ISOLATION AND CHARACTERIZATION OF THE CYANOGEN BROMIDE PEPTIDES FROM THE α2 CHAIN OF CALF SKIN COLLAGEN

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1. Introduction

Cleavage at the methionyl residues with CNBr has been proved to be a useful method for studies on the primary structure of the collagen molecule. CNBr (CB)-peptides of α1- and α2-chains of different species and tissues have been described [1–9]. Reuterberg and Kühn [3, 10] reported the isolation, characterization and ordering of the CB-peptides of the α1-chains of calf skin collagen. This paper describes the results of a similar investigation on the α2-chains of calf skin collagen. The methods applied were very similar to those used by Fietzek and Piez [6] to isolate the CB-peptides of the α2-chain of rat skin collagen. The peptides have been named on the basis of homology to six CB-peptides of the α2-chain of rat skin collagen.

2. Methods

Neutral salt-soluble and citrate buffer-soluble collagen from calf skin was isolated and purified as described earlier [11]. α2-chains were obtained by chromatography on carboxymethyl (CM)-cellulose as described by Piez et al. [12]. Rechromatography of α2-chains was performed under the same conditions on CM-cellulose and on Bio-Gel A-1.5 m, 200–400 mesh (Bio-Rad Laboratories) in 1 M $CaCl_2$ – 0.05 M Tris buffer, pH 7.5. Cleavage with CNBr was performed as described previously [1, 6]. The CNBr peptides were separated and purified as described before [6] with minor modifications. Fractions were lyophilized, desalted on Bio-Gel P-2, 100–200 mesh, and lyophilized. Amino acid analyses were performed on a Biochrom amino acid analyzer (Messrs. Bio-Cal, Munich, Germany). The molecular weights were determined on Bio-Gel A-1.5 m, 200–400 mesh [13]. The conditions for renaturation are given in the legend to fig. 2.

3. Results and discussion

Fig. 1 shows a typical chromatogram obtained by chromatography on phosphocellulose of a CNBr digest. Four peaks were eluted. The first, which eluted with the front, contained α2-CB0 and α2-CB1. α2-CB0 was separated from α2-CB1 by chromatography on Bio-Gel P-4, 200–400 mesh. α2-CB2 was rechromatographed on a Bio-Gel P-10, 200–400 mesh. α2-CB3 and α2-CB4, 5 were rechromatographed on CM-cellulose at pH 3.6 and further purified on Bio-Gel.

The amino acid compositions of the five CNBr peptides are shown in table 1. The values (residues per peptide) were calculated on the basis of amino acids present in low concentration and under the assumption of one residue of homoserine per peptide except for the COOH-terminal peptide. There were three small and two large peptides; each peptide contained one third glycine with the exception of α2-CB1, and had a characteristic amino acid composition by which it was readily distinguished from all the other peptides as well as from the whole α2-chain. Although the two large peptides had a similar composition, both exhibited some distinct features. For instance, α2-CB3 contained no tyrosine and one homoserine, α2-CB4,5 contained two tyrosine and no homoserine. The total

Table 1
Amino acid composition [a] of CB-peptides of the α2-chain of calf skin collagen.

Amino acid	α2-CB0	α2-CB1	α2-CB2	α2-CB3	α2-CB4,5	Total CB-peptides	α2
4-Hydroxyproline	0	0	2 (1.8)	32	49	83	88
Aspartic acid	0	2 (1.8)	2 (1.9)	14	33	51	49
Threonine	0	0	1 (0.9)	6 (5.6)	11	18	18
Serine	0	1 (1.2)	1 (1.2)	8 (7.5)	18	28	25
Glutamic acid	0	2 (2.1)	1 (1.4)	22	50	75	73
Proline	0	1 (1.4)	4 (3.7)	37	75	117	124
Glycine	1 (1.2)	3 (3.3)	10	111	207	332	339
Alanine	0	1 (0.9)	3 (3.0)	38	61	103	104
Valine	0	0	1 (0.9)	14	18	33	35
Isoleucine	0	0	0	5 (4.7)	11	16	18
Leucine	1 (1.0)	0	1 (1.1)	12	20	34	35
Tryosine	0	1 (0.9)	0	0	2 (2.1)	3	3 (2.7)
Phenylalanine	0	1 (0.9)	0	4 (4.3)	9 (8.9)	14	17
Hydroxylysine	0	0	0	4 (4.4)	7 (6.9)	11	11
Histidine	0	0	0	2 (1.8)	6 (6.1)	8	8 (8.2)
Lysine	0	1 (0.8)	0	6 (6.4)	13	20	22
Arginine	0	0	3 (3.1)	17	34	54	59
Homoserine	1 (0.9)	1 (0.9)	1 (0.9)	1 (0.8)	0	4	4 (3.5) [b]
Total	3	14	30	333	624	1004	1032

[a] Residues per peptide. Values are rounded off to the nearest whole number. Actual values are in parentheses in those cases where less than ten residues were found. A value of zero indicates less than 0.2 residue.
[b] Represent methionine in the case of α2.

