CHARACTERIZATION OF THE AMINO-TERMINAL SEGMENT IN PROCOLLAGEN pα2 CHAIN FROM DERMATOSPARACTIC SHEEP

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1. Introduction

The polypeptide chains of the biosynthetic precursor procollagen have additional segments at both ends as compared to collagen α-chains [1–4]. The amino-terminal extensions were recently characterized in type I procollagen pα1(I) chain which accumulates in tissues of dermatosparactic animals [5,6] and in type III procollagen pα1(III) chain from fetal bovine skin [7]. The data indicates that the precursor specific segment in both types of chain consists of three structural domains. A globular region of about 100 amino acids is particularly rich in cystine and is located at the amino end of the segment. The central portion consists of a collagen-like sequence of about 50 amino acids. Both precursor specific domains are joined to the amino-end of collagen α-chain by a short non-helical region.

The occurrence of collagen-like sequences in the precursor specific segments indicated that this portion may assume a triple helical configuration in native procollagen. Since type I procollagen consists of two pα1(I) and one pα2 chain it was of interest to show whether the amino-terminal segments of both chains have the same type of structure. Preliminary studies [5] indicated that the precursor specific segment of pα2 is destroyed by bacterial collagenase. In the presence study we isolated the respective region after cleavage with cyanogen bromide (CNBr) and showed that it consists mainly of a collagen-like sequence which lacks cysteine.

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2. Experimental

Procollagen pα2 chains were extracted from the skin of dermatosparactic sheep and purified by chromatographic methods as described previously [5]. Established procedures were used to purify collagen α2 chains from normal sheep skin [8,9]. These polypeptide chains were cleaved with CNBr in 70% formic acid [5]. The precursor specific peptide pα2-CB1 was further cleaved by bacterial collagenase (CLSPA, Worthington) at an enzyme/substrate ratio of 1:100 for 4 h at 37°C in 0.2 M ammonium bicarbonate, pH 7.9.

Chromatography of peptides was carried out on columns of Sephadex G-50 (2.5 × 145 cm) or Bio-Gel P-4 (1.5 × 100 cm) both equilibrates in 0.2 M ammonium bicarbonate, pH 8.5. Peptides α2-CB4 and α2-CB3.5 were separated on a column of agarose (Bio-Gel A-1.5) in 1 M $CaCl_2$, 0.05 M Tris–HCl, pH 7.5 [9]. For chromatography on phosphocellulose, the column (1 × 8 cm) was equilibrated in 0.001 M sodium acetate, pH 3.6 and eluted with a linear gradient from 0–0.3 M NaCl [5]. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate followed a previously described procedure [10]. Amino acid compositions were determined on a Durrum D-500 analyzer after hydrolyzing the samples with 6 M HCl (24 h, 110°C) under N_2.

3. Results

As shown previously [5] the pα2 chain obtained from procollagen of dermatosparactic sheep is larger

Table 1
Amino acid composition of CNBr peptides from α2 and pα2 chain and of collagenase fragments (Col) produced from pα2-CB1

	α2-CB0	α2-CB1	α2-CB2	α2-CB4	α2-CB3.5	pα2-CB1	pα2-CB1 Col 1	pα2-CB1 Col 2	pα2-CB1 Col 3	sum Col 1,2 and 3
hydroxyproline	-	-	2(1.9)	31	61	6(6.4)	2(2.0)	1(1.0)	4(3.7)	7
aspartic acid	-	1(1.0)	2(1.9)	13	34	6(6.1)	4(4.3)	1(1.2)	1(1.0)	6
threonine	-	-	1(0.9)	6(6.3)	16	1(1.2)	-	1(1.0)	-	1
serine	-	-	2(2.0)	11	20	3(2.8)	1(0.7)	2(1.6)	1(0.9)	4
homoserine	1(1.0)	1(1.1)	1(1.1)	1(0.6)	-	1(1.0)	1(0.7)	-	-	1
glutamic acid	-(0.3)	1(1.1)	1(1.4)	25	50	6(6.0)	2(1.7)	3(3.0)	1(1.4)	6
proline	-	2(2.0)	3(3.2)	38	80	11	3(2.8)	2(2.0)	5(5.4)	10
glycine	1(1.0)	5(4.9)	10(9.7)	108	208	22	10(9.8)	4(4.1)	7(7.4)	21
alanine	-	-	3(3.0)	37	68	5(5.0)	2(2.0)	2(2.2)	1(1.1)	5
valine	--	-	1(0.8)	12	21	-(0.3)	-	-	-	-
isoleucine	-	-	-	3(3.1)	10	1(1.1)	1(1.0)	-	-	1
leucine	1(0.7)	-(0.2)	1(1.0)	11	20	2(2.2)	1(1.0)	1(1.0)	-	2
tyrosine	-	-	-	-	4(3.6)	-	-	-	-	-
phenylalanine	-	1(0.9)	-	4(3.6)	11	2(1.9)	2(1.5)	-	-	2
histidine	-	-	-	2(2.0)	5(5.3)	-	-	-	-	-
hydroxylysine	-	-	-	4(4.1)	6(6.1)	-	-	-	-	-
lysine	-	1(0.5)	-	6(6.4)	15	2(1.7)	1(0.7)	1(0.7)	-	2
arginine	-	-	3(2.6)	16	36	5(4.9)	1(1.4)	2(2.0)	2(2.0)	5
total	3	12	30	328	665	73	31	20	22	73

