ISOLATION AND CHARACTERIZATION OF TWO COLLAGENOUS COMPONENTS FROM ANTERIOR LENS CAPSULE

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1. Introduction

Basement membranes are composed of collagen like protein(s), one or more glycoproteins and glycosaminoglycans [1–3]. The nature of collagenous components and its molecular organization remains undefined. At present, at least two opposing concepts are considered regarding the molecular organization of basement membranes. A single type of collagenous chain in triple helical structure [4] similar to other interstitial collagens, though different in amino acid composition and sequence, has been isolated by limited pepsin digestion of glomerular basement membrane, anterior lens capsule and Descemet's membrane. This collagenous portion is associated with non-collagenous portions in the membranes [5]. With the second hypothesis, alternating collagenous and non-collagenous regions linked through disulfide bonds [6,7] have been suggested, based on the results obtained by solubilization of basement membranes from glomeruli under denatured, reduced and alkylating conditions [2,8,9].

Recently, four collagenous components have been identified from bovine glomerular basement membrane [10]. In addition, collagenous chains A and B of app. mol. wt 110 000 have been found in skin and liver [11]. The same B chain and a collagenous component 50 000 mol. wt have been isolated from vascular medial layer and intima, respectively [11]. Furthermore, two similar αA and αB chains have been obtained from human placenta [12]. Since all these tissues are relatively rich in basement membranes [11], the possibility exists that more than one type of collagenous component may be present.

In this communication a different approach is described for the isolation of the collagenous components from anterior lens capsule. A high molecular weight material was isolated from the phosphate soluble fraction after limited pepsin digestion of lens capsule. This high molecular weight fraction on reduction and carboxymethylation under denaturing conditions yielded a total of four different fractions. The characterization of two fractions with mol. wt 110 000 and 50 000, respectively, is described.

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2. Materials and methods

Bovine eyes were obtained fresh from a local slaughter house. The anterior lens capsules were dissected and collected in cold 0.15 M sodium chloride. After sonification (Branson Model 185, Plainview, L.I.N.Y.) for 5–10 min to remove the adhering cells, the capsules were washed several times with cold water and lyophilized.

The lyophilized capsules (1 g) were pulverized in a freezer mill (Spex Industries, Metuchen, NJ) in liquid nitrogen and suspended in 150 ml 0.5 M acetic acid, pH 2.5. Pepsin (100 mg) was added and the mixture incubated at 4°C for 72 h with gentle shaking. At the end of incubation, the pepsin digest was centrifuged and the supernate was adjusted, to pH 8.0, with

NaOH. The collagens were precipitated by addition of NaCl to a concentration of 30% w/v. The precipitate was collected by centrifugation and redissolved in 0.2 M phosphate, pH 7.6 and was dialysed exhaustively against 0.01 M phosphate, pH 7.6. The precipitate again was removed by centrifugation and to the supernate NaCl was added to 30% concentration. The precipitated collagens were separated by centrifugation, dissolved in 0.5 M acetic acid, dialysed against 0.1 M acetic acid and lyophilized material (0.4 g) was recovered.

Molecular sieve chromatography was performed on Bio-Gel A-5 M columns essentially as described [13] using 1 M $CaCl_2$–0.01 M Tris, pH 7.4, as effluent. The fractions obtained from Bio-Gel A-5 M columns were purified by carboxymethyl cellulose chromatography [11,14]. Recoveries from molecular sieve and carboxymethyl cellulose columns were 70–80% and 40–50%, respectively.

The collagen component was reduced with 2-mercaptoethanol in 8 M urea and carboxymethylated with iodoacetate as described [15]. Amino acid analyses were performed using a single column method [16] on an automatic analyser (Beckman 121). Hydroxylysine glycosides were determined by procedure described [17,18].

