CYANOGEN BROMIDE PEPTIDES OF THE RABBIT COLLAGEN α1-CHAIN

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1. Introduction

Previous studies have revealed that cyanogen bromide (CNBr) cleavage of the α-chains of collagen provided an excellent tool to characterize these large polypeptides. The data on the α1-chain from various vertebrate species [1–6] demonstrated a remarkable invariance in the number and position of methionine residues as well as very similar amino acid compositions for most of the homologous CNBr peptides. Two regions at both ends of the α1-chain showed larger interspecies variations and were considered as nonhelical because of their low glycine content. These nonhelical sequences are important for cross-linking and exhibit a considerable antigenic activity (reviewed in [7]). Recently, some of these major antigenic determinants have been elucidated [8, 9] in their structure. Since rabbit antibodies have been used entirely in this kind of work, knowledge of the homologous sequences in rabbit collagen would help to approach the question of foreignness in conferring immunogenic activity to protein structures. As yet only a few CNBr peptides are characterized from rabbit collagen α1-chain [10]. A complete picture is given in the present work.

2. Experimental

Rabbits of three weeks age were made lathyritic by addition of 0.1% β-aminopropionitril fumarate to the drinking water. The rabbits were killed after four weeks and the skin was shaved and cleaned from adhering fat and muscle layers. After homogenizing, the tissue was extracted with 8 M urea (10 ml/g wet wt.) as described [9]. From the extracts the collagen α1-chains were purified on CM cellulose [11] after extensive dialysis at 4° against the starting buffer. The α1-chains were also prepared from urea extracts of nonlathyritic rabbits or from neutral salt-extracted collagen [12]. The purity of the chains was checked by disc electrophoresis [13]. Cleavage with CNBr was done as recommended [12] and the reagents were removed by lyophilization.

Molecular sieve chromatography on Bio-Gel P-4 (column 3 × 135 cm) was carried out at room temp. in 0.1 M acetic acid. Columns of Bio-Gel P-150 (2.5 × 110 cm) and Bio-Gel P-10 (1.5 × 120 cm) were equilibrated with 0.05 M sodium acetate pH 4.5 and run at 38°. For phosphocellulose chromatography a column (1.0 × 9 cm) equilibrated with 0.001 M sodium acetate pH 3.6 and a concave gradient from 0 to 0.3 M NaCl (100/100 ml) was used. Separation of α1-CB2, α1-CB4 and α1-CB5 was done on phosphocellulose according to [3]. Chromatography of CNBr peptides on CM-cellulose at pH 3.6 employed an earlier described method [1]. Purification of α1-CB0 on Dowex 50 × 2 (H^+ form) was done under conditions which did not retard peptides lacking basic groups [14].

The amino acid composition was determined as described [5]. Lysine-derived aldehyde (α-amino-adipic-ν-semi-aldehyde) was detected on the amino acid analyser after oxidation to α-amino-adipic acid by performic acid [15]. The size of the peptides was estimated from runs on Bio-Gel P-150 or P-10 [5] or by electrophoresis in the presence of sodium dodecyl-sulfate [13]. For calibration CNBr peptides from rat or calf collagen [1,5] were used. The values obtained were in good accordance with those given in table 1. The nomenclature of the CNBr peptides followed the suggestion of Miller et al. [2].

Table 1
Amino acid composition of CNBr peptides from the rabbit collagen α1-chain. [a]

	α1-CB0	α1-CB1	α1-CB2	α1-CB3	α1-CB4	α1-CB5	α1-CB6A	α1-CB6B	α1-CB7	α1-CB8	Total peptides	α1 [b]
3-Hydroxyproline	-	-	-	-	-	-	1.1	-	-	-	0.6	0.5
4-Hydroxyproline	-	-	5.4	14.8	5.7	2.7	15.7	-	25.1	29.0	98.4	103.6
Aspartic acid	-	1.1	-	7.2	3.1	3.0	9.0	1.1	13	13	50	47
Threonine	-	-	-	0.4	1.0	-	4.1	-	6.6	7.1	19	19
Serine	-	2.7	3.1	3.8	-	2.0	8.6	-	6.9	6.2	34	36
Homoserine	1.0	1.0	1.0	1.1	1.1	1.1	0.8	-	1.1	1.0	9	8.9 [c]
Glutamic acid	1.0	1.2	4.2	16	3.2	3.3	14	3.1	18	22	85	88
Proline	-	2.2	6.6	15.2	5.0	2.3	30.4	3.1	36.0	33.0	133.8	130.5
Glycine	-	3.2	12	49	16	12	66	3.4	90	94	345	331
Analine	-	1.2	1.3	19	4.0	4.2	19	2.0	32	36	118	123
Valine	-	1.8	-	4.3	-	-	2.8	-	7.8	5.8	23	21
Isoleucine	-	-	-	-	-	-	3.6	-	2.7	1.1	8	8.0
Leucine	-	-	1.1	3.2	2.0	1.1	4.1	-	4.7	4.2	20	20
Tyrosine	-	1.8	-	-	-	-	-	1.8	-	-	4	3.5
Phenylalanine	-	-	1.0	3.0	-	0.9	2.8	-	3.1	3.0	14	13
Hydroxylysine	-	-	-	0.3	-	0.9	1.5	-	0.6	0.6	3.9	4.0
Histidine	-	-	-	-	-	1.0	1.0	-	-	-	2	2.6
Lysine	-	0.9	-	4.9	2.0	1.9	3.7	0.8	10.6	9.3	34.1	30.9
Arginine	-	-	1.0	6.2	3.7	1.1	11	1.9	14	16	55	51
Total	2	17	36	147	47	37	200	17	273	281	1057	1043

