ISOLATION OF A COLLAGEN FROM BASEMENT MEMBRANES CONTAINING THREE IDENTICAL α- CHAINS

Nicholas A. Kefalides

Department of Medicine, University of Pennsylvania
and the Philadelphia General Hospital
Philadelphia, Pennsylvania 19104

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Summary: Chromatographic studies on CM-cellulose of collagens isolated from basement membranes of the glomerulus, lens capsule and Descemet's membrane indicate that the molecule is composed of three identical α-1 chains. The M. Wt. of the α-1 chains from basement membranes is 108,000; it is higher than that of α-1 chains of interstitial collagens by an amount attributed to the excess hexose. Chemical analyses reveal high hydroxylysine, hydroxyproline and glycine content and a low amount of alanine. There are 8 residues of half-cystine. Total carbohydrate is about 12%, consisting of equimolar amounts of glucose and galactose.

Introduction: Evidence from a number of laboratories indicates that mammalian tissues are capable of synthesizing more than one type of collagen molecule. A notable example is cartilage, where two types of collagen molecules have been found (1, 2). A unique feature of the new collagen molecule isolated from chick cartilage is that it is composed of three identical α-1 chains (2). Furthermore, it contains higher amounts of hydroxylysine and hexose than interstitial collagens examined thus far (3). A collagen rich in hydroxyproline, hydroxylysine and hexose was isolated some years ago from various basement membranes in our laboratory (4, 5, 6). One of our objectives was to isolate its component chains and compare them with those of other collagens.

Recently, we were able to demonstrate that the collagen from basement membranes of the glomerulus, lens capsule and Descemet's membrane is composed of a molecule having three identical alpha chains and possessing the chromatographic properties, on carboxymethyl cellulose, of α-1 chains isolated from interstitial collagen.

Materials and Methods: Human glomerular basement membrane, sheep anterior lens capsules and Descemet's membranes were isolated as described previously (6). The collagen component was isolated from the basement membranes after limited digestion with pepsin following a modification of a previously published method (6). Following digestion with pepsin, the supernatant solution was dialyzed against a solution of 0.05% acetic acid containing 0.025 M Na_2HPO_4. The collagen was precipitated from the retentate by the addition of solid KCl to a final concentration of 15% and solid Na_2HPO_4 to a final concentration of 0.02 M. The precipitation step was repeated three times.

The isolated collagen component was denatured and chromatographed on DEAE cellulose according to the method of Miller (2). Chromatography on carboxymethylcellulose was carried out under two separate conditions. In the first instance we used the method of Piez, Eigner and Lewis (7), where elution was carried out with 0.06 M sodium acetate buffer, pH 4.8. The second method was the one described by Miller (2) for characterization of the chick cartilage collagen. Elution was carried out with 0.02 M sodium acetate buffer, pH 4.8, in the presence of 1 M urea.

Amino acid and carbohydrate analyses were performed according to previously described methods (5). The hydroxylysine linked glycosides glucosyl-galactosyl-hydroxylysine (Glc-Gal-Holy) and galactosyl-hydroxylysine (Gal-Holy) were isolated and measured according to a previous method (8).

Molecular weight was determined by the method of Weber and Osborn (9) using sodium dodecyl sulfate - mercaptoethanol acrylamide electrophoresis with one modification - the amount of bisacrylamide was reduced by 50%. The molecular sieve chromatography of Piez (10) was used to compare the molecular weights of the α -1

chains and β components isolated from basement membranes and rat tail tendon collagen.

Sedimentation and diffusion constants, as well as intrinsic viscosity were measured as previously described (5). Specific optical rotation was measured at 313 mμ in an optical rotatory dispersion apparatus as described previously (5).

Results: The elution pattern of anterior lens capsule collagen on a DEAE-cellulose column appears in Figure 1. Over 98% of the protein emerged as a single component. No protein was eluted with 1 M NaCl.

