ISOLATION AND CHARACTERIZATION OF CYANOGEN BROMIDE PEPTIDES FROM BASEMENT MEMBRANE COLLAGEN

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Summary: Twelve peptides were isolated and characterized after cyanogen bromide cleavage of the α-chain of basement membrane collagen from bovine lens capsule. Glycine accounted for about 1/3 of all amino acids in each peptide. 4-Hydroxyproline was present in all; 3-hydroxyproline, in 7, and hydroxylysine, in 9 peptides. 54 to 100% of the hydroxylysine in these peptides was substituted with glucosyl-galactose; only 1 contained galactose. One peptide contained 2 residues of mannose, 1 of glucosamine, 4 of half-cystine, 11 of hydroxylysine, and 7 of glucosyl-galactose. The molecular weight of the chain is 100033 without the hexose and 113600 including the hexose.

Introduction: There is evidence to indicate that the collagen molecule isolated from various basement membranes is composed of three identical α-1 chains (1). Basement membrane collagen appears to contain a genetically distinct type of α-1 chain which has not been isolated from interstitial collagens found in skin, tendon, bone or cartilage. The unique features of this collagen include the high hydroxylysine content as well as the high sum of lysine plus hydroxylysine, the markedly decreased amounts of alanine and arginine and the high amounts of valine, leucine, isoleucine and phenylalanine. Basement membrane collagens examined to date contain from 4 to 8 residues of half-cystine. Hexose, in the form of glucosyl-galactose, accounts for 10-13%, while mannose and hexosamine account for less than 0.2% by weight (2, 3).

The α-1 chains of interstitial collagen from a number of tissues have been further characterized by examining the peptides ob-

tained by cleavage with cyanogen bromide (4, 5, 6), a method which has proven quite useful in carrying out sequence studies on collagen (7).

In the present study, we report on the isolation and characterization of peptides obtained by cleavage with cyanogen bromide of the α-1 chain of basement membrane collagen from bovine lens capsule.

Materials and Methods: The collagen from anterior lens capsules was prepared according to the method described previously with a slight modification (1). Prior to denaturation and chromatography on CM-cellulose, the collagen was further purified on a Bio-Gel P-300 column which was equilibrated and eluted with 0.1 M acetic acid at 4° C. The α-1 chains were prepared by chromatography on CM-cellulose according to the method of Piez, Eigner & Lewis (8) as described previously (1).

Cleavage with cyanogen bromide was performed essentially according to the methods described by Miller et al (6) and Epstein et al (9). For this purpose, samples of protein weighing 100 mg were dissolved in 20 ml of 70% formic acid, the solution flushed with nitrogen and a weight of CNBr (1.1 gm) equal to 150-fold molar excess relative to methionyl residues of the dissolved collagen was added. The mixture was incubated at 30° for 4 hrs, diluted 10-fold with distilled water and the volume reduced to one-half by rotary evaporation following which it was lyophilized, dissolved in water and relyophilized. A modification of the methods described (6, 9) involved a second treatment of the lyophilized material with CNBr to insure more complete cleavage of methionyl residues, followed again by evaporation and lyophilization as described above.

Small peptides were separated from the larger ones by

molecular sieve chromatography on a column (1.5 x 87 cm) of Bio-Gel P-4 (100-200 mesh) equilibrated with 0.1 M acetic acid. Thirty milligrams of the lyophilized peptides were dissolved in 1 ml 0.1 M acetic acid and applied on the column. Elution was carried out with the same solvent at a flow rate of 24 ml per hr. A representative elution pattern is shown in Figure 1. The peptides eluted in the three small peaks of the P-4 column were further chromatographed on a phosphocellulose column as described by Miller (6). The most retarded peak on P-4 was resolved into 2 components containing peptides 1 and 2 while the other 2 peaks contained essentially single components.

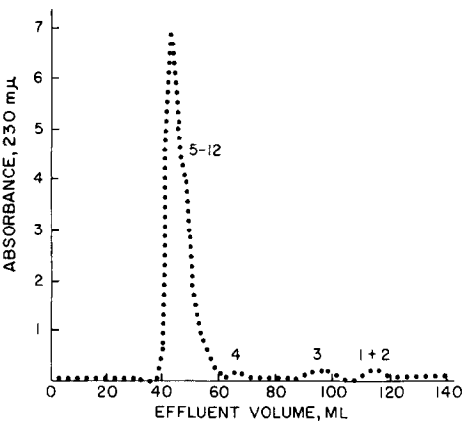

Figure 1. Elution pattern of CNBr peptides from the α-1 chain of bovine lens capsule collagen chromatographed on Bio-Gel P-4. Elution was carried out with 0.1 N acetic acid at a flow rate of 24 ml hr.

