AMINO ACID SEQUENCE OF CYANOGEN BROMIDE FRAGMENT CB3(Cys) OF HUMAN SERUM ALBUMIN

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The cyanogen bromide fragment studied represents the region of the polypeptide chain of human serum albumin between methionine No II and No III. It contains 175 amino acid residues and their sequence is the following: Cys-Thr-Ala-Phe-His-Asp-Asn-Gln-Glu-Thr-Phe-Leu-Lys-Lys-Tyr-Leu-Tyr-Glu-Ile-Ala-Arg-Arg-His-Pro-Tyr-Phe-Tyr-Ala-Pro-Glu-Leu-Leu-Phe-Phe-Ala-Lys-Arg-Tyr-Lys-Ala-Ala-Phe-Thr-Glu-Cys-Cys-Glu-Ala-Ala-Asp-Lys-Ala-Ala-Cys-Leu-Leu-Pro-Lys-Leu-Asp-Glu-Leu-Arg-Asp-Glu-Gly-Lys-Ala-Ser-Ser-Ala-Lys-Gln-Arg-Leu-Lys-Cys-Ala-Ser-Leu-Gln-Lys-Phe-Gly-Glu-Arg-Ala-Phe-Lys-Ala-Trp-Ala-Val-Ala-Arg-Leu-Ser-Gln-Arg-Phe-Pro-Lys-Ala-Glu-Phe-Ala-Glu-Val-Ser-Lys-Leu-Val-Thr-Asp-Leu-Thr-Lys-Val-His-Thr-Glu-Cys-Cys-His-Gly-Asp-Leu-Leu-Glu-Cys-Ala-Asp-Asp-Arg-Ala-Asp-Leu-Ala-Lys-Tyr-Ile-Cys-Glu-Asn-Gln-Asp-Ser-Ile-Ser-Ser-Lys-Leu-Lys-Glu-Cys-Cys-Glu-Lys-Pro-Leu-Leu-Glu-Lys-Ser-His-Cys-Ile-Ala-Glu-Val-Glu-Asn-Asp-Glu-Met. This structure was derived from the results of sequential degradation of the fragment, from the analyses of peptides from its tryptic and chymotryptic digest and of peptides from the tryptic digest of the maleyl derivative of fragment CB3(Cys).

Most of the sequential studies on fragments CB1(Asp), CB2(Ala), CB3(Cys), CB4(Pro) CB5(Phe), CB6(Pro), and CB7(Asp), arising from cyanogen bromide cleavage of human serum albumin, have been quoted in the paper reporting the complete amino acid sequence of the protein[1]. After this paper had appeared in the press, two concluding papers[2,3] were published describing experimental details of sequencing cyanogen bromide fragment CB5(Phe) and CB6(Pro). Studies on the remaining cyanogen bromide fragment CB3(Cys) have been reported in a preceeding paper describing the determination of the N-terminal sequence of the fragment and structures of tryptic peptides; these results were summarized in a partial sequence[4] accounting for 171 amino acid residues.

This paper provides the remaining experimental data on the sequencing of cyanogen bromide fragment CB3(Cys) containing residues No 124–298 of human serum albumin. The data obtained earlier[4] were complemented by an analysis of the chymotryptic digest of S-sulfonated fragment CB3(Cys) and by the characterization of selected peptides from the tryptic digest of the maleyl derivative of S-sulfonated fragment CB3(Cys). The results of the present study permitted the complete amino acid

sequence of fragment CB3(Cys) of human serum albumin to be determined. This paper also provides information on the reinvestigation of certain amino acid residues[5] of fragment CB1(Asp), the results of which have already been respected in the paper[1] reporting the complete amino acid sequence of human serum albumin.

EXPERIMENTAL

Material

Human serum albumin was from the Institute of Sera and Vaccines, Prague, Czechoslovakia. Cyanogen bromide fragments CB3(Cys) and CB1(Asp) and their S-sulfo derivatives were prepared as described in the preceding reports[4,5]. Chymotrypsin and TPCK-trypsin (trypsin treated with 1-chloro-4-phenyl-3-tosylamino-2-butanone[6]) were purchased from Worthington Biochemical Corporation, Freehold, N. J., U.S.A. Soy-bean trypsin inhibitor, B-grade, was a product of Calbiochem, San Diego, California, U.S.A. Maleic anhydride was from Koch-Light Laboratories, Colnbrook, Bucks, England, and 2,4,6-trimethylpyridine (purity 98%) from Downs Development Chemicals, Ruislip, England. Guanidine hydrochloride was a product purified in the Service Laboratory, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague. Silufol, aluminum sheets with a thin silica gel layer, were manufactured by Kavalier, Sázava, Czechoslovakia. Sephadex G-75, G-50 fine, G-25 fine, DEAE-Sephadex A-25, and SE-Sephadex C-25 were products of Pharmacia, Uppsala, Sweden. Dowex 1-X2, 200—400 mesh, was from FLUKA, Buchs, Switzerland.