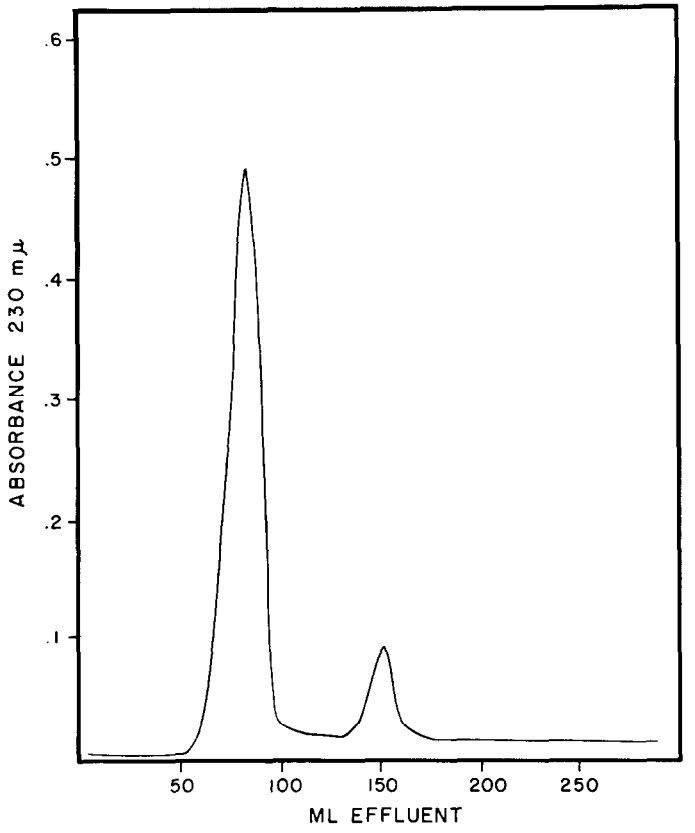

Figure 1. DEAE - cellulose chromatography of lens capsule collagen. Elution was performed with 0.2 M NaCl, 0.05 M Tris-HCl, pH 7.5. Elution with 1 M NaCl was begun at 180 ml. Column dimensions 17 x 2.5 cm.

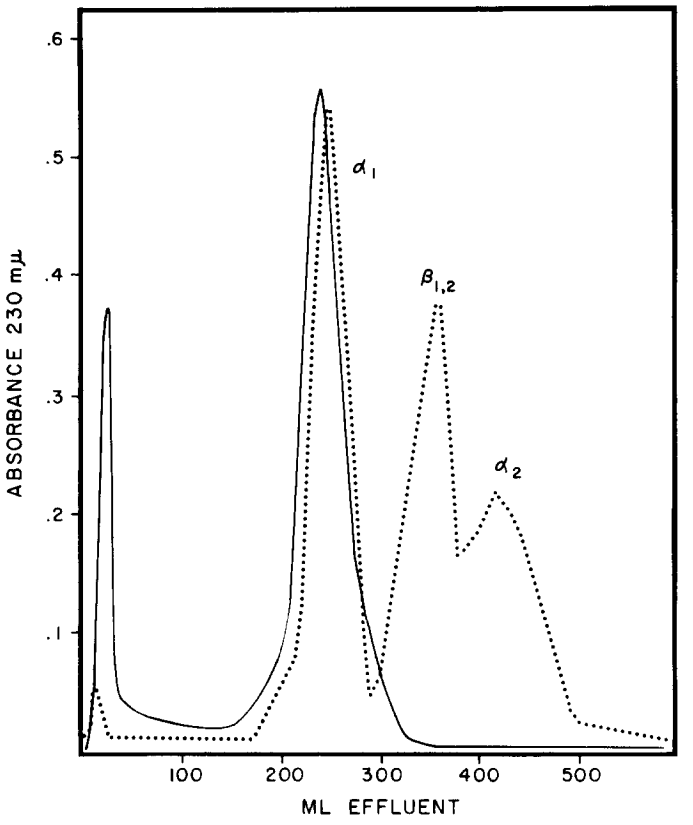

Figure 2. CM-cellulose chromatography of denatured collagens from anterior lens capsule (solid line), and rat tail tendon (dotted line). Column dimensions 14 x 1.5 cm. Elution performed with linear gradient of ionic strength from 0.06 to 0.1 M at pH 4.8.

Upon chromatography of either the native collagen after denaturation or of the material obtained after DEAE-cellulose chromatography on carboxymethylcellulose using the conditions of Piez, Einger and Lewis (7), the basement membrane collagen eluted with the volume characteristic of α-1 chains from interstitial collagen (Figure 2). However, no α-2 or β 1, 2 components were detectable. Rat tail tendon collagen chromatographed under the same conditions resolved into α-1 and α-2 chains and β 1, 2 components. When we used the conditions described by Miller (2), where the elution is carried out with 0.02 M sodium acetate buffer, pH 4.8 in 1 M urea,

the collagen emerged again as a single peak; this time, however, the protein was retarded longer, the peak emerging at 300 ml. The collagens isolated from human glomerular basement membrane and sheep Descemet's membrane chromatographed in a similar manner.