The peptides which eluted in the exclusion volume of the P-4 column were further resolved on a CM-cellulose column (1.5 x 17.5 cm) using the conditions described by Miller (6). A representative experiment appears in Figure 2.

Amino acid and hexosamine analyses were performed according to previously described methods (10). Neutral sugars were measured with the Technicon automatic sugar analyzer. The hydroxylysine linked

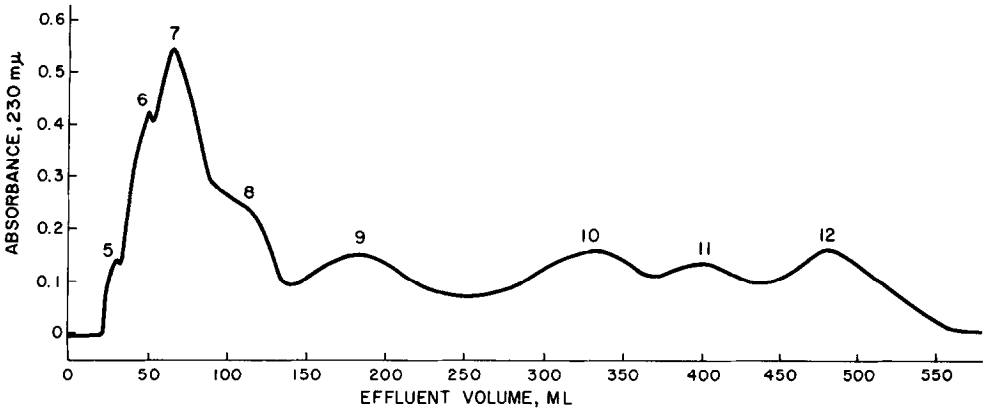

Figure 2. CM-cellulose chromatogram of the large CNBr peptides from the α-1 chain of bovine lens capsule collagen which are eluted in the exclusion volume of the P-4 column (Figure 1). Elution was accomplished in 0.02 M (Na^+) sodium citrate (pH 3-6) using a linear salt gradient of NaCl from 0.02 to 0.14 M in a total volume of 1000 ml.

glycosides glucosyl-galactose and galactose were isolated according to a previous method (11) and measured by the method of Askenasi & Kefalides (12) on an amino acid analyzer.

Results and Discussion: The CNBr cleaved peptides from lens capsule collagen were resolved into four fractions on Bio-Gel P-4 (Figure 1). Peptides 1 through 4 are designated as such according to their size, 1 having the smallest molecular weight. Peptides 5 through 12 were assigned numbers according to their position in the chromatogram (Figure 2).

The amino acid composition, as well as the molecular weights of the peptides, appear on Table I. No homologies have been noted between these peptides and peptides from interstitial collagens published so far. Glycine accounts for about 1/3 of the amino acid residues in all peptides. It will be noted that peptides 1-4 which were resolved on P-4 are the smallest. Peptides 1 and 2 contain no hydroxylysine or hexose. Peptides 3 and 4 contain 1 residue each of hydroxylysine and of glucosyl-galactose. Pep-

TABLE 1

Amino Acid Composition[a] of CNBr Peptides of the α-1 Chains of Bovine Lens Capsule Collagen

	1	2	3	4	5	6	7	8	9	10	11	12	Total CNBr Peptides
HOLysine[b]	0	0	1 (0.7)	1	0	4 (3.9)	7 (6.8)	7 (6.6)	9	8.5	13 (13.4)	8 (8.3)	58.5
Lysine[b]	0	0	1 (0.5)	1	2	0	1 (1.2)	1 (1.4)	3	1 (1.3)	3 (2.6)	2 (1.7)	15
Histidine	0	0	1	0	1	0	1	2	2	1	1	1	10
Arginine	0	0	0	0	0	0	1	1	2	5	5	5	19
3-HO-Proline[b]	0	0	0 (0.2)	0	0	1 (0.8)	1 (1.1)	1 (0.5)	1 (0.5)	1.5	2	2 (1.7)	9.5 9.5
4-HO-Proline[b]	1 (0.7)	1 (0.6)	1 (1.2)	3	2	8 (7.7)	13 (12.9)	10 (9.5)	14 (13.5)	27	31	21 (21.3)	132
Aspartic	0	0	1	2	5	2	5	4	5	8	9	9	50
Threonine	0	0	0	1	3	2	2	2	2	2	3	5	22
Serine	1	1	0	1	3	2	3	3	8	7	8	5	42
Glutamic	1	1	0	2	4	6	8	6	7	12	16	15	78
Proline[b]	0 (0.3)	0 (0.4)	1 (0.6)	1	4	4	7 (6.9)	5	7	12 (12.4)	14	13	68
Glycine	2	3	3	9	31	20	33	26	37	58	72	54	348
Alanine	0	0	0	1	4	2	3	4	4	7	8	6	39
Valine	0	1	1	1	3	1	3	2	2	5	5	5	29
1/2 Cystine	0	0	0	0	0	0	0	0	0	0	0	4	4
Isoleucine	0	0	0	1	2	2	2	2	3	6	7	4	29
Leucine	0	0	0	2	4	4	6	3	6	9	11	9	54
Tyrosine	0	0	0	0	1	0	0	0	0	0	0	2	3
Phenylal.	0	0	0	1	1	1	3	1	3	5	6	5	26
Homoserine	1	1	1	1	0	1	1	1	1	1	1	1	11
Total	6	8	11	28	70	60	100	81	116	176	215	176	1047
Mol. Wt.	593	749	1153	2704	6022	5718	9648	7817	11217	16906	19728	17196	100033

