AMINO ACID SEQUENCE OF CYANOGEN BROMIDE FRAGMENT CB6(Pro) OF HUMAN PLASMA ALBUMIN*

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The cyanogen bromide fragment studied represents the region of the polypeptide chain of human plasma albumin between methionine residues No V and VI. It contains 102 amino acid residues in the following sequence: Pro-Cys-Ala-Glu-Asp-Tyr-Leu-Ser-Val-Val-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-Pro-Cys-Phe-Ser-Ala-Leu-Glu-Val-Asp-Glu-Thr-Tyr-Val-Pro-Lys-Gln-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-Glu-Gln-Leu-Lys--Ala-Val-Met. This complete sequence was derived from the knowledge of peptides from the tryptic and chymotryptic digest of the maleyl derivative of fragment CB6(Pro) which provided overlaps of sequential regions determined earlier.

The molecule of human plasma albumin consists of one polypeptide chain which contains approximately 600 amino acid residues, of their number six methionines[1,2]. In the course of sequential studies on this protein, which have been pursued in this Laboratory, all the seven fragments resulting from cyanogen bromide cleavage of the protein have been isolated[3] and their order determined[4]: CB1(Asp)-CB2(Ala)-CB3(Cys)-CB4(Pro)-CB5(Phe)-CB6(Pro)-CB7(Asp). As yet, the complete amino acid sequences of cyanogen bromide fragments CB1(Asp), CB2(Ala), CB4(Pro), CB5(Phe), and CB7(Asp) have been determined in our Laboratory[5-10].

This paper describes experiments which led to the determination of the complete amino acid sequence of cyanogen bromide fragment CB6(Pro) which is penultimate in the polypeptide chain of human plasma albumin. In a preceding communication[11] the analysis of peptides derived from the tryptic and chymotryptic digest of fragment CB6(Pro) was described. These peptides covered the entire region of the fragment, did not permit, however, its complete amino acid sequence to be derived. Experiments with the tryptic and chymotryptic peptides of the maleyl derivative of fragment CB6 (Pro) which provided the necessary overlaps and led to the determination of its complete sequence are described here.

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EXPERIMENTAL

Material

Human plasma albumin was a product of the Institute for Sera and Vaccines (Prague, Czechoslovakia). TPCK-Trypsin and chymotrypsin were from Worthington Biochemical Corporation (Freehold, N. J., U.S.A.). Sephadex G-25, Sephadex G-75, DEAE-Sephadex A-25, and SE-Sephadex C-25 were purchased from Pharmacia (Uppsala, Sweden). Maleic anhydride was a product of Koch-Light Laboratories Ltd. (Colnbrook, Bucks., England). Polyamide layer sheets were from BDH Chemicals Ltd. (Poole, England) and Silufol sheets for thin layer chromatography from Kavalier (Sázava, Czechoslovakia).

Methods

Maleylation. The modification of lysyl residues by maleic anhydride was effected by the procedure of Butler and coworkers[12].

Demaleylation of peptides. The peptide material (10 mg) was dissolved in 9 ml of a mixture of pyridine and water (1 : 90) and 1 ml of acetic acid was added to the solution. The reaction mixture was heated at 60°C for 5 h; pyridine acetate was removed by lyophilization afterwards.

Purification of peptides. Small peptides from enzymatic digests were purified on Whatman No 3 paper using procedures S1—S3. These were descending electrophoresis[13] at pH 5·6 in the buffer water–pyridine–acetic acid (994 : 5 : 1, v/v) at 1400 V (procedure S1), horizontal high-voltage electrophoresis[14] at pH 1·9 in water–acetic acid–formic acid (16 : 3 : 1, v/v) at 6000 V (procedure S2), or chromatography (procedure S3) in the system[15] n-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 12, v/v).

Techniques of sequential analysis. The stepwise degradation of the peptides was effected by the phenylisothiocyanate procedure of Edman[16]; the phenylthiohydantoins released were identified by thin-layer chromatography[16,17] on Silufol sheets. Small amounts of peptides (70—90 nmol) were degraded by the dansyl-Edman method as modified by Gray and Smith[18]; the dansylamino acids were identified on polyamide layers[19] by the technique introduced by Hartley[20].

Other analytical methods. The quantitative amino acid analysis was carried out by the method of Spackman and coworkers[21]. The preparation on peptide maps was described in the preceding report[11]. The presence of amides in peptides was also judged by their net charge established electrophoretically (S2, ref.[13]).

