ORDER OF CYANOGEN BROMIDE FRAGMENTS IN THE POLYPEPTIDE CHAIN OF HUMAN PLASMA ALBUMIN. PARTIAL AMINO-ACID SEQUENCE OF THE PROTEIN

B. MELOUN, L. MORÁVEK and V. KOSTKA

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

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In a series of preceding studies, seven fragments were isolated which result from cyanogen bromide cleavage of human plasma albumin at its six methionine residues. Amino acid sequences around these methionine residues were determined in this study, which permit the unambiguous order of the fragments in the polypeptide chain to be defined. The present sequential information on human plasma albumin has been summarized in the form of a partial structure accounting for 403 amino-acid residues of the protein.

Cyanogen bromide cleavage of human plasma albumin with subsequent interruption of disulfide bonds gives rise to seven fragments[1-3]. The N- and C-terminal amino-acid sequences of these fragments have been determined in this Laboratory[3]. The available data on the order of these fragments in the chain are incomplete. The N-terminal 24-residue amino acid sequence of human plasma albumin has been known from the studies of Bradshaw and Peters[4], and Liu and coworkers[5]; the C-terminal amino-acid sequence of the protein represents fragment VII-Asp whose 37-residue[3] sequence has been determined completely in one of our earlier studies[6]. Lapresle and Bellon[7] demonstrated that this C-terminal fragment is preceded in the chain by our fragment V-Pro (ref.[3]).

This study was aimed at the isolation of methionine peptides which would provide experimental data necessary for unambiguous arrangement of all cyanogen bromide fragments; another aim of this paper was to summarize the present state of sequential studies on human plasma albumin carried out in this Laboratory. Preliminary information on the order of the cyanogen bromide fragments was a part of our recent communication[8].

EXPERIMENTAL

Material

Human plasma albumin was a product of Imuna, Šarišské Michalany, Czechoslovakia. TPCK-trypsin and chymotrypsin were purchased from Worthington Biochemical Corporation, Freehold,

N.J., U.S.A. Maleic anhydride and ethyleneimine were from Koch-Light Laboratories, Colnbrook, England, 2-mercaptoethanol from Fluka, A. G., Buchs, Switzerland. Sephadex G-25 fine, Sephadex G-75, SE-Sephadex C-25, and DEAE-Sephadex A-25 were products of Pharmacia, Uppsala, Sweden

Methods

The electrophoretic separation of peptides in mixtures was carried out by descending electrophoresis[9] in pyridine acetate buffer (water–pyridine–acetic acid, 994 : 5 : 1, v/v, pH 5·6) (system S1) or by high voltage electrophoresis[10] in water–acetic acid–formic acid (16 : 3 : 1, v/v, pH 1·8, (system S2). The isolation of methionine peptides after their alkylation with iodoacetamide was effected by the procedure of Tang and Hartley[11] using electrophoresis in system S1 and a load of 1—2 mg of peptides per 1 cm of Whatman No 3 paper. The relative mobilities of the alkylated peptides are expressed with respect to the mobility of lysine. Paper chromatography was carried out in system S3, butanol–pyridine–acetic acid–water (15 : 10 : 3 : 12, v/v). The amino acid analyses were performed by the method of Spackman and coworkers[12] on 20 h hydrolysates of the samples. Sequential analysis was effected by the phenylthiohydantoin method; PTH-amino acids were detected by chromatography on a thin layer of silica gel[13,14]. Oxidation of peptides by performic acid was carried out under the conditions developed by Hirs[15]. Enzymatic cleavage of peptides with trypsin or chymotrypsin was accomplished as follows: the solution (0·1%) of the

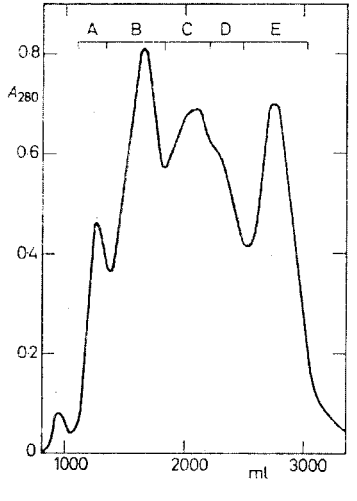

FIG. 1

Gel Filtration of Tryptic Digest of Maleylated S-sulfo-albumin on Column of Sephadex G-75

Column 5·7 . 120 cm. Eluted by 0·1M--NH_4HCO_3. Flow rate 108 ml/h. A—E, fractions containing methionine peptides.

