NON-HELICAL REGIONS IN RAT COLLAGEN α1-CHAIN

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1. Introduction

The triple-helical collagen molecule contains at both terminal sites short, non-helical areas being important for cross-linking [1, 2] and bearing the major antigenic activity [3–5]. Sequence studies of these regions [6, 7] have revealed that glycine does not occupy every third position which is considered a prerequisite for triple-helical assembly. Although the N-terminal regions were already characterized some years ago [1, 6], the demonstration of C-terminal counterparts in calf and rabbit collagen [8, 9] was achieved only recently. This is explained by the high susceptibility of these particular non-helical sequences to degradation by tissue proteases which can be prevented by extraction of the collagen α-chains under denaturating conditions [5, 7, 9].

As yet no evidence was available for a C-terminal, non-helical region in the rat collagen α1-chain. The amino acid composition reported for the C-terminal cyanogen bromide (CNBr) peptide α1-CB6 of neutral salt-extracted rat collagen [10] rather suggested its absence (cf. [5, 8, 11]). Furthermore, comparative sequence studies on the N-terminal region of rat tendon [12] and skin collagen [6] demonstrated the lack of four amino acid residues in the latter. Considering that these data may reflect extraction artefacts, the question on the occurrence and nature of non-helical regions was reinvestigated for this kind of collagen by improved methods recently established [5, 8, 9].

2. Experimental

Male Wistar strain rats (weight 100–150 g) were made lathyritic by adding 0.1% β-aminopropionitril fumarate to the drinking water for four weeks. The entire skin or the tail tendons were extracted with deionized 8 M urea or with 6 M guanidine hydrochloride (15 ml/g wet weight), respectively, for 2 hr at room temp. Following centrifugation (30 min, 24 000 *g*) the collagen α1-chains were isolated from the supernatant after dialysis at 4° against the starting buffer by CM-cellulose chromatography [13]. Prior to its application onto the column the dialysed extract was denatured for 30 min at 40° and a small precipitate removed by centrifugation. Neutral salt-soluble collagen was prepared [1] from the same sources. The α1-chains of this material were obtained analogously after denaturation of the collagen on CM-cellulose.

Cleavage with CNBr was carried out as described [1]. The C-terminal peptide α1-CB6 was purified by chromatography on Bio-Gel P-150 [11]. The small CNBr peptides α1-CB0 and α1-CB1 were first separated on Bio-Gel P-4 [4, 14] and α1-CB1 was further purified on phosphocellulose [1]. Chromatography of α1-CB0 on Dowex 50 X 2 (H^+ form) was carried out under conditions which did not retard peptides lacking basic groups [15]. Methods for chromatography on Bio-Gel P-10 or Sephadex G-50 are described elsewhere [5]. Digestion with trypsin or chymotrypsin was done as previously [5, 8].

The amino acid composition was determined as described [11]. Stoichiometric proportions between chymotryptic fragments were calculated from quantitative amino acid analyses after the separation of the peptides on Bio-Gel P-10. Molecular weights were determined by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate [16] employing gels of 11 cm length or by molecular sieve chromatography (cf. [11]). Cyanogen bromide pep-

tides from the calf collagen α1-chain served as calibrating substances.

3. Results and discussion

3.1. *Characterization of the C-terminal, non-helical region in rat collagen α1-chain*

The C-terminal CNBr peptide α1-CB6 was prepared from the α1-chains of skin and tendon obtained either by denaturating extraction or from neutral salt-extracted collagen. Comparison in polyacrylamide gel electrophoresis (fig. 1) revealed a size of approx. 200 amino acids for the peptides from neutral salt-extracted collagen. However, α1-CB6 from guanidine-extracted tendon had a distinctly slower mobility suggesting a molecular weight about 10% higher. Two bands corresponding to those described were found

