CHARACTERIZATION OF PEPTIDE CHAINS RESULTING FROM INTERRUPTION OF DISULFIDE BONDS IN CYANOGEN BROMIDE FRAGMENTS OF HUMAN PLASMA ALBUMIN

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The cyanogen bromide hydrolysis of human plasma albumin with intact disulfide bonds afforded three fragments. By the interruption of the disulfide bonds of these fragments seven chains were obtained which were characterized by the determination of their N-terminal amino acid sequences as follows. Chain I-Asp : Asp-Ala-His, II-Ala : Ala-Asp-Cys-Cys-Ala-, III-Cys : Cys-Thr-Ala-Phe-Ala-, IV-Phe : Phe-Leu-Tyr-Glu-Tyr-, V-Pro : Pro-Cys-Ala-Glu-Asp-Tyr-, VI-Pro : Pro-Ala-Asp-Leu-Pro-, VII-Asp : Asp-Asp-Phe-Ala-Ala-. C-Terminal peptides containing homoserine were isolated from the individual chains. Chain I-Asp contains the only free SH-group of the albumin whereas the only tryptophan residue is present in chain III-Cys. The obtained results have made it possible to propose a scheme of the human plasma albumin molecule at the level of cyanogen bromide chains: this scheme, containing also the partial structure of certain regions, was compared with an analogous scheme of the bovine plasma albumin molecule.

During the past few years the structure of plasma albumins has been studied. After the cyanogen bromide fragments of bovine plasma albumin[1] had been isolated, papers reporting on the primary structure of bovine and porcine albumin[2] appeared. In the preceding study[3], we isolated from the cyanogen bromide hydrolysate of human plasma albumin with intact disulfide bonds two fragments, designated N and C and determined their amino acid composition. Fragment C has four N-terminal amino acids (Phe, Pro, Pro, and Asp) and consists of four chains linked together by disulfide bonds. Fragment N has three N-terminal amino acids (Asp, Ala, and Cys). The relatively large elution volume of fragment N on gel chromatography indicated that one of the chains is not linked covalently by disulfide bonds.

We have shown in the present study that only two chains (Ala and Asp) of original fragment N are linked by disulfide bonds. This fragment will be referred to as N_1. The other fragment (Cys) with intrachain disulfide bonds is designated N_2. The designation of the third fragment remains unaltered. After the interruption of the disulfide bonds of these fragments, the individual chains were isolated and their amino acid composition and N-terminal amino-acid sequences of several residues were determined. Homoserine peptides, characterizing the C-terminal regions of the chains, were isolated from the tryptic digests of the oxidized chains. The results of this study made is posible to determine the differences in the position of methionine residues of human and bovine plasma albumin and to outline partly the homologous

regions in both proteins. While this study was in its final stage, the fragmentation of human plasma albumin by cyanogen bromide was reported[4]. The authors isolated the same number of chains as we did. Both studies differ in methodical approach and in certain analytical data. Our study provides moreover certain additional observations.

EXPERIMENTAL

Human plasma albumin was a product of Imuna, Šarišské Michaľany. Fragments N and C were isolated from the cyanogen bromide hydrolysate of mono S-(β-aminoethyl)cysteinyl derivative of albumin[3] with intact disulfide bonds. Trypsin free of chymotryptic activity was prepared from crystalline trypsin[5] by the reaction with L(1-tosylamido-2-phenyl)ethyl chloromethyl ketone[6]. The solutions of the fragments were concentrated in an Amicon Ultrafiltration Cell (Amicon N.,V., The Hague, Holland), equipped with a UM-10 filter. The oxidative cleavage of the chains leading to the chains was effected by performic acid[7].

The peptide maps of tryptic digests of oxidized chains were prepared by cleavage of the chains (0·15 μmol) by trypsin at a molar enzyme to substrate ratio of 1 : 50, 16h at 23°C. The digests were separated in the first direction by descending electrophoresis[8] in pyridine–acetate buffer at pH 5·6, in the second direction by descending chromatography in the system butanol–pyridine–acetic acid–water (15 : 10 : 3 : 12). Arginine-containing peptides were detected on these maps by the Sakaguchi reaction using 8-hydroxyquinoline and hypobromite. For certain maps high-voltage electrophoresis[9] at pH 1·9 was also used.