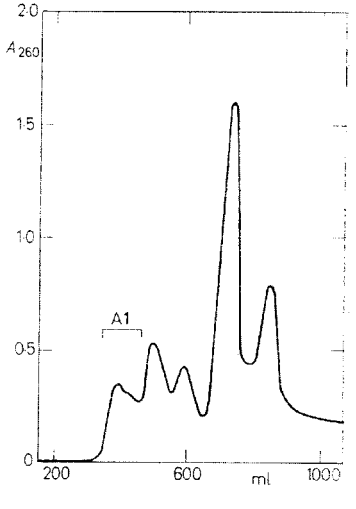

FIG. 2

Gel Filtration of Peptides Contained in Fraction A (Fig. 1) on Column of Sephadex G-25

Column 2·8 . 145 cm. Eluted by 0·05 M--HCOOH. Flow rate 54 ml/h. A1, fraction containing methionine peptides.

peptide in 0·05M-NH_4HCO_3 was digested with the enzyme at a weight ratio of 1 : 100, 1 h at 37°C. An equal quantity of the enzyme was subsequently added and digestion was allowed to proceed for additional 3 h. The solution of the digest was lyophilized.

Qualitative tests for the presence of methionine in peptides. The peptide material (1 mg) was dissolved in 50 µl of 70% HCOOH containing 1 mg of cyanogen bromide and digested 16 h at 25°C. The reaction mixture was subsequently taken to dryness in a desiccator over KOH. The dry residues were dissolved in 100 µl of 6M-HCl and hydrolyzed 16 h at 110°C. Hydrochloric acid was dried off and the hydrolysates were analyzed electrophoretically in system S1. The electropherograms were stained with ninhydrin. A yellow spot of homoserine lactone, whose mobility is higher than that of lysine, indicated the presence of methionine in the original fractions.

The preparation of S-*sulfo-albumin* was based on the procedure of Pechère and coworkers[16]. Albumin (1·5 g) and urea (144 g) were dissolved in 115 ml of 0·001M-HCl. The pH was kept at 3·0 during the dissolving by the addition of 1M-HCl. Sodium sulfite (30 ml of a solution containing 5·7 g of the anhydrous salt) and a mixture of 6 ml of 2M-$Cu(NO_3)_2$ and 6 ml of concentrated ammonium hydroxide were added to the albumin solution. The pH of the reaction mixture was adjusted to 10·2 by ammonium hydroxide and the mixture was set aside for 1 h at 25°C.

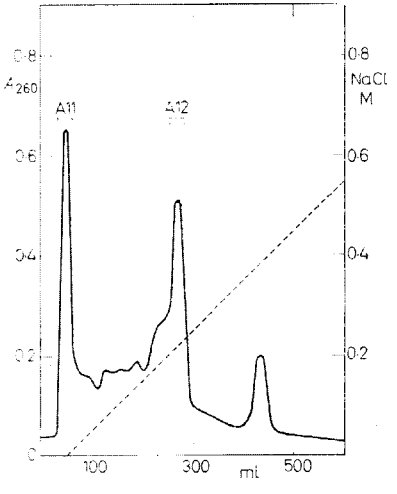

FIG. 3

Chromatography of Peptides Contained in Fraction A1 (Fig. 2) on Column of SE-Sephadex C-25

Column 1·6 . 50 cm. Eluted by 600 ml of 0·005M ammonium formate in 8M urea, pH 3. A linear elution gradient of 0—0·6M-NaCl was used. Flow rate 20 ml/h. A11, A12, fractions containing methionine peptides.

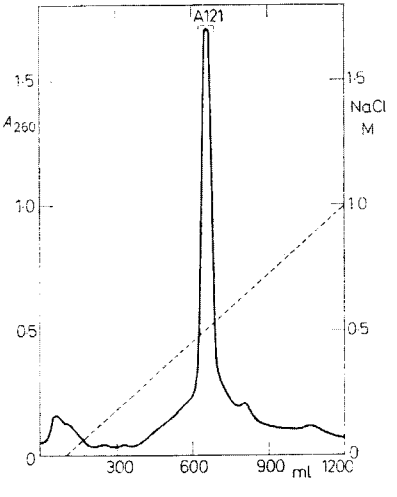

FIG. 4

Chromatography of Peptides Contained in Fraction A12 (Fig. 3) on Column of QAE-Sephadex A-25

Column 1·23 cm. Eluted by 120 ml of 0·01M sodium acetate in 8M urea, pH 5. A linear gradient of 0—1M-NaCl was used. Flow rate 12 ml/h. A121, methionine peptide.

The modified protein was separated from the reaction products by gel filtration on a column of Sephadex G-25 (6 . 75 cm) equilibrated with 0·1M-NH_4HCO_3. Fractions of 20 ml were collected at 5 min intervals. The protein-containing fraction afforded 1·46 g of material after lyophilization. A total of 4 g of S-sulfo-albumin was prepared.