Table I shows the amino acid composition of the α-1 chains isolated by carboxymethyl cellulose chromatography from various basement membrane collagens. It will be noted that they are all characterized by unusually high amounts of hydroxyproline and hydroxylysine. The sum of lysine and hydroxylysine is higher than

TABLE I. Amino Acid Composition of α-1 Chains Isolated from Basement Membrane Collagens.

	Residues/1000 Residues		
	Human	Sheep	
	Glomerulus	Anterior Lens Capsule	Descemet's Membrane
Hydroxylysine	44.6	67.4	43.0
Lysine	10.0	8.3	15.2
Histidine	10.4	7.1	7.8
Arginine	33.0	24.0	30.0
3-Hydroxyproline	11.0	15.4	8.0
4-Hydroxyproline	130.0	175.4	156.0
Aspartic	51.0	42.0	30.0
Threonine	23.0	18.2	18.0
Serine	37.0	31.2	25.0
Glutamic	84.0	76.0	78.0
Proline	61.0	70.3	90.0
Glycine	310.0	310.0	320.0
Alanine	33.0	30.3	32.0
1/2 Cystine	8.0	8.0	8.0
Valine	29.0	21.5	25.0
Methionine	10.0	9.0	9.5
Isoleucine	30.0	20.0	24.0
Leucine	54.0	43.0	52.0
Tyrosine	6.0	2.0	3.0
Phenylalanine	27.0	21.0	22.0

that of interstitial collagens (3). However, the sum of proline and hydroxyproline is closer to the one observed for interstitial collagens. A significant percentage of the total hydroxyproline is 3-hydroxyproline. Glycine accounts for 1/3 of all the amino acid residues. Alanine, however, is low and corresponds to only 25% of the amount found in interstitial collagen. Another unusual feature is the presence of 8 residues of half-cystine. Table II shows the carbohydrate composition of the α-1 chains. The chains from all three types of basement membranes contain from 12.0 to 12.5% hexose composed of equimolar amounts of glucose and galactose. About 95% of all the hexose is in the form of the disaccharide unit glucosyl-galactosyl-hydroxylysine; the remainder exists as galactosyl-hydroxylysine.

The peak obtained after carboxymethyl cellulose chromatography

TABLE II. Carbohydrate Composition of α-1 Chains Isolated from Basement Membrane Collagens.

	g/100 g		
	Human	Sheep	
	Glomerulus	Anterior Lens Capsule	Descemet's Membrane
Hexose	12.0	12.5	10.0
Glucose	5.5	6.0	5.0
Galactose	6.0	6.3	5.2
Mannose	Trace	Trace	0
Fucose	0	0	0
Hexosamine	0	0	0
	μmoles/μmole α -chain		
Glc-Gal-Holy	34	34	26
Gal-Holy	2	2	1.8

was further characterized by molecular sieve chromatography on Bio-Gel A-1.5. Figure 3 shows the elution patterns of the collagen components from anterior lens capsule and rat tail tendon. It will be noted that the α-1 chains and the β1, 1 component from anterior lens capsule collagen elute earlier than the corresponding α- chain and β component of rat tail tendon collagen. Calculation of the molecular weight on the basis of the ratio of the elution volume of the protein to that of tritiated water gave a value of 108,000 for the α-1 chain and 212,000 for the dimer. The increase in molecular weight over that of α-1 chains of interstitial collagen is accounted for by the 12.5% hexose.

