sessing relative substrate specificities of different nucleases. For example, the bovine RNase hydrolysate of RNA eluted the chicken nuclease because the specificities of the two enzymes differ.

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Characterization of the Cyanogen Bromide Peptides from the α1 Chain of Chick Skin Collagen*

Andrew H. Kang,† Karl A. Piez, and Jerome Gross

ABSTRACT: The α1 chain of chick skin collagen was cleaved at the methionyl residues with cyanogen bromide and the resulting peptides were separated and characterized. Ten peptides of varying molecular weight ranging from 242 to 25,000 were obtained. The sum of the molecular weights of these peptides as determined by gel filtration was 91,567, and that determined by amino acid analysis was 90,721, values which are in good agreement with the known molecular weight of the α1 chain.

The sum of the amino acid compositions also agrees with the composition of the whole α1 chain. These data indicate that the ten peptides account for the entire amino acid sequence represented in the original α1 chain. The finding of only ten unique peptides from a chain containing nine methionyl residues indicates that the two α1 chains of chick skin collagen are identical or very similar in their amino acid sequence. These peptides are similar and clearly homologous to the cyanogen bromide peptides previously isolated from the α1 chain of rat skin collagen. They are identical in every respect examined with the cyanogen bromide peptides from the α1 chain of chick bone collagen except for the degree of hydroxylation of lysine. This result suggests that chick bone and skin collagens are derived from the same structural genes and, therefore, that the different properties of the two tissues cannot be explained on this basis.

The amino acid sequence of the collagen molecule, a rigid three-chain rod-shaped structure (3000 Å long and 13 Å wide), has been difficult to establish because of its great size. It has been possible to separate and isolate the three chains by gentle heat denaturation and chromatography on CM-cellulose which, in the case of most vertebrate collagens, provides two α1 chains identical in amino acid composition and one α2 chain with a different amino acid composition and chromatographic behavior. Both α1 and α2 have a molecular weight of about 95,000. CNBr cleavage of the seven methionyl bonds of the α1 chain of rat skin collagen has produced eight peptides of different sizes, ranging from 1,400 to 25,000 in molecular weight. These have been separated, purified, and characterized as to their molecular weight and amino acid composition (Butler *et al.*, 1967) and the amino acid sequence has been determined for two of the smaller ones (Kang *et al.*, 1967; Bornstein, 1967). In addition, by a combination of chemical analyses and localization of one of the larger peptides by electron microscopy, Piez *et al.* (1969) have succeeded in tentatively ordering all cyanogen bromide peptides along the length of the α1 chain of rat skin collagen.

In order to obtain a more general picture of the primary structure of collagen, it was deemed essential to study a collagen of another class, which can be purified as well as that from mammals, can be readily solubilized, and is stable under usual laboratory conditions. We report here the isolation and characterization of the CNBr peptides from the α1 chain of chick skin collagen. The recent study on chick bone collagen (Miller *et al.*, 1969) provides an opportunity

* From the Developmental Biology Laboratory, Departments of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts (A. H. K. and J. G.), and the Laboratory of Biochemistry, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland (K. A. P.). *Received November 12, 1968.* This is publication 473 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities. This work has been supported by grants from the U. S. Public Health Service (AM-3564) and from the RGK Foundation.

† Senior Investigator of the Arthritis Foundation.

for a careful comparison of two collagens from different tissues of one species. The comparison is particularly valid since the two studies were done independently and comparisons were not made until both studies were nearly complete. The nomenclature applied to the CNBr peptides has been discussed (Miller *et al.*, 1969). The preparation of native collagen from chick skin and some of its properties with particular regard to the cross-linking site will be reported separately (A. H. Kang, K. A. Piez, and J. Gross, in preparation).

Experimental Section

Preparation of α1. Neutral salt and acid-extracted collagens were prepared from the skins of 3-week-old, White Leghorn chicks. All operations were performed at 4°. The chicks were sacrificed by decapitation and the feathers were carefully removed from the skin. The skin was cut into small pieces, ground with a mechanical meat grinder with chips of Dry Ice, and washed with a large volume of cold water. The ground skin was extracted with five volumes of 0.05 M Tris buffer (pH 7.0) containing 1 M sodium chloride for 48 hr with occasional gentle stirring. The extract was filtered through cheesecloth and further clarified by centrifugation at 13,000*g*. The residue was washed with a large volume of cold water and extracted with five volumes of 0.5 M acetic acid overnight. The neutral salt and acid extracts were then purified by the methods previously described (Kang *et al.*, 1966). Lathyritic collagen was prepared from 3-week-old chicks (White Leghorn) which had been fed a commercial diet containing 0.1% β-aminopropionitrile fumarate. The only difference between the lathyritic and normal collagens was in the relative amounts of the lysine- and aldehyde-containing forms of the peptides involved in the formation of interchain covalent cross-links as previously shown for rat skin collagen (Bornstein *et al.*, 1966; Bornstein and Piez, 1966). Since the source of the collagen did not influence the results reported here, the source is not specified. Undegraded α1 chains were obtained from solubilized, heat-denatured collagen by chromatography on 2.5 × 15 cm columns of CM-cellulose (Whatman CM-32) at 43° as previously described and desalted on columns of Sephadex G-25 equilibrated with pyridine acetate buffer (pH 4.8) (Piez *et al.*, 1963; Bornstein and Piez, 1966).