Maleylated S-sulfo-albumin was prepared according to Butler and coworkers[17], using Tang's[18] modification of the procedure. S-sulfo-albumin (4 g) was dissolved in 400 ml of 5M guanidine hydrochloride and the solution was treated by turns with maleic anhydride (a total of 12 g) and solid sodium carbonate to keep the pH of the solution at 8·5 ± 0·1. The reaction was allowed to proceed at 5°C. After completion of the substitution, the solution was dialyzed against 10 l of 0·05M-NH_4HCO_3 and desalted in two portions on a column of Sephadex G-25 (6 . 79 cm), equilibrated with the same ammonium bicarbonate solution. The material was lyophilized (yield 3·8 g).

The preparation and fractionation of the tryptic digest of maleylated S-sulfo-albumin were carried out as follows. Substituted albumin (3·8 g) was dissolved in 0·1M-NH_4HCO_3 to a 1% solution and digested with trypsin under the conditions described for enzymatic cleavage of peptides. The digest was separated in two portions on a column of Sephadex G-25 (Fig. 1) into fractions A (356 mg), B (1357 mg), C (918 mg), D (814 mg), and E (511 mg).

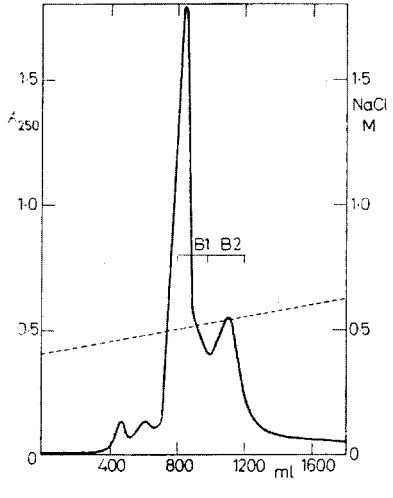

FIG. 5

Chromatography of Peptides Contained in Fraction B (Fig. 1) on Column of DEAE--Sephadex A-25

Column 3·2 . 55 cm. Eluted by 2 liter of 0·05M Tris-HCl buffer, pH 7·5. A linear elution gradient of 0·4—0·65M-NaCl was used. Flow rate 60 ml/h. B1 and B2, fractions containing methionine peptides.

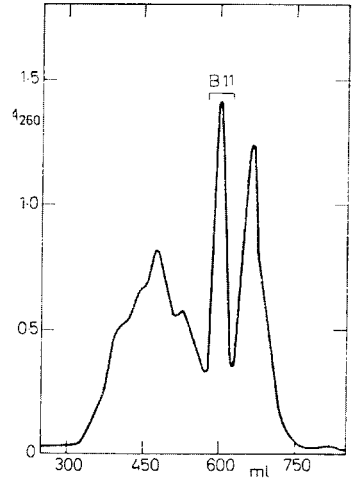

FIG. 6

Gel Filtration of Tryptic Digest of Demaleylated Fraction B1 (Fig. 5) on Column of Sephadex G-25.

Column 2·8 . 145 cm. Eluted by 0·05M--HCOOH. Flow rate 54 ml/h. B11, fraction containing methionine peptides.

Demaleylation[17] *of peptide material* was effected as follows. The substituted peptides (100 mg) were dissolved in 90 ml of a mixture of pyridine and water (1 : 90), the solution was treated with 10 ml of acetic acid and heated 5 h at 60°C. After the treatment the buffer was rotary evaporated, the demaleylated peptides were dissolved in water, and the solution was lyophilized.

Treatment of Individual Fractions

Fraction A. The peptide material (324 mg) was demaleylated and digested with trypsin. The resulting mixture was subjected to gel filtration on a column of Sephadex G-25 (Fig. 2). Methionine-containing peptides were detected in fraction A1 (72 mg). A part of this fraction (61 mg) was dissolved in 3 ml of 0·005M ammonium formate containing 8M urea (pH 3·0) and separated on a column of SE-Sephadex C-25 (Fig. 3). The main fractions, A11 and A12 were made alkaline by the addition of ammonium hydroxide, desalted on a column of Sephadex G-25 (2·8 . 145 cm) equilibrated with 0·02M-NH_4OH, and lyophilized.

A part of fraction A11 (8 mg) was digested with chymotrypsin. A methionine-containing peptide A111 was isolated by diagonal electrophoresis from the digest in the form of sulfonium (mobility 0·44).