a. Residues per peptide, rounded off to the nearest whole number.

b. Actual values for lysine, hydroxylysine, proline and hydroxyproline are given in parentheses because of the possibility of partial hydroxylation giving rise to nonintegral values.

tide 5, with a molecular weight of 6022, contains no homoserine and must represent the carboxyl terminal peptide.

Hydroxyproline is present in all peptides and the ratio of 4-hydroxyproline to glycine is almost constant except in the C-terminal peptide where it is significantly lower. The degree of hydroxylation of proline is almost identical in all peptides and varies between 60 and 75%, except in the C-terminal peptide where it is 30%. The average percent hydroxylation of proline in most interstitial collagens is about 42. 3-Hydroxyproline is present in only 7 of the peptides and represents about 8% of the hydroxyproline. No interstitial collagen contains as much 3-hydroxyproline as does basement membrane collagen.

Hydroxylysine and hydroxylysine-bound glucosyl-galactose appear to be distributed throughout the peptide chain since they are found in 9 of the 12 peptides (Table II). The degree of hydroxylation of lysine in those peptides containing hydroxylysine varies between 50% and 100% with an average of 82%. The degree of glycosylation varies between 50 and 100% with an av-

TABLE II

Carbohydrate Composition of CNBr Peptides of the α-1 Chains of Bovine Lens Capsule Collagen

	1	2	3	4	5	6	7	8	9	10	11	12	Total CNBr Peptides
Glc-Gal-HOLys	0	0	0.85	0.9	0	3	5	5	6	6	7	6	39.5
Gal-HOLys	0	0	0	0	0	0	0	0	0	1	0	0	1
Percent Glycosylation of HOLys	-	-	100	100	-	78	73.5	76	78	75	54	87.5	80.2
Mannose	0	0	0	0	0	0	0	0	0	0	0	2	2
Glucosamine	0	0	0	0	0	0	0	0	0	0	0	1	1

erage of 80%. Again a similarity among peptides is noted with respect to degree of glycosylation, a feature not observed with other interstitial collagens. It should be pointed out that the amount of arginine in these peptides does not correlate with the degree of glycosylation of hydroxylysine, although the presence of arginine in a triplet vicinal to glycosylated hydroxylysine has been reported for rat skin collagen (13).

A very interesting observation was the presence of all the half-cystine in a single peptide (Table II, peptide 12). This fact, along with the presence of all the mannose and glucosamine in the same peptide, suggest the possibility that a peptide, probably non-collagen, which contains a trisaccharide mannosyl-mannosyl glucosamine is linked to the collagen polypeptide via disulfide bonds (3, 14). Digestion of the lens capsule with pepsin cleaves-off the non-collagen polypeptide leaving only the linkage region between it and the collagen chain.

Other important observations noted in previous studies (1, 2, 3) and confirmed here include the low arginine and alanine content. Although the alanine content is about 1/3 of that present in most interstitial collagens, the sum of the non-polar amino acids is almost the same by virtue of the marked increases in the content of valine, isoleucine, leucine and phenylalanine.

The molecular weight of the α-1 chain calculated from the amino acid content is 100033; if we add the contribution of glucosyl-galactose, galactose, mannose and glucosamine, we obtain a molecular weight of 113600. This is consistent with the weight for intact α-1 chains of sheep lens capsule collagen (1) and the newly synthesized collagen by chick embryo lenses (15).

The data indicate that the peptides of basement membrane collagen from lens capsule are derived from a genetically distinct

α-1 chain. On the basis of the differences noted between basement membrane collagens from the glomerulus and Descemet's membrane and interstitial collagens, it can be stated that basement membrane collagens in general form a genetically distinct group of proteins.

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