Detection of homoserine-containing peptides. The oxidized chains (1 μmol) dissolved in 0·05M-NH_4HCO_3 to a 1% solution were digested by trypsin at a molar enzyme to substrate ratio of 1 : 50, 16 h at 23°C. The digests were lyophilized and separated by electrophoresis[8] at pH 5·6. The separation of the mixture of peptides was checked by the staining of marginal strips 8 and 20 mm wide with ninhydrin. The position of the peptides was corroborated on a narrower marginal strip by the chlorination test[10]; the peptides from the wider strip stained with ninhydrin were eluted by the buffer used for electrophoresis. After the buffer had been dried off, the peptides were hydrolyzed 16 h at 105°C. Hydrochloric acid was removed by the usual technique. The digests of the peptides were separated by descending electrophoresis[8]. After the hydrolysis of homoserine-containing peptides, homoserine lactone[11] yields on the electropherograms a spot stained yellow with ninhydrin and showing a considerably higher mobility than arginine and lysine. The yellow stain turns blue after some time.

The amino-acid analyses were performed by the method of Spackman and coworkers[12] on 20- and 70-h hydrolysates of the samples. Homoserine lactone contained in the hydrolysates of peptides was converted into homoserine by the reaction with piperidine[13]. The sequential analysis was performed by the phenylthiohydantoin technique combined with chromatographic identification of PTH-amino acids on a thin layer of silica gel[14,15]. Alternatively the dansyl technique[1] combined with the identification of amino acid derivatives on polyamide layer sheets[17] was used.

Cleavage of Disulfide Bonds of Fragment N and Isolation of Arising Chains

Reduction of fragment N *and carboxymethylation*[18] *of chains.* Lyophilized fragment N (560 mg) and urea (19·7 g) were dissolved in 17 ml of water and 84 mg of ethylenediaminetetraacetic acid in 3 ml of water was added. The solution was made alkaline by the addition of 0·95 g of 2-amino-(hydroxymethyl)-1,3-propanediol; 3·35 ml of β-mercaptoethanol was added during the dissolving

TABLE I

Amino-Acid Composition of Human Plasma Albumin and of Products of Its Cyanogen Bromide Hydrolysis

Chains I—VII, total number of amino acids in chains I—VII, fragments N_1, N_2, and C, unhydrolyzed plasma albumin PA. The values are average values from three analyses, the number of residues is given. Amide groups are not included.

