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THE COLLAGENOUS PROTEIN WITH ELASTIN CROSSLINKS FROM DESCEMET'S MEMBRANE IS NOT RELATED TO TYPE VIII COLLAGEN

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A collagen-like insoluble protein containing the elastin cross-links (desmosine and isodesmosine) has been isolated from Descemet's membrane. Recently type VIII collagen (endothelial collagen) has been shown to be a major constituent of this membrane. Biochemical studies suggest that these two proteins are unrelated. The cyanogen bromide peptide maps show negligible similarity. Antiserum raised against oxalic acid digests of elastin (α-elastin) did not react against an oxalic acid digest of type VIII collagen but did show some reaction against the cross-linked preparation. Immunofluorescent localization has demonstrated the presence of type VIII collagen in trachea but a desmosine cross-linked collagen could not be isolated from this tissue.

Type VIII collagen was first detected and isolated from cell cultures - in particular several endothelial cell and tumour cell lines (1-3). Subsequently its purification from a tissue (bovine Descemet's membrane) was described (4). Its presence and localization in a number of other tissues including aorta, trachea and ear cartilage have been reported (5-7).

Treatment of connective tissues with phenol: acetic acid: water (1:1:1 w/v/v) (PAW) dissolves most of the proteins but not the elastin component (8). When Descemet's membrane is subjected to treatment with PAW, a collagen-like residue which contained the characteristic elastin crosslinks, desmosine and isodesmosine, was found (9). As the Descemet's

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Abbreviation: PAW (phenol: acetic acid: water, 1: 1: 1, w/v/v).

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membrane is a specialized type of basement membrane, it has been suggested that this insoluble protein material is the tissue form of the pepsin resistant fragment known as type VIII collagen (4,10).

Several experiments have been performed in an attempt to determine the validity of such a proposition. Evidence is presented here which suggests that type VIII collagen is not a component of this PAW insoluble residue.

MATERIALS AND METHODS

Preparation of Tissue. PAW extracts were prepared from ovine Descemet's membrane and from the perichondrium of tracheal cartilage as described previously (8,9). Type VIII collagen was isolated from ovine Descemet's membrane by the method of Kapoor et al (4).

Digestion of Proteins. Samples of the purified type VIII collagen and of the PAW insoluble material were digested under nitrogen with cyanogen bromide at a ratio of 1:1 by weight at 37°C for 4 h. After dilution with water, the digests were dried. Oxalic acid digestions of the protein preparations were performed according to Partridge et al (11).

Analytical Procedures. The ELISA experiments were based on the method of Wrenn and Mecham (12). Each well of the microtitre plate was coated with 1μg of α-elastin (11). Incubations of the oxalic acid digests and of standard α-elastin with anti α-elastin (Elastin Products, Pacific, MO) were performed at 4°C for 18 h. One hundred μl of this mixture was added to the coated wells and the plates were incubated at room temperature for 30 min. After washing, the plates were incubated with anti-rabbit IgG-peroxidase (Sigma Chemicals, St Louis, MO) for 2 h, washed and assayed for bound peroxidase with o- phenylenediamine (13).

SDS-slab gel electrophoresis of the cyanogen bromide peptides was performed on a 4-20% gradient of acrylamide. Visualization of the protein bands was by silver staining (14).

Amino acid analysis was performed on samples that had been hydrolysed in 6N HCl for 24h at 110°C in vacuo. The analysis was undertaken on either a Beckman 119CL amino acid analyzer or on a Water Pico Tag Analyser using the dansyl chloride derivatization procedure (15).

RESULTS

Type VIII collagen and PAW-insoluble material were prepared from sheep Descemet's membrane. The type VIII collagen fraction obtained by chromatography of the 1.5M NaCl precipitate on agarose 1.5mm (4) appears to be free of type V collagen (figure 1). The PAW-insoluble material cannot be dissolved in the gel electrophoresis buffers. The amino acid composition of each is comparable to published analysis for bovine preparations (Table 1) (4, 9). They both contain hydroxyproline and hydroxylysine suggesting a collagenous nature. Additionally the insoluble residue has the desmosine crosslinks characteristic of elastin.