Preparation of Cyanogen Bromide Fragment CB6(Pro)

Human plasma albumin was converted into monoaminoethyl-albumin which was subsequently treated with cyanogen bromide. The products of the cleavage were resolved on a Sephadex G-100 column. This procedure has been reported in detail earlier[2,5].

One of the products isolated, fragment C, served as starting material in the present study. It consists of four fragments, CB4(Pro), CB5(Phe), CB6(Pro), and CB7(Asp), joined together by disulfide bonds. Fragment C was subjected to performic acid oxidation[22] as follows. The material (7·8 g) was dissolved in a mixture of 98% formic acid (160 ml) and methanol (32 ml) and maintained 30 min at −5°C. A performic acid oxidation mixture was prepared from 20 ml of 30% H_2O_2 and 320 ml of 98% formic acid which were mixed together and allowed to stand 2 h at 25°C. The performic acid was then cooled down to −5°C and added to the solution of fragment C. The oxidation was allowed to proceed 150 min at this temperature. The excess of performic acid was eliminated by saturated solution of sodium sulfite added dropwise until the mixture gave

a negative reaction with tolidine and potassium iodide[23]. The solution of the product in formic acid was neutralized to pH 6 with cooling ($T < 20°C$) by concentrated ammonium hydroxide. A precipitate was formed. The latter was separated by filtration and dissolved in 60 ml of 5M guanidine hydrochloride. This solution was passed over a column of Sephadex G-100. The course of the fractionation is shown in Fig. 1. The material designated CB6(Pro) was rechromatographed on the same column and lyophilized. Yield 1850 mg of fragment CB6(Pro).

Preparation of Maleylated Fragment CB6(Pro)

Oxidized fragment CB6(Pro) (800 mg) was dissolved in 80 ml of 5M guanidine hydrochloride and treated stepwise with 2·4 g of maleic anhydride in 36 ml of redistilled dioxane. The pH of the reaction mixture was kept in the range 8·5—9·0 by the addition of 5M-NaOH. The reaction was allowed to proceed at 3—10°C for 60 min. The resulting clear solution was desalted on a Sephadex G-25 column (9·5 . 91 cm), equilibrated with 5mM-NH_4HCO_3. Fractions of 200 ml were collected at 10 min intervals. The protein-containing fractions were pooled and lyophilized. (Yield 796 mg).

Preparation and Fractionation of Tryptic Digest of Maleylated Fragment CB6(Pro)

The fragment (550 mg) was dissolved in 50 ml of 0·05M-NH_4HCO_3 and digested with trypsin at a ratio of 1 : 50 (w/w), at pH 8·8 and 37°C, 10 min. The digest was placed on top of a column of

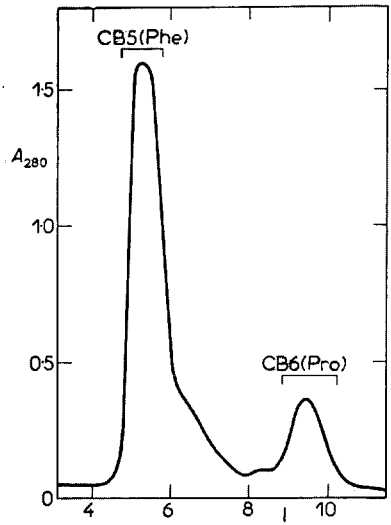

FIG. 1

Fractionation of Products of Oxidation of Fragment C by Gel Filtration on Sephadex G-100 Column

Column, 16 × 95 cm, eluted by 0·1M-NH_4HCO_3. Fractions, 220 ml per 30 min. Fragments CB5(Phe) and CB6(Pro) were isolated.

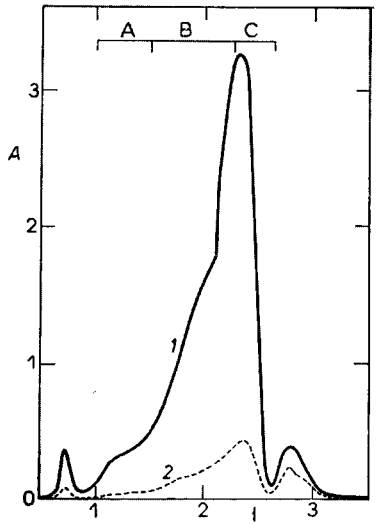

FIG. 2

Gel Filtration of Tryptic Digest of Maleylated Fragment CB6(Pro) on Sephadex G-75

Column, 4·5 × 195 cm, eluted by 5mM-NH_4HCO_3. Fractions, 25 ml per 15 min. *1* Absorbance at 250 nm, *2* absorbance at 280 nm. Fractions A, B and C were isolated.