Peptides contained in fraction A12 (30 mg) were dissolved in 2 ml of 0·01M sodium acetate

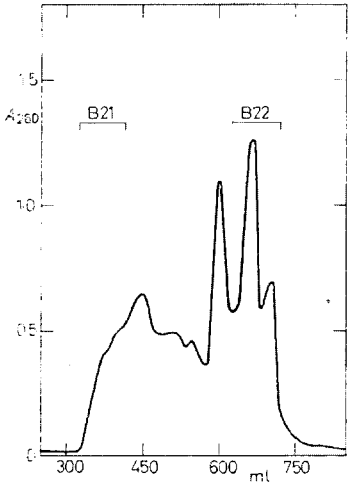

FIG. 7

Gel Filtration of Tryptic Digest of Demaleylated Fraction B2 by Gel Filtration on Column of Sephadex G-25

Column 2·8 . 145 cm. Eluted by 0·05M-HCOOH. Flow rate 54 ml/h. B21 and B22, fractions containing methionine peptides.

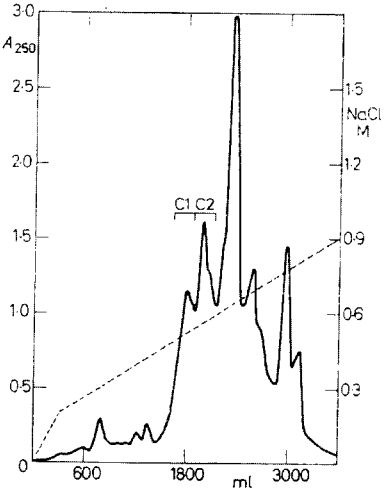

FIG. 8

Chromatography of Peptides Contained in Fraction C (Fig. 1) on Column of DEAE-Sephadex A-25

Column 3·2 . 55 cm. Eluted by 4 liters of 0·05M Tris-HCl buffer, pH 7·5 A linear elution gradient of 0·2—1M-NaCl was used. Flow rate 60 ml/h. C1 and C2, fractions containing methionine peptides.

TABLE I

Amino-Acid Composition of Peptides Isolated and their Cyanogen Bromide Fragments

The values are not corrected. The analyses were performed on 20 h hydrolysates of peptides. None of the peptides analyzed contained tryptophan.

Designation of peptide	Lys	Aec[a]	His	Arg	Asp	Met SO_2	Thr	Ser	Hse[b]
A111									1·0
A121[c]	1·2		0·8		3·7	0·9		2·3	
A121—CB1[c]			0·1		2·1			0·3	1·0
A121—CB2	0·8				2·2			1·9	
B111					2·2				
B211		0·9		1·0	1·2				
B221	0·2				0·7				
C111				1·0	1·1	0·7			
C211	1·0				1·2		0·9		
D111[c]				0·9	1·0	0·7			
D211	1·2				1·0		0·9		
D211—CB1							1·0		0·8
D211—CB2[c]	1·1				1·1				

in 8M urea (pH 5·0) and chromatographed on a column of QAE-Sephadex A-25 (Fig. 4). Peptide A121 (18·9 mg) was obtained. This peptide was cleaved by cyanogen bromide. Excess reagents were dried off and the reaction products were oxidized by performic acid; the resulting peptides A121—CB1 and A121—CB2 were isolated by descending electrophoresis.

Fraction B. The peptides (1370 mg) were dissolved in 30 ml of 0·05M Tris-HCl buffer at pH 7·5 and chromatographed on a column of DEAE-Sephadex A-25 (Fig. 5). Methionine was found in fractions B1 and B2 only. These fractions were demaleylated, digested with trypsin, and lyophilized. Peptides arising from fraction B1 were dissolved in 15 ml of 70% HCOOH and separated on a column of Sephadex G-25 (Fig. 6). Methionine was detected in peak B11. After lyophilization, peptide B111 was isolated by electrophoresis in systems S1 and S2. Fraction B2 was subjected to the same separation as fraction B1. The course of gel filtration is shown in Fig. 7. The mixture of peptides emerging in the hold-up volume from the Sephadex G-25 column was designated B21 (51 mg). This material was dissolved in 5 ml of 8M urea and a solution of 10 mg of EDTA in 0·4 ml of water and 120 ml of Tris were added. The S-sulfo-cysteine residues of peptides were reduced by the addition of 0·42 ml of 2-mercaptoethanol at pH 8·6—8·0. Reduction was allowed to proceed 4 h at 23°C in the atmosphere of nitrogen. The reaction mixture was subsequently treated with 0·65 ml of ethyleneimine added in parts. The pH of the solution was kept at 8·6 by the addition of 4M-HCl. The completion of the substitution was checked by the nitroprusside test. The reaction mixture was subjected to gel filtration on a column of Sephadex G-25 equilibrated with 0·05M-HCOOH. Peptide material was digested with trypsin, the digest substituted by iodoacetamide and separated by the diagonal technique. Peptide B211 (mobility 0·73) was isolated.