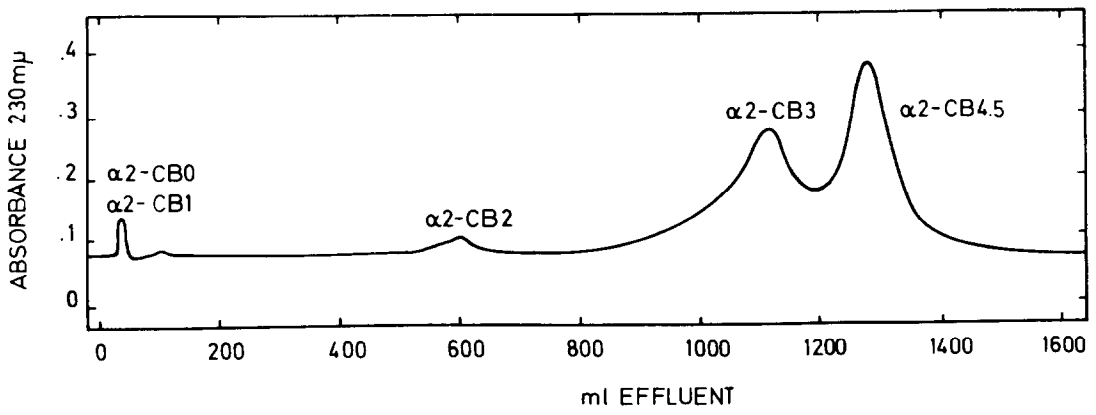

Fig. 1. Phosphocellulose elution pattern of the CNBr peptides (50 mg) derived from the α2-chain of calf skin collagen. Elution was performed in 0.001 M sodium acetate, pH 3.6, using a linear gradient of sodium chloride from 0.0 to 0.5 M. The total volume of the gradient was 2000 ml.

Table 2
Molecular weight of the CB-peptides of the α2-chain of calf skin collagen.

Peptide	Number of amino acids	Amino acid analysis	Molecular sieve chromatography [a]
α2-CB0	3	289	289
α2-CB1	14	1588	1588
α2-CB2	30	2743	2743
α2-CB3	333	30280	30000
α2-CB4,5	624	57659	59000
Total	1004	92559	93620

[a] The molecular weights of α2-CB0, α2-CB1 and α2-CB2 were calculated from their amino acid composition only.

number of amino acid residues of the five CNBr peptides account within experimental error, for all of the amino acids in the α2-chain (table 1). Table 2 shows conformity of molecular weights as determined by amino acid analysis and molecular sieve chromatography. These results indicate that the five CB-peptides isolated account for all of the amino acids and for the molecular weight of the α2-chain.

In order to localize α2-CB3 and α2-CB4,5, they were renatured to triple helical structures and precipitated as segment long spacing fragments. Their electronmicrographs are shown in fig. 2. α2-CB3 corresponds to the NH_2-terminal part of the molecule and α2-CB4,5 to the COOH-terminal part. α2-CB4,5 must be the COOH-terminal peptide, since it did not contain homoserine. The order of the remaining small peptides is not yet clear. In analogy to data from rat skin collagen [1, 6], α2-CB1 should be the NH_2-terminal peptide. The localization of α2-CB0 and α2-CB2 is under investigation.

It is noteworthy that there are four methionyl residues in the α2-chain of calf skin collagen as compared to five in the α2-chain of rat skin, chick bone or chick skin collagen. In the α2-chain of calf skin collagen, the methionyl residues are distributed in such a way, that the two large peptides account for about 95% of the total amino acids and molecular weight. A comparison of the results with known data of rat skin and chick bone and skin collagen reveals that there is a considerable homology in size and amino acid composition of the peptides.

Fig. 2. Correlation between long spacing segments from native calf skin collagen molecules (2b) and from renatured α2-CB3 (2a) and α2-CB4,5 (2c). Renaturation was performed in citrate buffer pH 3.7 for 160 hr between 15 and 6°. For preparation and staining of segments see [3]. Segments obtained from α2-CB3 are aggregated at their NH_2-terminal ends.

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