a) Residues per peptide, rounded off to the nearest whole number. Actual values are given in those cases where less than 10 residues were found and for hydroxyproline, proline, hydroxylysine and lysine since there might be incomplete hydroxylation giving rise to nonintegral values. A dash indicates less than 0.2 residues. Totals were calculated from rounded off values.
b) Values calculated for a molecular weight of 95,000.
c) As methionine.

3. Results

As suspected with the nine methionine residues found in the urea-extracted α1-chains, ten peptides could be identified after CNBr cleavage. The amino acid composition of the individual peptides is listed in table 1. In the first purification step the entire mixture was passed over Bio-Gel P-4 (fig. 1A) and the material emerging from the column was divided into three pools. The peptides of the first pool were subsequently separated on Bio-Gel P-150 (fig. 1B). Two peaks contained only partially cleaved material (mainly α1-CB3-7 and α1-CB4-5, [5]) and were not investigated further. The other peaks except the last were rechromatographed under identical conditions and subsequently subjected to CM-cellulose chromatography. Two peptides, α1-CB7 and α1-CB8, could thus be obtained from the second peak and only a single peptide, α1-CB6A and α1-CB3, respectively, from the following two. The last peak of the Bio-Gel P-150 run was separated on phosphocellulose into three main components, α1-CB2, α1-CB4 and α1-CB5, which were further purified on Bio-Gel P-10 (not shown).

The second pool of the Bio-Gel P-4 run was composed of two peptides (fig. 2A) which were identical in their amino acid composition except for the replacement of lysine found in α1-CB1 by lysine-derived aldehyde in α1-CB1^{Ald}. After chromatography of the third pool (fig. 1A) on phosphocellulose a complex pattern appeared (fig. 2B). The first peak contained the dipeptide α1-CB0 (Glx-Hse) in addition to traces of other unidentified material. Since α1-CB0 was not retarded on Dowex 50 × 2, a modified glutamic acid (e.g. pyrollidone-5-carboxylic acid) is suggested as already demonstrated for the amino-terminal end of the rat collagen α2-chain [16].
In addition to contamination by α1-CB1^{Ald} three

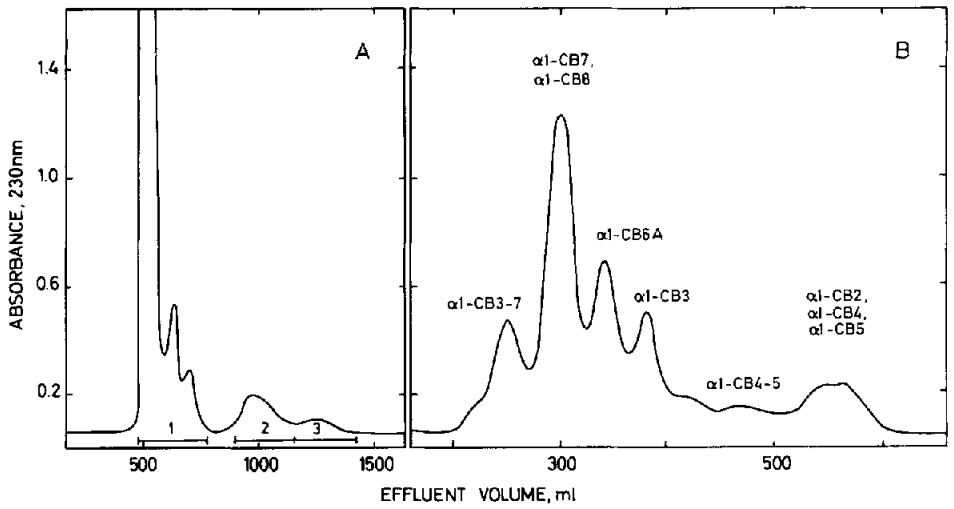

Fig. 1. Chromatography of 200 mg CNBr peptides from the rabbit collagen α1-chain on Bio-Gel P-4 (A) and rechromatography of pool 1 on Bio-Gel P-150 (B).

