ISOLATION AND CHARACTERIZATION OF TWO α CHAIN SIZE COLLAGENOUS POLYPEPTIDE CHAINS C AND D FROM GLOMERULAR BASEMENT MEMBRANE

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1. Introduction

Basement membranes are extracellular structures composed of collagenous protein(s), one or more glycoproteins and glycosaminoglycans [1–3]. At the present time, the chemical structure of the collagenous component and its molecular organization remain controversial. The collagen component(s) of basement membrane constitutes a genetically distinct class of collagen differing from types I, II and III collagen by the presence of higher contents of 3-hydroxyproline, hydroxylysine, leucine, and carbohydrate and low contents of alanine. Kefalides [4–6] described an α chain size basement membrane collagenous component (type IV), obtained from the pepsin digest of lens capsule, glomerulus, and descemet's membrane. Timpl et al. [7] have estimated that molecular weight of type IV collagen is >400 000. Other investigators [8–10] have published results indicating the heterogenous nature of the collagenous component of glomerular basement membrane.

α Chain size polypeptide chains A and B have been isolated from placenta, liver, skin, and the medial layer of vascular tissues [11–13]. Similar chains A and B have also been reported from placenta [14], bovine skeletal muscle [15], synovial membrane and gingiva [16], and whole chick embryos [17]. The low content of alanine and high content of hydroxylysine in the A and B chains somewhat resemble basement membrane collagen described [4–6]. However, it has not been definitely established whether these chains are derived from basement membranes. Recently, the evidence for the presence in human placenta of at least two distinct chains designated C and D* which are structurally different have also been published [18–21].

This communication presents the isolation and partial characterization of two approximately α chain size polypeptides, C and D chains from human glomeruli and from homogenates of human and porcine kidney corteces.

2. Materials and methods

2.1. *Collagen Preparation*

2.1.1. From human glomeruli

Human glomeruli were prepared essentially by the procedure in [22] with minor modifications. Small thin slices of cortex were forced through an 80 mesh sieve with the bottom surface of a beaker. The material that emerged was collected on a 150 mesh sieve. Cold 0.1 M acetic acid containing a mixture of protease inhibitors (4 mM EDTA, 1 mM phenylmethylsulfonyl fluoride and 5 mM *N*-ethylmaleimide) was used for the wash of the tissue and preparation of glomeruli. The glomeruli that remain on the 150 mesh sieve were washed freely with cold 0.1 M acetic acid in the presence of inhibitors and then lyophilized.

* *Nomenclature:* The nomenclature of C and D chains presented in this paper corresponds to one used by Kresina, Rhodes and Miller (1978) Fed. Proc. FASEB 37(6), 1528. The C and D chains have also been referred to as α1(IV) and α2(IV) chains, respectively, by Dr Paul Bornstein

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The lyophilized glomeruli were pulverized in a freezer mill (Spex Industries, Metuchen, NJ) in liquid nitrogen and then suspended in 0.5 M acetic acid. Pepsin was added in the ratio of 1:10 (dry wt) and the mixture was shaken gently for 62 h at 4°C in the presence of the above inhibitors. After the incubation period, the pepsin digest was centrifuged (18 000 × *g* for 1 h) and collagen was precipitated from the supernate by the addition of NaCl to 10% final conc. The precipitate obtained after centrifugation was redissolved in 0.5 M acetic acid and reprecipitated with 10% NaCl. The precipitated collagen was collected by centrifugation, redissolved in 0.01 M Tris/1 M NaCl (pH 7.4). The solution was adjusted to pH 7.4 by the addition of cold 1 M NaOH. Solid NaCl was added to the clear solution to 30% and the precipitate was collected by centrifugation. The precipitate was redissolved in 0.01 M Tris/0.1 M NaCl (pH 7.4) and dialyzed versus 0.01 M Tris/0.02 M NaCl (pH 7.4) with several changes of the same buffer. The precipitated material was centrifuged and the supernate was passed through a column of diethylaminoethyl-cellulose column equilibrated with 0.01 M Tris/0.02 M NaCl (pH 7.4) and was eluted with the same buffer. The collagen fraction which eluted unretarded from the column was collected and collagen was precipitated by addition of NaCl to 30% final conc. Collagen was isolated by centrifugation, dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid and lyophilized. Lyophilized material was used for column chromatography. All procedures were performed at 4°C.