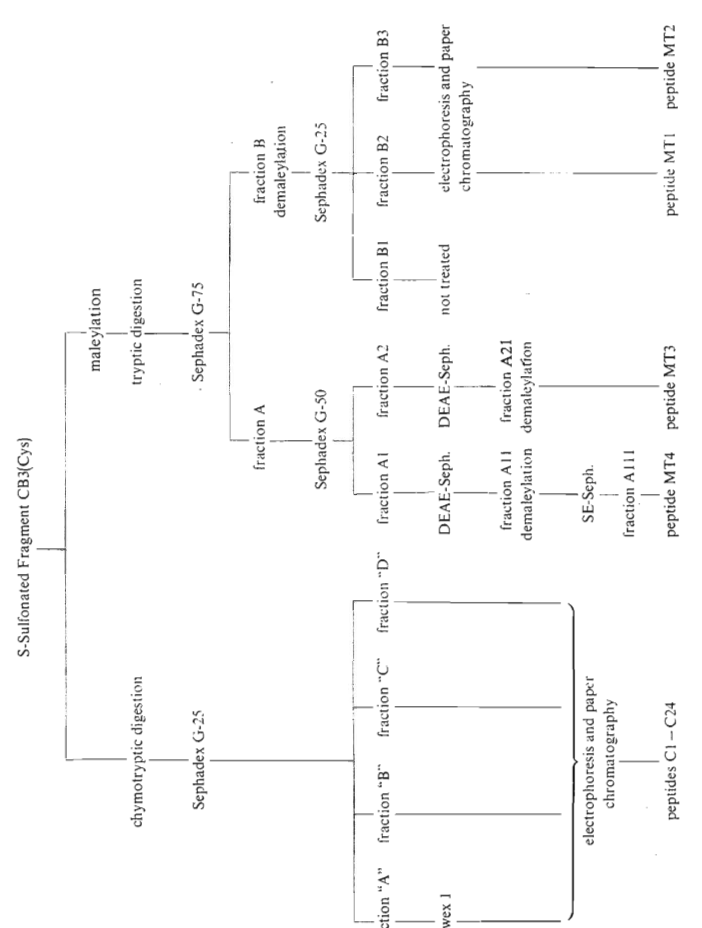
Methods

Separations of peptide mixtures were effected by paper chromatography in the system[7] n-butyl alcohol–pyridine–acetic acid–water (15 : 10 : 3 : 12, v/v). Descending electrophoresis[8] at pH 5·6 (30 V/cm) was carried out in the system pyridine–acetic acid–water (5 : 1 : 494, v/v) and horizontal high voltage electrophoresis[9] at pH 1·9 (100 V/cm) in the system acetic acid–formic acid–water (3 : 1 : 16, v/v). The techniques of amino acid analysis[10] have been described in the preceding paper[4]. Sequential analyses of peptides were effected by Edman degradation[11] and the phenylthiohydantoins of amino acids were identified by thin-layer chromatography on silica gel[11,12]. The treatment of lysine peptides by maleic anhydride was carried out according to Butler and coworkers[13]. For the demaleylation of peptides, 50 mg of material was dissolved in 45 ml of a mixture of pyridine and water (1 : 90); 5 ml of glacial acetic acid was added to this solution and the mixture was heated 5 h at 60°C. The peptide material was dried and freed of pyridine acetate by lyophilization.

The fragmentation and fractionation procedures used to obtain the individual peptides are shown in Fig. 1.

Preparation and Fractionation of Chymotryptic Digest of S-Sulfonated Fragment CB3(Cys)

S-Sulfonated fragment CB3(Cys) (1·1 g) was dissolved in 60 ml of 0·2M-NH_4HCO_3, 11 mg of chymotrypsin and 1·1 mg of soy bean trypsin inhibitor dissolved in 1 ml of water were added and the reaction mixture was incubated 2 h at 37°C. Another addition of 11 mg of chymotrypsin and 1·1 mg of trypsin inhibitor was made afterwards and the digestion was allowed to proceed for 2 more hours at 37°C. The chymotryptic digest without any additional treatment was applied onto a column of Sephadex G-25 (2·8 × 150 cm) which was subsequently eluted by 0·05M-


S-Sulfonated Fragment CB3(Cys)
maleylation
tryptic digestion
Sephadex G-75
fraction A
fraction B
demaleylation
Sephadex G-25
Sephadex G-50
fraction A1
fraction A2
fraction B1
fraction B2
fraction B3
DEAE-Seph.
DEAE-Seph.
not treated
electrophoresis and paper chromatography
fraction A11 demaleylation
fraction A21 demaleylation
SE-Seph.
fraction A111
peptide MT4
peptide MT3
peptide MT1
peptide MT2
chymotryptic digestion
Sephadex G-25
fraction "A"
fraction "B"
fraction "C"
fraction "D"
Dowex 1
electrophoresis and paper chromatography
peptides C1–C24