TABLE I
(*Continued*)

Glu	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe
		1·3						0·7		1·0
4·0	1·9		3·5	1·3[d]	1·9		0·8	2·4		0·9
2·6			1·0	0·7[d]	0·9		0·8			
1·2	2·2		3·0		1·0			2·0		1·0
			3·0		1·1	0·9				2·3
1·3	1·0				2·8	0·8		1·0		
1·8		0·8	1·8		0·7	0·6		2·0	1·9	2·1
1·2	1·0				2·8			1·3		
2·2		0·9	2·1	1·8		0·9			0·8	
1·0					2·6			0·6		
2·0		1·0	2·0	1·7		0·9			0·8	
2·0		1·1							0·9	
			2·1	2·0[d]						

[a] Aminoethyl-cysteine [b] homoserine [c] sample oxidized by performic acid [d] determined as cysteic acid.

TABLE II
Amino-Acid Sequences of Methionine Peptides

Designation of peptide	Amino-acid sequence
A111	Leu-Gly-Met-Phe
A121	Ser-His-Cys(Ile,Asx,Asx,Glx,Glx,Glx,Ala,Val)Met-Pro-Ala-Asp-Leu-Pro-Ser--Leu(Ala,Ala,Asx,Phe,Val,Glx,Ser)Lys
B111	Ala-Val-Met-Asp-Asp-Phe-Ala-Ala-Phe
B211	Leu(Val,Arg,Pro,Glx,Val,Asx,Val)Met-Aec
B221	Ala-Glx-Ala(Lys,Asx,Val,Phe,Leu,Gly,Met,Phe,Leu,Tyr,Glx,Tyr)
C111	Leu(Val,Arg,Pro,Glx,Val,Asx,Val)Met
C211	Glu(Thr,Tyr,Gly,Glx,Met,Ala,Asx,Cys,Cys,Ala)Lys
D111	Leu(Val,Arg,Pro,Glx,Val,Asx,Val,$MeSO_2$)
D211	Glu-Thr-Tyr-Gly-Glu-Met-Ala-Asp-Cys-Cys-Ala-Lys

The mixture of peptides contained in peak B22 (Fig. 7) was subjected to electrophoretic separation in systems S1 and S2 and methionine-containing peptide B221 was isolated.

Fraction C. The peptides (850 mg) were dissolved in 50 ml of 0·05M Tris-HCl buffer at pH 7·5 and chromatographed on a column of DEAE-Sephadex A-25 (Fig. 8). Fractions C1 and C2 were demaleylated and digested with trypsin; the digests were lyophilized. The lyophilisates were dissolved in 5 ml of 70% HCOOH and fractionated by gel filtration on columns of Sephadex G-25 (Fig. 9 and 10). Methionine-containing peptides were found in fractions C11 and C21. From these fractions, peptides C111 and C211 were isolated by electrophoresis in system S2 and by paper chromatography in system S3.

Fraction D. The peptides (414 mg) were dissolved in 7 ml of 0·05M Tris-HCl buffer at pH 7·5 and the pH of the solution was adjusted to 9·5 by ammonium hydroxide. The hydrolysate was fractionated on a column of DEAE-Sephadex A-25 (Fig. 11). Methionine was detected in fractions D1 and D2. Each of these fractions was lyophilized. The lyophilisates were dissolved in 4 ml of 70% HCOOH and desalted on columns of Sephadex G-25 (Fig. 12 and 13). Peptides D11 and D21 were obtained. Peptide D211 was treated with cyanogen bromide. The resulting mixture of peptides was resolved by electrophoresis in system S1 into peptides D211—CB1 and D211—CB2.

Fraction E. No methionine-containing peptides were isolated from this fraction.

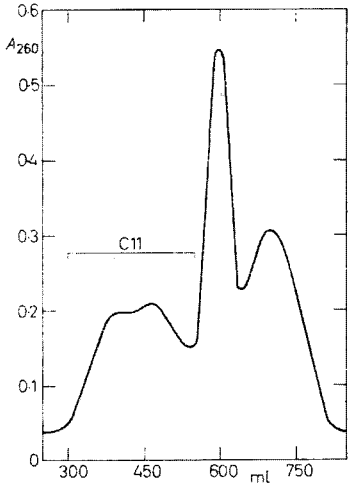

FIG. 9

Gel Filtration of Tryptic Digest of Demaleylated Fraction C1 (Fig. 8) on Column of Sephadex G-25

Column 2·8 . 145 cm. Eluted by 0·05M-HCOOH. Flow rate 54 ml/h, fraction containing methionine peptides.

