THE COVALENT STRUCTURE OF COLLAGEN: THE TRYPTIC, THERMOLYTIC AND CHYMOTRYPTIC PEPTIDES OF α1-CB3 FROM CALF SKIN COLLAGEN

Peter WENDT*, Peter P. FIETZEK and Klaus KÜHN
Max-Planck-Institut für Biochemie, Abteilung Kühn, 8033 Martinsried bei München, Germany

Received 10 July 1972

1. Introduction

As part of our investigations on the amino acid sequence of collagen we have recently published the sequence of the 217 residues of the C-terminal peptide α1-CB6, derived from cyanogen bromide cleavage of the α1-chain of calf skin collagen [1–4]. We now report about the peptide α1-CB3. This peptide comprises 149 residues and is derived from the central area of the α1-chain where it occupies positions 421 through 570 of the 1055 residues of the entire chain. Previous information on α1-CB3 was sparse and restricted to the peptide from rat skin collagen. Thus, Butler [5] had succeeded in locating one of the hydroxylamine sensitive bonds in α1-CB3. At the same time, he isolated the C-terminal and the lysine containing tryptic peptides and elucidated the sequences of positions 1–5 and 118–123.

In the present communication we describe the isolation of trypsin-, chymotrypsin-, and thermolysin-derived peptides of α1-CB3. The entire α1-CB3 [6] as well as α1(125)-CBN, a peptide comprising only the 56 C-terminal residues of αl-CB3 [7] served as starting materials. Sequence analysis was to be accomplished by automated stepwise degradation according to Edman and Begg [8]. This method preferentially requires long, overlapping peptides, therefore, obviating the isolation of the complete set of all tryptic and thermolytic peptides. The sequence work itself will be described in a following communication [9].

* Present address: Institut für Experimentelle Chirurgie an der Chirurgischen Klinik der Technischen Universität, 8 München 80, Ismaninger Str. 22, Germany.

2. Experimental

The peptide α1-CB3 was isolated as described by Rauterberg and Kuhn [6], α1(125)-CBN according to v.d. Mark et al. [7].

Treatment of α1-CB3 and α1(125)-CBN with trypsin, thermolysin and chymotrypsin resembled that described by Fietzek et al. [4]. The ratio of collagen to enzyme was 50:1 on a weight basis in all cases. The substrates were incubated at 37° with trypsin for 4 hr, with thermolysin for 6 hr and with chymotrypsin for 1 hr.

Molecular sieve chromatography of the degradation peptides was accomplished on Sephadex G-50 superfine (conditions see fig. 1). Ion exchange chromatography on Aminex A-6, spherical beads (BioRad Laboratories) followed the procedure of Wendt et al. [3].

For ion exchange chromatography on phosphocellulose (Whatman, P11, fibrous form) 30 mg of the peptide mixture were dissolved in 5 ml distilled water and applied to a column, 1.0 × 8.5 cm, maintained at 40°. The chromatogram was developed with a linear gradient of 0–0.5 M NaCl in 0.001 M sodium acetate in a total volume of 500 ml. The rate of elution was 180 ml per hour.

3. Results and discussion

3.1. *Isolation of the tryptic (T) peptides*

Fig. 1a depicts the separation on Sephadex G-50s of the tryptic peptides of α1(125)-CBN. For clarity, the peptides are designated already here according to

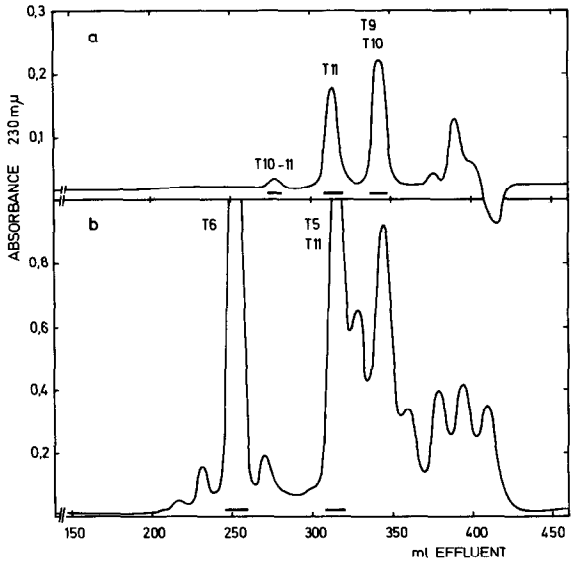

Fig. 1. Molecular sieve chromatography on Sephadex G-50s of the tryptic peptides from α1(125)-CBC (1a) and from α1-CB3 (1b). Approx. 50 mg of the digest, dissolved in 2 ml were applied to the column, 2 × 140 cm, and eluted at 35° with 0.03 M sodium acetate pH 4.8 at a rate of 20 ml per hr. The bars indicate the fractions collected.