NH_4HCO_3. Fractions of 23 ml were collected at 20-min intervals. Aliquots (0·2 ml) of the fractions were taken to dryness, applied to Whatman No 3 paper and separated electrophoretically at pH 5·6. According to the results of the electrophoretic separation individual fractions were pooled and the pooled material lyophilized. The following pooled fractions were thus obtained: "A" (tubes No 16–21; 510 mg) "B" (tubes No 22–27; 275 mg), "C" (tubes No 28–33; 234 mg), and "D" (tubes No 34–40; 34 mg).

Fraction "A" was chromatographed on a column of Dowex 1-X2. The sample (500 mg) was dissolved in 5 ml of water, its pH adjusted to 10 by ammonium hydroxide, and the solution placed onto a column of Dowex 1(1·1 × 51 cm), equilibrated with 1% solution of *sym*-collidine (pH 9·6). The chromatography was carried out essentially according to Guest and coworkers[14]. The following eluants were allowed to flow into a mixing device (volume 280 ml) connected with the column: 1% collidine starting from fraction No 1, 0·1M-CH_3COOH from fraction No 6, 0·3M-CH_3COOH from fraction No 21, 1M-CH_3COOH from fraction No 36, and 5M-CH_3COOH from fraction No 51. The mixing device was then disconnected and starting from fraction No 66 the column was eluted by 5M-CH_3COOH and from fraction No 71 by glacial acetic acid. Fractions of 10 ml were taken at 10 min intervals; 0·1 ml aliquots of each fraction were withdrawn and analyzed by descending electrophoresis (pH 5·6). Effluent fractions from the Dowex 1 column containing peptides of identical electrophoretic characteristics were pooled and the pooled material lyophilized. Individual peptides were isolated from the pooled fractions by paper chromatography and electrophoresis.

Fractions "B", "C", and "D" of the chymotryptic digest of S-sulfo-CB3(Cys) were worked up by paper techniques.

Maleylation of S-Sulfonated Fragment CB3(Cys)

S-Sulfonated fragment CB3(Cys) (2 g) was dissolved in 200 ml of 5M guanidine hydrochloride. This solution was treated with 6 g of maleic anhydride, dissolved in 90 ml of dioxane and added in parts within 30 min. The reaction mixture was maintained at pH 8·5–9·0 by the addition of 2M-NaOH for 1 h at 3–5°C and then desalted by gel filtration on a column of Sephadex G-25 (10 × 90 cm), equilibrated in 0·005M-NH_4HCO_3. The course of the gel filtration was monitored by absorbance measurement of the effluent at 280 nm and fractions of 200 ml were collected. The protein moiety was lyophilized.

Preparation and Fractionation of Tryptic Digest of Maleylated S-Sulfo Derivative of Fragment CB3(Cys)

Substituted fragment CB3(Cys) (2·1 g) was dissolved in 110 ml of 0·1M-NH_4HCO_3 and 40 mg of trypsin, dissolved in 2 ml of water, was added to this solution. The reaction mixture was incubated 30 min at 37°C and applied onto a column of Sephadex G-75 afterwards. The course of the fractionation is shown in Fig. 2*a*. By this approach, fraction A, containing larger peptides, and fraction B, containing a mixture of small peptides, were obtained.

Fraction A was lyophilized, the material (750 mg) was dissolved in 15 ml of 0·02M-NH_4OH and fractionated further on a column of Sephadex G-50. Fraction A1 (179 mg) and fraction A2

FIG. 1

Fragmentation and Fractionation Procedures Yielding Peptides Used for Sequential Determination of Fragment CB3(Cys)

(132 mg) were isolated (Fig. 2*b*). Fraction A1 was chromatographed on DEAE-Sephadex A-25 at pH 7·5 (Fig. 3*a*) and afforded material designated A11 (56 mg). The latter was demaleylated, dissolved in 1 ml of 8M urea and fractionated on a column of SE-Sephadex C-25 at pH 3·0 (Fig. 3*b*). The material obtained was designated A111, desalted on a column of Sephadex G-25 (2·5 × × 61 cm), equilibrated in 0·02M-NH_4OH, and lyophilized; peptide MT4 was obtained.

Fraction A2 was chromatographed on a column of DEAE-Sephadex A-25 (Fig. 3*c*). The material from peak A21 (50 mg) gave peptide MT3 after demaleylation and desalting. Peptides MT3 and MT4, dissolved to a 1% solution in 0·1M-NH_4HCO_3, were digested with trypsin at a molar ratio of 1 : 100 at 37°C for 3 h. Individual peptides were isolated by techniques of paper chromatography and electrophoresis (pH 5·6).