CNBr Cleavage. Approximately 100–200 mg of protein was dissolved in 0.1 N HCl at a concentration of 5 mg/ml and reacted with a 100-fold molar excess (relative to methionine) of CNBr at 30° for 4 hr under nitrogen as described previously (Bornstein and Piez, 1966; Kang *et al.*, 1967). The reaction was terminated by dilution with ten volumes of cold water and lyophilization.

Chromatography of CNBr Peptides. The lyophilized CNBr peptides were first chromatographed on phosphocellulose as described by Bornstein and Piez (1966) with modification. Columns (2.5 × 25 cm) of phosphocellulose were equilibrated with 0.001 M sodium formate buffer (pH 3.6) at 40°. Samples of CNBr digest (100–200 mg) were dissolved in 10 ml of this buffer and pumped onto the column. Chromatography was performed by superimposing a linear gradient of NaCl from 0 to 0.6 M over a total volume of 1600 ml. The optical density of the effluent was monitored at 230 mμ in a Gilford or Beckman DB spectrophotometer. Fractions of 10 ml were collected. In the initial fractionation of the CNBr peptides on phosphocellulose, six peptides (α1-CB0–α1-CB6A) were resolved (see Figure 1). After the elution of α1-CB6A, the remaining peptides were eluted as one peak with 1 M NaCl solution. The peptides were desalted on columns of Bio-Gel P-2 (200–400 mesh) equilibrated with 0.15 M acetic acid or on Sephadex G-15 equilibrated with pyridine acetate (pH 4.8).

The peptides eluted with 1 M NaCl from the phosphocellulose columns were fractionated further on columns of CM-cellulose as described by Butler *et al.* (1967) with modification. Columns (2.5 × 15 cm) of CM-cellulose were equilibrated at 40° with 0.01 M sodium formate (pH 3.4) containing 0.05 M NaCl. Samples of CNBr peptides were dissolved in 10 ml of the same buffer and applied to the column. The elution was carried out with a linear gradient of NaCl from 0.05 to 0.15 M superimposed on a total volume of 1600 ml. Resolution of two small peptides (α1-CB4 and α1-CB5) which did not separate on CM-cellulose was accomplished by rechromatography on phosphocellulose. Two of the larger peptides obtained from CM-chromatography at pH 3.4 (α1-CB7 and α1-CB8) were further purified by chromatography on CM-cellulose columns at pH 4.8 as described earlier (Bornstein and Piez, 1965). A concave gradient prepared by a constant level device consisting of 1000 ml of 0.02 M sodium acetate (pH 4.8) in a 1-l. beaker (mixing chamber) and 740 ml of the same buffer containing 0.14 M NaCl in a 750-ml erlenmeyer flask was utilized.

Molecular Weight Determination. Molecular weights of the peptides were determined by molecular sieve chromatography as described by Piez (1968). A column (2 × 110 cm) was packed with Sephadex G-150 suspended in 1 M $CaCl_2$ containing 0.01 M Tris-HCl (pH 7.5). The samples were applied to the column in 2 ml of the buffer containing 0.1 μCi of tritiated water. The effluent was monitored at 230 mμ with a Beckman DB spectrophotometer. A standard curve of log molecular weight of peptides *vs.* the ratio of the elution volume of the peptides, V_E, to the elution volume of the excluded peptide, V_0, in this case α1, was first determined by using the CNBr peptides derived from rat skin collagen.[1] The molecular weights of the CNBr peptides of the α1 chain of rat skin collagen used here had been previously determined by the sedimentation equilibrium method of Yphantis (Butler *et al.*, 1967). Thus by measuring the V_E/V_0 of a given peptide, its molecular weight could be determined from the standard chart.

Amino Acid Analysis. Samples were hydrolyzed in constant-boiling HCl at 108° for 24 hr in an atmosphere of nitrogen. Analyses were performed on a single-

[1] The α1 CNBr peptides of rat skin collagen used here were generous gifts of Dr. William T. Butler.