peptides with an unique amino acid composition could be identified. Their main characteristics were the absence of homoserine and a low glycine content. Two peptides (α1-CB6B and α1-CB6B^{Ald}) resembled each other except for the occurrence of lysine-derived aldehyde instead of lysine in the latter. The third peptide α1-CB6B* was identical to α1-CB6B but lacked one residue of arginine and alanine. Fragmentation by trypsin indicated that the loss must have occurred at the carboxyterminal site (unpublished).

The peptide pattern was not different when urea-extracted α1-chains from non-lathyritic rabbits were investigated. The CNBr peptides were also characterized from the α1-chains of neutral salt-extracted rabbit collagen which corresponds to the material used by Bornstein and Nesse [10]. Most of the peptides, within the limits of analytical error, were identical to those obtained from the urea-extracted α1-chains. However, no α1-CB6B was found and α1-CB6A though not remarkably different in size to that given in table 1 lacked at least the homoserine and one residue of phenylalanine and isoleucine.

4. Discussion

Most of the CNBr peptides from the rabbit collagen α1-chains resembled those from other mammalian species [1,4,5] and the data suggested only a few amino acid substitutions. Even the aminoterminal region (see also [10]), as comprised by the peptides α1-CB0 and α1-CB1, is probably identical to those from rat tendon [17] or skin [18] although a higher interspecies variation is usually observed in this part [7]. The most prominent differences are located in the carboxyterminal area. The peptide α1-CB6A was somewhat shorter than the corresponding calf collagen α1-CB6 [5] and contained homoserine. Accordingly, a small peptide α1-CB6B was identified which lacked homoserine. Its amino acid composition resembled quite good although not completely the carboxyterminal, nonhelical sequence of the calf collagen α1-chain elucidated recently [19]. Additional evidence for the order 6A – 6B was obtained from the characterization of an overlapping tryptic peptide (cf. [9]) which contained methionine (unpublished). The chicken collagen α1-chain has also an additional methionine in the carboxyterminal region. The two CNBr peptides obtained were, however, of about equal size [2,3].

The general nature of a carboxyterminal, nonhelical region in the collagen α1-chain is supported by earlier findings for the calf [19, 20] and by a recent study on rat collagen [18]. As in the calf the rabbit contains in this region a lysine residue which can be oxidized to an aldehyde, pointing to

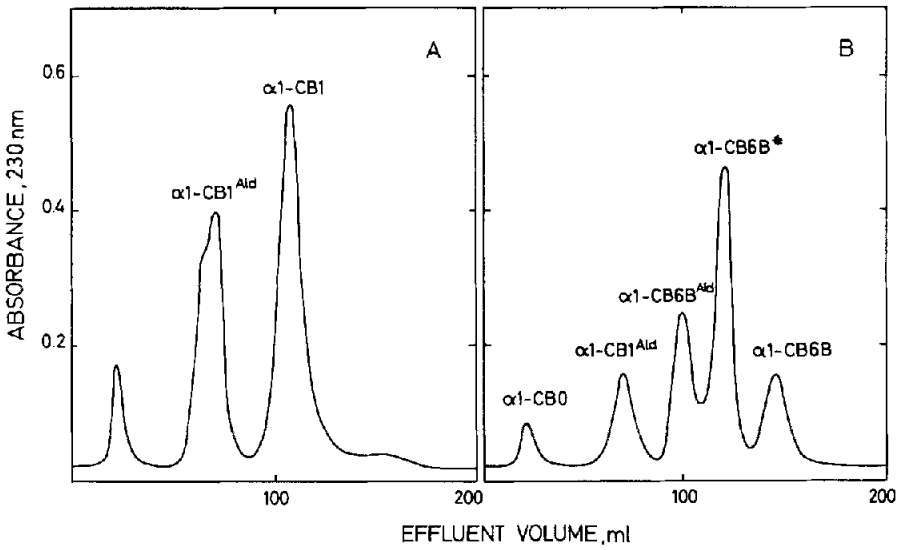

Fig. 2. Phosphocellulose chromatography of pool 2 (A) and pool 3 (B) obtained from the Bio-Gel P-4 run (see fig. 1A).

its importance in the cross-linking reaction [7]. These sequences are very susceptibile to an artificial shortening probably by tissue proteases (see also [9]) which can be prevented by the extraction of the α-chains under denaturating conditions.

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