Amino acids	I-Asp	II-Ala	III-Cys	IV-Phe	V-Pro	VI-Pro	VII-Asp	I-VII	N_1	N_2	C[a]	PA[a]
Lys	6·9 7	2·0 2	17·1 17	9·9 10	11·8 12	3·0 3	4·9 5	56	9·0 9	16·9 17	27·2 27	54
His	3·8 4	0·9 1	4·2 4	2·6 3	2·8 3	— —	— —	15	4·3 4	3·8 4	6·0 6	14
Arg	2·1 2	2·9 3	8·1 8	4·7 5	4·0 4	— —	— —	22	4·5 5	8·3 8	10·2 10	23
Asp	8·7 9	7·0 7	13·0 13	6·7 7	7·9 8	4·8 5	4·3 4	53	17·0 17	13·2 13	25·8 26	54
Thr	5·8 6	— —	5·7 6	5·1 5	9·1 9	— —	1·0 1	27	5·9 6	5·7 6	14·5 15	28
Ser	3·1 3	— —	7·8 8	3·1 3	5·1 5	1·8 2	1·2 1	22	3·1 3	7·8 8	11·1 11	22
Glu	13·0 13	5·9 6	22·1 22	13·0 13	16·8 17	2·5 2	5·1 5	78	21·2 21	22·9 23	40·0 40	80
Pro	1·7 2	3·7 4	5·0 5	4·8 5	4·8 5	1·8 2	— —	23	4·6 5	4·8 5	14·0 14	26
Gly	3·0 3	— —	3·0 3	1·9 2	— —	1·1 1	2·0 2	11	3·2 3	2·9 3	6·1 6	13
Ala	8·8 9	2·1 2	21·4 21	7·0 7	7·8 8	5·1 5	8·0 8	60	11·4 11	21·0 21	29·2 29	61
Cys[b]	3·2 3	2·7 3	10·0 10	4·9 5	6·5 7	1·2 1	2·9 3	32	6·0 6	10·0 10	17·0 17	35
Val	6·8 7	2·8 3	4·9 5	7·6 8	11·0 11	3·0 3	2·3 2	39	10·2 10	4·8 5	22·0 22	40
Met	— —	— —	— —	— —	— —	— —	— —	—	— —	— —	— —	6
Ile	0·9 1	— —	3·6 4	0·9 1	2·0 2	— —	— —	8	0·9 1	3·4 4	3·0 3	8
Leu	9·2 9	3·2 3	18·1 18	11·0 11	11·1 11	3·1 3	3·3 3	58	11·7 12	17·4 18	30·0 30	61
Tyr	1·8 2	— —	5·9 6	4·9 5	1·9 2	0·8 1	— —	16	1·6 2	5·8 6	9·6 10	18
Phe	5·7 6	1·1 1	10·0 10	4·0 4	4·6 5	2·0 2	3·0 3	31	6·8 7	9·0 9	14·8 15	31
AE-Cys[c]	0·7 1	— —	— —	— —	— —	— —	— —	1	0·7 1	— —	— —	—
Hse	1·0 1	1·0 1	0·9 1	0·9 1	1·1 1	1·0 1	— —	6	2·0 2	1·0 1	3·0 3	—
Trp[d]	— —	— —	— 1	— —	— —	— —	— —	1	— —	— 1	— —	1
Sum	88	36	162	95	110	31	37	559	125	162	284	575

[a]Analytical values from the preceding study; [b]determined as cysteic acid or S-(β-carboxymethyl)cysteine, [c]aminoethylcysteine, [d]tryptophan was determined in the chains qualitatively.

of the product. The pH of the reaction mixture was kept at the value 8·6—8·8 for 4 h at 23°C under a nitrogen barrier. To the solution of the reduced protein were added in turns a solution of iodoacetate (prepared from 9·25 g of iodoacetic acid dissolved in 6·45 ml of 5M-NaOH) and additional 5M-NaOH to maintain the pH at 8·6. The reaction was allowed to proceed at +5°C. The completeness of the substitution was judged by the negative reaction for free SH-groups using the nitroprusside solution. The mixture of the carboxymethylated chains was freed of salts by gel filtration on a Sephadex G-25 column equilibrated with 0·02M-NH_4OH. The protein solution was lyophilized. The yield was 550 mg of S-carboxymethylated chains of fragment N.

Maleylation of carboxymethylated chains. The lyophilized material (250 mg) was dissolved in 25 ml of 5M guanidine hydrochloride. The protein solution was treated in turns with finely ground maleic anhydride (750 mg) and solid sodium carbonate[19,20] with stirring. The maleylation was allowed to proceed 10 min at pH 8—9. An equal volume of 1M hydroxylamine hydrochloride at pH 9·0 was then added to the reaction mixture. The mixture was set aside for 10 min at room temperature to remove the maleyl residues bound to the hydroxyl groups. The chains were desalted on a Sephadex G-25 column equilibrated with 0·02M NH_4OH. The yield of the protein material after lyophilization was 255 mg. To isolate the individual chains, a part of the modified chains of fragment N (25 mg) was dissolved in 2 ml of 0·1M-NH_4HCO_3 and fractionated on a column of Sephadex G-100 (Fig. 1). The yield was 7 mg of chain I-Asp, 4 mg of chain II-Ala, and 12 mg of chain III-Cys. Their amino-acid composition is given in Table I.

