ISOLATION OF THREE COLLAGENOUS COMPONENTS OF PROBABLE BASEMENT MEMBRANE ORIGIN FROM SEVERAL TISSUES*

Endy Chung, R. Kent Rhodes, and Edward J. Miller

Departments of Pathology and Biochemistry, and Institute of Dental Research, University of Alabama Medical Center, Birmingham, Alabama 35294

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SUMMARY: Fractionation of pepsin-solubilized collagens from several human tissues has shown that substantial quantities of collagen-like protein remain in solution under conditions leading to the precipitation of Type I, II, and III collagens. Characterization of the more soluble collagens has led to the isolation of three unique collagenous components each of which exhibit compositional features indicative of their origin from basement membranes. One of these has an apparent molecular weight of 55,000 daltons and appears to originate in endothelial basement membranes. The other two components (A chain and B chain) are somewhat larger than collagen α chains and appear to be derived from the collagen of epithelial and smooth muscle basement membranes, respectively.

Early data on the amino acid composition of glomerular basement membrane (GBM)(1,2), lens capsule (3,4), and other basement membranes (3) suggested that these structures contain a collagen-like protein as well as noncollagenous proteins. These findings were corroborated in additional studies showing that the carbohydrate units of GBM (5) and lens capsule (6) are comprised of glucosylgalactosyl disaccharide units linked to hydroxylysine in collagen-like sequences, and larger heteropolysaccharide units linked to asparaginyl residues in predominantly noncollagenous sequences.

Studies designed to elucidate the molecular organization of basement membranes have led to disagreement with respect to the na-

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ture and number of the collagenous components as well as the relationship of these components to the glycoprotein constituents. Thus, collagen solubilized by limited pepsin digestion from lens capsule, GBM, and Descemet's membrane was reported to contain a single type of chain similar in size to the constituent α chains of interstitial collagens, but markedly different from the latter chains in compositional features (7). Subsequent studies suggested that collagen molecules comprised of these chains were associated in the membranes with large noncollagenous sequences to achieve a subunit structure resembling the procollagen molecule (8). However, a more recent study on pepsin-solubilized collagen of GBM has reported the presence of at least four collagenous components, two of which were similar in size to α chains (95,000 daltons) and two of which appeared to be considerably larger (140,000 daltons)(9). In addition, the marked heterogeneity of components solubilized in detergent-containing solvents from reduced and alkylated GBM (10,11) has suggested a different molecular organization in which the membrane is comprised of disulfide-linked chains containing alternating collagenous and noncollagenous regions of varying length (12).

The present communication describes our approach to the isolation of collagenous components from basement membranes. The technique involves selective fractionation of these components from the total pepsin-solubilized collagen of a given tissue and has the advantage of not requiring prior isolation of an anatomically defined membrane.

MATERIALS AND METHODS: Collagen was solubilized by limited pepsin digestion from samples of human liver, placenta, uterus, and the intimal and medial layers of several major vessels as previously described (13, 14). Following centrifugation to remove undigested material, Type I and III collagens were precipitated from the supernate by the addition of NaCl to a concentration of 0.9 M (14). The precipitate was removed by centrifugation and the supernatent was dialysed extensively against 0.02 M Na_2HPO_4. The precipitate formed during dialysis was redissolved in 2.0 M guanidine-HCl, pH 7.5 (0.05 M Tris) and subsequently dialyzed against 0.5 M acetic acid. Following the latter dialysis procedure, material which had precipitated during dialysis was removed by centri-

fugation and the supernatant fluid was lyophilized. All of the above procedures were performed at 4°.

Agarose molecular sieve chromatography was performed on 1.5 x 155 cm columns of Sepharose 4B (Pharmacia) equilibrated and eluted with 2.0 M guanidine-HCl, pH 7.5 (0.05 M Tris). Collagen samples were dissolved in 1-2 ml of the latter solvent and denatured by warming to 40° for 30 min prior to chromatography. In certain experiments, the samples were dissolved in 5.0 M urea and reduced with 2-mercaptoethanol at pH 8.0 prior to chromatography (14).

Collagenous components eluted from agarose columns were rechromatographed on carboxymethyl (CM-) cellulose as previously described (15) with minor modifications as noted in the legend for Figure 2.

Amino acid analyses were performed on an automatic amino acid analyzer (13) following hydrolysis of the samples in 6N HCl at 105° for 24 hr.

For the determination of hexosamines, samples were hydrolyzed in 4 N HCl at 100° for 6 hr. Hydroxylysine glycosides were determined following hydrolysis in 2 N NaOH at 105° for 24 hr. Analyses for hexosamines and hydroxylysine glycosides were performed on the amino acid analyzer (13) employing elution with 0.35 M (Na^+) sodium citrate buffer at pH 5.3 for the initial 240 min of each run followed by elution with buffer D (13) for an additional 100 min.