Sephadex G-75, equilibrated with 5mM-NH_4HCO_3. Dry Sephadex, which absorbed completely the sample solution, was added before elution. The course of the fractionation of the digest is shown in Fig. 2. Fractions A, B, and C were cut and lyophilized.

Fraction A was not treated further.

Fraction B was dissolved in 10 ml of water. This solution was adjusted to pH 10 by ammonium hydroxide and subjected to chromatography on DEAE-Sephadex A-25. The elution profile is shown in Fig. 3. Fractions B1 and B2 were taken. These fractions were desalted on Sephadex G-25 (column 2·5× 60 cm), equilibrated with 0·02M-NH_4OH. Fractions of 15 ml were collected at 10-min intervals. The desalted peptide material was lyophilized. Fraction B1 afforded 76 mg of a peptide designated MT4. This peptide (2 μmol) was digested with chymotrypsin at a ratio of 1 : 100 (w/w) in 0·01M-NH_4HCO_3, at 37°C, 4 h. The digest was lyophilized, the resulting mixture of peptides was demaleylated, and individual peptides (MT4C1—MT4C5) were isolated by procedures S1, S2, and S3. Fraction B2 (180 mg of desalted material) was demaleylated before further treatment. The demaleylated material was dissolved in 3 ml of 5mM sodium formate in 8M urea, pH 3·0, and fractionated on a column of SE-Sephadex C-25 (Fig. 4). Fractions B21, B22, and B23 containing peptides MT1, MT3a, and MT3, respectively, were isolated. Peptide MT3a

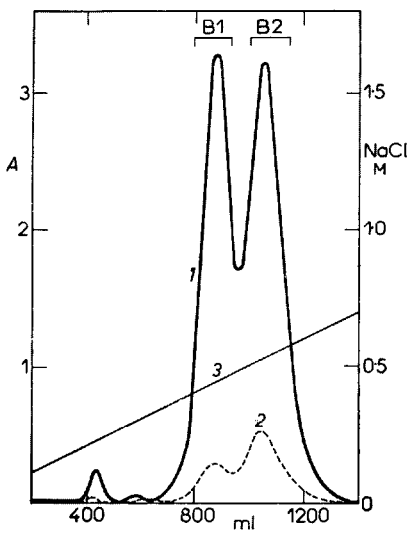

FIG. 3

Chromatography of Fraction B (Fig. 2) on DEAE-Sephadex A-25

Column, 2·5 × 39 cm, eluted by 0·05M Tris-HCl buffer, pH 7·5. Temperature, 23°C. Fractions, 20 ml per 10 min. *1* absorbance at 250 nm, *2* absorbance at 280 nm. A linear gradient of 0—1M-NaCl *3* was used. Fractions B1 and B2 were collected.

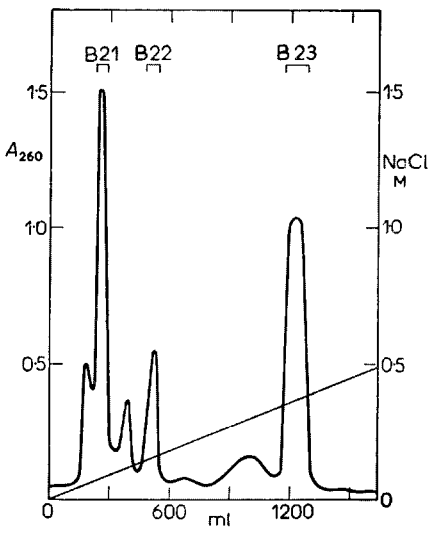

FIG. 4

Chromatography of Demaleylated Fraction B2 (Fig. 3) on SE-Sephadex C-25

Column, 2·7 × 45 cm, eluted by 1 300 ml of 5mM sodium formate in 8M urea, pH 3·0. A linear gradient of 0—1M-NaCl was used. Temperature, 23°C. Fractions, 13·7 ml per 20 min. Fractions B21, B22, and B23 were taken.