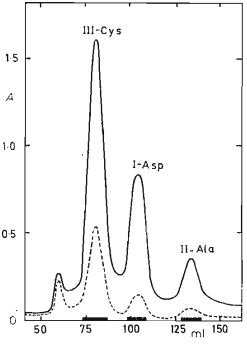

FIG. 1

Fractionation of Mixture of Carboxymethylated and Maleylated Chains of Fragment N on Sephadex G-100 Column in 0·1M-NH_4HCO_3

Column 1 × 250 cm. Flow rate 12 ml/h, ——— absorbance at 250 nm, — — — absorbance at 280 nm. Chains I-Asp II-Ala, and III-Cys.

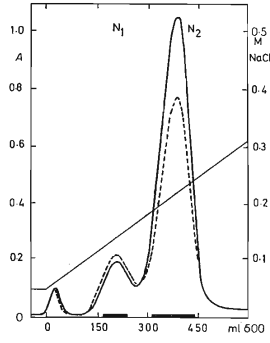

FIG. 2

Chromatography of Fragment N with Intact Disulfide Bonds on SE-Sephadex C-25 Column

Column 4 × 7 cm. Flow rate 46 ml/h. Eluted by 1400 ml of 0·01M acetate buffer, 8M in urea, pH 5·0, with linear gradient of 0·05—0·65M-NaCl. ——— absorbance at 280 nm, — — — absorbance at 260 nm. Fragments N_1 and N_2.

Demalеylation of chains. Substituted chain I-Asp (1 μmol) was dissolved in 1 ml of 80% formic acid and set aside for 72 h at 37°C. The solution was made alkaline to pH 10 and then desalted in 0·01M-NH_4OH. Substituted chains II-Ala and III-Cys (1 μmol) were dissolved in 0.1M potassium formate[21], 6M in guanidine hydrochloride, pH 3·5, to a 1% protein solution. The mixture was set aside for 72 h at 37°C. After this period an equal volume of concentrated formic acid was added and the mixture was allowed to stand 5 min at 23°C. The desalting was carried out on a Sephadex G-25 column equilibrated with 0·2% formic acid. The protein solution was lyophilized and used for the N-terminal analysis of the individual chains.

Chromatography of Fragment N on SE-Sephadex C-25

The solution of fragment N prepared from 1 g of plasma albumin[3] was diluted with the same volume of water and applied on a SE-Sephadex C-25 column equilibrated in 0·01M sodium acetate[22] containing 0·05M-NaCl at pH 5·0. The column was then washed with 120 ml of the equilibrating buffer and with an equal volume of the equilibrating buffer containing 8M urea.

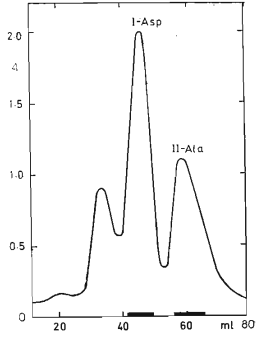

FIG. 3

Gel Filtration of Oxidized Fragment N_1 on Sephadex G-50 Column in 0·1M-NH_4OH

Column 1·6 × 100 cm. Flow rate 16 ml/h. ——— absorbance at 230 nm. Chains I-Asp and II-Ala.

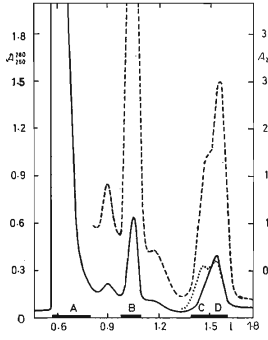

FIG. 4

Fractionation on Sephadex G-75 Column of Mixture of Peptide Chains Resulting from Oxidation of Fragment C of Human Plasma Albumin

Column 3·9 × 190 cm. Eluted by 0·1M-NH_4HCO_3. Flow rate 42 ml/h. Volume of effluent in l. ——— absorbance at 280 nm; ... absorbance at 260 nm, — — — absorbance at 230 nm. Fractions A, C, and D were purified by chromatography on QAE — Sephadex A-25 (Fig. 5, 7, and 8), fraction B by chromatography on SE-Sephadex C-25 (Fig. 6).