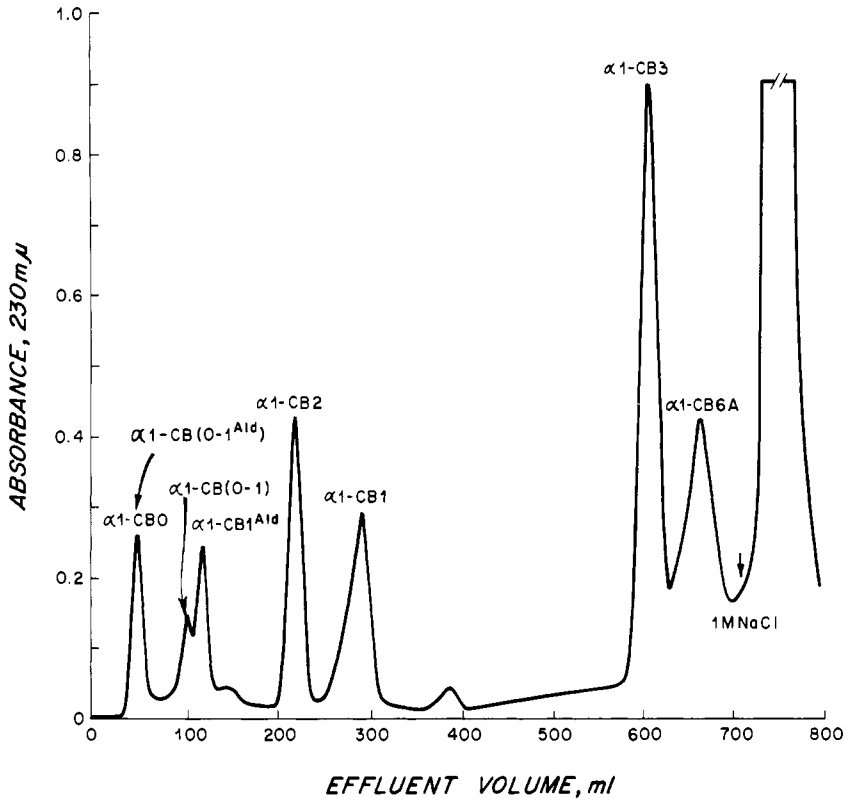

FIGURE 1: Chromatography of a CNBr digest of the $\alpha 1$ chain of chick skin collagen on phosphocellulose at pH 3.6, 40°. Elution was with a linear gradient of 0.001 M sodium formate (pH 3.6) to 0.001 M sodium formate and 0.6 M NaCl (pH 3.6) over a total volume of 1600 ml.

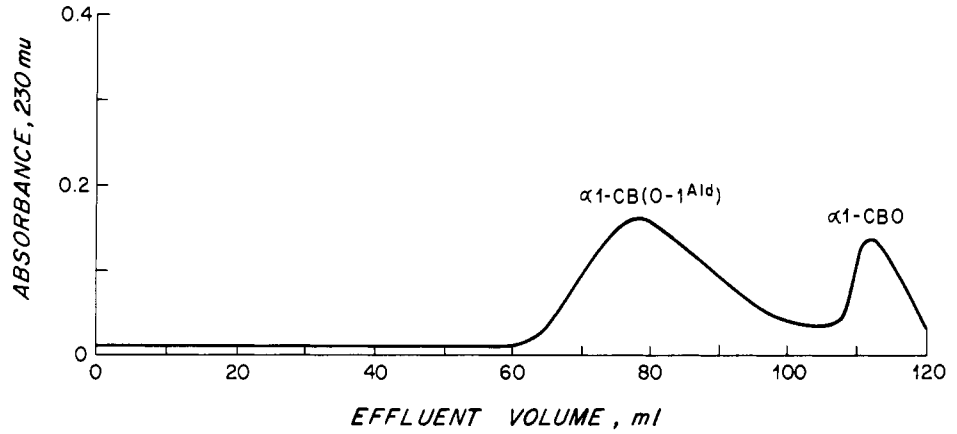

FIGURE 2: Chromatography of the peak containing $\alpha 1$-CB0 and $\alpha 1$-CB(0-1^{Ald}) (see Figure 1) on Bio-Gel P-2, 200-400 mesh in 0.15 M acetic acid. The column size was 2 × 50 cm.

column automatic amino acid analyzer modified for high-speed analysis (Miller and Piez, 1966). Correction factors for hydrolytic losses of threonine, serine, methionine, and tyrosine and incomplete release of valine were used as previously determined (Piez *et al.*, 1960). Where methionine sulfoxide was found, it was added to the value of methionine.

Results

Chromatography. A representative elution pattern from a phosphocellulose column of the mixture of peptides formed by cleavage of $\alpha 1$ with CNBr is illustrated in Figure 1. The identity of the peptides was established by amino acid composition and molecular weight (presented later) and by homology to the CNBr peptides from the $\alpha 1$ chain of rat skin collagen. The peptides remaining on the column after the elution of $\alpha 1$-CB6A were eluted as one peak by increasing the NaCl concentration of the elutrient to 1 M. The peak eluting at the start of the salt gradient was comprised of largely nonprotein ultraviolet-absorbing material as