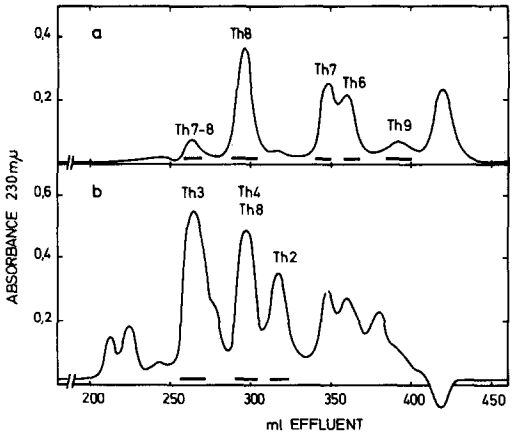

Fig. 2. Molecular sieve chromatography on Sephadex G-50s of the thermolysin derived peptides from α1 (125)-CBN (2a) and from α1-CB3 (2b). Elution conditions as in fig. 1. The bars indicate the fractions collected.

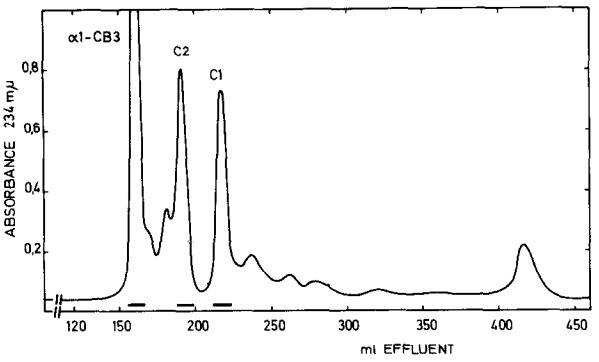

Fig. 3. Molecular sieve chromatography on Sephadex G-50s of the chymotryptic peptides of α1-CB3. Elution conditions as in fig. 1. The bars indicate the fractions collected.

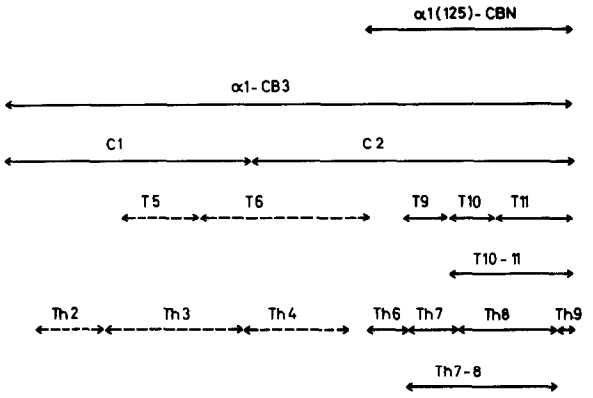

Fig. 4. Order of some of the peptides derived by tryptic-, thermolytic- and chymotryptic cleavage of α1(125)-CBN and α1-CB3. Peptides represented by dashed lines could only be assigned to their respective positions after sequencing of the corresponding regions of α1-CB3 [9].

their later determined position within the polypeptide chain of α1-CB3. The peptides contained in the first three peaks are indicated. The peptides T9 and T10 which were eluted together were separated by chromatography on Aminex A-6. The last two peaks were not investigated. The elution pattern on Sephadex G-50s of the tryptic peptides of the entire α1-CB3 is depicted in fig. 1b. The peptides T6, T5 and T11 were isolated. T5 and T11 which eluted in a common peak were separated by rechromatography on phosphocellulose. The amino acid analyses of the isolated tryptic peptides are compiled in table 1.

Table 1
Comparison of amino acid composition of the tryptic peptides from α1-CB3, determined by amino acid analysis, A, and by sequence analysis, SA [9].

