is present in peptides T3 and T8. Peptide C9 contains two arginine residues. Assuming a total content of four arginines in fragment CB6(Pro), the N-terminal arginine residue of peptide C9 overlaps either the arginine of peptide T8 (Arg No 38) or that of peptide T4. Peptide C9 results from the cleavage of the peptide bond at the amino side of the arginine residue. The existence of peptide C14, obtained in a high yield, indicates that the bond involving the amino group of its arginine residue was not cleaved by chymotrypsin. We therefore link tentatively arginine No 38 to arginine No 39. Overlapping peptides permit us to extend the sequence up to phenylalanine No 61. Tryptic cleavage at the carboxyl side of this phenylalanine residue indicates that this bond is extremely sensitive to the residual chymotryptic activity of the trypsin preparation used. The subsequent sequence represents the region contained in peptide T4. The C-terminal arginine of peptide C14 demonstrates that this residue is involved in a bond extremely sensitive to the residual tryptic activity of the chymotrypsin preparation used and that this activity was not eliminated in this case even by the presence of soy-bean trypsin inhibitor added. The remaining part of the molecule of fragment CB6(Pro) is represented by five short sequences of which peptides T15 and C16 contain the C-terminus of the fragment.

The peptides isolated from the tryptic and chymotryptic digest of fragment CB6(Pro) cover almost the entire region of its chain[3], yet the sequential data obtained are insufficient for the determination of the complete amino-acid sequence of the fragment, the reason being mainly the distribution of certain lysine residues along the chain which gives rise to small tryptic peptides (T10–T17). Relevant chymotryptic peptides provided in some cases one-residue overlaps of lysine, insufficient

for unambiguous arrangement of the tryptic peptides. In an effort to complete the amino acid sequence of the fragment, studies are in progress in this laboratory on peptides from a maleylated derivative of fragment CB6(Pro) with lysine residues protected against tryptic activity[15].

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