2.1.2. From kidneys

Human kidneys were obtained at autopsy of patients who had died of causes other than diseases involving kidneys and kept frozen. Porcine kidneys were obtained from a local slaughterhouse and transported to the laboratory on ice. Kidneys were defatted and freed of the capsule and vascular tissue as well as the medula by dissection. The cortex slices were washed thoroughly with cold 0.5 M acetic acid containing protease inhibitors as described above and homogenized. The homogenate was adjusted to pH 2.5. Pepsin was added in 1:200 ratio (wet wt) and the homogenate was shaken gently for 62 h at 4°C and then centrifuged. Basement membrane-like collagens in the supernates were purified by the procedure outlined in fig.4. All of the operations were performed at 4°C. The lyophilized collagen fraction so obtained was used for column chromatography.

2.2. *Column chromatography and amino acid analysis*

The initial fractionation of the collagen preparations was performed on a calibrated column of Agarose A-5M equilibrated with 0.01 M Tris/1 M $CaCl_2$, (pH 7.4) essentially as in [23]. Carboxymethyl cellulose chromatography of collagen chains was performed on a column equilibrated with 0.02 M sodium acetate/1 M urea (pH 4.8) at 44°C [12,24].

Samples for amino acid analysis were hydrolyzed in constant boiling HCl acid for 24 h under an atmosphere of nitrogen at 108°C. Analyses were performed on an automatic analyzer (Beckman 121) using a single column method [25]. Hydroxylysine glycosides (Glc–Gal–Hyl and Gal–Hyl) were determined by the procedure in [26].

2.3. *Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)*

The polyacrylamide gel electrophoresis of various chains were performed on 7.5% slab gels in the presence of mercaptoethanol as in [27]. The CNBr digests of C and D chains were electrophoresed on 15% slab gels.

3. Results and discussion

Lyophilized collagen obtained from human glomeruli was initially fractioned on an Agarose A-5M column. The typical elution pattern is presented in fig.1. The fraction represented by solid bar (C and D) in fig.1 was pooled, desalted and lyophilized. The lyophilized material was subjected to carboxymethyl cellulose chromatography when two major peaks designated as C and D were obtained (fig.2). The SDS–PAGE pattern of C and D from glomeruli is shown in fig.3A. The amino acid composition of C and D chains is presented in table 1.

The procedure outlined in fig.4 describes the preparation of basement membrane collagen from the pepsin digest of human and procine corteces. The interstitial collagens types I and III were separated as heat gel [28] on incubation at 37°C for 16 h. In the next step, the dialysis of collagen solution against 0.01 M Tris/0.02 M NaCl (pH 7.4) precipitated the

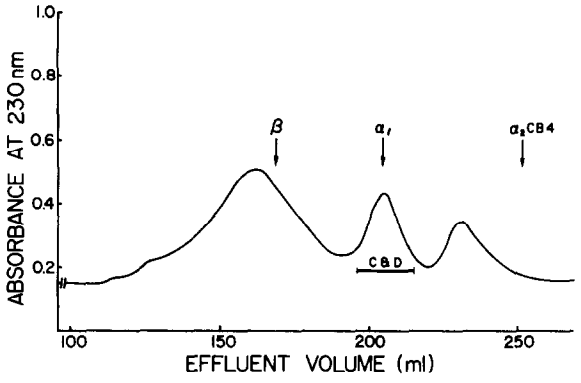

Fig.1. Molecular sieve chromatography of human glomerular basement membrane collagen (30 mg) on Agarose A-5M column (2.0 × 120 cm). The column was eluted with 0.01 M Tris/1 M $CaCl_2$ (pH 7.4) at a flow rate of 12 ml/h.