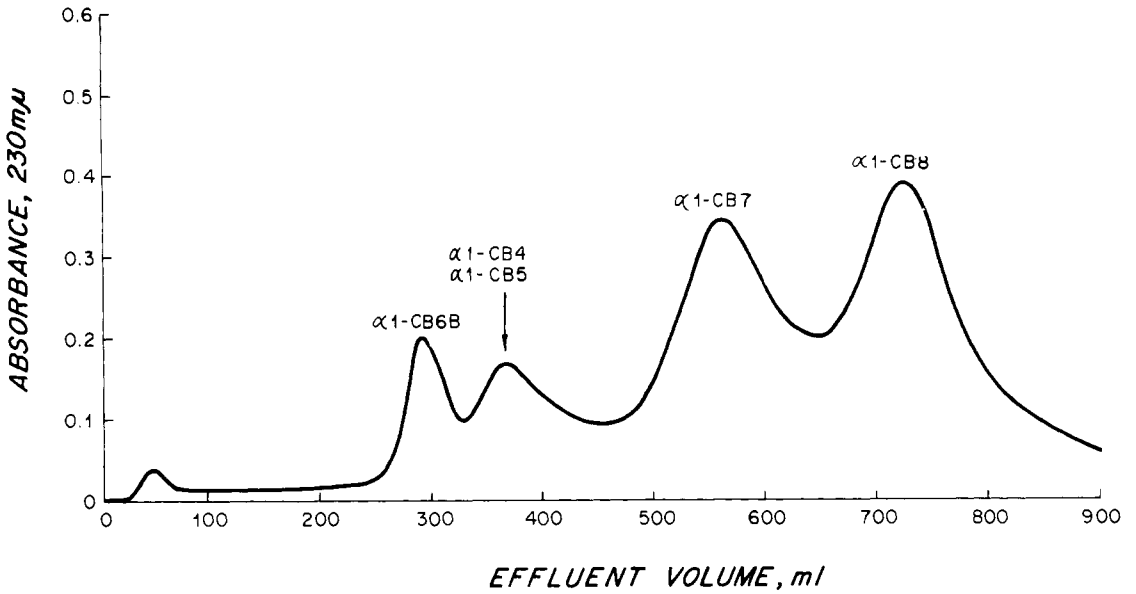

FIGURE 3: Chromatography of the CNBr peptides eluted from phosphocellulose with 1 M NaCl solution (see Figure 1) on CM-cellulose at pH 3.4, 40°. Elution was with a linear gradient of 0.01 M sodium formate, 0.05 M NaCl (pH 3.4) to 0.01 M sodium formate, and 0.15 M NaCl (pH 3.4) over a total volume of 1600 ml.

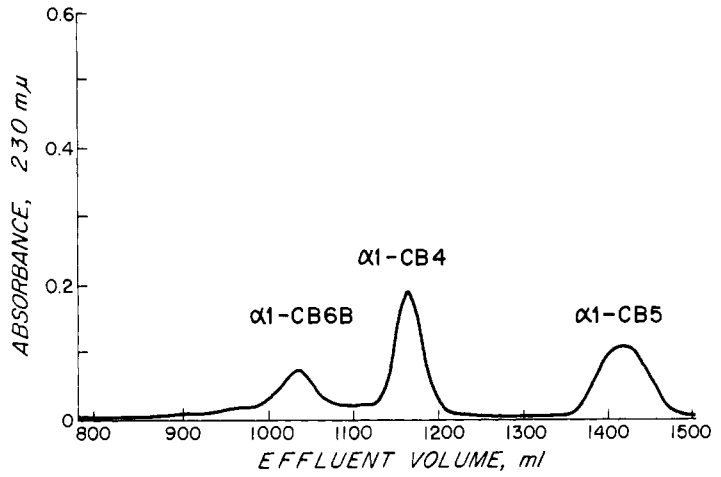

FIGURE 4: Chromatography of the peak containing α1-CB4 and α1-CB5 (see Figure 3) on phosphocellulose. The conditions are identical with that described in Figure 1 except that elution was continued for a longer time.

well as a dipeptide, α1-CB0, and a second peptide, α1-CB(0–1^{Ald}). When rechromatographed on Bio-Gel P-2 (200–400 mesh), α1-CB(0–1^{Ald}) was eluted well ahead of α1-CB0, which was eluted in an effluent volume corresponding to the fluid volume of the column (Figure 2). α1-CB0 so obtained was free of other peptide material but was contaminated with salt. However, the salt did not interfere with subsequent amino acid analysis after hydrolysis. α1-$CB1^{Ald}$ is the aldehyde-containing form of α1-CB1 whose single lysyl residue has been converted into an aldehyde preliminary to the formation of interchain covalent cross-links (A. H. Kang, K. A. Piez, and J. Gross, in preparation). Similar events have been shown to occur in rat skin collagen (Bornstein *et al.*, 1966; Bornstein and Piez, 1966; Piez *et al.*, 1966). α1-CB(0–1) and α1-$CB1^{Ald}$ can be separated from each other by rechromatography on phosphocellulose.

The peptides eluted with 1 M NaCl from phosphocellulose were desalted and then factionated on columns of CM-cellulose at pH 3.4. Figure 3 illustrates a typical pattern. The first peak appearing at the start of the salt gradient consisted of nonpeptide ultraviolet-absorbing material. Two small peptides, α1-CB4 and α1-CB5, were not resolved in this system, but were resolved by rechromatography on phosphocellulose (pH 3.6) (Figure 4). The peptides α1-CB7 and α1-CB8 were further purified by chromatography of each on CM-cellulose at pH 4.8. By this procedure, the α1-CB7 peak was separated into one major (α1-CB7) and two minor (α1-CB7′ and α1-CB7″) components (Figure 5). Similarly, chromatography of α1-CB8 yielded one major (α1-CB8) and two minor (α1-CB8′ and α1-CB8″) components (Figure 6). However, molecular weight studies, amino acid analyses, and acrylamide disc electrophoresis of the two minor components did not

