CLEAVAGE OF HUMAN PLASMA ALBUMIN BY CYANOGEN BROMIDE AND CHARACTERIZATION OF THE FRAGMENTS

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After cyanogen bromide hydrolysis of the mono-S(β-aminoethyl)cysteinyl derivative of human plasma albumin two fragments with intact disulfide bonds, containing 287 and 284 amino acid residues, respectively, were isolated. The total number of amino acids in the fragments corresponds to the amino-acid composition of human plasma albumin. The first fragment consists of three peptide chains with N-terminal aspartic acid, alanine and half-cystine. One of these chains is probable not covalently bound to the remaining ones. This fragment contains also the only tryptophan residue of human plasma albumin. The second fragment is composed of four covalently bound chains with N-terminal aspartic acid, phenylalanine, and proline (two residues).

The microheterogeneity of plasma albumin has been reported in papers[1-4] which, however, do not show whether this microheterogeneity is caused by the variability of the primary structure of the protein. As regards human plasma albumin, only the composition of some smaller fragments has been determined[5-7] while selective cleavage of the molecule to a small number of defined fragments has not been carried out so far. We performed therefore the fractionation of the cyanogen bromide digest of human plasma albumin. The results were compared with the data on cyanogen bromide fragments of bovine plasma albumin[8].

EXPERIMENTAL

Human plasma albumin was a product of Imuna, Šarišské Michaľany, ČSSR. The amino-acid analyses were carried out by the method of Spackman and coworkers[9] with 20 and 70 h hydrolysates of the samples.

Hydrolysates containing homoserine lactone were rotary evaporated, dissolved in a few drops of water, again rotary evapotared to dryness, and then dissolved in 1 ml of 0·1M piperidine[10]. The solutions were kept 1 h at 37°C, taken to dryness, dissolved in 0·2M citrate buffer and applied to the column of the analyzer. The separation of the amino acids was carried out at 48°C until homoserine emerged, then the temperature was raised to 53°C and the analysis was completed. The content of tryptophan was determined after the reaction of the protein with 2-hydroxy-5-nitrobenzylbromide[11].

Other methods. The molar extinction coefficients of the fragments were calculated from their amino-acid compositions using the data of Edelhoch[12]. For quantitative N-terminal end group analysis the cyanate method was employed[13]. The qualitative determination of N-terminal end groups was effected by the phenylthiohydantoin[14,15] and dansyl techniques[16,17]. The mobility of human plasma albumin and of its fragments was examined by gel electrophoresis[18,19].

The preparation of the monomer[18] from commercial preparation of albumin was effected by gel filtration on a column of Sephadex G-150, equilibrated with 0·1M-Tris-HCl and 0·2M-NaCl buffer, pH 8·1, as proposed by Pedersen[20]. Protein-containing fractions were dialyzed against water and lyophilized.

Preparation of half-aminoethyl plasma albumin. Lyophilized human plasma albumin (1 200 mg, 17 μmol) was dissolved in 11 ml of water. To the sample 73 μl of ethylene imine was added (a 50-fold molar excess per —SH group) and the alkalinity of the sample was kept at pH 8·6 ± 0·1 for 20 min by the addition of 1M-HCl. An aliquot of the solution was withdrawn for amino acid analysis and the volume of the sample was made up to 17 ml with water.

Cyanogen bromide hydrolysis of half-aminoethyl plasma albumin. The solution of substituted human plasma albumin was mixed with 51 ml of 99% HCOOH in which 0·55 g of cyanogen bromide had been dissolved. The hydrolysis was allowed to proceed 22 h at 22°C. The solution was concentrated to a volume of approximately 20 ml in the rotary evaporator. The fractionation of the cyanogen bromide hydrolysate was effected on a column of Sephadex G-75 equilibrated with formate buffer[8]. Fractions indicated in Fig. 1 were rechromatographed under identical conditions. The samples were desalted on a column of Sephadex G-25 equilibrated with 0·2M-HCOOH.

RESULTS AND DISCUSSION

The homogeneity of the isolated monomer of human plasma albumin was established by the phenylthiohydantoin method which showed the presence of the N-terminal amino-acid sequence Asp-Ala. Only traces of the dimer in addition to the main zone of the monomer were observed when the sample was subjected to disc electrophoresis.

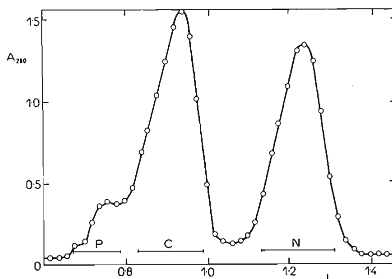

FIG. 1

Gel Filtration of Cyanogen Bromide Hydrolysate (15·5 μmol) of Half-Aminoethyl Human Plasma Albumin on Sephadex G-75

Column dimensions 3·9 × 190 cm. Eluted by 0·174M formic acid and 0·026M ammonium formate at pH 2·86. Flow rate 40 ml per hour. Volume of effluent in liters.