(4 μmol) was digested with chymotrypsin and the digest was treated in the same manner as the digest of peptide MT4.

Fraction C was separated repeatedly by electrophoresis (S1) and by paper chromatography and peptides MT2 and MT3b were isolated.

Preparation and Fractionation of Chymotryptic Digest of Maleylated Fragment CB6(Pro)

Maleylated fragment CB6(Pro) (245 mg) was dissolved in 30 ml of 0·05M-NH_4HCO_3 and the pH of the solution was adjusted to 8·1 by ammonium hydroxide. Chymotryptic digestion was allowed to proceed at an enzyme to substrate ratio of 1 : 50 (w/w) 90 min at 37°C. The digest was fractionated on a column of Sephadex G-25 (3·1 × 150 cm), equilibrated with 5mM-NH_4HCO_3. Fractions of 16 ml were collected at 15-min intervals. The fractionation was monitored by absorbance measurement of the effluent at 280 nm and by paper chromatography of aliquots (1%) of individual fractions. Arginine-containing fractions were demaleylated and purified by procedures S1, S2, and S3. Two arginine-containing peptides, designated MC1 and MC2 were isolated. A flow chart showing the isolation of all peptides described under "Experimental" is given in Fig. 5.

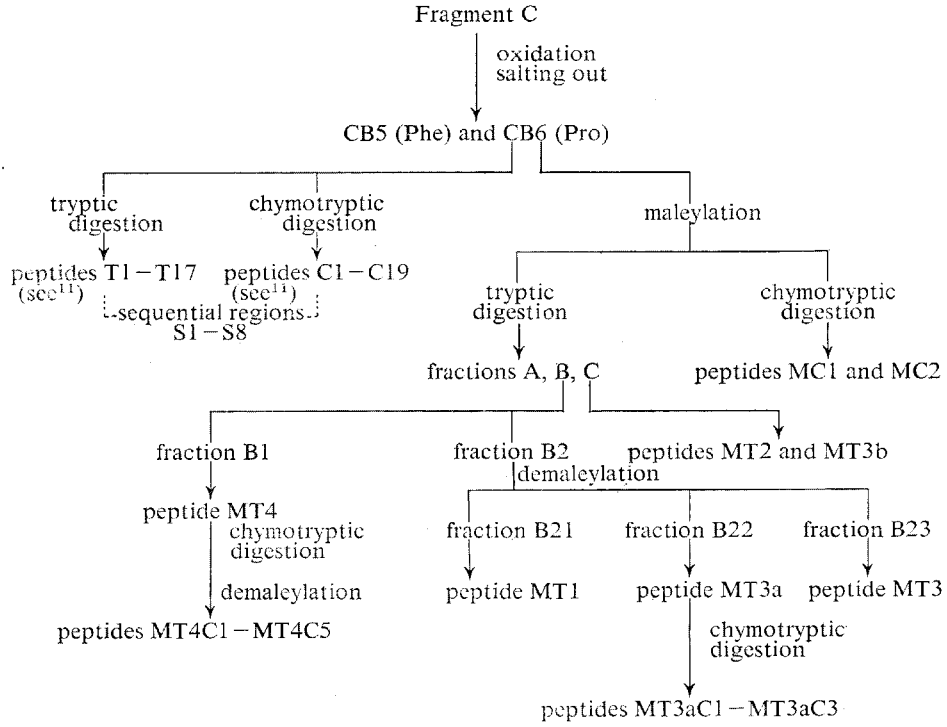

FIG. 5

Scheme of Isolation of Peptides Used for the Determination of the Complete Amino Acid Sequence of Fragment CB6(Pro)

The individual procedures are described in detail in the text.

RESULTS AND DISCUSSION

The preparation of fragment CB6(Pro) was improved compared to the original procedure[3]. We found that the mixture resulting from the oxidation of fragment C can be fractionated by increasing the concentration of ammonium formate at pH 6: large cyanogen bromide fragments CB5(Phe) and CB6(Pro) separate in the form of a precipitate whereas the smaller fragments, CB4(Pro) and CB7(Asp), remain soluble. Fragments CB5(Phe) and CB6(Pro) can easily be resolved by gel filtration on Sephadex G-100 (Fig. 1).