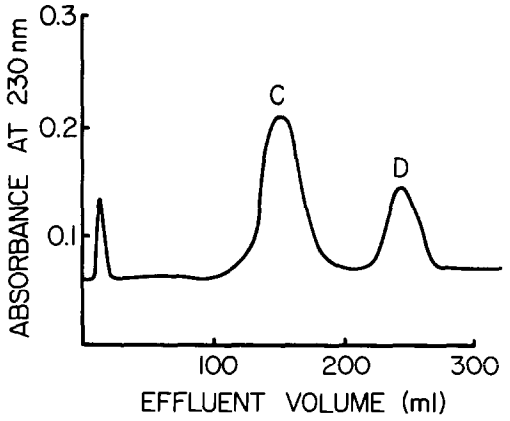

Fig.2. Carboxymethyl cellulose chromatography of 6 mg fraction represented by solid bar from fig.1. The column (1 × 10 cm) was equilibrated with 0.02 M sodium acetate/ 1 M urea (pH 4.8) at 44°C and was eluted at a flow rate of 100 ml/h with a linear gradient of NaCl from 0–0.12 M containing 1 M urea over 500 ml total vol.

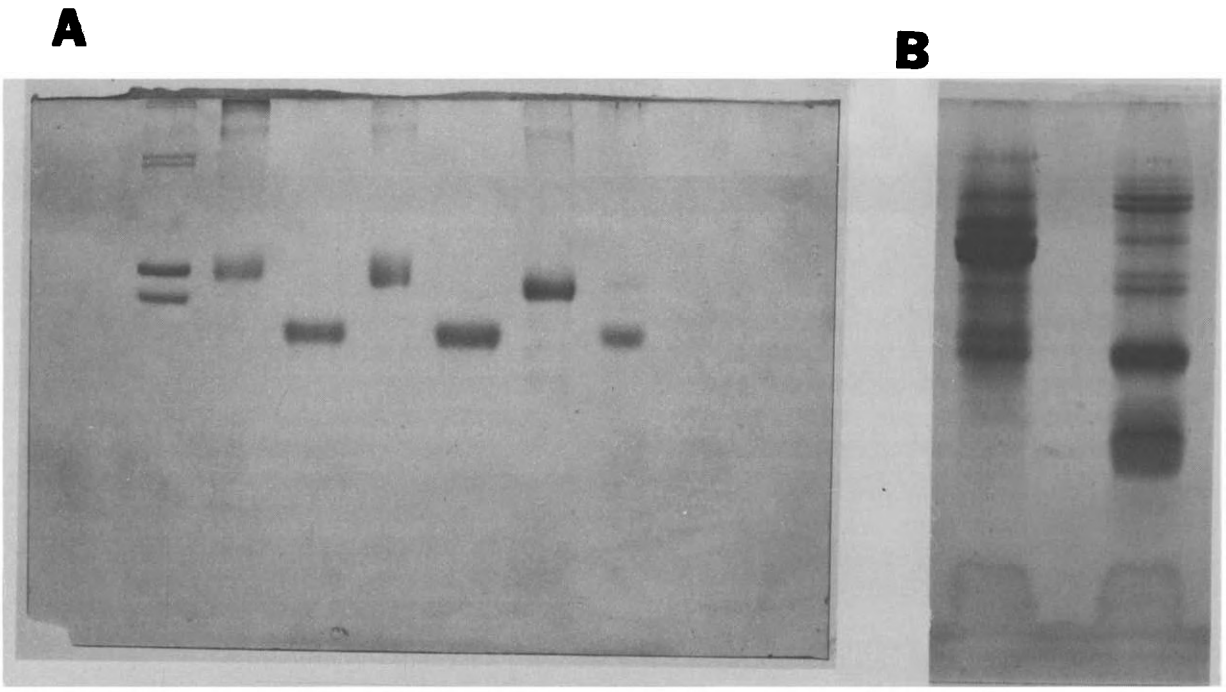

Fig.3A. SDS–polyacrylamide gel electrophoresis of: lane 1 (from left), type I collagen of chick skin showing α1(I), α2, β_{11}, and β_{12}; lanes 2 and 3, the C and D chain, respectively, from glomeruli; lanes 4 and 5, the C and D chain from human kidney corteces and lanes 6 and 7, the C and D chains from porcine kidney corteces. Gels (7.5%) were electrophoresed in the presence of mercaptoethanol for 2 h at 50 mA.