Fraction B (1080 mg) was demaleylated and lyophilized. The material was dissolved in 100 ml of water whose pH had been adjusted to 9·5 by ammonium hydroxide and the solution was placed on top of a column of Sephadex G-25 (6 × 45 cm), equilibrated in 0·01M-NH_4HCO_3. The column was eluted by the same solution and 22 ml fractions were taken at 5 min intervals. The fractions were analyzed by electrophoresis (pH 5·6) of aliquots taken from each fraction and corresponding to 1/220 of the fraction volume. The effluent fractions were pooled according to the results of electrophoretic analysis to fractions B1—B3 which were lyophilized. Fraction B1 contained some of the peptides found in fraction A1 and was not treated further. Fraction B2 and B3 were separated by paper electrophoresis (pH 5·6) and chromatography. The peptides isolated by these techniques were designated MT1 and MT2.

Preparation and Fractionation of Tryptic Digest of S-Sulfonated Fragment CB1(Asp)

The tryptic digest of the S-sulfonated fragment (420 mg) was prepared as described earlier[5]. The three peptides sought were isolated by preparative paper chromatography of the tryptic digest and subsequent electrophoretic separation (pH 5·6) based on the known R_F-values and mobility values, resp. of the peptides.

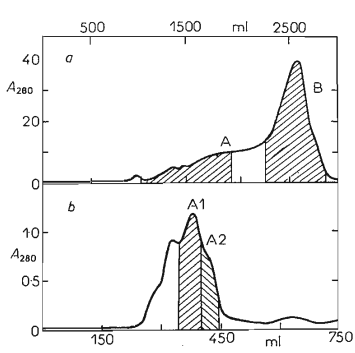


FIG. 2

Fractionation of Tryptic Digest of S-sulfonated and Maleylated Fragment CB3(Cys)

The areas marked by hatching were pooled for further treatment. *a* First fractionation of the digest on a column of Sephadex G-75. The sample in 112 ml of 0·1M-NH_4HCO_3 was fractionated on a column (5·0 × 130 cm) eluted by 0·05M-NH_4HCO_3. Fractions of 22 ml were collected at 20 min intervals. *b* Additional separation of fraction A on Sephadex G-50. The sample in 15 ml of 0·02M-NH_4OH was fractionated on a column (2·8 × 145 cm) eluted by 0·05M-NH_4HCO_3. Fractions of 10 ml were collected at 20 min intervals.

RESULTS AND DISCUSSION

The first fractionation of the chymotryptic digest of S-sulfonated fragment CB3(Cys) was effected by gel filtration on a column of Sephadex G-25 and afforded four fractions. The first of these fractions, containing large peptides, was chromatographed on a column of Dowex 1; the subsequent treatment of the material contained in the effluent as well as the fractionation of the remaining three fractions were carried out by techniques of paper chromatography and electrophoresis. A total of 24 peptides were isolated. Their amino acid composition is given in Table I and the results of sequential analysis of the individual peptides are marked by horizontal bars in Fig. 4. In parallel experiments the tryptic digest of the maleylated S-sulfo derivative of fragment CB3(Cys) was also analyzed. The fractionation of this digest on Sephadex G-75 (Fig. 2*a*) afforded fraction A, containing large peptides, and fraction B composed of small peptides. Fraction A was separated further on Sephadex G-50 (Fig. 2*b*) and the fractions obtained were subjected to additional purification on columns of DEAE-Sephadex A-25 and SE-Sephadex C-25 (Fig. 3*a*,*b*,*c*); these

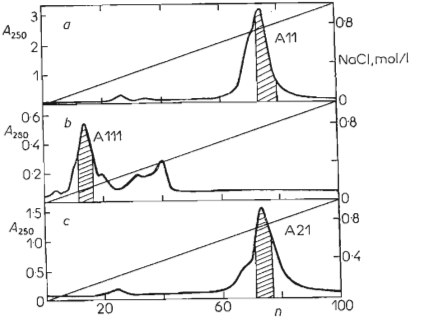


FIG. 3

Chromatography of Fractions A1 and A2 (Fig. 2*b*) on Sephadex Ion Exchangers

a Chromatography of fraction A1. A DEAE-Sephadex A-25 column (2·7 × 40 cm) was eluted by a linear gradient of 0—1·0M-NaCl in 0·05M Tris-acetate buffer (pH 7·5), total volume 2000 ml. Fractions (n) of 20 ml were collected at 15 min intervals.

b Chromatography of demaleylated fraction A11 on SE-Sephadex C-25. The column (2·2 × × 19 cm) was eluted by a linear gradient of 0—1M-NaCl in 0·005M sodium formate buffer (pH 3·0), total volume 500 ml. The buffer contained 8M urea. Fractions (*n*) of 5 ml were collected at 10 ml intervals. *c* Chromatography of fraction A2 (Fig. 2*b*) on a DEAE-Sephadex A-25 column. The same conditions of separation as in the case of fraction A1 were used.

procedures gave peptides MT3 (35 amino acid residues) and MT4 (45 amino acid residues). Peptides MT1 and MT2 were isolated from fraction B (Fig. 2*a*) by paper chromatography and electrophoresis. The amino acid composition of these peptides is given in Table II together with the composition of products obtained by tryptic digestion of demaleylated MT-peptides. The N-terminal amino acids of peptides MT1 to MT4, were determined and the partial structures of these peptides are given in Fig. 4.