In earlier work[11], cyanogen bromide fragment CB6(Pro) was digested with trypsin and chymotrypsin and the resulting peptides were sequenced. The sequential data obtained, however, were insufficient for the determination of the complete amino acid sequence of the fragment. This was caused mainly by (*a*) the distribution of certain lysine residues along the C-terminal region of the chain, a distribution which gave rise to a number of small tryptic peptides, and (*b*), the fact that the chymotryptic peptides provided mostly ambiguous one-residue overlaps. For these reasons the

TABLE I

Amino Acid Composition of Tryptic Peptides of Maleylated Fragment CB6(Pro)

The values were obtained with 20-h hydrolysates of the peptides and are not corrected.

Amino acid	Number of residues in peptide							Found in sequence[a]
	MT1	MT2	MT3a	MT3b	MT3	MT4	CB6(Pro)	
Lysine	1·0	1·1	1·3	1·0	1·9	7·1	10·7	11
Histidine	0·9			0·8	0·8	1·1	2·6	3
Arginine	1·0	1·0	1·2	0·9	1·8		3·9	4
Cysteic acid	2·0	1·7	1·2	1·0	2·3		5·9	6
Aspartic acid	2·9	1·0	2·0	1·1	2·7		7·3	7[b]
Threonine	1·1	1·7	2·0	1·8	3·8	1·8	8·7	9
Serine	1·7	0·9	1·0	1·1	1·0		4·8	5
Glutamic acid	3·1	1·2	3·8	1·8	5·9	4·8	15·4	15[b]
Proline	2·0		1·8		2·2	0·9	4·6	5
Alanine	1·1		1·8	1·0	3·0	2·9	7·4	7
Valine	3·5	1·7	2·1		2·3	3·0	10·6	11
Isoleucine				0·9	0·8	0·8	1·8	2
Leucine	3·7	1·0	1·3	1·1	2·2	3·0	10·0	10
Tyrosine	0·8		0·8		0·8		1·6	2
Phenylalanine			2·7	1·0	4·0		4·1	4
Homoserine						1·0	1·1	1
Total	26	12	23	14	37	27	(102)	102

[a] Given in Fig. 6; [b] total of acid and amide form.

present study was started with the maleyl derivative of fragment CB6(Pro) in which lysine residues had been protected against tryptic attack. All peptides resulting from specific cleavage at arginine residues (MT1 through MT4) were isolated from the tryptic digest of maleylated fragment CB6(Pro). Of the chymotryptic peptides of maleylated fragment CB6(Pro) only those containing arginine and providing overlaps of the MT-peptides were analyzed. The amino acid composition of fragment CB6(Pro) is given together with that of the MT-peptides in Table I; the composition of the remaining peptides characterized in this study is given in Table II.

The tryptic digest of maleylated fragment CB6(Pro) was resolved on the Sephadex G-75 column (Fig. 2) into three fractions, A, B, and C. Aliquots (3 mg) of these fractions wer demaleylated, cleaved with chymotrypsin, and analyzed by the method of peptide maps. These maps were compared with the tryptic map[11] of oxidized fragment CB6(Pro). One part of the material contained in fraction A was found also in fraction B. The peptide map of fraction C was completely different. Fractions B and C only were treated further (Fig. 5).

TABLE II

Amino Acid Composition of Peptides from Chymotryptic Digest of Maleylated Cyanogen Bromide Fragment CB6(Pro) and Peptides Resulting from Chymotryptic Cleavage of Maleylated Tryptic Peptides

The values were obtained with 20-h hydrolysates of the peptides and are not corrected.

Amino acid	Number of residues in peptide									
	MC1	MC2	MT3aC1	MT3aC2	MT3aC3	MT4C1	MT4C2	MT4C3	MT4C4	MT4C5
Lysine		3·1		1·2		2·0			4·0	1·0
Histidine		0·7							1·2	
Arginine	2·0	0·9								
Cysteic acid	1·1	1·0								
Aspartic acid	0·7	1·0	1·0		1·0					
Threonine		1·6	1·1		1·0		1·0		0·9	
Serine		1·0	0·9							
Glutamic acid		4·0	1·9	1·0	1·0	2·1		1·0	2·0	
Proline	1·1			0·9					1·3	
Alanine		1·2	1·0		1·0		1·0		1·0	0·9
Valine	0·7	0·5	1·0	1·0	0·9			0·9	0·8	1·0
Isoleucine		1·7				1·0				
Leucine		1·3	1·0				1·0	1·0	1·0	
Tyrosine			0·6							
Phenylalanine	1·1			0·9						
Homoserine										1·0