The separation of the components was achieved by a concentration gradient of NaCl and 0·01M-sodium acetate in 8M urea (Fig. 2). After desalting and lyophilization, 54 mg of fragment N_1 and 125 mg of fragment N_2 was obtained.

Oxidation of fragment N_1. The sample (30 mg) was oxidized by performic acid[7]. The volume of the reaction mixture was concentrated from the original 2 ml to 0·5 ml and to this solution 2 ml of water was added. Performic acid was reduced by a few drops of saturated solution of sodium sulfite. When the pH of the solution was adjusted to pH 10 by ammonium hydroxide, a gel had formed which was dissolved by the addition of a small volume of water. The reaction mixture was fractionated on a Sephadex G-50 column (200 ml) in 0·1M-NH_4OH (Fig. 3). The yield was 12 mg of chain I-Asp and 8·5 mg of chain II-Ala. The oxidation of fragment N_2 was carried out by a procedure analogous to that employed for fragment N_1. The material gave only one peak when chromatographed on the Sephadex G-50 column.

Cleavage of Disulfide Bonds of Fragment C and Fractionation of Arising Chains

Lyophilized fragment C (1 254 mg) was oxidized by performic acid[7]. After the oxidation had been completed, the solution was concentrated to 25 ml in the rotary evaporator, diluted with 75 ml of water, and lyophilized. The residue was dissolved in 2 ml of 5M guanidine hydrochloride and the solution diluted with 25 ml of 0·1M-NH_4HCO_3. The pH of the solution was adjusted to 9 by ammonium hydroxide. The fractionation of the mixture of oxidized chains was effected on

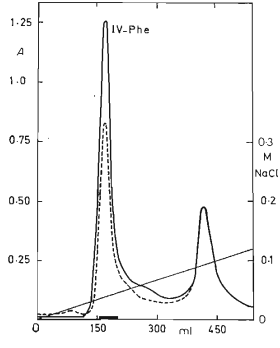

FIG. 5

Isolation of Chain IV-Phe from Fraction A (Fig. 4) on QAE-Sephadex A-25 Column

Column 3 × 10 cm. Eluted by 900 ml of 0·01M sodium acetate, 8M in urea, pH 5, with linear gradient of 0—0·2M-NaCl, 23°C. Flow rate 60 ml/h, ——— absorbance at 280 nm, — — — absorbance at 260 nm.

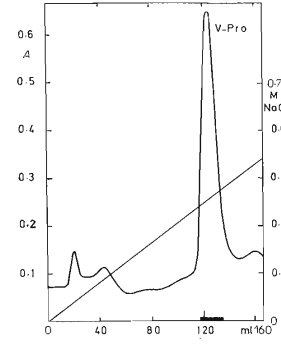

FIG. 6

Isolation of Chain V-Pro from Fraction B (Fig. 4) on SE-Sephadex C-25 Column

Column 1·4 × 20 cm. Eluted by 180 ml of 0·005M ammonium formate, 8M in urea, pH 3, with linear gradient of 0—0·6M-NaCl, 23°C. Flow rate 15 ml/h. Absorbance at 280 nm.

a Sephadex G-75 column equilibrated with 0·1M-NH_4HCO_3. Fractions A through D (Fig. 4) were lyophilized and purified further by chromatography on SE-Sephadex and QAE-Sephadex. The amino-acid composition of the chains is given in Table I.