RESULTS: Amino acid analyses revealed that depending on the tissue un-

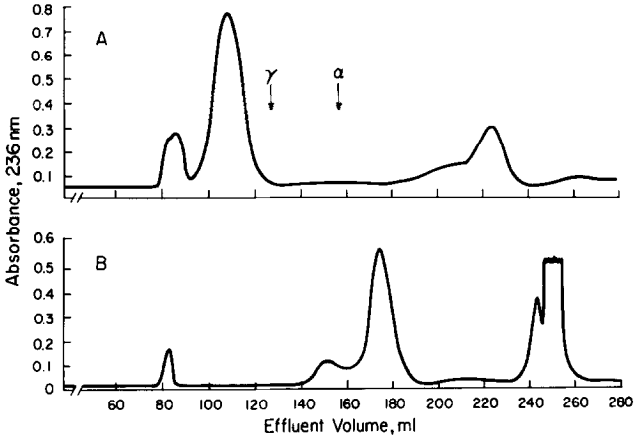

Figure 1: (A) Agarose molecular sieve (Separose 4B) elution profile of the collagen-like protein isolated as described in the text from samples of human aortic antima. (B) Elution profile of the major high molecular weight fraction (eluted at 110 ml in A) following reduction with 2-mercaptoethanol. The column was equilibrated and eluted at a flow rate of 10 ml/hr with 2.0 M guanidine-HCl, pH 7.5 (0.05 M Tris). Arrows indicate the elution position of γ-components (285,000 daltons) and α chains (95, 000 daltons).

der study, as much as one-third of the total hydroxyproline-containing material in the initial pepsin digest failed to precipitate under conditions leading to the precipitation of Type I and III collagens (14). The supernatant collagen was considerably purified and quantitatively recovered in the acid-soluble fraction following dialysis against 0.02 M Na_2HPO_4 and the subsequent procedures outlined above.

Figure 1A depicts the agarose molecular sieve elution profile of the material thus obtained from digests of aortic intima. Only the major high molecular weight component eluting at 110 ml contained hydroxylproline. When this component was reduced with 2-mercaptoethanol and rechromatographed on agarose, it was recovered largely as collagen-like subunits with an apparent molecular weight of 55,000 daltons (Figure 1B). The reduced subunits chromatographed on CM-cellulose as a single somewhat broad peak (Figure 2) in a region coincident with the elution position of α1(III) chains when Type III collagen is denatured and chromatographed in the same system.

The compositional features of the subunit as recovered following CM-cellulose chromatography are presented in Table I. These data clearly show that it represents a collagen-like protein containing a

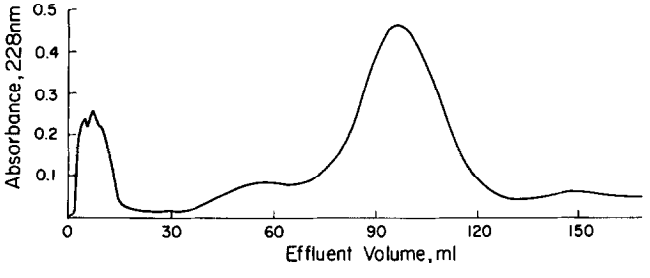

Figure 2: CM-cellulose elution pattern of the reduced material eluted from agarose as shown in Figure 1B. Chromatography was performed on a 0.9 x 9.0 cm column in 0.02 M (Na^+) sodium acetate buffer containing 1.0 M urea at pH 4.8. Elution was achieved by employing a linear gradient from 0 to 0.12 M NaCl over a total volume of 200 ml.

TABLE I

Amino Acid Composition of the Collagenous Components Isolated from Human Aortic Intima and Skin.

	Residues/1000 Amino Acid Residues		
		Skin	
Amino Acid	Aortic Intima	A Chain	B Chain
3-Hydroxyproline	0	7	10
4-Hydroxyproline	65	113	105
Aspartic Acid	78	49	49
Threonine	16	29	21
Serine	27	34	25
Glutamic Acid	104	86	95
Proline	92	98	120
Glycine	318	346	334
Alanine	41	54	45
Half-cystine	20	0	0
Valine	21	28	22
Methionine	8	11	9
Isoleucine	20	12	15
Leucine	24	33	38
Tyrosine	18	0	3
Phenylalanine	15	10	11
Hydroxylysine	48	22	39
Histidine	3	8	6
Lysine	18	12	13
Arginine	64	48	40
Glc-Gal-Hydroxylysine	(41)	(5)	(29)
Gal-Hydroxylysine	(4)	(3)	(5)
Glucosamine	(22)	0	0

relatively low level of 4-hydroxyproline and unusually large amounts of cysteine and tyrosine. Although it does not contain 3-hydroxyproline, it exhibits several other compositional features commonly recognized as characteristic for basement membrane collagen (16,17). Most notable among these are the relatively low alanine content, the relatively large amount of hydroxylysine plus lysine, and the prevalence of the disaccharide derivative of hydroxylysine. In addition, the occurence of glucosamine in the subunit suggests the presence of heteropolysaccharide units (5,6).