A part of the experimental section of this paper deals also with the preparation of peptides used for the reinvestigation of the positions of residues No 44, 59–63, and 73–75 which fall into cyanogen bromide CB1(Asp) (ref.[5]). The numbers of the residues denote their positions in the molecule of albumin[1]. The following peptides were isolated from the tryptic digest of S-sulfonated fragment CB1(Ala): T6, $Lys_{1.0}$, $Asp_{1.0}$, $Thr_{1.0}$, $Glu_{1.9}$, $Ala_{0.9}$, $Val_{1.8}$, $Leu_{0.9}$, $Phe_{1.0}$; T 7, $Lys_{1.0}$, $Cys_{2.0}$, $Asp_{2.9}$,

TABLE I

Amino Acid Composition of Cyanogen Bromide Fragment CB3(Cys) and of Peptides (C1–C24) Isolated from Chymotryptic Digest of S-sulfo Derivative of Fragment CB3(Cys)

The values are not corrected, tryptophan was determined only qualitatively.

Amino acid	Fragment CB3(Cys)		Number of residues in peptide							
	number of residues[a]	found in sequence[b]	C1	C2	C3	C4	C5	C6	C7	C8
Lysine	18·4	19		2·3			1·1	1·0	1·5	2·3
Histidine	4·5	5	0·8		0·9					
Arginine	8·7	9			2·0		1·0			0·9
Cysteic acid	10·7	12							3·1	
Aspartic acid	13·9	14[c]	2·0						1·0	2·2
Threonine	6·1	6	1·0						1·0	
Serine	8·4	9								1·0
Glutamic acid	23·8	24[c]	2·0		1·0	1·0			2·1	2·1
Proline	5·4	5			1·0	1·1				1·1
Glycine	3·2	3								1·0
Alanine	23·0	23			1·0	1·0	0·8	2·0	4·0	1·0
Valine	5·2	5								
Isoleucine	3·9	4			0·9					
Leucine	19·4	19		1·0		1·9			0·8	2·8
Tyrosine	6·3	6		0·9	0·8	0·6	0·8			
Phenylalanine	10·7	10	0·7			1·0		1·0		
Homoserine	1·0	1								
Tryptophan	+	1								

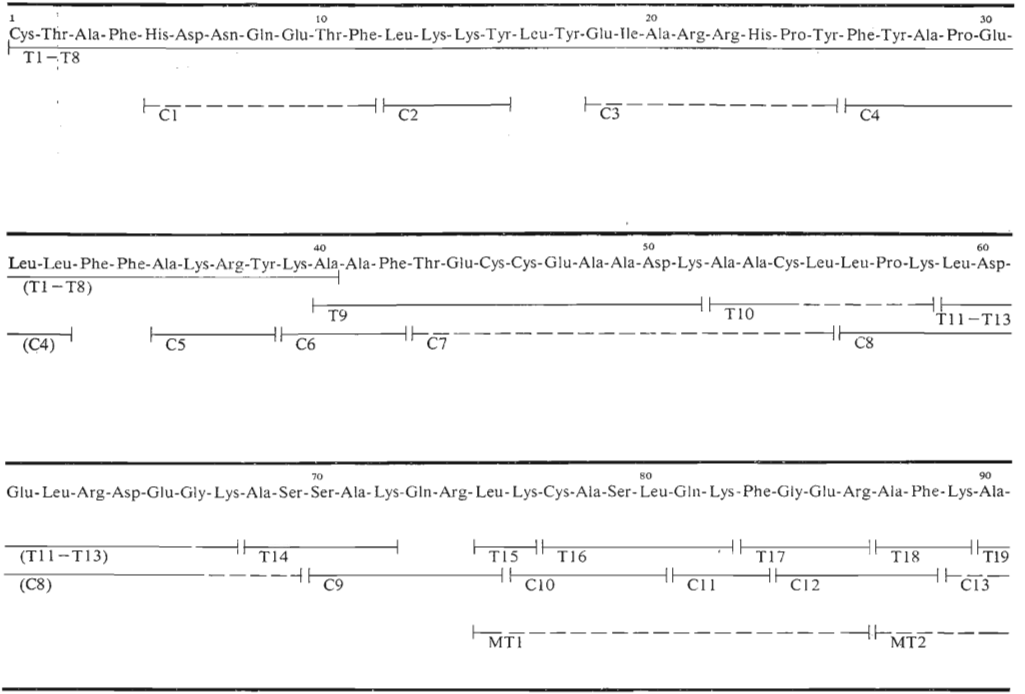
[a] For the recalculation of the analysis (ref.[15]) alanine was taken to represent 23·0 residues;