Isolation of chains. Chain IV-Phe: A part of fraction A (300 mg) was dissolved in 10 ml of 0·01M ammonium acetate, 8M in urea[22], the solution was diluted with 10 ml of water and with 50 ml of buffer containing 8M urea. The pH of the solution was adjusted to 9·5 by ammonium hydroxide and fractionated on a QAE Sephadex A-25 column (Fig. 5). The main fraction was desalted on a Sephadex G-25 column equilibrated with 0·02M-NH_4OH. The yield of lyophilized chain IV-Phe was 75·1 mg. Chain V-Pro: A part of fraction B (25 mg) was dissolved in 2 ml of 0·005M ammonium acetate, 8M in urea, pH 3, and fractionated on a SE-Sephadex C-25 column (Fig. 6). The main fraction was subsequently desalted on a 1·6 × 50 cm column of Sephadex G-25 equilibrated with 0·02M-NH_4OH and lyophilized. The yield was 22·7 mg of chain V-Pro. Chain VI-Pro: A part of fraction D (30 mg) was dissolved in 3 ml of 0·01M sodium acetate at pH 5·0 and the pH of the solution was adjusted to 8 by NH_4OH. The fraction was purified on a column of QAE-Sephadex A-25 (Fig. 7). The main fraction was desalted on a column of Sephadex G-10 equilibrated with 0·02M-NH_4OH, lyophilized, and separated on Sephadex G-25 in the same solvent. The yield of lyophilized chain VI-Pro was 16·2 mg. Chain VII-Asp: A part of fraction C (30 mg) was fractionated by chromatography on a QAE-Sephadex A-25 column (Fig. 8). The main fraction was desalted on Sephadex G-25 equilibrated with 0·02M-NH_4OH. The yield was 14·6 mg of chain VII-Asp.

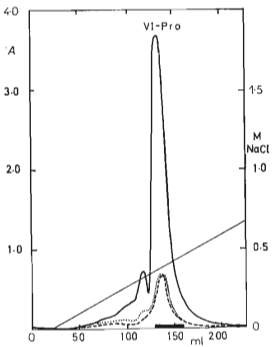

FIG. 7

Isolation of Chain VI-Pro from Fraction D (Fig. 4) on QAE-Sephadex A-25 Column

Column 1·4 × 20 cm. Eluted by 300 ml of 0·01M sodium acetate, pH 5·0, with linear gradient of 0—1M-NaCl, at 23°C. Flow rate 20 ml/h. ——— absorbance at 230 nm, . . . absorbance at 260 nm, — — — absorbance at 280 nm.

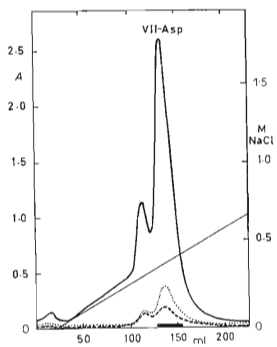

FIG. 8

Isolation of Chain VII-Asp from Fraction C (Fig. 4) on QAE-Sephadex A-25 Column

Column 1·4 × 20 cm. Eluted by 300 ml of 0·01M sodium acetate, pH 5, with linear gradient of 0—1M-NaCl, 23°C. Flow rate 21 ml/h. —— absorbance at 230 nm, ····· absorbance at 260 nm, - - - - absorbance at 280 nm.

RESULTS AND DISCUSSION

The specific hydrolysis of human plasma albumin by cyanogen bromide at the methionine residues has given rise to seven peptide fragments designated in this study I – VII. When the albumin with intact disulfide bonds is hydrolyzed, larger fragments[1–3] are formed in which the individual chains are linked together by cystine residues.

The disulfide bonds of fragment N of human plasma albumin were reduced by β-mercaptoethanol and the arising chains with free SH-groups were substituted by iodoacetic acid. Attempts to fractionate this mixture of chains by gel filtration were unsuccessful since the chains remained in aggregated state even in 8M urea. Therefore the carboxymethylated chains were maleylated. The obtained material was fractionated on a column of Sephadex G-100, equilibrated with 0·1M-NH_4HCO_3 (Fig. 1). Chains I-Asp, II-Ala, and III-Cys were selectively separated. The maleylation of the lysine residues, which eliminates polar interactions and decreases the tendency to aggregate, makes easier also the identification of the chains by the measurement of absorbance at 250 nm. Some of the chains, especially chain II-Ala,

TABLE II

Amino Acid Composition of Homoserine Peptides Isolated from Tryptic Digests of Oxidized Chains I–VI

The number of residues is given.