Fraction B was resolved on DEAE-Sephadex A-25 (Fig. 3) into fractions B1 and B2. Fraction B1 contained one peptide only, MT4, Gln-Ile(Lys_7,His,Thr_2,Glx_4,Pro, Ala_3,Val_3,Leu_3)Hse. From the chymotryptic digest of the latter peptides MT4Cl, (Glx_2,Ile,Lys_2), MT4C2,(Thr,Ala,Leu), MT4C3(Val,Glu,Leu),MT4C4, Val-Lys-His--Lys-Pro(Lys,Ala,Thr,Lys,Glx_2,Leu), and MT4C5, (Lys,Ala,Val)Hse, were isolated. Fraction B2 was demaleylated and resolved on SE-Sephadex C-25 (Fig. 4) into fractions B21, B22, and B23 (Fig. 5b. Fraction B21 afforded a 26-residue peptide, MT1,(Lys,His,Cys_2,Asx_3,Thr,Ser_2,Glx_3,Pro_2,Ala,Val_4,Leu_4,Tyr)Arg, which was characterized by its tryptic map. Fraction B22 afforded peptide MT3a, Arg-Pro-Cys--Phe-Ser-Ala-Leu-Glu-Val-Asp-Glu-Thr-Tyr-Val(Pro, Lys, Glx_2, Phe_2, Ala, Thr, Asx), whose partial structure was determined by Edman degradation. Peptide MT3a was subjected to chymotryptic digestion and peptides MT3aCl, (Ser,Ala,Leu,Glx_2,Val, Asp,Thr)Tyr, MT3aC2(Val, Pro, Lys, Gln)Phe, and MT3aC3(Asx, Ala, Glx,Thr)Phe were isolated. Fraction B23 contained peptide MT3, Arg(Lys_2,His,Cys_2,Asx_3,Thr_4, Ser,Glx_6,Pro_2,Ala_3,Val_2,Ile,Leu_2,Tyr,Phe_4)Arg.

Fraction C was resolved by procedures S1–S3 into peptides MT2, Val-Thr-Lys--Cys-Cys-Thr-Glu-Ser-Leu-Val-Asn-Arg and MT3b, Thr-Phe-His-Ala-Asp-Ile-Cys--Thr-Leu-Ser-Glu-Lys-Glu-Arg.

From the chymotryptic digest of maleylated cyanogen bromide fragment CB6(Pro) arginine-containing peptides MC1(Val,Asx,Arg,Arg,Pro,Cys,Phe) and MC2,(Lys_3, His,Arg,Cys,Asx,Thr_2,Ser,Glx_5,Ala_2,Val,Ile_2,Leu_3) were obtained.

The data from which the complete amino acid sequence of fragment CB6(Pro) was derived are represented schematically in Fig. 6. In a preceding study[11], the primary structure of the fragment was presented in the form of 8 sequential regions which are marked S1–S8 in Fig. 6. The amino acid sequence of peptide T1 (ref.[11]), residues No 7–10, -Leu-Ser-Val-Val-) was determined later. The tryptic digestion of maleylated fragment CB6(Pro) cleaved its chain specifically at 3 arginine residues and four main tryptic peptides MT1-MT4 were isolated.

Peptide MT1 is derived from the N-terminal region of the chain and its amino acid composition corresponds to residue No 1–26 in sequential region S1. The peptides from the tryptic digest of demaleylated peptide MT1 occupy on peptide maps positions identical with those of peptides T1 and T9 from the N-terminal region, which have been isolated earlier[11].

The amino acid sequence of peptide MT2 is identical with that of residues No 27 to 38 in sequential region S1.