3. Results and discussion

The collagenous precipitate from pepsin digests obtained on addition of 30% NaCl was removed by centrifugation. When this precipitate was dissolved in 0.2 M phosphate pH 7.6 and dialysed exhaustively against 0.01 M phosphate, pH 7.6, a fraction of the collagen was precipitated. After removal of the insoluble material by centrifugation, the phosphate soluble collagen fraction was obtained by addition of 30% NaCl to the supernate. This precipitated collagen was isolated, dissolved in 0.5 M acetic acid, dialysed against 0.1 M acetic acid and lyophilized. The initial fractionation of the lyophilized material was performed on Bio-Gel A-5 M column. The elution pattern is depicted in fig.1. Two major peaks (fig.1) were consistently obtained. Peak 2 (fig.1) has an app. mol. wt 220 000. The amino acid analysis of this component (table 1, column 1) definitely showed it to be basement membrane-like collagen. This fraction was reduced with 2-mercaptoethanol and was carboxymethylated with iodoacetate. The elution pattern of this reduced and carboxymethylated material on Bio-Gel A-5 M column is presented in fig. 2. Four major fractions were obtained. Peak 2A and peak 2B (fig.2) have app. mol. wt 110 000 and 50 000, respectively,

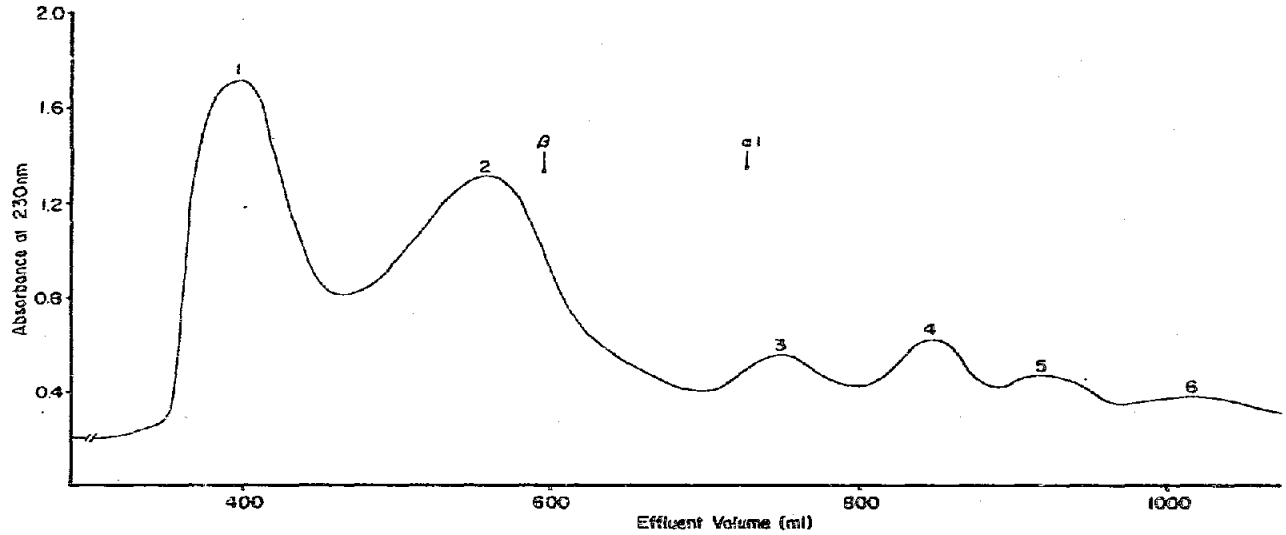

Fig.1. Gel filtration elution pattern of phosphate soluble fraction (200 mg) of pepsin solubilized lens capsule collagen on Bio-Gel A-5 M column (4 × 110 cm). The column was eluted with 0.01 M Tris/1 M $CaCl_2$, pH 7.4 at a flow rate of 35 ml/h. Fractions 1 and 2 were pooled as shown in the figure.

Table 1
Amino acid composition[a] in residues/1000

	Collagenous component (app. mol. wt)		
	220 000	110 000	50 000
3-Hydroxyproline	4.4	3.2	2.8
4-Hydroxyproline	125	129	114
Aspartic acid	51	52	50
Threonine	16	17	18
Serine	33	36	40
Glutamic acid	84	89	72
Proline	67	57	54
Glycine	339	344	351
Alanine	33	33	42
Half-cystine	4	2.9[b]	1.8[b]
Valine	16	18	19
Methionine	11	13	17
Isoleucine	16	19	23
Leucine	52	51	60
Tyrosine	5.2	3.0	2.6
Phenylalanine	30	29	35
Hydroxylysine	50	64	45
Lysine	6.2	7.3	4.1
Histidine	6.1	6.2	5.4
Arginine	26	21	45
Glc–Gal–Hydroxylysine	ND	52	28
Gal–Hydroxylysine	ND	6	6

[a] Values for residues present in number greater than 10 were rounded off to the nearest whole number
[b] Half-cystine calculated as *S*-carboxymethylcysteine
ND denotes values not determined

as determined by precalibrated Bio-Gel A-5 M column.