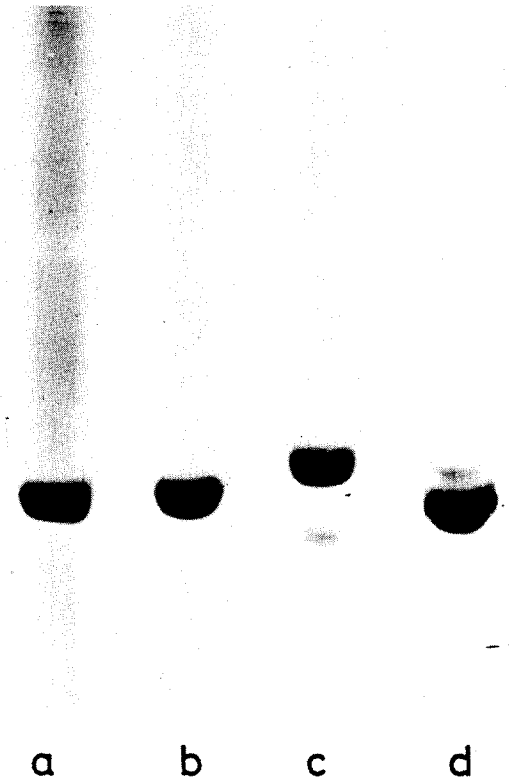

Fig. 1. Comparison of rat collagen α1-CB6 obtained from different sources by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate. a) From neutral salt-extracted skin; b) from neutral salt-extracted tendon; c) from guanidine extracted tendon; d) from urea-extracted skin.

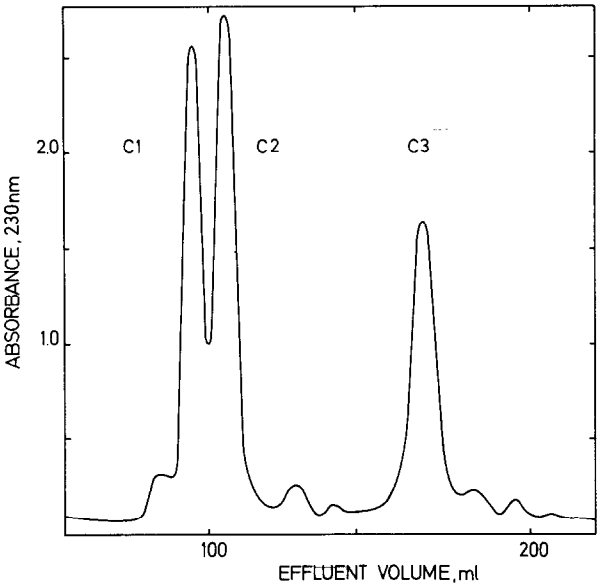

Fig. 2. Separation of chymotryptic peptides (C1 to C3) of α1-CB6 from guanidine-extracted rat tendon collagen on Bio-Gel P-10. Elution was carried out at 38°with 0.05 M sodium acetate pH 4.5, column size 1.5 × 120 cm.

in α1-CB6 from urea-extracted skin but the faster one predominated distinctly. Quite significant differences between these peptides were also observed in their amino acid composition (table 1) especially in tyrosine and phenylalanine content.

Chymotrypsin cleavage of α1-CB6 from guanidine-extracted tendons rendered three peptides, C1, C2 and C3, which occurred in a molar proportion of 1.0:0.9:0.6 after separation on Bio-Gel P-10 (fig. 2) and accounted for all of the amino acids in α1-CB6 (table 1). Because of their close resemblance to chymotryptic fragments obtained from calf collagen α1-CB6 [8] the order C1–C2–C3 is indicated. The peptide C3 must be considered as non-helical since it had a low glycine content. In comparison to calf collagen C3 [7, 8] at least one residue of leucine, alanine and histidine is replaced by phenylalanine, serine and glutamic acid (or glutamine) in the rat collagen sequence. This relatively high degree of interspecies variability corresponded to findings for the aminoterminal region of collagen (reviewed in [17]).

Studies on α1-CB6 from urea-extracted skin revealed similar findings to guanidine-extracted α1-CB6 except for a decreased yield of C3 (about 0.1 moles per mole C1 or C2) and the occurrence of some small-

Table 1

Amino acid composition of CNBr peptides and proteolytic fragments from the terminal regions of the collagen α1-chain from rat tendon and skin [a].