Peptide MT3, the largest of all peptides isolated in this study, consists of amino acid residues No 39–75. This peptide is N-terminated by sequence Arg-Pro-, involving an arginine bond not cleaved by trypsin, and contains as the only one of the tryptic peptides two arginine residues. Peptide MC1, from the chymotryptic digest of maleylated fragment CB6(Pro), joins together sequential regions S1 and S2 and

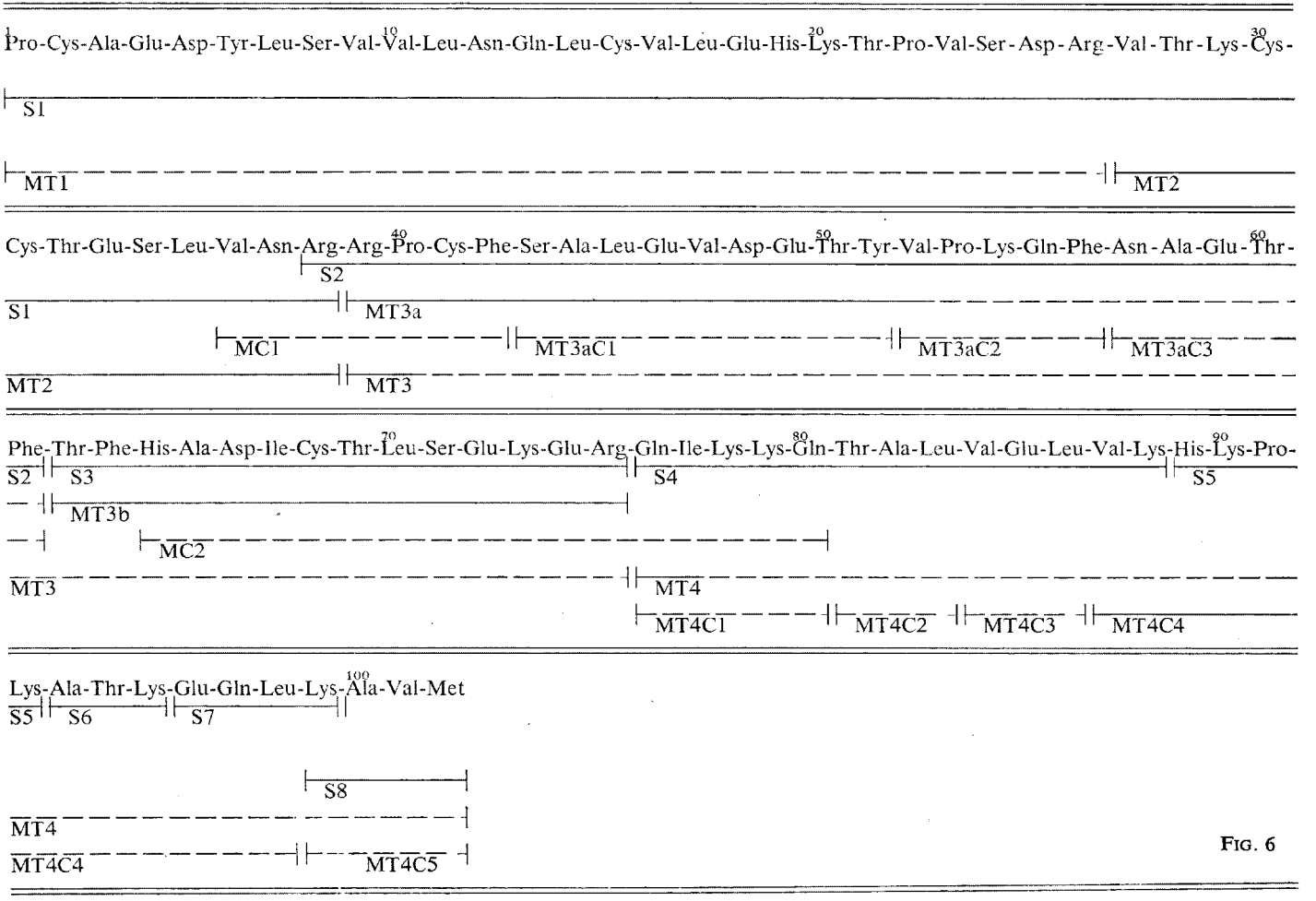
Pro-Cys-Ala-Glu-Asp-Tyr-Leu-Ser-Val-Val-Leu-Asn-Gln-Leu-Cys-Val-Leu-Glu-His-Lys-Thr-Pro-Val-Ser-Asp-Arg-Val-Thr-Lys-Cys-
S1
MT1
MT2
Cys-Thr-Glu-Ser-Leu-Val-Asn-Arg-Arg-Pro-Cys-Phe-Ser-Ala-Leu-Glu-Val-Asp-Glu-Thr-Tyr-Val-Pro-Lys-Gln-Phe-Asn-Ala-Glu-Thr-
S2
S1
MT3a
MC1
MT3aC1
MT3aC2
MT3aC3
MT2
MT3
Phe-Thr-Phe-His-Ala-Asp-Ile-Cys-Thr-Leu-Ser-Glu-Lys-Glu-Arg-Gln-Ile-Lys-Lys-Gln-Thr-Ala-Leu-Val-Glu-Leu-Val-Lys-His-Lys-Pro-
S2
S3
S4
S5
MT3b
MC2
MT3
MT4
MT4C1
MT4C2
MT4C3
MT4C4
Lys-Ala-Thr-Lys-Glu-Gln-Leu-Lys-Ala-Val-Met
S5
S6
S7
S8
MT4
MT4C4
MT4C5