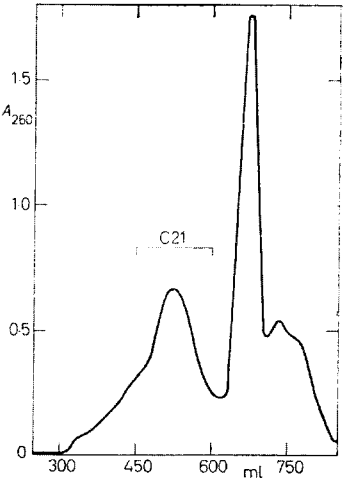

FIG. 10

Gel Filtration of Tryptic Digest of Demaleylated Fraction C2 (Fig. 8) on Column of Sephadex G-25

Column 2·8 . 145 cm. Eluted by 0·05M-HCOOH. Flow rate 54 ml/h. C21, fraction containing methionine peptides.

RESULTS AND DISCUSSION

The tryptic digest of maleylated S-sulfo-albumin was resolved on a Sephadex G-75 column (Fig. 1) into fractions A through E.

Fraction A could not be separated into additional components by chromatography on DEAE- or QAE-Sephadex. It was demaleylated and digested with trypsin. The high molecular weight part of the digest contained a mixture of two larger methionine-containing peptides A11 and A12. These peptides were isolated (Fig. 2) and subjected to additional cleavage to smaller peptides whose composition would permit the immediate neighborhood of the methionine residue to be defined. Fraction A11 was digested with chymotrypsin and peptide A111, Leu-Gly-Met-Phe, was isolated from the digest by the diagonal technique. The amino acid composition of this peptide and of the other peptides isolated as well as of their cyanogen bromide fragments are given in Table I. The amino-acid sequences of the methionine peptides are shown in Table II. Fraction A12 afforded after rechromatography on a column of QAE-Sephadex A-25 peptide A121. The cleavage of the latter by cyanogen bromide

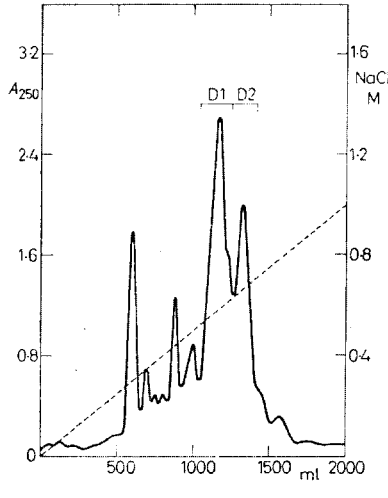

FIG. 11

Chromatography of Peptides Contained in Fraction D (Fig. 1) on Column of DEAE--Sephadex A-25

Column 2·7 . 45 cm. Eluted by 2 liters of 0·05M Tris-HCl buffer, pH 7·5. A linear elution gradient of 0—1M-NaCl was used.. Flow rate 60 ml/g. D1 and D2, fractions containing methionine peptides.

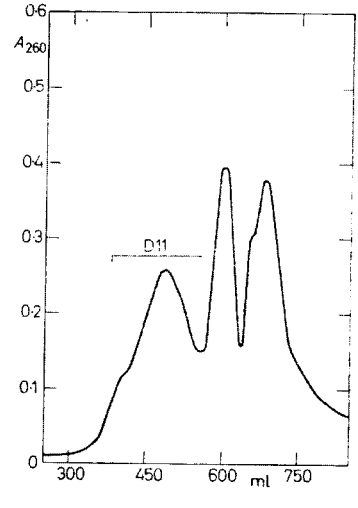

FIG. 12

Gel Filration of Tryptic Digest of Demaleylated Fraction D1 (Fig. 11) on Column of Sephadex G-25

Column 2·8 . 145 cm. Eluted by 0·05M--HCOOH. Flow rate 108 ml/h. D11, fraction containing methionine peptides.

gave peptide A121 – CB1, Ser(His,Cys,Ile,Asx,Asx,Glx,Glx,Glx,Ala,Val)Hse and peptide A121 – CB2 Pro-Ala-Asp-Leu-Pro-Ser-Leu(Ala,Ala,Asx,Phe,Val,Glx,Ser)Lys.

Fraction B yielded methionine peptides B111, B211, and B221. Peptide B111 does not contain any basic amino acid and resulted obviously from nonspecific cleavage caused by residual chymotryptic activity of the trypsin preparation. Peptide B221 also results from nonspecific cleavage. Peptide B211 was identified as Leu (Val,Arg,Pro,Glu,Val,Asp,Val)Met-Aec.

Fraction C afforded two methionine peptides, C111 and C211 (Table II).

Fraction D yielded peptides D111 and D211. Peptide D111 is probably a result of a secondary cleavage of a larger peptide contained in fraction B. Peptide D211 (which is identical with peptide C211) was subjected to cyanogen bromide cleavage and both resulting fragments, D211 – CB1 and D211 – CB2 were isolated and sequenced: D211 – CB1, Glu-Thr-Tyr-Gly-Glu-Hse, D211 – CB2, Ala-Asp-Cys-Cys--Ala-Lys. The amino acid sequences of the peptides isolated are summarized in Table II.