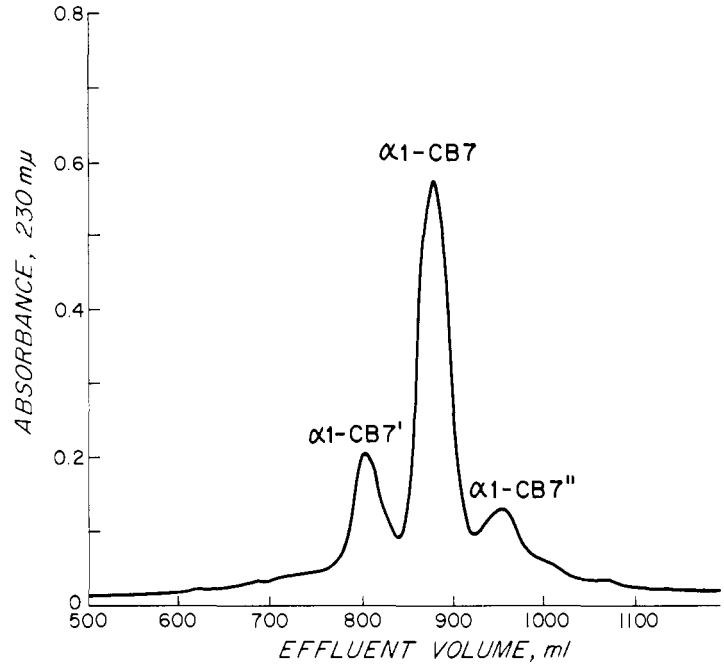

FIGURE 5: Rechromatography of α1-CB7 (Figure 2) on CM-cellulose at pH 4.8, 40°. Elution was with a concave gradient of 1000 ml of 0.02 M sodium acetate (pH 4.8) to 740 ml of 0.02 M sodium acetate and 0.14 M NaCl (pH 4.8). See text for details.

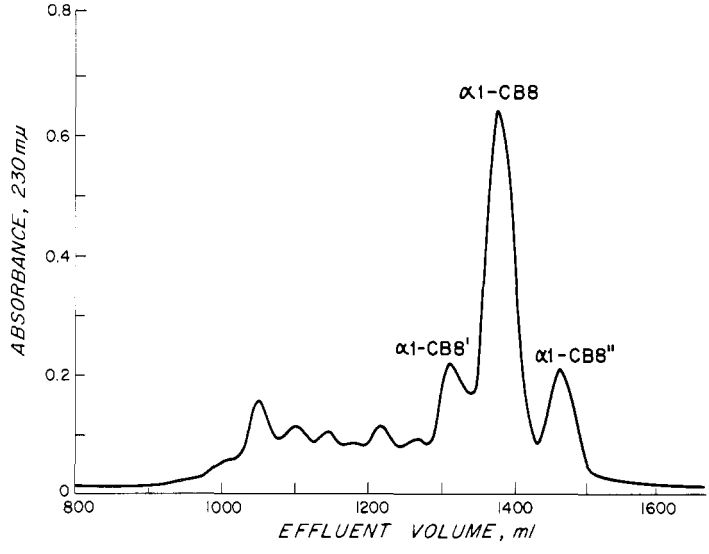

FIGURE 6: Rechromatography of α1-CB8 on CM-cellulose at pH 4.8, 40°. See legend to Figure 5.

reveal any differences from the respective major components. Identical findings have been reported for the larger CNBr peptides from the α1 chain of rat skin collagen (Butler *et al.*, 1967). The more acidic component was shown to differ from the major component in that it contained homoserine as the free acid rather than the lactone. The more basic component gave the same tryptide map as the major component but its chromatographic difference remains unexplained.

By a combination of these procedures, then, a total of ten unique CNBr peptides has been obtained. This is consistent with the fact that the α1 chain of chick skin collagen contains nine residues of methionine. Miller *et al.* (1969) have also found the same number of CNBr peptides from the α1 chain of chick bone collagen and, furthermore, each of the CNBr peptides isolated from the α1 chains of chick bone collagen is identical with or very similar to the corresponding peptide from chick

TABLE 1: Amino Acid Composition of the CNBr Peptides from the α1 Chain of Chick Skin Collagen.[a]