$Thr_{0.9}$, $Ser_{0.9}$, $Glu_{2.0}$, $Ala_{1.9}$, $Val_{0.9}$; T (8 + 9), $Lys_{1.1}$, $His_{1.0}$, $Arg_{1.0}$, $Cys_{1.0}$, $Asp_{1.0}$, $Thr_{2.8}$, $Ser_{1.0}$, $Gly_{1.0}$, $Ala_{0.9}$, $Val_{1.0}$, $Leu_{3.8}$, $Phe_{0.9}$. The peptides designated according to paper ref.[5] were sequenced by the phenylisothiocyanate method. In peptide T 6 (residues No 42–51): Leu-Val-Asn-Glu(Val,Thr,Glu,Phe,Ala,Lys) the third residue, originally reported[5] as aspartic acid was found to be an asparagine residue. Peptide T 7 (residues No 52–64): Thr-Cys-Val-Ala-Asp-Glu-Ser-Ala-Glu-Asn-Cys--Asp-Lys permits us to correct the residues in positions No 59–63, originally reported[5] as Asp-Ala-Gln-Cys-Asn. Peptide T (8 + 9) (residues No 65–81): Ser-Leu--His-Thr-Leu-Phe-Gly-Asp-Lys-Leu-Cys(Thr,Val,Ala,Thr,Leu,Arg) determines the sequence of residues No 73–75, reported earlier[5] as Leu-Cys-Lys. These changes have been included in the paper[1] reporting the complete amino acid sequence of human serum albumin.

TABLE II
(*Continued*)

Number of residues in peptide															
C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
1·2	0·9	0·8		1·3		1·1	1·1		1·0	1·1		1·1	1·0	2·0	1·3
									1·6	1·4					1·0
0·9			1·0		1·0	1·0				0·9	1·0				
	0·8								1·9	2·7	1·0		1·0	1·9	1·2
								1·0	2·0	3·9	3·0		2·2		2·2
									2·7	1·6					
0·8	0·9					0·7	0·9	0·9					2·7		1·0
1·0		1·0	1·0			1·6	1·1		1·1	2·2	1·0		2·1	2·2	4·0
						0·8								0·6	
			0·9						1·0	0·8					
1·2	1·0		1·0	1·0	1·8	1·0	1·1			1·7	2·0	1·0			1·2
					0·9		1·0	0·9	1·7	1·0					0·9
													1·8		0·9
0·9	1·0				1·0		1·2	1·2	3·0	3·0	1·0		1·0	0·8	1·2
												0·8			
		1·0	1·0			1·6									0·7

[b] shown in Fig. 4; [c] sum of acid and amide form.


1
Cys-Thr-Ala-Phe-His-Asp-Asn-Gln-Glu-Thr-Phe-Leu-Lys-Lys-Tyr-Leu-Tyr-Glu-Ile-Ala-Arg-Arg-His-Pro-Tyr-Phe-Tyr-Ala-Pro-Glu-
10
20
30
T1 – T8
C1
C2
C3
C4
Leu-Leu-Phe-Phe-Ala-Lys-Arg-Tyr-Lys-Ala-Ala-Phe-Thr-Glu-Cys-Cys-Glu-Ala-Ala-Asp-Lys-Ala-Ala-Cys-Leu-Leu-Pro-Lys-Leu-Asp-
40
50
60
(T1 – T8)
T9
T10
T11 – T13
(C4)
C5
C6
C7
C8
Glu-Leu-Arg-Asp-Glu-Gly-Lys-Ala-Ser-Ser-Ala-Lys-Gln-Arg-Leu-Lys-Cys-Ala-Ser-Leu-Gln-Lys-Phe-Gly-Glu-Arg-Ala-Phe-Lys-Ala-
70
80
90
(T11 – T13)
T14
T15
T16
T17
T18
T19
(C8)
C9
C10
C11
C12
C13
MT1
MT2