The sulfhydryl group[21,22] of human plasma albumin was converted into the mixed S-(β-aminoethyl)cysteinyl disulfide by the reaction with ethylene imine. In the hydrolysate of half-aminoethyl human plasma albumin (Table I), 0·7 residue of S-(β-aminoethyl)cysteine was found. The number of methionine residues in human plasma albumin has been reported[17,23] to be four to six residues. The value of methionine found in half-aminoethyl human plasma albumin indicates the presence of six methionine residues, in accordance with the results of the analysis of the unfractionated cyanogen bromide hydrolysate in which the same number of homoserine residues

TABLE I

Amino Acid Composition of Human Plasma Albumin and its Fragments

Half-aminoethyl human plasma albumin AE-HPA, its cyanogen bromide hydrolysate AE-HPA(BrCN), and isolated fragments N and C. Average values of three analyses, numbers of residues are given. Amide groups are not included.

Amino acid	Determined				Best integers		
	AE-HPA	AE-HPA(BrCN)	N	C	HPA	N	C
Lysine	53·9	55·3	25·9	27·2	54	26	27
Histidine	13·8	14·4	9·0	6·0	14	9	6
Arginine	22·2	22·7	13·3	10·2	23	13	10
Aspartic acid	53·9	54·0	29·2	25·8	54	29	26
Threonine	27·9	27·6	11·6	14·5	28	12	15
Serine	21·8	22·1	12·1	11·1	22	12	11
Glutamic acid	79·5	77·2	41·0	40·0	80	41	40
Proline	25·8	25·8	12·4	14·0	26	12	14
Glycine	12·6	12·6	6·9	6·1	13	7	6
Alanine	61·2	61·4	32·1	29·2	61	32	29
Half-cystine[a]	30·9	32·8	15·9	17·0	35	16	17
Valine	39·6	39·8	14·6	22·0	40	15	22
Methionine	5·7	—	—	—	6	—	—
Isoleucine	7·4	7·7	4·4	3·0	8	5	3
Leucine	61·3	61·0	28·9	30·0	61	29	30
Tyrosine	17·6	17·9	7·8	9·5	18	8	10
Phenylalanine	30·9	30·9	16·3	14·8	31	16	15
Tryptophan[b]	1·0	N.D.[c]	0·8	—	1	1	—
Homoserine	—	5·7	3·0	3·0	—	3	3
Aminoethyl-cysteine	0·7	0·7	0·3	—	—	1	—
Total					*575*	*287*	*284*
Mol. weight[d]					65 100	32 400	32 100

[a] In S-carboxymethyl-cysteinyl human plasma albumin 35·2 residues of S-carboxymethyl-cysteine were found. [b] Independent determination. [c] N.D. not determined. [d] The molecular weights were calculated from the determined compositions.

was found. From the cyanogen bromide hydrolysate of half-aminoethyl human plasma albumin, fragments N and C were isolated in a yield of 65 and 62%, respectively (Fig. 1) after rechromatography on the column of Sephadex G-75, in addition to a small quantity of the high molecular weight amount (P). We have not determined experimentally as yet which one of these two fragments represents the N-terminal region of human plasma albumin. Fragment N shows the presence of three N-terminal amino acids, aspartic acid (0·7 μmol), alanine (1·0 μmol) and half-cystine (quantitatively not determined). Four N-terminal end-groups, aspartic acid (1·0 μmol), proline (1·7 μmol, 2 residues), and phenylalanine (0·7 μmol), were found in fragment C. The same N-terminal end groups were found in the unfractionated cyanogen bromide hydrolysate. The number of homoserine residues (Table I) is also in agreement with the number of N-terminal end groups. The tryptophan residue is localized in the N fragment together with the S-(β-aminoethyl)cysteine residue. The results obtained by amino-acid analysis of half-aminoethyl human plasma albumin, and of the isolated fragments were correlated. The probable number of residues in fragments N and C, together with the amino acid composition of the whole molecule of human plasma albumin shown in Table I, will be determined more exactly after individual peptide chains of the two fragments have been analyzed.

The elution volumes of fragments C and N correspond to molecular weights of 40000 ± ± 4000 and 20000 ± 2000, respectively. The values calculated from the amino acid composition are 32100 for fragment C and 32400 for fragment N. Greater differences in the found values of molecular weight of fragment N could be explained by the assumption that one of its chains is not covalently bound to the remaining ones. This would result in a shift of its elution volume toward volumes corresponding to lower molecular weights. The found values of molar extinction coefficients, *i.e.* 14900 for fragment N and 13900 for fragment C, are roughly in agreement with the values 16900 and 13700 respectively, calculated from the amino-acid composition of the fragments.

The corresponding fragments N of human plasma albumin (this paper) and of bovine plasma albumin[8], isolated from the cyanogen bromide hydrolysates of the proteins, have identical N-terminal amino acids, alanine and aspartic acid, but in human plasma albumin one half-cystine residue more was found. Fragment C isolated from human plasma albumin has one N-terminal aspartic acid residue, one phenylalanine and two prolines, compared to arginine, proline, and glutamic acid which are the N-terminal-amino acid residues of fragment C of bovine plasma albumin[8].

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