THE ORDER OF THE CNBr PEPTIDES FROM THE α1 CHAIN OF COLLAGEN

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Summary:

Chemical and electron microscopic evidence has established the order of the CNBr peptides from the α1 chain of rat skin and tendon collagen and chicken skin and bone collagen. This order is 0-1-2-4-5-8-3-7-6 for the rat. For the chicken, 6 is replaced by 6A-6B as a result of one extra methionyl residue.

In the case of the collagen molecule the determination of the complete amino acid sequence is a particularly difficult task since each of the three α chains in the molecule contains about 1000 amino acids and there are generally two kinds of α chains, α1 and α2 (1). The problem has been approached by the use of cyanogen bromide (CNBr) cleavage at methionyl residues. It has been possible to isolate six to ten peptides (per chain) from CNBr digests of the α1 and α2 chains of rat skin and tendon and chick bone and skin collagen (2-6) which account for all of the amino acids and all of the weight of the chain. A tentative order for the peptides in the α1 chain has been suggested (7). We present evidence here which confirms this order with one correction.

The α1 chains from rat skin and tendon collagen appear to have identical primary structures except for the absence of four amino acids at the N-terminal end of the α1 chain of rat skin collagen (2,3) and other alterations such as hydroxylation and aldehyde formation that must occur after the amino acids are assembled into poly peptide chains. The same conclusion holds for the α1 chains

of chick skin and bone collagen except that there are no missing residues at the N-terminal end of the chick skin collagen (4,5). Furthermore the CNBr peptides from rat and chick collagen show a close homology (7). The only major difference is that α1-CB6 from the rat is represented by two peptides, α1-CB6A and α1-CB6B, from the chick consistent with the presence of an extra methionyl residue in the α1 chain of the chick collagen. Therefore the α1 chains of these four collagens can be considered together for purposes of ordering the CNBr peptides.

The order of the peptides has been deduced from a variety of evidence. Since CNBr cleavage converts methionine to homoserine which appears as the C-terminal residue on each peptide except the C-terminal peptide and α1-CB6 contains no homoserine, α1-CB6 must be C-terminal. In the case of chick collagen, α1-CB6B contains no homoserine and is therefore C-terminal. α1-CB6A must immediately preceed α1-CB6B since the two together are clearly homologous by molecular weight and amino acid composition to α1-CB6 (4).

The presence in CNBr digests of products of incomplete cleavage permits the isolation of fragments containing a methionyl residue and therefore representing two adjacent CNBr peptides. From the isolation and characterization of α1-CB(0-1) and α1-CB(1-2) it has been shown that α1-CB0, 1, and 2 are N-terminal and have the order 0-1-2. These data have been published (4,5,8,9). α1-CB(3-7) and α1-CB(4-5) have recently been isolated. The former chromatographs after α1-CB7 on CM-cellulose and was purified by molecular sieve chromatography on 8% agarose (4). The latter was isolated by molecular sieve chromatography followed by phosphocellulose chromatography as described (4), except that a gradient from 0.1 to 0.5 N NaCl over a total volume of 600 ml was used. In each case the uncleaved peptides have the molecular weight (10) and the amino acid composition predicted from the composition of the individual peptides (4). Data for the peptides from chick bone collagen α1 are presented in Table I. Similar data have been obtained for the homologous peptides from rat skin collagen α1. That α1-CB4 precedes α1-CB5 is shown by

the existence of an overlap peptide isolated from an enzymatic digest containing the C-terminal two residues of α1-CB4 and the N-terminal residues of α1-CB5 in the same sequence as in the individual peptides (11). The identification is unequivocal since the overlap peptide contains O-glucosylgalactosyl hydroxylysine which occupies the N-terminal position of α1-CB5 and represents the only position in the α1 chain of rat skin collagen containing a significant amount of carbohydrate (2,12). The order of the peptides in α1-CB(4-5) has been confirmed by N-terminal analysis. Glycine was shown to be the N-terminal of α1-CB(4-5) by dansylation. Glycine is also the N-terminal of α1-CB4 but not of α1-CB5 (12).