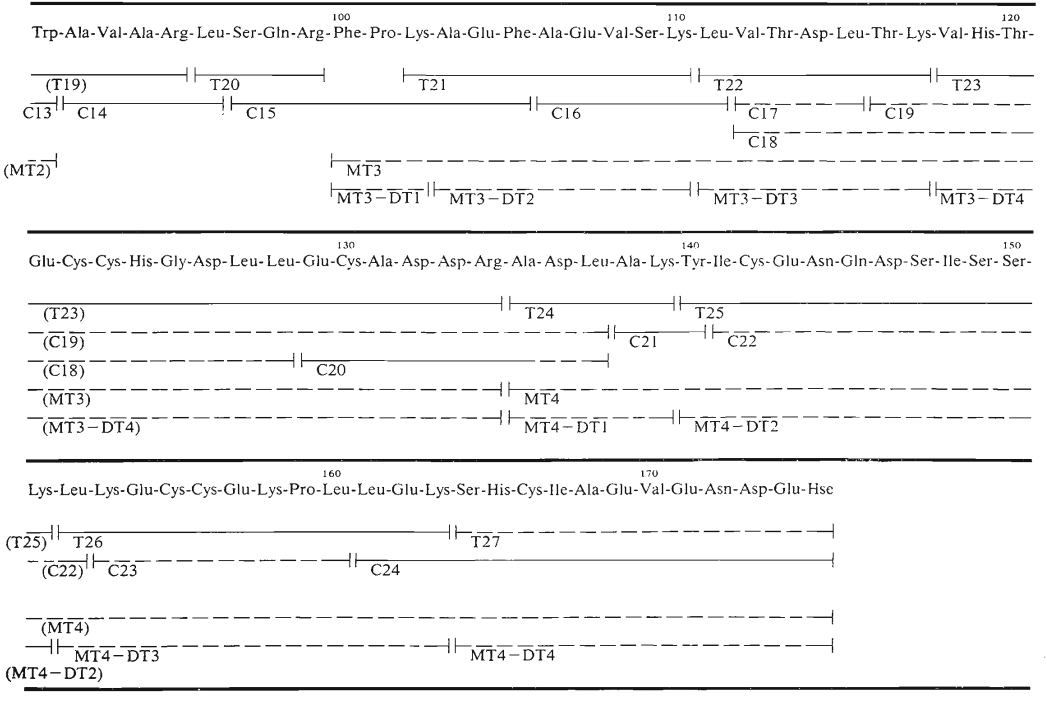

100
110
120
Trp-Ala-Val-Ala-Arg-Leu-Ser-Gln-Arg-Phe-Pro-Lys-Ala-Glu-Phe-Ala-Glu-Val-Ser-Lys-Leu-Val-Thr-Asp-Leu-Thr-Lys-Val-His-Thr-
(T19)
T20
T21
T22
T23
C13
C14
C15
C16
C17
C19
C18
(MT2)
MT3
MT3-DT1
MT3-DT2
MT3-DT3
MT3-DT4
130
140
150
Glu-Cys-Cys-His-Gly-Asp-Leu-Leu-Glu-Cys-Ala-Asp-Asp-Arg-Ala-Asp-Leu-Ala-Lys-Tyr-Ile-Cys-Glu-Asn-Gln-Asp-Ser-Ile-Ser-Ser-
(T23)
T24
T25
(C19)
C21
C22
(C18)
C20
(MT3)
MT4
(MT3-DT4)
MT4-DT1
MT4-DT2
160
170
Lys-Leu-Lys-Glu-Cys-Cys-Glu-Lys-Pro-Leu-Leu-Glu-Lys-Ser-His-Cys-Ile-Ala-Glu-Val-Glu-Asn-Asp-Glu-Hse
(T25)
T26
T27
(C22)
C23
C24
(MT4)
MT4-DT3
MT4-DT4
(MT4-DT2)

TABLE II

Amino Acid Composition of Peptides (MT1 – MT4) Isolated from Tryptic Digest of Maleylated Cyanogen Bromide Fragment CB3(Cys) and of Peptides Resulting from Tryptic Digestion of Demaleylated Peptides MT3 and MT4

The values are not corrected, tryptophan was determined only qualitatively.

Amino acid	Number of residues in peptide				
	MT1	MT2	MT3	MT3–DT1	MT3–DT2
Lysine	1·9	1·0	3·1	1·0	1·0
Histidine			1·7		
Arginine	1·0		1·0		
Cysteic acid	1·0		2·6		
Aspartic acid			4·0		
Threonine			2·7		
Serine	0·9		1·0		0·9
Glutamic acid	2·0		4·1		1·9
Proline			0·8	1·1	
Glycine	1·0		1·0		
Alanine	1·1	2·0	3·2		1·9
Valine			2·8		1·0
Isoleucine					
Leucine	1·9		4·0		
Tyrosine					
Phenylalanine	1·0	1·2	1·7	1·0	1·0
Homoserine					
Tryptophan		+			

All peptides isolated in this study are given in Fig. 4 which also shows how the amino acid sequence of fragment CB3(Cys) has been derived. The sequential data obtained permit us to divide this part of the molecule into three continuous, well defined regions. The N-terminal region (residues No 1 – 75) has been sequentially determined in the study on the tryptic digest[4] of fragment CB3(Cys) up to residue No 40 in the form of a continuous sequence T (1 – 8); chymotryptic peptides C1 – C5

FIG. 4

Amino Acid Sequence of Cyanogen Bromide Fragment CB3(Cys)

The sequential regions determined earlier[4] are marked by symbol T. The peptides isolated in this study are marked C, peptides from the tryptic digest of the maleyl derivative are marked MT. Peptides resulting from additional tryptic cleavage of the demaleylated MT peptides are designated MT-DT. Full lines denote sequences determined completely, unsequenced regions are marked by dashed lines.