The reduction and carboxymethylation of peak 1 (fig.1) gave an identical elution pattern when fractionated on Bio-Gel A-5 column and obviously represents the higher mol. wt aggregates 110 000, 50 000 and the other two fractions shown as peaks 2C and 2D (fig.2).

Peak 2A (fig.2) on a column of CM-cellulose eluted as a single peak (fig.3). Under similar conditions the peak 2B (fig.2) on CM-cellulose chromatographed as one major peak (fig.4) after the start of the gradient. The fractions represented by the bars were pooled, desalted and analyzed for amino acid composition. These are presented in table 1 under column 2 and 3, respectively.

Homogeneity of the two above carboxymethylated collagen components was established by polyacrylamide gel electrophoresis using the procedure in [19], when a single band was obtained.

The data presented here show that the phosphate soluble fraction obtained from anterior lens capsule by limited pepsin digestion produced two major fractions, a higher molecular weight fraction and a 220 000 mol. wt component. The two fractions appear to be aggregates of at least more than one type of chain subunit held together by disulfide linkage of cystine. The amino acid composition of the two collagenous components (110 000 daltons and 50 000 daltons) presented in table 1 show that they are of basement membrane origin [1,20] with high contents of hydroxylysine, leucine, low content of alanine and presence of 3-hydroxyproline. Glycosylation of hydroxylysine to an extent of 90% in the 110 000 dalton component and 75% in the 50 000 dalton component was noted. It appears unlikely that a 50 000 dalton component may arise from the same chain but due to use of pepsin in the extraction procedure, this possibility cannot be ruled out.

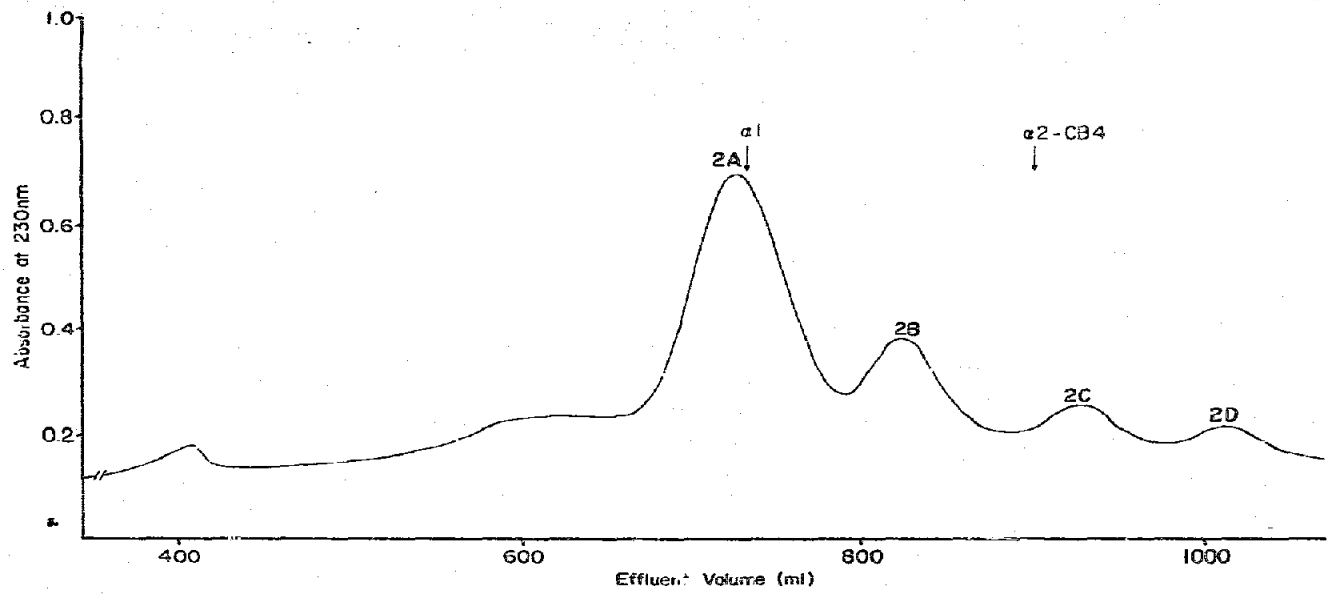

Fig.2. Gel filtration elution pattern of reduced and carboxymethylated peak 2 (100 mg) from fig.1 on Bio-Gel A-5 M column. Conditions were identical with those used for fig.1. Fractions 2A and 2B were pooled as shown. The elution positions of α1(I) and α2-CB4 are indicated which were used as markers.

