SEQUENCE STUDIES CONCERNING HUMAN SERUM TRANSFERRIN: THE PRIMARY STRUCTURE OF TWO CYANOGEN BROMIDE FRAGMENTS

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1. Introduction

In the course of our studies devoted to human serum transferrin, we prepared and purified several cyanogen bromide fragments. The present paper deals with the establishment of the sequence of two of these fragments.

2. Materials and methods

Human serum transferrin was obtained from Cohn's fraction IV following the procedure of Roberts et al. [1]. Trypsin (EC 3.4.4.4) and chymotrypsin (EC 3.4.4.5) were purchased from Worthington. All reagents (analytical grade) were obtained from Merck or Prolabo except those employed for the Sequencer which were purchased from Socosi (94100 St. Maur, France), 4-sulfophenylisothiocyanate from Pierce and dithiothreitol from Calbiochem. Sephadex G-75 (fine) was obtained from Pharmacia.

Preparation of the cyanogen bromide fragments. Human serum transferrin was submitted to the action of cyanogen bromide according to Steers et al. [2]. The fragments were purified on Sephadex G-75 following Jeppson's procedure [3].

Preparation and purification of the tryptic digest of peptide CN-C. 20 mg (3.3 μM) of the peptide were digested during 22 hr. with 0.66 mg trypsin at 37°C in 0.1 M NH_4HCO_3. Trypsin was pretreated during 16 hr with 0.0625 M HCl at 37°C. The tryptic peptides were obtained by preparative paper electrophoresis (Whatman No. 1) at pH 6.5 (pyridine–water–acetic acid, 100:900:4, v/v/v) and 50 V/cm and when necessary, purified by preparative descending paper chromatography (Whatman No. 1; *n*-butanol–pyridine–acetic acid–water, 15:10:3:12, v/v/v/v).

Chymotryptic digestion of peptide CN-C. 6 mg (1 μM) of peptide were digested with 0.2 mg chymotrypsin at 37°C in 0.1 M NH_4HCO_3 during 16 hr. The purification of the chymotryptic peptides was achieved as indicated above for the tryptic peptides.

The amino acid compositions of the peptides were established with a Technicon Autoanalyzer after total hydrolysis (6 M HCl; 18 hr; under vacuum).

Determination of the structure of the peptides. Automated Edman degradation was carried out in a Socosi Sequencer, Model PS-100, by the quadrol double-cleavage method [4] for peptide CN-C and by the dimethylallylamine simple-cleavage method [5] for the tryptic split peptides. Dithiothreitol was added in 1-chlorobutane (30 mg/liter) [6] in order to avoid the degradation of thiazolinones. The lysine containing peptides were modified with 4-sulfophenylisothiocyanate according to Braunitzer et al. [7]; the N-terminal amino acid was identified on an aliquot before modification by the dansylation procedure. The thiazolinones were converted into PTH-amino acids which were identified by thin-layer chromatography, gas–liquid chromatography and chromatography on an amino

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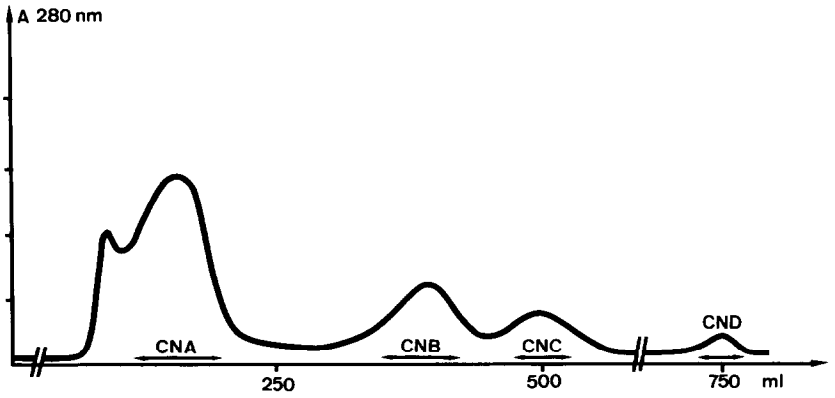


Fig. 1. Filtration of a CNBr-human serum transferrin mixture (250 mg) on Sephadex G-75 (130 × 3 cm) in 0.1 M formic acid.

acid Autoanalyzer after regeneration of the free amino acid as previously indicated [8].