Fig.3B. SDS–polyacrylamide gel electrophoresis of CNBr peptides pattern of C (left) and D chain isolated from porcine kidney corteces.

Table 1
Amino acid composition of C and D chains
(residues/1000 amino acid residues)[a]

	Human glomeruli		Human kidney corteces		Porcine kidney corteces	
	C	D	C	D	C	D
3-Hydroxyproline	6.5	5.8	6.8	5.5	6.8	6.1
4-Hydroxyproline	126	121	119	114	126	115
Aspartic acid	41	52	45	52	52	57
Threonine	16	27	16	27	17	21
Serine	36	31	32	31	34	29
Glutamic acid	85	70	83	68	73	56
Proline	61	66	56	62	64	60
Glycine	342	335	360	337	359	349
Alanine	30	38	33	42	35	40
Valine	25	21	24	23	21	32
Methionine	13	12	14	12	13	12
Isoleucine	28	30	26	32	23	23
Leucine	53	55	52	51	55	59
Tyrosine	4.1	6.0	5.8	5.5	3.6	3.9
Phenylalanine	26	30	25	29	25	31
Hydroxylysine	57	45	60	46	60	43
Lysine	6.2	6.8	8.3	8.8	7.6	6.0
Histidine	5.9	7.6	6.4	8.2	6.6	9.7
Arginine	24	39	24	42	21	47
Glc–Gal–Hyl	n.d.	n.d.	42	29	48	27
Gal–Hyl	n.d.	n.d.	1.9	0.7	2.0	1.7

[a] Values for residues present in numbers greater than 10 were rounded off to the nearest whole number
n.d., denotes not determined

residual type I and III and A and B chains [13], leaving the soluble basement membrane collagen in solution. The Agarose A-5M column chromatography of lyophilized collagen (fig.4) obtained from kidney corteces is presented in fig.5. When peak 3 (fig.5), which eluted in the position of α1(I) chain, was subjected to carboxymethyl cellulose chromatography, two chains C and D were consistently obtained (figure not shown) in the same chromatographic elution position of C and D chains obtained from glomeruli (fig.2). The amino acid composition of C and D chains isolated from human and porcine kidney corteces is tabulated in table 1 and their SDS-PAGE pattern is shown in fig.3A.

The data presented here describes the isolation of two distinct collagenous chains, C and D, from the pepsin digest. The two chains are different in their chromatographic properties (fig.2) and their electrophoretic mobility (fig.3A). The amino acid composition of the two chains are significantly different in contents of glutamic acid, hydroxylysine and arginine residues. As shown in fig.3B, their CNBr peptide pattern on SDS–PAGE is also significantly different. The apparent molecular weight of the C and D chain estimated by gel filtration on Agarose A-5M column is 95 000, whereas on SDS–PAGE the estimated molecular weight of C and D is 95 000 and 75 000, respectively (fig.3A). The reason for the anomalous electrophoretic migration of D chain is not clear although it is known that the α1(I) and α2 chains migrate with different mobility during SDS–PAGE (fig.3A) despite their identical molecular weight. The presence of two bands on SDS–PAGE corresponding to mobility of C and D chains were reported [29] recently from pepsin-solubilized glomerular basement membrane collagen. In addition, similar chains C and D have been shown to be present in the anterior lens capsule [30,31]. The C chain described here has a close similarity with the earlier work on glomeruli [4], anterior lens capsule [6,24,32] and the α chain