TABLE II
(*Continued*)

Number of residues in peptide						
MT3–DT3	MT3–DT4	MT4	MT4–DT1	MT4–DT2	MT4–DT3	MT4–DT4
1·0		4·8	1·0	1·1	3·0	
	1·7	0·9				0·8
	0·9					
	2·8	3·9		1·0	2·1	1·1
1·0	3·0	5·0	1·0	2·2		2·2
1·7	1·0					
		3·9		2·4		1·1
	2·0	8·0		2·1	3·1	3·2
		1·2			1·1	
	1·0					
	1·0	2·6	1·9			1·0
1·0	0·9	1·3				0·9
		2·5		1·7		1·0
1·9	1·9	3·9	1·0		3·0	
		0·7		0·2		
		0·9				0·9

can be aligned with this sequence. The amino acid sequence of peptides T9–T14 and C6–C9 extends this region up to residue No 75. The structure of the middle region (residues No 75–96) of fragment CB3(Cys) follows from overlaps of tryptic and chymotryptic peptides T15–T19 and C10–C14. Peptide MT1 supports the correctness of allocation of peptides T15 and C10 and of the one-residue overlap. Since peptide MT1 was isolated from the tryptic digest of the maleyl derivative we can expect that tryptic digestion had attacked an arginine bond preceding peptide MT1. Peptide MT2 contains the only tryptophan residue present in albumin; the isolation of this peptide from maleylated material extends the overlap given by the relatively short peptides T18 and C13. The presence of the tryptophan residue at the C-terminus points to residual chymotryptic activity of the trypsin preparation used. The C-terminal part (residues No 96–175) of fragment CB3(Cys) consists of three regions: A (residues No 96–111, peptides T20–T21 and C15–C16), B (residues No 111–152, peptides T22–T25 and C17–C22), and C (residues No 152–175,

peptides T26–T27 and C23–C24), linked together by two one-residue overlaps at positions No 111 and 152. The order of region A, B, and C emerged from amino acid analysis of maleylated peptides MT3 and MT4. Peptide MT3 covers the region between residues No 100 and 134, its terminal amino acids (Phe and Arg) determine the order of regions A and B. The homoserine-containing fragment MT4 involves the region between residues No 135 and 175 at the C-terminus of the fragment and provides the link between regions B and C. The amino acid sequence of fragment CB3(Cys) proposed here contains two one-residue overlaps (leucines) at positions No 75 and 96. The correctness of this sequence is supported by the agreement in the number of amino acid residues contained in the sequence and the number determined by amino acid analysis of the fragment[15].

The sequence of 175 amino acid residues of fragment CB3(Cys) determined by us is shown in Fig 4; it extends between residues No 124 and 298 of human serum albumin[1]. This part of the albumin molecule has been determined also by Behrens and coworkers[16] in their sequence studies on human serum albumin. Likewise Gambhir and coworkers[17,18] investigating the location of the indole binding site sequentially determined a 107-residue N-terminal region which falls into cyanogen bromide fragment CB3(Cys). Certain differences will emerge if our data are compared with the results of these authors:

Residue, No[a]	7	8	34	36	37	44	47	54	63	64
This study	Asn	Gln	Phe	Lys	Arg	Glu	Glu	Cys	Arg	Asp
Behrens and coworkers[16]	Asp	Glu	—[b]	Thr	Lys	Glx	Glx	Cys	Arg	Asp
Gambhir and coworkers[18]	Asn	Gln	Phe	Lys	Arg	Glu	Gln	Ser	Asp	Gly[c]

Residue, No[a]	76	98	143	144	145	146	147	156	158	159	172
This study	Lys	Gln	Glu	Asn	Gln	Asp	Ser	Cys	Lys	Pro	Asn
Behrens and coworkers[16]	Arg	Glu	Glx	Asx	Glx	Asx	Thr	Lys	Pro	Cys	Asx

[a] The residues are numbered according to this study. [b] This residue is missing in sequence[16]. [c] An additional comparison with this sequence would require the insertion of 4 residues at the carboxyl side of residue No 64.

This paper concludes the series of papers from this Laboratory on the determination of the amino acid sequence and disulfide bonds[19] of human serum albumin.

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