	α1-CB0	α1-CB1	α1-CB2	α1-CB3	α1-CB4	α1-CB5	α1-CB6A	α1-CB6B	α1-CB7	α1-CB8	Total	α1[b]
3-Hydroxyproline	0	0	0	1	0	0	0	1 (0.6)	0	0	1	1 (0.9)
Hydroxyproline	0	0	6 (5.8)	14	6 (5.7)	4 (4.0)	7 (7.2)	9 (8.9)	29	31	106	111
Aspartic acid	0	1 (1.0)	0	7 (6.8)	3 (3.1)	2 (1.9)	5 (5.2)	4 (3.9)	11	10	43	44
Threonine	0	0	0	1 (1.0)	1 (0.9)	0	3 (2.6)	2 (1.7)	7 (6.8)	6 (6.2)	20	20
Serine	0	2 (1.9)	1 (0.9)	0	0	2 (1.8)	0	4 (3.7)	6 (6.4)	9 (9.1)	24	28
Glutamic acid	1 (1.0)	1 (1.1)	4 (4.0)	16	3 (3.0)	4 (3.8)	8 (7.8)	5 (5.0)	16	20	78	80
Proline	0	2 (2.1)	6 (6.1)	14	5 (5.4)	2 (2.2)	14	12	34	30	119	125
Glycine	0	3 (3.2)	12	46	16	12	32	26	89	88	324	341
Alanine	0	2 (2.1)	3 (3.1)	22	4 (4.1)	3 (3.0)	14	7 (7.4)	33	39	127	134
Valine	0	2 (2.0)	0	3 (3.1)	0	0	1 (1.2)	1 (2.0)	4 (4.0)	3 (3.1)	10	15
Methionine	0	0	0	0	0	0	0	0	0	0	0	9 (8.7)
Isoleucine	0	0	0	0	0	0	0	1 (1.0)	4 (4.0)	2 (2.0)	7	7 (6.7)
Leucine	0	0	1 (1.0)	2 (2.1)	2 (2.1)	1 (1.1)	1 (1.0)	3 (2.9)	6 (6.1)	4 (4.0)	20	21
Tyrosine	0	2 (1.9)	0	0	0	0	0	0	0	0	2	2 (1.8)
Phenylalanine	0	0	0	3 (2.8)	0	1 (1.0)	0	2 (1.7)	3 (3.2)	3 (3.0)	13	13
Hydroxylysine	0	0	0	0.4	0.1	1.3	0.8	0.2	0.8	0.8	4.4	5.4
Lysine	0	1 (0.9)	0	4.4	1.9	1.7	1.9	1.8	8.9	9.0	30.5	31.2
Histidine	0	0	0	0	0	1 (1.0)	0	1 (0.8)	0	0	2	2 (2.0)
Arginine	0	0	1 (1.0)	6 (6.0)	4 (4.0)	1 (1.0)	6 (6.1)	4 (4.0)	13	15	50	52
Homoserine[c]	1 (1.0)	1 (1.0)	1 (1.1)	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)	0	1 (1.0)	1 (1.0)	9	
Total	2	17	36	140	47	37	95	85	266	271	996	1041

[a] Residues per peptide. Values are rounded off to the nearest whole number except for lysine and hydroxylysine when both are present since they may not be integers (Miller *et al.*, 1969; Butler, 1968). Where less than ten residues were found actual values are shown in parentheses. A value of zero indicates less than 0.1 residue. [b] Values are computed for a molecular weight of 95,000 with an average residue weight of 91.2. [c] Includes homoserine lactone.

skin collagen with respect to amino acid composition and molecular weight.

Amino Acid Composition. The amino acid compositions of the purified CNBr peptides are presented in Table I. The data are presented as residues per peptide using those amino acid residues present in small amounts to calculate a minimum molecular weight and assuming one residue of homoserine (plus homoserine lactone) per peptide. Each peptide has a unique amino acid composition. α1-CB0 was found to contain one residue each of glutamic acid and homoersine (plus homoserine lactone). This peptide did not react with ninhydrin to yield the characteristic color. No NH_2-terminal residue could be detected using the dinitrophenylation and isothiocyanate method (A. H. Kang, K. A. Piez, and J. Gross, in preparation), suggesting that the glutamic acid residue may be in the form of pyrrolidone-5-carboxylic acid. A similar conclusion has been reached (Miller *et al.*, 1969) from other evidence for the same residue in chick bone collagen. The amino acid composition of α1-CB1 is similar to that of α1-CB1 from rat skin collagen (Bornstein *et al.*, 1966; Bornstein and Piez, 1966; Kang *et al.*, 1967) except that it has one more residue of tyrosine and alanine. It is identical with that of α1-CB1 from chick bone collagen (Miller *et al.*, 1969) except the lysyl residue is not hydroxylated in chick skin collagen. α1-CB2 is high in imino acid content and α1-CB3 is very rich in glutamic acid residues. α1-CB4 and α1-CB5 are small peptides like α1-CB2 but contain more basic amino acid residues. α1-CB5 has one of the two histidine residues. α1-CB6B contains no homoserine and thus must contain the COOH terminus of the α1 chain. It also contains the one residue of 3-hydroxyproline and one of the two histidine residues. In each of the peptides except α1-CB0 and α1-CB1, glycine represents approximately one-third of the residues.

Table I also compares the total amino acid contents accounted for by the ten CNBr peptides and those present in the original α1 chain. All of the approximately 1000 residues have been accounted for within experimental error suggesting that the ten peptides represent all of the α1 chain.

Amino Acid Compositions of α1-CB1^{Ald}, α1-CB(0–1), and α1-CB(0–1^{Ald}). The amino acid compositions of these peptides are given in Table II. α1-CB1^{Ald} has an amino acid composition identical with α1-CB1 except that it lacks a residue of lysine. However, it has been shown to contain a residue of lysine-derived aldehyde, δ-semialdehyde of α-aminoadipic acid (A. H. Kang, K. A. Piez, and J. Gross, in preparation). Identical findings have been reported for the corresponding peptides in rat skin collagen (Bornstein *et al.*, 1966; Bornstein and Piez, 1966). The amino acid composition of α1-CB(0–1) is identical with the sum of the amino acid compositions of α1-CB0 and α1-CB1 plus one residue of methionine, indicating that it arose from incomplete cleavage at the methionyl residue joining α1-CB0 and α1-CB1. An identical deduction can be made for α1-CB(0–1^{Ald}).