The individual cyanogen bromide fragments of human plasma albumin were isolated in an earlier study[3]. All these fragments were characterized by analyses of amino-acid composition and terminal sequences[3] and marked by working symbols I-Asp, II-Ala, III-Cys, IV-Phe, V-Pro, VI-Pro, and VII-Asp, without respect to their actual order in the polypeptide chain.

It follows from the identity of the N-terminal sequence of fragment I-Asp with the N-terminal sequence of the human plasma albumin molecule that this fragment represents the N-terminus of the polypeptide chain of the protein. The C-terminal

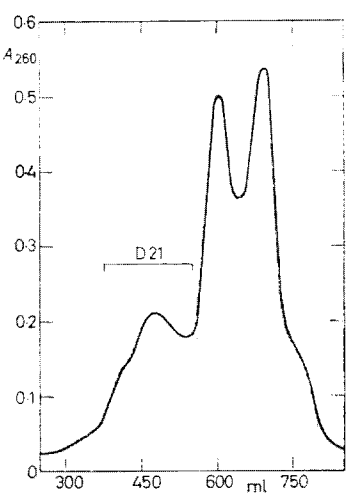

FIG. 13

Gel Filtration of Tryptic Digest of Fraction D2 (Fig. 11) on Column of Sephadex G-25 Column 2·8 . 145 cm. Eluted by 0·05M--HCOOH. Flow rate 108 ml/h. D21, fraction containing methionine peptides.

sequence of fragment I-Asp involves therefore methionine residue No I. This C-terminal sequence, *i.e.*. Glu-Thr-Tyr-Gly-Glu-Met, is contained in methionine peptide D211 Glu-Thr-Tyr-Gly-Glx-Met-Ala-Asp-Cys-Cys-Ala-Lys obtained in this study.

Asp-Ala-His-Lys-Ser-Glu-Val-Ala-His-Arg-Phe-Lys-Asp-Leu-Gly-Glu-Glu-Asn-Phe-Lys-
Ala-Leu-Val-Leu-Ile-Ala-Phe-Ala-Gln-Tyr-Leu-Gln-Gln-Cys-Pro-Phe-Glu-Asp-His-Val-
Lys-Leu-Val-Asp-Glu-Val-Thr-Glu-Phe-Ala-Lys-Thr-Cys-Val-Ala-Asp-Glu-Ser-Asp-Ala-
Gln-Cys-Asn-Lys-Ser-Leu-His-Thr-Leu-Phe-Gly-Asp-Leu-Cys-Lys-Thr-Val-Ala-Thr-Leu-
Arg-*Glu-Thr-Tyr-Gly-Glu*-**Met**-*Ala-Asp-Cys-Cys-Ala-Lys*-Glu-Gln-Pro-Glu-Arg-Asn-Glu-
Cys-Phe-Leu-Gln-His-Lys-Asp-Asp-Asn-Pro-Asn-Leu-Pro-Arg-*Leu-Val-Arg-Pro-Glu-Val-*
Asp-Val-**Met**-*Cys*-Thr-Ala-Phe.....Ala-Trp-Ala-Val-Ala-Arg.....
*Ser-His-Cys-Ile(Asx,Asx,Glx,Glx,Glx,Ala,Val)***Met**-*Pro-Ala-Asp-Leu-Pro-Ser-Leu-Ala-*
Ala-Asp-Phe-Val-Glu-Ser-Lys-Asp-Val-Cys-Lys-Asn-Tyr-Ala-Glu-Ala-Lys-Asp-Val-Phe-
Leu-Gly-**Met**-*Phe*-Leu-Tyr-Glu-Tyr-Ala-Arg/Leu-Ala-Lys/Phe-Gln-Asn-Ala-Leu-Leu-Val-
Arg/Thr-Tyr-Glu-Thr-Leu-Glu-Lys-Cys-Cys-Ala-Ala-His-Asp-Pro-Tyr(Glx,Cys,Ala,Ala)
Lys/Tyr-Thr-Lys-Lys-Val-Pro-Gln-Val-Ser-Thr-Pro-Thr-Leu-Val-Glu-Val-Ser-Arg/....
(Leu,Leu,Glx,Glx,Glx,Glx,Cys,Phe,Asx,Tyr,Gly)Lys/Asn-Leu-Gly-Lys-Val-Gly-Ser-
Lys-Cys-Cys-Lys-His-Pro-Glu-Ala-Lys-Arg-**Met**-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-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*-**Met**-*Asp-Asp-Phe-Ala-Ala-*
Phe-Val-Glu-Lys-Cys-Cys-Lys-Ala-Asp-Asp-Lys-Glu-Thr-Cys-Phe-Ala-Glu-Glu-Gly-Lys-
Lys-Leu-Val-Ala-Ala-Ser-Gln-Ala-Ala-Leu-Gly-Leu

FIG. 14

Partial Amino-Acid Sequence of Human Plasma Albumin

This scheme summarizes data obtained in this Laboratory. The regions of the chain whose amino acid sequence is not known from our experiments are marked by periods (regardless of the actual number of amino-acid residues). Complete sequences whose positions inside an individual fragment are interchangeable are separated by symbol /. The methionine peptides determined in this study are set in italics. Methionine residues (I—VI) are set in boldface type. The first 127 residues of the N-terminal part of the polypeptide chain are numbered.