	T5 33–51*		T6 52–96*		T9 106–117*		T10 118–129*		T11 130–149*		T10–11 118–149*	
	A	SA	A	SA	A	SA	A	SA	A	SA	A	SA
4-Hydroxyproline	0.6	1	4.6	6	1.0	1	0.9	1	2.7	3	3.7	4
Aspartic acid	0.9	1	1.0	1	–	–	3.1	3	1.0	1	3.8	4
Threonine	–	–	–	–	–	–	–	–	–	–	–	–
Serine	–	–	2.0	2	–	–	–	–	1.0	1	0.8	1
Homoserine	–	–	–	–	–	–	–	–	1.5	1	1.3	1
Glutamic acid	2.8	3	7.1	6	1.0	1	–	–	2.3	2	2.3	2
Proline	3.1	3	4.8	4	3.0	3	–	–	0.3	–	0.8	–
Glycine	6.3	6	15	15	4.1	4	4.0	4	7.0	7	11	11
Alanine	4.0	4	5.0	5	1.0	1	3.0	3	4.1	4	7.0	7
Valine	–	–	0.7	1	0.8	1	–	–	–	–	–	–
Leucine	–	–	1.7	2	–	–	–	–	1.0	1	1.0	1
Phenylalanine	–	–	0.7	1	–	–	–	–	–	–	–	–
Hydroxylysine	–	–	0.1	–	–	–	–	–	–	–	0.7	–
Lysine	–	–	0.9	1	–	–	0.9	1	–	–	0.3	1
Arginine	0.8	1	1.0	1	1.0	1	–	–	–	–	–	–
Sum		19		45		12		12		20		32

* Positions along the peptide chain of α1-CB3.

3.2. *Isolation of the thermolytic (Th) peptides*

Separation of the thermolytic peptides of α1(125)-CBN is shown in fig. 2a. Each peak contained a single peptide. It can be seen from table 2 that the sum of amino acids of Th6, Th7, Th8 and Th9 corresponded to the amino acid composition of α1(125)-CBN. In addition, there appeared small amounts of the double peptide Th 7–8. Three additional thermolytic peptides (Th2, Th3 and Th4) were isolated from the digest of α1-CB3 whose chromatogram is shown in fig. 2b. Th4 which was eluted together with Th 8 was isolated by chromatography on phosphocellulose. The amino acid composition of these peptides is included in table 2.

3.3. *Isolation of the chymotryptic (C) peptides*

Only one bond in α1-CB3 is cleaved by chymotrypsin under the conditions employed. The chromatogram of the digest (fig. 3) revealed two peptides, C1 and C2, in addition to uncleaved α1-CB3.

3.4. *Order of the peptides (fig. 4)*

Comparison of the amino acid composition of the tryptic and thermolytic peptides served as a basis for determining the order of some of the peptides. The tryptic peptide T11 is C-terminal since it contains homoserine. Due to the double peptide T10–11, T10 precedes T11. The fact that T10 contains the only lysine residue of α1(125)-CBN and no arginine has implications for the position of the thermolytic peptides. Th9 occupies, judged from its homoserine, a C-terminal position. It is preceded by Th8 since this peptide contains one lysine but no arginine. The double peptide Th7–8 determines the position of Th7. Th6, therefore, must be the N-terminal peptide of α1(125)-CBN. T9, consequently, should precede T10. The order of the remaining tryptic and thermolytic peptides could only be determined by sequencing of α1-CB3 [9]. The chymotryptic peptide C2 must, on the basis of its homoserine content, be attributed to the C-terminal position.

The appearance of the double peptide T10–11 can be explained by partial hydroxylation of the C-terminal lysine residue in T10. Thus, T10 contained one lysine residue, whereas 0.7 hydroxylysine and 0.3 lysine were present in T10–11 (table 1). The molar ratio between T10 and T11 on the one hand and the double peptide T10–11 on the other hand permit the

Table 2

Comparison of amino acid composition of the thermolytic peptides from α1-CB3, determined by amino acid analysis, A, and by sequence analysis, SA [9].