NH_2-terminal analyses by the Edman method have shown that serine is the NH_2-terminal residue of α1-CB1 and that α1-CB(0–1) has no available NH_2-terminal residue (A. H. Kang, K. A. Piez, and J. Gross, in preparation). These results demonstrate that α1-CB0 is on the amino side of α1-CB1. An identical conclusion has been reached for chick bone collagen from evidence based on tryptic digestions of α1-CB1 and α1-CB(0–1) (Miller *et al.*, 1969).

Molecular Weights. Figure 7 presents the plot of log molecular weight *vs.* V_E/V_0 of the rat skin CNBr peptides used to standardize the Sephadex G-150 column and shows the positions at which the CNBr peptides from chick skin collagen were eluted. Table III compares the molecular weights of the CNBr peptides as determined by amino acid analysis and by molecular sieve chromatography. The values obtained by the two methods are in good agreement in all instances. The sum of the molecular weights of these peptides, 90,721 by amino acid analysis, and 91,567 by

TABLE II: The Amino Acid Compositions[a] of Peptides α1-CB1^{Ald}, α1-CB(0–1), and α1-CB(0–1^{Ald}).

	α1-CB1^{Ald}	α1-CB(0–1)	α1-CB(0–1^{Ald})
Aspartic acid	1 (1.0)	1 (1.0)	1 (1.0)
Serine	2 (1.9)	2 (1.9)	2 (1.8)
Glutamic acid	1 (1.2)	2 (1.9)	2 (2.0)
Proline	2 (2.0)	2 (2.1)	2 (2.1)
Glycine	3 (3.0)	3 (3.1)	3 (3.2)
Alanine	2 (2.0)	2 (2.1)	2 (2.1)
Valine	2 (2.0)	2 (2.2)	2 (2.0)
Methionine[b]	0	1 (0.7)	1 (0.6)
Tyrosine	2 (1.8)	2 (1.8)	2 (1.8)
Lysine	0	1 (1.0)	0
Homoserine[c]	1 (1.0)	1 (1.1)	1 (1.1)

[a] Residues per peptide. [b] Includes methionine sulfoxide. [c] Includes homoserine lactone.

TABLE III: Molecular Weights of the CNBr Peptides of α1 as Measured by Amino Acid Analysis and by Molecular Sieve Chromatography.

Peptide	Amino Acid Anal.	Molecular Sieve Chromatography
α1-CB0	242	(242)[a]
α1-CB1	1,725	(1725)[a]
α1-CB2	3,322	3,200
α1-CB3	12,650	12,900
α1-CB4	4,447	4,600
α1-CB5	3,563	3,300
α1-CB6A	8,573	8,700
α1-CB6B	7,910	7,600
α1-CB7	23,995	24,300
α1-CB8	24,294	25,000
Total	90,721	91,567

[a] These values obtained only by amino acid analysis.

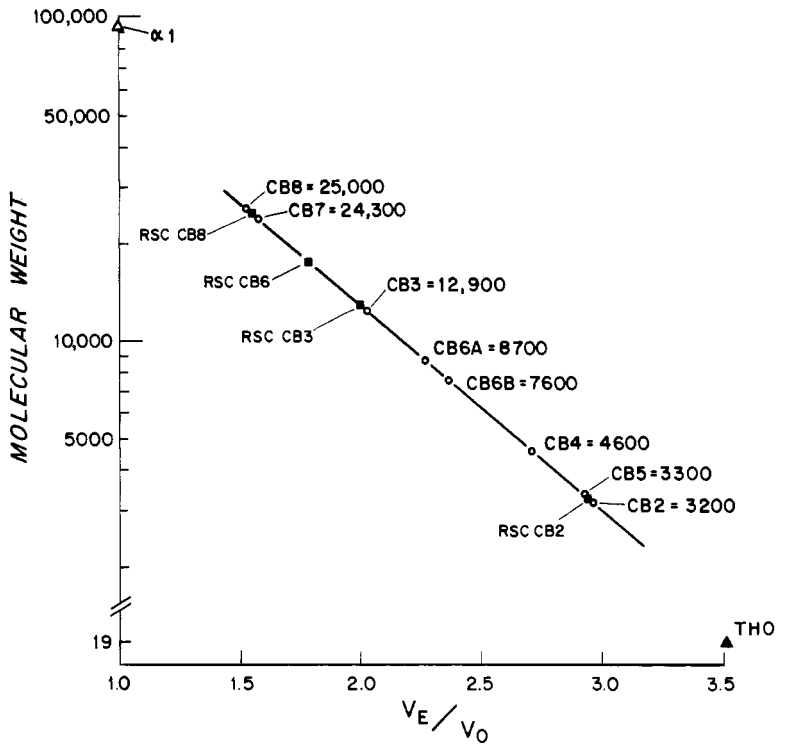

FIGURE 7: Plot of log molecular weight *vs.* elution volume, V_E/V_0, from Sephadex G-150 of standard peptides from rat skin collagen (RSC α1-CB8, α1-CB6, α1-CB3, and α1-CB2) and CNBr peptides from chick skin collagen α1. Uncleaved α1 and tritiated water (THO) were included as markers.

molecular sieve chromatography agrees well with the reported value of about 95,000 for the α1 chain (Lewis and Piez, 1964; Piez, 1965; Kang *et al.*, 1966).