Amino acids given as residues per peptide rounded off to the nearest whole number. Actual values are given in brackets for values less than 10 residues. A dash denotes less than 0.2 residues.

by about 7000 daltons when compared with collagen α2 chain obtained from normal sheep skin. In order to identify the precursor specific portion of pα2 we isolated CNBr peptides from both the pα2 and α2 chain and characterized them by amino acid analysis (table 1). Chromatography of the CNBr digest of α2 on Sephadex G-50 (fig.1) showed two large peptides α2-CB4 and α2-CB3.5 which eluted in the void volume of the column and three smaller peptides α2-CB0, α2-CB1 and α2-CB2*. These five peptides closely resemble in amino acid composition and size those CNBr peptides described previously for calf collagen α2 chain [12,13].

Examination of the CNBr digest of pα2 chain showed that it contained the peptides α2-CB0, α2-CB2, α2-CB4 and α2-CB3.5. As shown in fig.1 the digest essentially lacked the peptide α2-CB1. However, the digest contained a peptide pα2-CB1 which eluted shortly after the void volume of the Sephadex G-50 column and which was missing in the CNBr cleaved α2 chain. We frequently observed a further peak eluting just before the position of α2-CB2 (fig.1). This peak consisted of small amounts of several peptides which presumably arose from a contamination with cross-linked pα1(I) chains [5].

Peptide pα2-CB1 could be eluted as a single peak from phosphocellulose and migrated as a single band in polyacrylamide gel electrophoresis (data not shown). The molecular weight of pα2-CB1 was estimated by chromatography on agarose [9] as about 9000. The peptide was composed of 73 amino acid residues (table 1) and about one third of them were glycine. It contained one residue of homoserine but lacked cysteine. By using peptide α2-CB2 as an internal standard the stoichiometric recovery of pα2-CB1 was calculated to be 0.9 mol/mol pα2 chain.

For further characterization of pα2-CB1 we cleaved the peptide with bacterial collagenase and separated the digest into three peaks on Bio-Gel P-4 (fig.2). Amino acid analyses (table 1) indicated that the first peak (Col 1) consisted of a peptide of about 30 amino

*The nomenclature used for the CNBr peptides follows earlier suggestions [11]

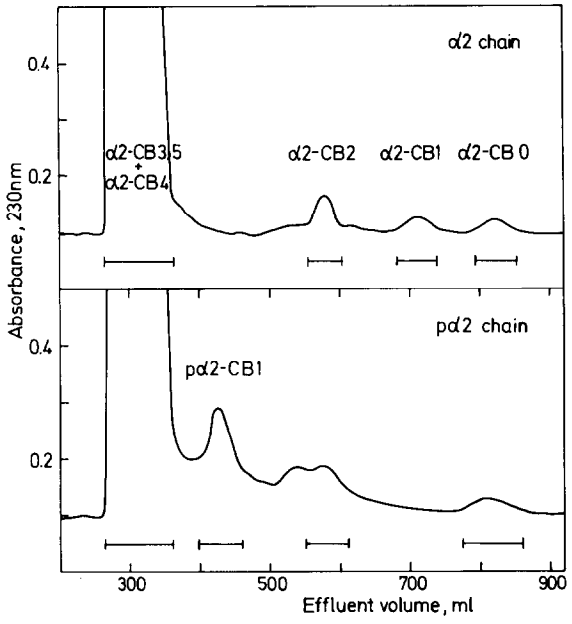

Fig. 1. Chromatography of CNBr digests of collagen α2 chain (top) or of procollagen pα2 chain (bottom) on Sephadex G-50. The column (2.5 × 145 cm) was equilibrated in 0.2 M ammonium bicarbonate, pH 8.5. The lines at the base line of both diagrams indicate the pools used to isolate individual CNBr peptides. Peptides α2-CB4 and α2-CB3.5 which eluted together in the front peak were separated by a second chromatography on agarose [9].