Amino acids	Homoserine peptides isolated from chains											
	I		II		III		IV		V		VI	
Lysine	—	—	—	—	—	—	—	—	—	—	—	—
Histidine	—	—	—	—	0·8	1	—	—	—	—	—	—
Arginine	—	—	0·8	1	—	—	0·9	1	—	—	—	—
Cysteic acid	—	—	—	—	1·0	1	—	—	—	—	—	—
Aspartic acid	—	—	0·8	1	2·2	2	—	—	—	—	1·1	1
Threonine	1·0	1	—	—	—	—	—	—	—	—	—	—
Serine	—	—	—	—	0·8	1	—	—	—	—	—	—
Glutamic acid	2·1	2	1·0	1	3·1	3	—	—	—	—	—	—
Proline	—	—	1·0	1	—	—	—	—	—	—	—	—
Glycine	1·0	1	—	—	—	—	—	—	—	—	1·0	1
Alanine	—	—	—	—	1·0	1	—	—	0·8	1	—	—
Valine	—	—	2·7	3	1·0	1	—	—	0·9	1	1·0	1
Isoleucine	—	—	—	—	1·0	1	—	—	—	—	—	—
Leucine	—	—	0·9	1	—	—	—	—	—	—	1·0	1
Tyrosine	0·8	1	—	—	—	—	—	—	—	—	—	—
Phenylalanine	—	—	—	—	—	—	—	—	—	—	0·8	1
Homoserine	1·0	1	1·0	1	1·1	1	1·0	1	1·0	1	1·0	1

show a very low absorbance at 280 nm. An advantage of this procedure is the relatively quick preparation of all three chains in one column operation. The behavior of the individual chains during demaleylation is different. The demaleylation of chain I-Asp proceeds best in 80% formid acid; under other conditions most of the material remains insoluble in gel form. After the demaleylation, the reaction mixture must be made alkaline before the desalting since chain I-Asp is more soluble in alkaline media. The demaleylation of chain II-Ala and III-Cys proceeds easily.

TABLE III

Amino Acid Sequences of N-Terminal and C-Terminal Regions of Chains I—VII
S Number of amino acids in chains.

Chain	S	N-terminal region and C-terminal region
I-Asp	88	Asp-Ala-His-.....-Glx-Thr-Tyr-Gly-Glx-Hse
II-Ala	36	Ala-Asp-Cys-Cys-Ala-..... -Leu-Val-Arg-Pro(Val_2,Glx,Asx)Hse
III-Cys	162	Cys-Thr-Ala-Phe-Ala-.....-Ser-His-Cys-Ile(Asx_2,Glx_3,Ala,Val)Hse
IV-Phe	95	Phe-Leu-Tyr-Glu-Tyr-..... -Arg-Hse
V-Pro	110	Pro-Cys-Ala-Glu-Asp-Tyr-..... -Ala-Val-Hse
VI-Pro	31	Pro-Ala-Asp-Leu-Pro-.....-Asp-Val-Phe-Leu-Gly-Hse
VII-Asp	37	Asp-Asp-Phe-Aja-Ala-.....[a]

[a] The chain does not contain homoserine.

The chromatography of fragment N with intact disulfide bonds on SE-Sephadex C-25 (Fig. 2) afforded components N_1 and N_2 and thus experimental proof was obtained of the assumption[3] that one of the chains of fragment is not covalently bound to the remaining two chains. The N-terminal end group analysis of the material from peak N_1 revealed two groups, Asp and Ala. The material from peak N_2 shows the presence of only one N-terminal end group, Cys, and thus represents an individual chain, III-Cys. On gel filtration on Sephadex G-75 fragments N_1 and N_2 emerged in one peak[3], designated N. Fragments N_1 and N_2 do not differ markedly in their molecular weights. From fragment N_1 chains I-Asp and II-Ala can be obtained after oxidation and chromatography on the Sephadex G-50 column (Fig. 3). The strong tendency to aggregate of the chain terminated with aspartic acid is the reason for the existence of a relatively high peak emerging at the beginning of the elution profile, and decreases the yield of the monomer of chain I-Asp. The amino-acid composition of the chains is given in Table I, together with the amino-acid composition of the fragments.