FIG. 6

also peptides MT2 and MT3. The peptide bond between amino acid residues No 61 and 62 is obviously very sensitive to traces of chymotryptic activity and was cleaved even with the preparation of TPCK-trypsin used. This is evidenced by the existence of two smaller fragments MT3a and MT3b; the former corresponds to sequential region S2, yet it is shorter by the N-terminal arginine. The link between peptides MT3 and MT4 is provided by peptide MC2. Its content of one arginine and both isoleucine residues of fragment CB6(Pro) clearly indicates that peptide MC2 is derived from this region of the chain even though it has not been obtained in perfectly pure state (Thr 1·6, Val 0·5) in this study.

Peptide MT4 contains homoserine and represents therefore the C-terminal region of fragment CB6(Pro). The demaleylated peptide cannot be degraded by the Edman procedure; exposure to low pH during demaleylation obviously brings about the conversion of N-terminal glutamine into pyrrolidonecarboxylic acid not reacting with phenylisothiocyanate. The part of the chain between residues No 76 and 102 is fully covered by sequential regions S4 through S8, determined earlier[11]. In this study, the chymotryptic fragments of peptide MT4, *i.e.* MT4C1 through MT4C5, were isolated. The most important of these peptides is peptide MT4C4 whose partial amino acid sequence Val-Lys-His-Lys-Pro(Lys,Ala,Thr,Lys,Glu,Gln,Leu) fully defines the order of sequential regions S4 through S7. The homoserine-containing peptide MT4C5 comes from the C-terminus of fragment CB6(Pro).

A comparison of the amino acid composition of fragment CB6(Pro) with the number of amino acid residues found in its sequence is made in Table I. There is good agreement between both data. This study continues earlier work in which the order of cyanogen bromide fragments CB4(Pro)-CB5(Phe)-CB6(Pro)-CB7(Asp) (ref.[6]) and their amino acid sequences[7–10] were determined. The sequence of fragment CB6(Pro) permits us to extend the amino acid sequence of the C-terminal region of human plasma albumin determined in this Laboratory to 287 residues.

Recently, the communication by Behrens and coworkers[24] reporting on the structure of human serum albumin became available to us. An alignment of the complete amino acid sequence of fragment CB6(Pro) (Fig. 6) with the corresponding portion of this structure (res. No 446–547) of human serum albumin reveals several differences. From our data in fragment CB6(Pro) position No 9 is occupied by Val,

FIG. 6

Amino-Acid Sequence of Cyanogen Bromide Fragment CB6(Pro) of Human Plasma Albumin

——— sequential regions determined in full, – – – – unsequenced regions. S1–S8, sequential regions determined earlier[11]. MT, peptides isolated from the tryptic digest of the maleylated fragment. MC, peptides isolated from the chymotryptic digest of the maleylated fragment. MTC, peptides from the tryptic digest of the maleylated fragment which were subjected to additional cleavage by chymotrypsin (Fig. 5).

No 18 and 19 by Glu-His, No 24 and 25 by Ser-Asp, and No 55 by Gln; Behrens and coworkers[24] place at the corresponding sites Leu (No 454 of their structure), His-Glu (No 463 and 464), Asp-Ser (No 469 and 470), and Glu (No 500). The sequence -Ser-Glu-Lys-Glu-Arg-, determined for residues No 69–73 of fragment CB6(Pro) by us (Fig. 6) is in disagreement with -(Asp,Thr,Pro,Glx)Lys-, proposed by these authors for the corresponding site (positions No 516–519 of their structure).

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