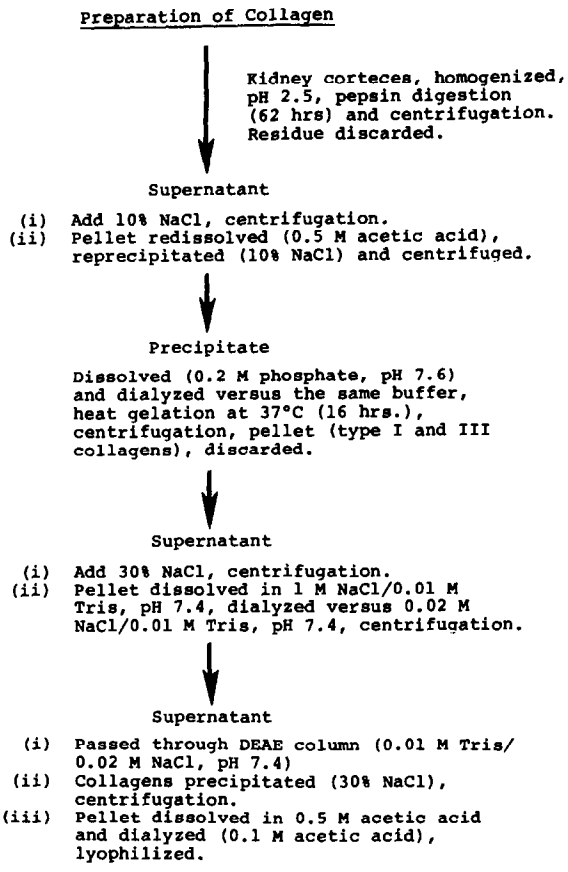

Fig.4. Outline of procedure for isolation and purification of basement membrane collagens from homogenates of kidney corteces.

size component reported recently from human placenta [18–21]. The structurally different D chain is probably similar to one described from human placenta [18–21].

The procedure described in fig.4 for preparation of collagen from whole kidney corteces has merit as C and D chains can be isolated in gram quantities needed for further structural studies. The molecular organization of C and D chains is not clear at present. They may possibly be represented by separate $[C]_3$ and $[D]_3$, or as subunit structures of $[C]_2D$ or $[D]_2C$ composition.

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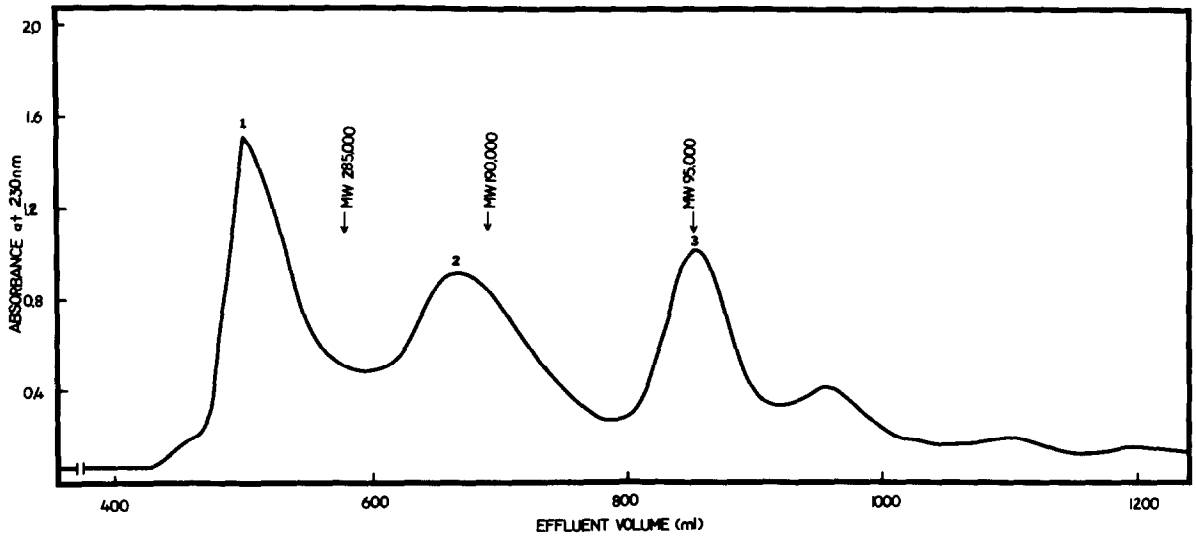

Fig.5. Molecular sieve chromatography of basement membrane collagens (200 mg) from fig.4 on Agarose A-5M column (4.0 × 120 cm). The column was eluted with 0.01 M Tris/1 M $CaCl_2$ (pH 7.4) at a flow rate of 36 ml/h. Peak 3 was resolved on carboxymethyl cellulose into C and D chains.

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