Different results were observed when similarly prepared collagenous components from skin were chromatographed on agarose. In this

case, the hydroxyproline-containing material was eluted as a single peak in a region corresponding to a molecular weight of approximately 110,000

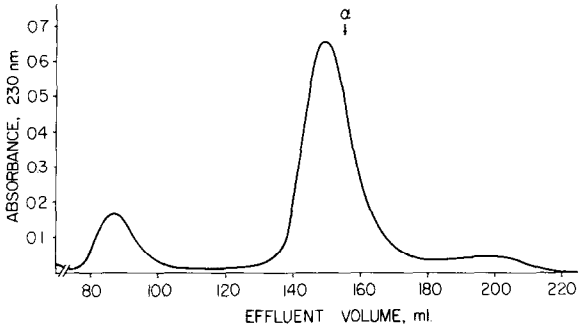

Figure 3: Agarose molecular sieve elution profile of the collagenous components isolated as described in the text from samples of human skin. Chromatography was performed as described in Figure 1.

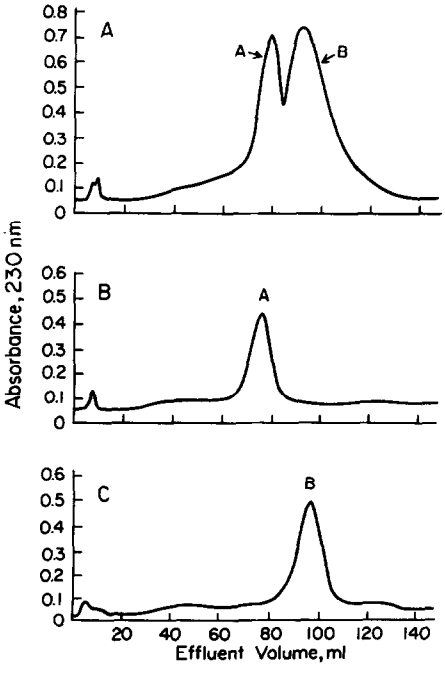

Figure 4: (A) CM-cellulose elution pattern of the material eluted from agarose as shown in Figure 3. Chromatography was performed as described in Figure 2. (B) Rechromatography of selected fractions containing the A chain. (C) Rechromatography of selected fractions containing the B chain.

daltons (Figure 3). Rechromatography of this material on CM-cellulose showed the presence of two incompletely resolved components designated A and B (Figure 4A). Appropriate fractions containing each component were selected for rechromatography and purification as illustrated in Figures 4B and 4C.

The amino acid composition of these chains is likewise presented in Table I. The data show that the A and B chains have distinctive compositional features, but both chains share many of the features characteristic of basement membrane collagen (16,17), including relatively high levels of 3-hydroxyproline.

Similar results to those described above were obtained with other tissues. Only the B chain, however, was found in digests of aortic media, whereas digests of placenta yielded the 55,000 molecular weight component plus the B chain, and digests of liver contained both A and B chains.

DISCUSSION: Our results show that in addition to Type I and III collagens and their constituent α chains, three additional collagenous components can be isolated from pepsin-solubilized collagen of several tissues. As indicated above, the compositional features of each of these components support their probable origin in basement membrane structures. Moreover, these components can be isolated only from tissues relatively rich in basement membranes, with tissues such as cartilage and bone yielding none of the components described here.

It is likely that each of the collagenous components described here has a unique cellular origin. The 55,000 molecular weight component, for instance, is found in vascular intima and highly vascularized tissues but is not detected in skin, suggesting its derivation from endothelial basement membranes. The A chain may originate in epithelial basement membranes since it is abundant in skin and liver

where epithelial structures predominate. Similar considerations indicate that smooth muscle cells may be the source of the B chain since it is the exclusive basement membrane-like component detected in the medial layers of vascular tissue.

In this regard, the present data are particularly informative with respect to some of the conflicting views concerning the molecular organization of basement membranes in the current literature. The cellular origin of GBM, for example, indicates that it would contain a mixture of collagenous components some of which may be similar to or identical to the components described here. This would account for the heterogeneity of collagenous components from GBM observed by Daniels and Chu (9) and further suggests that the α1-like component observed in similar studies (7) is actually a mixture of several components. In addition, the characteristics of the 55,000 molecular weight component isolated in the present study support the concept that at least some of the collagen-like protein in basement membranes occurs in relatively short sequences (12), as also indicated in the recent study of Ohno et al. (19).

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