	Th2 8–25*		Th3 26–61*		Th4 62–68*		Th6 95–106*		Th7 107–118*		Th8 119–145*		Th7–8 107–145*		Th9 146–149*		α1(125)-CBN 94–149*	
	A	SA	A	SA	A	SA	A	SA	A	SA	A	SA	A	SA	A	SA	A	SA
4-Hydroxyproline	2.0	3	2.5	2	3.2	4	0.9	1	1.0	1	3.2	4	4.3	5	–	–	5.0	6
Aspartic acid	–	–	2.0	1	1.4	1	–	–	–	–	4.2	4	4.0	4	–	–	4.3	4
Threonine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Serine	–	–	1.0	1	–	–	–	–	–	–	1.0	1	1.2	1	–	–	1.0	1
Homoserine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.2	1	1.2	1
Glutamic acid	2.0	2	4.1	5	4.3	4	2.1	2	1.0	1	1.0	1	2.1	2	1.0	1	4.9	5
Proline	1.5	1	4.8	5	2.4	2	0.3	–	3.0	3	0.4	–	3.5	3	–	–	4.0	3
Glycine	6.3	6	12	12	7.6	8	3.7	4	4.1	4	8.7	9	12	13	1.0	1	19	19
Alanine	3.4	3	7.4	7	2.3	2	1.1	1	1.1	1	7.0	7	8.3	8	–	–	9.1	9
Valine	0.7	1	1.1	1	0.9	1	–	–	0.8	1	–	–	0.7	1	–	–	1.0	1
Leucine	–	–	–	–	0.9	1	–	–	–	–	–	–	–	–	0.8	1	0.9	1
Phenylalanine	–	–	–	–	1.0	1	1.0	1	–	–	–	–	–	–	–	–	1.0	1
Hydroxylysine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–
Lysine	0.9	1	0.8	1	0.9	1	–	–	–	–	0.9	1	0.8	1	–	–	0.9	1
Arginine	0.9	1	1.0	1	–	–	3.0	3	1.0	1	–	–	1.0	1	–	–	4.2	4
Sum:		18		36		25		12		12		27		39		4		56

* Positions along the peptide chain of α1-CB3.

Table 3
Comparison of amino acid composition of the chymotryptic peptides from α1-CB3, determined by amino acid analysis, A, and by sequence analysis, SA [9].

	C1 1–62*		C2 63–149*		α1-CB3 1–149*	
	A	SA	A	SA	A	SA
4-Hydroxyproline	4.5	6	9.5	11	15	17
Aspartic acid	2.1	1	4.8	5	7	6
Threonine	0.5	–	0.2	–	–	–
Serine	1.2	1	2.2	2	3	3
Homoserine	–	–	–	1	1	1
Glutamic acid	6.5	7	9.1	9	16	16
Proline	8.6	7	7.6	6	14	13
Glycine	21	21	24	29	50	50
Alanine	9.0	10	12	12	22	22
Valine	2.0	2	2.1	2	4	4
Leucine	0.2	–	3.1	3	3	3
Phenylalanine	2.3	2	1.2	1	3	3
Hydroxylysine	0.2	–	0.1	–	–	–
Lysine	3.4	3	1.8	2	5	5
Arginine	2.4	2	3.9	4	6	6
Sum:		62		87		149

* Positions along the peptide chain of α1-CB3.

degree of hydroxylation to be determined as approx. 5%. This hydroxylation of the lysine residue in the present case rendered the corresponding peptide bond resistant to the attack of trypsin. The sequence of this area was determined as Ala–Hyl–Gly–Asp [9]. Comparison with the two hydroxylysine residues present in α1-CB6 [1, 3] suggests a dependence on the immediate vicinity of the susceptibility to trypsin of a hydroxylysine bond. Thus, the sequence Asp–Hyl–Gly–Glu is resistant to trypsin while Ile–Hyl–Gly–His is completely digestible. More detailed statements will have to be based on experiences gained in sequencing additional hydroxylysine containing peptides.

Acknowledgement

The authors wish to thank the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 51) for supporting this work.

References

[1] K. v.d. Mark, P. Wendt, F. Rexrodt and K. Kühn, FEBS Letters 11 (1970) 105–108.
[2] J. Rauterberg, P.P. Fietzek, F. Rexrodt, U. Becker, M. Stark and K. Kühn, FEBS Letters 21 (1972) 75–79.
[3] P. Wendt, M. v.d. Mark, F. Rexrodt and K. Kühn, European J. Biochem., in press.
[4] P.P. Fietzek, F. Rexrodt, P. Wendt, M. Stark and K. Kühn, European J. Biochem., in press.
[5] W.T. Butler, J. Biol. Chem. 244 (1969) 3415–3417.
[6] J. Rauterberg and K. Kühn, European J. Biochem. 19 (1971) 398–407.
[7] K. v.d. Mark, F. Rexrodt, P. Wendt and K. Kühn, European J. Biochem. 16 (1970) 143–151.
[8] P. Edman and G. Begg, European J. Biochem. 1 (1967) 80–91.
[9] P.P. Fietzek, P. Wendt, I. Kell and K. Kühn, FEBS Letters 26 (1972) 74.