3. Results

3.1. *Methionine content of human serum transferrin*

A methionine content of 7.8 residues/mole was established by usual analytical techniques with an Autoanalyzer.

3.2. *Separation of the cyanogen bromide fragments*

4 fractions (CN-A, CN-B, CN-C and CN-D) were obtained by filtration on Sephadex G-75 (fig. 1). Similar results were obtained by Jeppson [3] and Sutton and Brew [9] with one exception, concerning fraction CN-D, which was not described by these authors. Fraction CN-C was constituted by a single chain as only one N-terminal amino acid, Gly, was characterized (yield = 90%). Its sequence is described below. Fraction CN-D was further purified by paper electrophoresis; two methionine-containing peptides were characterized, peptide CN-D1 (m = + 0.6 at pH 6.5; yield 35%; see section 3.3.1) and peptide CN-D2 (m = 0 at pH 6.5; yield 30%): its structure is under investigation.

3.3. *Sequence determinations*

3.3.1. Peptide CN-D1

Its amino acid composition was: Asp (1.00); Ala (1.00), HSer (0.80), Lys (0.90). The sequence of this tetrapeptide was established by classical methods: Asn–Ala–Lys–HSer.

3.3.2. Fragment CN-C

(a) The N-terminal sequence of peptide CN-C. Automated Edman degradation permitted the establishment of the N-terminal sequence up to the 32nd amino acid (Table 3).

(b) Study of the tryptic digest of peptide CN-C. 7 main peptides (T1–T4; T5a, T5b; T6) were characterized. In table 1, the amino acid compositions, yields, R_f and mobility (m) values at pH 6.5 of all the tryptic peptides are indicated; these latter account for all the amino acids present in peptide CN-C. The structures of peptides T2, T3, T4 and T5b were established by automated Edman degradation. Peptide T6 as well as the two dipeptides T1 and T5a were included in the long N-terminal sequence of peptide CN-C. Peptide T6 contained two lysine residues; the presence of two acidic residues prevented the tryptic split after the first lysine residue.

(c) Study of the chymotryptic digest of peptide CN-C. 4 main chymotryptic peptides (C1-C4) containing basic amino acids and thus constituting overlaps between tryptic peptides were studied in detail. Table 2 indicates their R_f and mobility (m) values as well as their yields, amino acid compositions and N-terminal residues.

(d) Alignment of the tryptic peptides. Automated Edman degradation of peptide CN-C allowed the alignment of tryptic peptides T6 → T5a → T1 → T4; peptide C2 is in accordance with this sequence. The chymotryptic peptides C4, C3 and C1 allowed to join tryptic peptides T4 → T5b; T5b → T3; T3 → T2, respectively. The complete structure of peptide CN-C with the different overlaps is indicated in Table 3.

Table 1
Amino acid composition of the tryptic peptides of peptide CN-C

Amino acid	T 1	T 2	T 3	T 4	T 5a	T 5b	T 6	Total	CN-C
Asp			1.00 (1)		1.00 (1)	0.88 (1)	2.17 (2)	5	5
Ser	0.92 (1)		1.00 (1)	1.55 (2)				4	4
Glu				1.45 (2)			4.32 (5)	7	7
Pro		2.00 (2)		0.77 (1)				3	3
Gly			1.05 (1)	1.07 (1)			2.75 (3)	5	5
Ala			0.93 (1)				1.07 (1)	2	2
Val		1.00 (1)						1	1
HSer		0.70 (1)						1	1
Ile							0.85 (1)	1	1
Leu			1.00 (1)	0.89 (1)		1.73 (2)	2.67 (3)	7	7
Phe			0.95 (1)	1.45 (2)		1.00 (1)	1.08 (1)	5	5
Trp*							+	1	1
Lys	1.00 (1)		1.06 (1)	1.00 (1)	1.08 (1)	1.07 (1)	2.00 (2)	7	7
His			0.76 (1)	0.78 (1)			0.72 (1)	3	3
Arg		0.85 (1)						1	1
TOTAL	2	5	8	11	2	5	20	53	53
yield %	35	40	26	37	30	46	28		
R_f	0.19	0.47	0.38	0.45	0.12	0.70			
m**	+ 0.75	+ 0.45	+ 0.22	+ 0.12	0	0	− 0.10		
N-terminal amino acid	Ser	Val	Asp	Glu	Asp	Asp	Gly		Gly