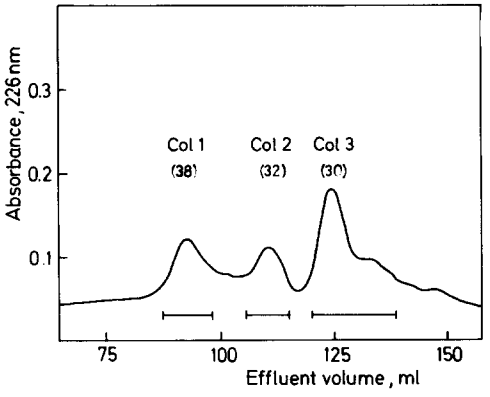

Fig. 2. Separation of a collagenase digest of peptide pα2-CB1 on a Bio-Gel P-4 column (1.5 × 100 cm) which was equilibrated in 0.2 M ammonium bicarbonate, pH 8.5. Material recovered from the column was collected into three pools (Col 1–3). Bracketed numbers refer to the relative amount (in %) of peptide material in each pool.

acids including the homoserine residue. The second peak (Col 2) accounted for about 20 amino acid residues and had less than one third of glycine. The third peak (Col 3) showed a collageneous composition as indicated by the high glycine and hydroxyproline content. Presumably the peaks Col 2 and Col 3 consisted of a mixture of small peptides. When taken together the composition of Col 1, 2 and 3 agreed well with the composition found for pα2-CB1 (table 1).

4. Discussion

Previous studies have indicated that the pα2 chain of intact type I procollagen has a molecular weight of about 140 000 and differs from collagen α2 chain (molecular weight 95 000) by extra peptide segments connected to both ends of the α-chain [1–4]. Type I procollagen, as it accumulates in the tissue of dermatosparactic animals, is already a degraded form of the precursor. Studies on the pα1(I) chain from this procollagen showed the presence of an additional amino-terminal segment but failed to show extra peptide sequences at the carboxyl-end [5]. The difference in size previously observed between pα2 chain from dermatosparactic procollagen (molecular weight 102 000) and collagen α2 chain could be attributed in the present study to a single CNBr peptide pα2-CB1. Amino acid analysis and the failure to demonstrate in CNBr cleaved pα2 the peptide α2-CB1, which is known to originate from the amino-end of α2 chain [13], locate pα2-CB1 to the amino-end of pα2. Thus, the data show pα2-CB1 consists of 12 amino acid residues from the amino-terminal region of α2 and of about 60 amino acid residues unique to pα2.

Amino acid analysis and the susceptibility towards bacterial collagenase indicate that the precursor specific part of pα2-CB1 consists largely of a collagen-like sequence of the type Gly–*X*–*Y*. Since similar sequences have been demonstrated in pα1(I) chain [5], it is likely that a part of the amino-terminal segment in type I procollagen exists in triple-helical conformation. Small portions of peptide pα2-CB1, which could be recovered in the fragments Col 1 and Col 2, may also contain other types of sequences. Because of its homoserine content, fragment Col 1 is apparently

derived from the region where the precursor specific sequence is joined to the amino-end of the α2 chain. Previous data on pα1(I) chain [11] suggest that the precursor specific part of this segment may contain a short non-helical sequence in which glycine does not occur at every third position. The other fragment(s) of pα2-CB1 found in the peak Col 2 may also in part contain a non-helical sequence since it has less than one third glycine. Perhaps Col 2 contains a short globular segment of pα2-CB1. Similar segments at the amino-end of pα1(I) and pα1(III) chain have a size of about 100 amino acids [5,7]. It is, however, possible that the failure to demonstrate a large globular segment in pα2 is due to proteolytic degradation ocurring in situ or during isolation of the amino-terminal fragment.

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