This peptide is identical also with peptide C211 (Table II). The sequence at the carboxyl side of methionine No I in peptide D211 is identical with the N-terminal sequence of fragment II-Ala (ref.[3]). This fragment therefore represents the region of chain between methionine No I and methionine No II, contained in the sequence Leu-Val--Arg-Pro-Glu-Val-Asp-Val-Met (ref.[19]). The latter sequence can be correlated with methionine peptide B211, Leu(Val,Arg,Pro,Glx,Val,Asx,Val)Met-Aec. One fragment only, III-Cys, is N-terminated by a half-cystine residue. Hence, fragment III-Cys is linked to fragment II-Ala at methionine No II. The C-terminal methionine No III of fragment III-Cys is contained in the sequence. ... Ser-His-Cys-Ile(Asx_2,Glx_3,Ala, Val)Met. The latter is compatible with methionine peptide A121, Ser-His-Cys(Ile, Asx_2, Glx_3, Ala,Val)Met-Pro-Ala-Asp-Leu-Pro-Ser-Leu(Ala_2, Asx, Phe, Val, Glx, Ser) Lys. The sequence around methionine No III in this peptide is identical with the N-terminal sequence of fragment VI-Pro. This fragment therefore is located in the chain at the carboxyl side of methionine No III. The C-terminal sequence of fragment VI-Pro, *i.e.* Ala-Glu-Ala-Lys-Asp-Val-Phe-Leu-Gly-Met, involving methionine No IV is reconcilable with methionine peptides B221, Ala-Glx-Ala(Lys,Asx,Val,Phe, Leu,Gly,Met,Phe,Leu,Tyr,Glx,Tyr) and A111, Leu-Gly-Met-Phe. A phenylalanine residue is N-terminal in frament IV-Phe. The N-terminal sequence of this fragment, Phe-Leu-Tyr-Glu-Tyr, is compatible with peptide B221. Fragment IV-Phe thus represents the part of the human plasma albumin chain between methionine No IV and methionine No V. It is C-terminated by the sequence Asn-Leu-Gly-Lys-Val-Gly--Ser-Lys-Cys-Cys-Lys-His-Pro-Glu-Ala-Lys-Arg-Met (ref.[20]), containing methionine No V. The corresponding methionine peptide derived from this site of chain was not obtained in the present study.

Since, however, methionine peptide B111, Ala-Val-Met-Asp-Asp-Phe-Ala-Ala-Phe provides an unambiguous link between the C-terminus of fragment VI-Pro (ref.[21]), *i.e.* Ala-Val-Met, and the N-terminus of fragment VII-Asp, *i.e.* Asp-Asp-Phe-Ala--Ala-Phe, which represents the C-terminal region of the whole human plasma albumin chain, the order of all fragments can be defined unequivocally as I-Asp/II-Ala / / III-Cys/VI-Pro/IV-Phe/V-Pro/VII-Asp. On the basis of the knowledge of the order of the cyanogen bromide fragments in the chain, we replace the above working symbols by a rational nomenclature, expressing the order of the fragments and their N-terminal amino acids:

New designation	Earlier working symbol	New designation	Earlier working symbol
CB1(Asp)	I-Asp	CB5(Phe)	IV-Phe
CB2(Ala)	II-Ala	CB6(Pro)	V-Pro
CB3(Cys)	III-Cys	CB7(Asp)	VII-Asp
CB4(Pro)	VI-Pro		

The present state of sequential studies on human plasma albumin carried out in our Laboratory is summarized in the form of partial amino acid sequence of the protein, shown in Fig. 14. This summary contains the complete amino-acid sequences of fragment (CB1(Asp) (ref.[22]), CB2(Ala) (ref.[19]), CB4(Pro) (ref.[23]), and CB7(Asp) (ref.[6]). Our sequential information on fragment CB6(Pro) (ref.[21]) and CB5(Phe) (ref.[20]) has also achieved an advanced stage; the remaining fragment CB3(Cys), can be characterized as yet merely by terminal sequences and by the amino acid sequence around the only tryptophan[3] residue of human plasma albumin which falls into the region of fragment CB3(Cys). The partial structure of the whole polypeptide chain of human plasma albumin involves 403 amino-acid residues.

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