That α1-CB7 immediately precedes α1-CB6 is shown by a comparison of the CNBr peptides with the products produced by the action of tadpole collagenase on native collagen. This enzyme cuts through all three chains of the collagen molecule one-quarter of the way in from the C-terminal end producing a C-terminal piece from α1 that by composition and molecular weight (13) must consist

Table I

Amino acid composition and molecular weight of uncleaved CNBr peptides from chick bone collagen α1. Results are given as residues per peptide

	α1-CB(3-7)		α1-CB(4-5)	
Amino Acid	Predicted	Found	Predicted	Found
Hydroxyproline	47	46	10	9.8
Aspartic acid	18	18	5	4.8
Threonine	8	8.3	1	1.1
Serine	8	8.2	2	2.2
Glutamic acid	35	34	7	6.8
Proline	48	47	7	7.4
Glycine	141	141	28	28
Alanine	59	59	7	6.8
Valine	6	6.0	-	-
Methionine	1	0.7	1	0.7
Isoleucine	4	4.0	-	-
Leucine	8	8.2	3	3.0
Phenylalanine	6	5.8	1	1.2
Hydroxylysine	2.6	2.8	1.5	1.7
Lysine	13.4	13.0	3.5	3.0
Histidine	-	-	1	1.2
Arginine	19	19	5	5.1
Homoserine	1	1.0	1	1.0
Total Residues	425	422	84	84
Molecular Weight	37300	38000	7750	7600

of α1-CB6 plus a portion of α1-CB7 (7). The isolation of α1-CB(3-7), discussed above, requires that α1-CB3 and 7 be adjacent, and since 7 precedes 6, 3 must precede 7. The order in the C-terminal portion of the α1 chain is then 3-7-6 or 3-7-6A-6B in the case of the chick collagens.

The position of α1-CB8 has been determined by renaturating the peptide to form a shortened, collagen-like molecule and comparing the segment-long-spacing (SLS) form with the SLS form of whole collagen by electron microscopy (7). This comparison is possible since alternate charged and uncharged regions along the molecule can be seen after appropriate staining as a band pattern which is unique throughout the length of the molecule. The pattern is clearly visible since the collagen molecules in the SLS form lie side-by-side in bundles. The bands produced by renatured α1-CB8 could be uniquely matched to a portion of the pattern of whole collagen lying between the positions of α1-CB0, 1 and 2 at the N-terminal end and α1-CB3, 7 and 6 at the C-terminal end. The position of α1-CB8 was difficult to determine exactly and it was first suggested (7) that α1-CB4 preceded and α1-CB5 followed α1-CB8. However, the isolation of α1-CB(4-5) indicates that α1-CB4 and 5 must be adjacent. Furthermore, Rauterberg and Kühn (14) have renatured α1-CB8, 3, 7 and 6 and have shown from the SLS patterns that they have the order 8-3-7-6. They have also isolated a fragment identified as α1-CB(8-3) by electron microscopy and amino acid composition which rules out any possibility that a small peptide such as α1-CB4 or 5 could lie between α1-CB8 and 3 (15). α1-CB4 and 5 must then precede α1-CB8 giving a complete order for the α1 chain of the rat collagens of

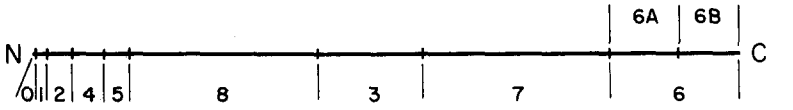

Fig. 1. A linear representation of the α1 chain of rat (skin or tendon) and chick (skin or bone) collagen showing the order and relative sizes of the CNBr peptides. The peptides from the two species are homologous; α1-CB6A and 6B from the chick are equivalent in sum to α1-CB6 from the rat. In rat skin α1-CB0 (a dipeptide) and two residues of α1-CB1 are absent.

0-1-2-4-5-8-3-7-6. In the case of the chick collagens, 6A-6B replaces 6. The four missing residues in the α1 chain from rat skin collagen include the residues comprising α1-CB0 (a dipeptide, pyrrolidonecarboxyl homoserine) and the first two residues of α1-CB1 (3). The order of the peptides is summarized in Fig. 1 where the length of each peptide is proportional to its size (7).

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