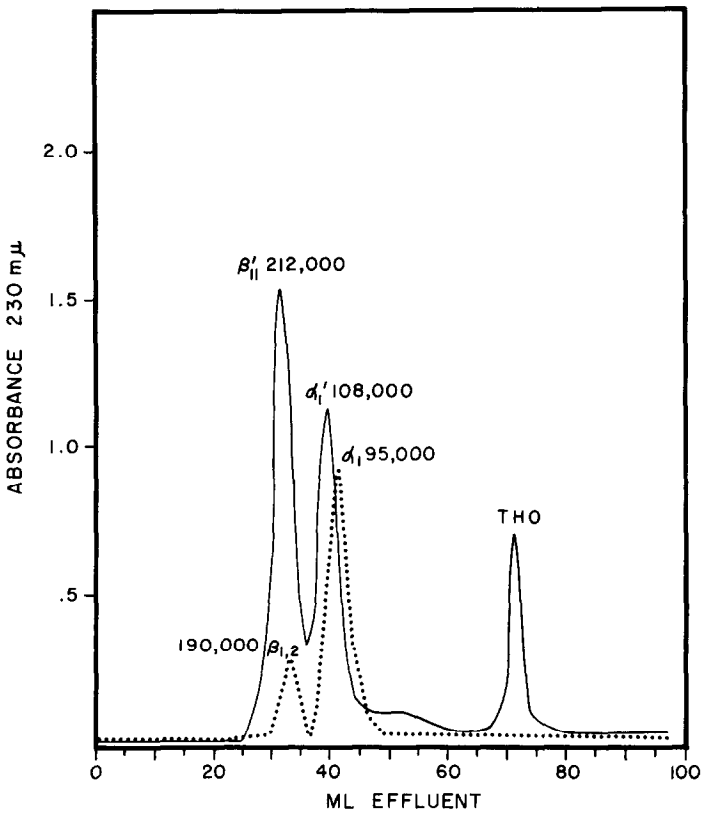

Figure 3. Molecular sieve chromatography on Bio-Gel A-1.5 of denatured collagens from anterior lens capsule (solid line), and rat tail tendon (dotted line). Column dimensions 95 x 1.5 cm. Elution performed with 0.05 M Tris-acetate, 1 M $CaCl_2$ at pH 7.5. THO = Tritiated water.

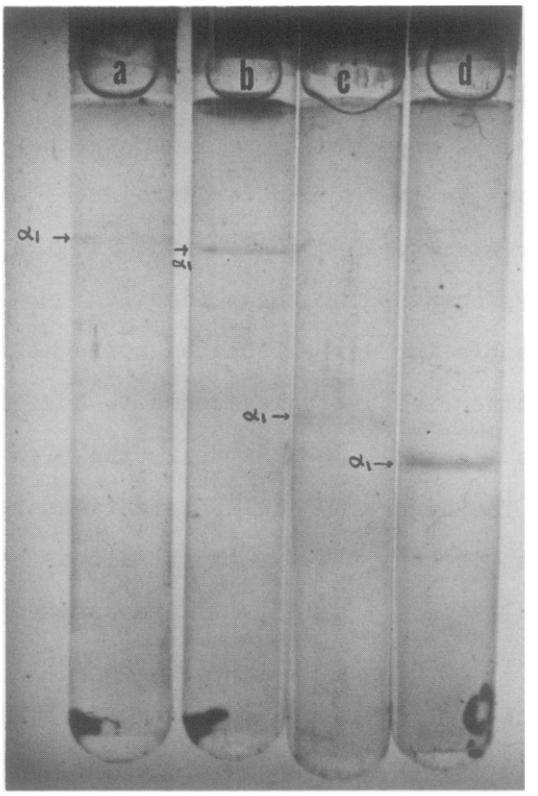

Figure 4. Disc acrylamide gel electrophoresis in SDS - mercaptoethanol of α-chains from anterior lens capsule collagen (a, c), and rat tail tendon collagen (b, d). a and b 7.5% gels; c and d 5% gels.

Further evidence that the α-1 chains isolated from basement membranes are heavier than α-1 chains from tendon collagen was obtained by acrylamide disc gel electrophoresis in sodium dodecyl sulfate (SDS). Figure 4 shows α-1 chains from anterior lens capsule collagen run in 7.5% and 5% gel (gels a & c) and rat tail tendon collagen ran similarly (gels b & d). In each instance the α-chains from basement membrane collagen migrate slower. Their molecular weight calculated from the R_f values was 110,000. The native collagen from anterior lens capsule had values for intrinsic viscosity, diffusion, and sedimentation velocity of 13.1 dl/gm, 0.9×10^{-7} cm^2/sec, and 3.5 S respectively. The molecular weight

calculated from the sedimentation and diffusion constants is 325,000. The specific optical rotation at 313 mμ was estimated as -2460°.

Discussion: The data presented here indicate that basement membrane are not only unique in their amino acid and carbohydrate composition but also in their structural organization. Unlike most interstitial collagens, they are composed of three identical α-1 chains. In this respect they resemble the newly isolated collagen from chick cartilage (1, 2). Another feature which seems to correlate with the presence of three identical α- chains is the high hydroxylysine and hexose content. The higher molecular weight observed for basement membrane collagens is consistent with the excess hexose which accounts for about 12% of dry weight. In an earlier report, Kefalides and Winzler (11) isolated a fraction from glomerular basement membrane by reduction and alkylation of disulfide bonds in the presence of 8 M urea. This fraction had a molecular weight of 132,000 (11) and could represent the collagen molecule in association with the pepsin susceptible polypeptide which according to Bellamy and Bornstein (12) has a molecular weight of 25,000 in newly synthesized collagen by rat calvaria.

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