Discussion

After cleavage of the α1 chain of chick skin collagen we have isolated ten unique peptides and characterized them with regard to amino acid composition and molecular weight. The number of peptides obtained is consistent with the known methionine content of the α1 chain of chick skin collagen and suggests that α1 of chick skin collagen is a single component. Although codfish skin collagen has been shown to contain three different α chains (Piez, 1964, 1965), other reports that calf skin collagen (Heidrich and Wynston, 1965; Francois and Glimcher, 1967a) and chick bone collagen (Francois and Glimcher, 1967b) contain three distinct α chains have been questioned (Miller *et al.*, 1967, 1969). Differences in primary structure sufficient to give rise to chromatographic heterogeneity of the whole α1 chain would have been detected in the present study. The finding of only ten unique peptides in the CNBr digests of α1 strongly suggests that the two α1 chains of chick skin collagen are identical. Similar conclusions have been reached by Butler *et al.* (1967) for rat skin collagen, and by Miller *et al.* (1969) for chick bone collagen

The ten CNBr peptides obtained in this study account for the amino acid content and the known molecular weight of the whole α1 chain from chick skin collagen. This is an important step toward the elucidation of the primary structure of a very large protein such as collagen. In the case of rat skin collagen, the amino acid sequences of two of the eight CNBr peptides from α1 have been determined (Kang *et al.*, 1966; Bornstein, 1967). Recently, it has been possible to assign a tentative order to the CNBr peptides of α1 from rat skin collagen (Piez *et al.*, 1969). This is α1-CB(1-2-4-8-5-3-7-6). Although some of the peptide locations need confirmatory evidence, they are probably correct and form a useful basis for comparison with other collagens.

Of the ten peptides obtained from chick skin collagen in this study, seven (α1-CB1, α1-CB2, α1-CB3, α1-CB4, α1-CB5, α1-CB7, and α1-CB8) are clearly homologous to peptides from the α1 chain of rat skin collagen. Each pair from the two sources have identical molecular weights and very similar unique characteristics in amino acid composition. Therefore, it is likely that the order is the same as in rat skin collagen. Of the remaining three (α1-CB0, α1-CB6A, and α1-CB6B), α1-CB0 has no homologous counterpart in α1 of rat skin collagen but it has been shown that α1-CB0 is located NH_2 terminal to α1-CB1 in α1 of chick skin collagen. α1-CB6B must be COOH terminal as it contains no homoserine. Since the COOH-terminal peptide of rat skin collagen α1, α1-CB6, has a molecular weight of 16,000, and since the sum of the molecular weights of α1-CB6A and α1-CB6B from chick is 16,300, it would seem reasonable to assume a homology between the sum of α1-CB6A and α1-CB6B of chick skin collagen and α1-CB6 of rat skin collagen. A comparison of

the amino acid compositions is also consistent with this conclusion. On the basis of homology, then, the probable order of the CNBr peptides of chick skin collagen $\alpha 1$ is $\alpha 1$-CB(0-1-2-4-8-5-3-7-6A-6B).

The studies of Miller *et al.* (1969) on the CNBr peptides of the $\alpha 1$ chain of chick bone collagen yielded ten peptides which are identical within experimental error in amino acid composition and molecular weight with those found in chick skin collagen. Thus, at least the $\alpha 1$ chains of the two collagens presumably have identical primary structures. Although the identity of the $\alpha 2$ chains of the two collagens has not yet been established in the same manner as has been accomplished for $\alpha 1$, the two $\alpha 2$ chains have the same amino acid composition and, therefore, it is likely that they also have identical primary structures. If these indications are correct, it would seem that the two collagens derive from the same structural genes. This suggests that modification of collagen after the biosynthesis of the polypeptide chains rather than a difference in primary structure is responsible for different properties of collagens from functionally different tissues. These modifications might include the formation of aldehydes and subsequently of covalent cross-links, the addition of carbohydrate moieties, the hydroxylation of prolyl and lysyl residues, and perhaps other subtle variants which have not yet been identified. An inspection of amino acid compositions of the CNBr peptides as presented in Table I shows that lysyl residues are hydroxylated to various extents in different peptides. $\alpha 1$-CB1 is the only lysine-containing peptide which did not contain hydroxylysine. The peptides $\alpha 1$-CB3, $\alpha 1$-CB4, $\alpha 1$-CB5, $\alpha 1$-CB6A, $\alpha 1$-CB6B, $\alpha 1$-CB7, and $\alpha 1$-CB8 all contain nonintegral numbers of residues of hydroxylysine. The lysine content of these peptides are such that the sum of the two residues is consistent with a whole number of residues. This has also been pointed out by Miller *et al.* (1969) for chick bone collagen $\alpha 1$ and by Butler (1968) for rat skin collagen $\alpha 1$. In the latter case it was shown that some positions containing lysine are hydroxylated about 5% of the time. The lysyl residue in $\alpha 1$-CB1 of chick bone collagen is hydroxylated about 50% of the time (Miller *et al.*, 1969) whereas the same residue in chick skin collagen is apparently never hydroxylated. Small but probably significant differences in the degree of hydroxylation of the two chick collagens can also be seen by a comparison of some of the other peptides. Whether there are differences in the degree of hydroxylation of prolyl residues or not, as has been shown for rat skin and tail tendon collagen (Bornstein, 1967), cannot be determined from the present data.

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