	Rat tendon (guanidine extract)						Rat skin (urea extract)				
	C1	C2	C3	Sum C1-3	α1-CB6	α1-CB6[b]	α1-CB6[b]	α1-CB6	α1-CB1	α1-CB0	T 1
3-Hydroxyproline	–	2(1.9)	–	2	2(2.0)	2(1.7)	1(1.0)	1(0.9)	–	–	–
4-Hydroxyproline	7(7.1)	9(8.7)	–	16	16	16	15	15	–	–	–
Aspartic acid	6(6.3)	3(3.3)	2(2.0)	11	11	10	10	10	1(1.0)	–	1(1.1)
Threonine	3(3.0)	1(1.2)	–	4	4(4.2)	4(4.2)	4(4.0)	4(4.1)	–	–	–
Serine	3(3.3)	8(7.7)	2(1.9)	13	13	10	10	11	3(2.6)	–	1(1.0)
Homoserine	–	–	–	–	–	–	–	–	1(1.2)	1(1.0)	–
Glutamic acid	9(9.5)	4(4.0)	5(5.1)	18	17	13	13	14	1(1.1)	1(1.0)	2(2.1)
Proline	15	14	3(3.1)	32	32	30	31	30	2(2.0)	–	–
Glycine	38	28	2(2.1)	68	68	65	66	65	3(3.2)	–	1(1.4)
Alanine	14	5(5.0)	–	19	20	20	19	19	1(1.1)	–	–
Valine	1(1.2)	–	–	1	1(1.4)	2(1.7)	2(1.6)	2(1.6)	2(1.9)	–	–
Methionine	–	–	–	–	–	–	–	–	–	–	1(0.9)
Isoleucine	2(1.9)	1(1.0)	–	3	3(2.7)	3(3.1)	3(2.9)	3(2.9)	–	–	–
Leucine	1(1.1)	3(3.1)	1(1.2)	5	5(5.0)	4(4.2)	4(3.9)	4(4.4)	–	–	–
Tyrosine	–	1(0.9)	2(1.8)	3	2(2.4)	1(1.0)	1(0.8)	1(1.2)	2(1.7)	–	2(1.8)
Phenylalanine	1(0.8)	–	2(1.9)	3	3(3.1)	2(1.7)	1(1.2)	2(2.2)	–	–	–
Hydroxylysine	2(1.7)	–	–	2	2(1.6)	2(2.0)	2(1.7)	2(1.8)	–	–	–
Histidine	1(1.0)	–	–	1	1(1.0)	1(1.4)	1(0.9)	1(1.0)	–	–	–
Lysine	2(2.4)	1(1.0)	1(0.9)	4	4(4.2)	3(2.7)	3(3.3)	4(3.6)	1(0.9)	–	1(0.9)
Arginine	8(7.8)	3(3.2)	1(1.0)	12	12	11	11	11	–	–	–
Total	113	83	21	217	216	199	197	199	17	2	9

[a]Given as residues per peptide rounded off to the nearest whole number. Actual values are given in brackets for values below 10. A dash denotes less than 0.2 residues. The abbreviations C1 to C3 denote chymotryptic peptides from α1-CB6 and T 1 a tryptic peptide of the α1-chain.

[b]Obtained from neutral salt-extracted collagen.

er degradation products as yet not identified. However, only C1 and C2 could be demonstrated in chymotryptic digests of α1-CB6 if obtained from neutral salt-extracted rat skin collagen. Besides variable amounts of the tripeptide Arg–Gly–Phe which is derived from a further, limited cleavage in C1 (cf. [8]) no additional peptide which might represent a shorter C3 could be detected. The peptide α1-CB6 from neutral salt-extracted tendon collagen had a similar size and amino acid composition. Although not subjected to the same fragmentation procedure it is unlikely that this peptide should contain substantial amounts of C3.