The fractionation of the oxidized chains of fragment C on the Sephadex G-75 column (Fig. 4) was satisfactory and the material was resolved into fractions A through D. The eluting buffer used, 0·1M-NH_4HCO_3, enabled us to measure the absorbance even at 230 nm; this was important especially for the detection of smaller chains, present in fractions C and D, whose absorbance at 280 nm is relatively low. The greatest difficulties caused us the further purification of fraction A. When this material was chromatographed on QAE-Sephadex in buffers containing 8M urea (Fig. 5), chain IV-Phe was isolated. The second peak contains material showing a higher aspartic acid content. The peptide maps of its tryptic digest indicate the presence of chain III-Cys, V-Pro, and of a small quantity of original, incompletely hydrolyzed plasma albumin. Chain IV-Phe is present in aggregated form under the described experimental conditions (Fig. 4). Chain IV-Pro was isolated in pure form from fraction B by chromatography in buffers containing 8M urea. The remaining two chains, IV-Pro and VII-Asp appear on gel chromatography in fraction C and D (Fig. 4) and are separated only incompletely. They can easily be isolated from these fractions by chromatography on QAE-Sephadex A-25 without using urea. The amino-acid composition of chains I–VII (Table I) was based on the presence of one homoserine residue and on the number of peptides observed on peptide maps of the tryptic digests of oxidized chains. The relation between the number of amino acids in the chains, fragments, and in the original plasma albumin is in good agreement within experimental error of the methods used.

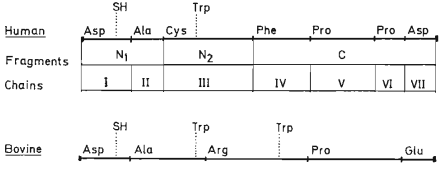

FIG. 9

Comparison of Human Plasma Albumin and Bovine Plasma Albumin N-Terminal Amino Acids after BrCN Cleavage

From the tryptic digests of the individual chains homoserine peptides, representing the C-terminal regions of the chains, were isolated by a combination of chromatographic and electrophoretic methods. The amino-acid composition of the homoserine peptides is given in Table II. The amino-acid sequences of the N-terminal and C-terminal regions of the isolated chains, given in Table III, were determined by a combina-

tion of the phenylthiohydantoin technique with the dansyl method. Six chains C-terminated with homoserine and one chain lacking homoserine and representing the C-terminal region of plasma albumin were isolated.

Concluding this study we compared the location of methionine residues in human plasma albumin and in bovine plasma albumin[1]. The length of the peptide regions and the N-terminal amino acids appearing after the hydrolysis of plasma albumin by cyanogen bromide are shown in Fig. 9. The positions of the tryptophan residues and the free SH-group of the albumins are also shown. The presented order of chains has not been confirmed in full experimentally. Chain I-Asp has the same N-terminal amino-acid sequence as human plasma albumin[3,23] and thus characterizes the N-terminus of the whole molecule of human plasma albumin. Chain I-Asp contains one residue of AE-cysteine and thus characterizes the site of the original free SH-group. An analogous location of SH-groups has been observed also with bovine plasma albumin[1]. The only tryptophan residue of human plasma albumin is present in chain III-Cys and is characterized by the amino-acid sequence Ala-Trp-Ala-Val-Ala-Arg. A peptide of this composition has been isolated from the tryptic digest of aminoethylated human plasma albumin[24]. Its composition is identical with the amino-acid sequence reported by Swaney and Klotz[25], yet it does not agree with the amino-acid sequence described by Sugae and Jirgensons[26].

It can be thus concluded that the hydrolysis of human plasma albumin by cyanogen bromide gives rise to seven peptide chains I–VII, which represent regions composed of 88, 36, 162, 95, 110, 31 and 37 amino-acid residues, respectively. These chains were isolated and characterized by the analysis of N- and C-terminal regions and thus a basis for the determination of the exact order of cyanogen bromide fragments of human plasma albumin was established.

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