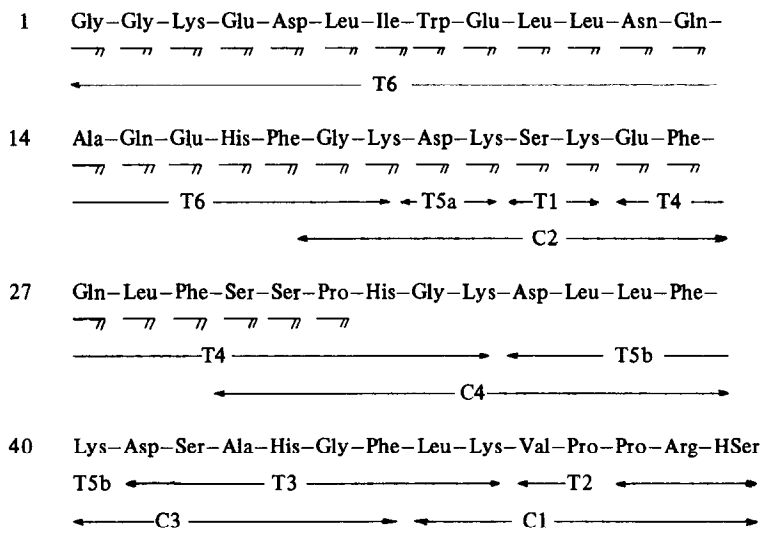
Residue number in parentheses, nearest integer based on hydrolysis for 18 hr.
* Characterized by the Ehrlich reagent.
** Mobility m at pH 6.5; m = + 1 for Arg; m = 0 for Gly; m = −1 for $CySO_3H$.

Table 2
Study of 4 main chymotryptic peptides of peptide CN-C, overlaps between tryptic split peptides

Amino acid	C 1		C 2		C 3		C 4	
Asp			0.80	(1)	1.00	(1)	0.95	(1)
Ser			0.81	(1)	0.95	(1)	1.45	(2)
Glu			0.96	(1)				
Pro	1.52	(2)					1.04	(1)
Gly			0.94	(1)	1.08	(1)	1.05	(1)
Ala					1.05	(1)		
Val	0.68	(1)						
HSer	0.45	(1)						
Leu	0.70	(1)					1.87	(2)
Phe			0.80	(1)	0.81	(1)	0.90	(1)
Lys	1.10	(1)	3.00	(3)	1.00	(1)	1.00	(1)
His					0.76	(1)	0.78	(1)
Arg	0.80	(1)						
Total	7		8		7		10	
Yield %	47		60		36		14	
R_f	0.53		0.12		0.22		0.53	
m at pH 6.5	+ 0.50		+ 0.27		+ 0.17		+ 0.17	
N-terminal amino acid	Leu		Gly		Lys		Ser	

Residue number in parentheses, nearest integer based on hydrolysis for 18 hr.

Table 3
Primary structure of peptide CN-C



—n = Amino acid residues determined by automated Edman degradation directly on peptide CN-C.

4. Conclusion

The presence of eight methionine residues in human serum transferrin was established suggesting the presence of 9 cyanogen bromide fragments. Sutton and Brew [9] described recently the isolation of 7 fragments; our results are in accordance with these data and complete them as an eighth fragment could be sequenced; the last and ninth fragment was also isolated and its structure is under investigation.

Acknowledgements

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