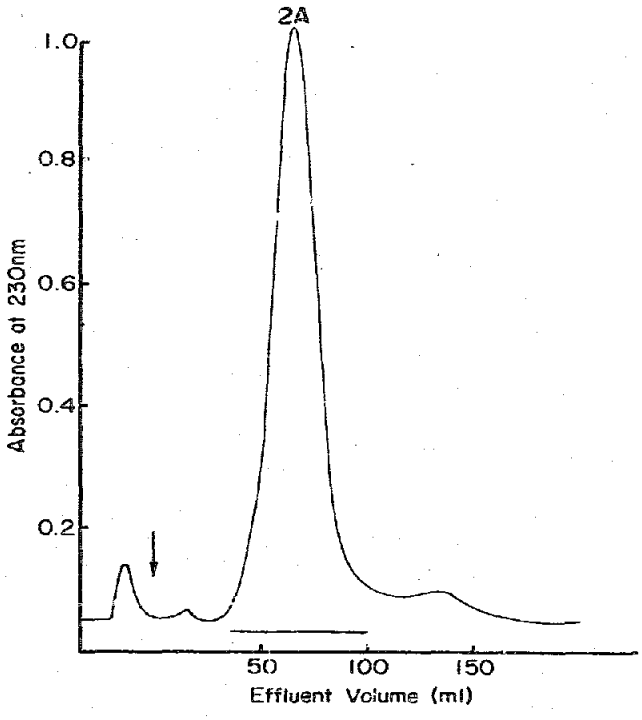

Fig.3. Rechromatography of peak 2A (30 mg) from fig.2 on carboxymethyl cellulose on 1 × 6 cm column equilibrated with 0.02 M acetate/1 M urea (pH 4.8) at 42°C. The column was eluted with a linear gradient consisting of 0.02 M acetate and 0–0.1 M NaCl in 1 M urea over total vol. 250 ml at a flow rate of 60 ml/h. The arrow indicates the start of gradient.

The 110 000 dalton collagen component described here apparently is similar to one reported [21,22] but is different from basement membrane collagen chain A and B from skin [11] and from placenta [12]. The 50 000 dalton component has been isolated and characterized for the first time. A 55 000 dalton component has been reported for human vascular intima [11] but the differences in the amino acid composition (4-Hyp, Glu, Leu, Tyr, Phe, Lys and Arg) are striking when compared to the 50 000 dalton component (table 1), although the variation may be due to the use of different species and tissues. The present study further indicates that basement membrane collagens form a unique class of collagens and should await classification at a future date.

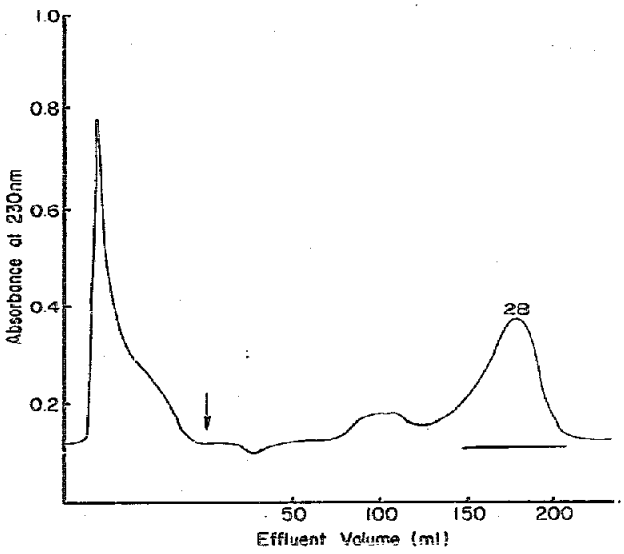

Fig.4. Carboxymethyl cellulose chromatography of peak 2B (10 mg) from fig.2. Conditions are identical to those for fig.3. Arrow indicates the start of gradient. The fraction represented by solid horizontal bar was pooled.

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