The data clearly demonstrate that denaturation during collagen extraction is essential for preserving the full C-terminal, non-helical sequence. These findings are similar to earlier experiences for calf and rabbit collagen [5, 9, 11]. It is suggested that the shortening occurs by the action of tissue proteases considered to be more active in skin than in tendon. Even 8 M urea obviously could not completely prevent their action. Nevertheless, part of the non-helical region still exists in the neutral salt-extracted collagen since the only tyrosine found in C2 is located four residues beyond the end of the helical region in calf collagen [18].

3.2. *Aminoterminal region of the α1-chain of rat skin collagen*

Amino terminal, non-helical sequences which differ in size have been reported for the α1-chains of neutral salt-extracted rat skin and tendon collagen. After cleavage with CNBr two N-terminal peptides α1-CB0 and α1-CB1, which contain 2 and 17 amino acid residues, respectively, were demonstrated in the tendon collagen [12]. However, only an α1-CB1 two amino acid residues shorter than that found in tendon was obtained from skin collagen [1]. In the present study essentially the same results as described for tendon were observed for skin if the collagen α1-chains were obtained upon urea extraction (table 1). The earlier findings must therefore be considered as an extraction artefact. However, it is further indicated that the amino terminal region is much less affected by tissue proteases than the carboxyterminal sites.

To clarify the order of the CNBr peptides an overlapping tryptic nonapeptide T1 was prepared from the urea-extracted rat skin α1-chain. It could be entirely purified by molecular sieve chromatography on Sephadex G-50 followed by Bio-Gel P-2 and P-4 (fig. 3) since tyrosine-containing peptides under these conditions emerged in retarded positions [3, 4]. The peptide T1 contains methionine (table 1) and was cleaved

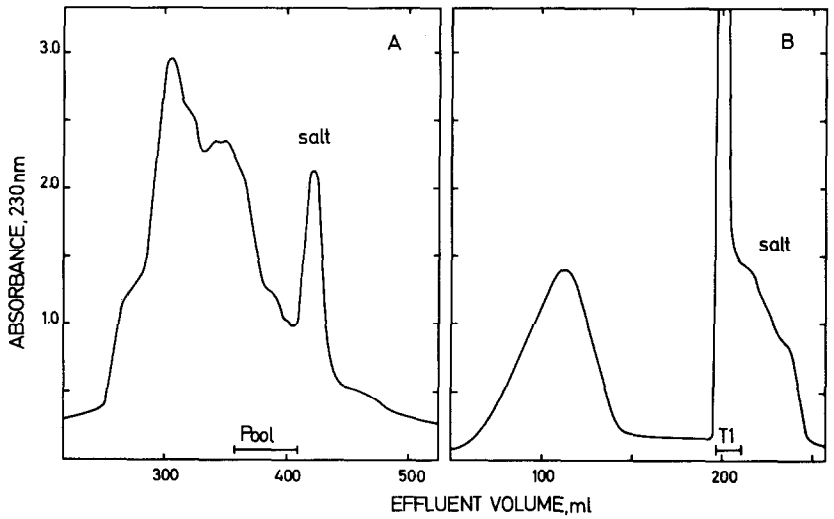

Fig. 3. Purification of the tryptic peptide T1 from urea-extracted α1-chains of rat skin collagen on Sephadex G-50 (A) and rechromatography of the pool indicated on Bio-Gel P-2 (B). Residual salt was removed by a final run on Bio-Gel P-4 (not shown). Elution was carried out in (A) at 38° with 0.05 M sodium acetate pH 4.5 (column size 2.0 × 144 cm) and in (B) at room temp. with 0.1 M acetic acid (column size 1.5 × 115 cm).

with CNBr. Passage of the digest over Dowex 50 × 2 at pH 2 revealed the peptide α1-CB0 in the effluent. Elution of the column by dilute ammonia yielded a heptapeptide which accounted for the remaining amino acids and resembled the N-terminal tryptic peptide already known from rat tendon collagen α1-CB1 [12]. The order α1-CB0–α1-CB1 suggested earlier [12] is therefore unequivocally established by these results.

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