Cyanogen bromide peptide mapping of both preparations shows that the digestion of type VIII collagen produces a number of peptides (Figure 2 lane 3). These are similar to those reported for the individual chains of

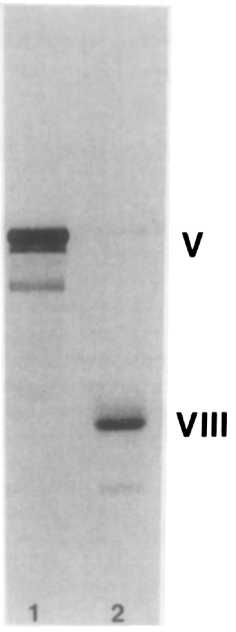

FIGURE 1
SDS Polyacrylamide gel electrophoresis of type V collagen (lane 1) and type VIII collagen (lane 2). The gel is a 4-15% linear gradient of polyacrylamide.

TABLE I

AMINO ACID COMPOSITION OF TYPE VIII COLLAGEN AND PAW-INSOLUBLE FRACTION

(Residues/1000 Residues)

	TVIII COLLAGEN		PAW-INSOLUBLE FRACTION		
	1 Sheep	2 Bovine (Ref 4)	3 Sheep	4 Bovine (Ref 9)	5 Sheep
Lysine	27.0	22.8	31.5	28	27.5
Histidine	10.9	6.3	18.1	14	6.8
Arginine	24.0	27.4	46.2	25	29.1
Aspartic Acid	37.5	31.1	50.3	50	12.3
Threonine	29.7	16.3	38.4	24	14.2
Serine	35.5	15.4	68.5	20	12.7
Glutamic Acid	82.4	84.2	76.1	84	36.4
Proline	111.2	99.9	93.9	124	90.7
Glycine	319.6	342.4	223.1	229	285.5
Alanine	72.2	45.3	50.4	61	194.2
Half Cystine	1.7	4.2	-	-	-
Valine	30.2	31.9	61.5	55	115.3
Methionine	2.8	3.8	19.8	22	-
Isoleucine	35.7	25.5	29.3	29	31.6
Leucine	54.4	69.8	74.2	75	81.6
Tyrosine	6.8	9.2	35.5	47	5.5
Phenylalanine	15.7	18.9	28.7	30	39.0
Isodesmosine	-	-	1.1	1.8	1.1
Desmosine	-	-	2.3	3.8	1.2
Hydroxyproline	87.1	134.7	45.4	71	11.7
Hydroxylysine	15.6	22.2	5.7	13	0.4
Lysinonorleucine	-	-	-	-	3.3

Columns 1-4: Descemet's membrane Column 5: Tracheal perichondrium

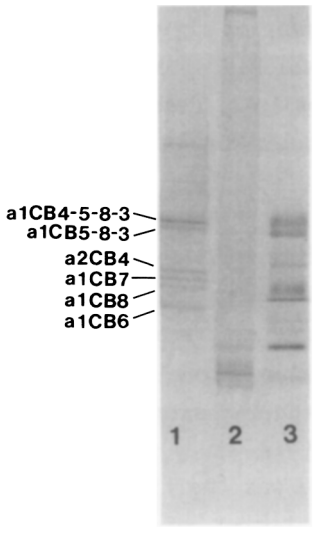

FIGURE 2
SDS - Polyacrylamide gel electrophoresis of cyanogen bromide peptides of type I collagen from rat tail tendon (lane 1), of PAW-insoluble residue (lane 2) and of type VIII collagen (lane 3) from ovine Descemet's membrane. The gel is a 4-20% linear gradient of polyacrylamide.

bovine type VIII collagen (4). In contrast there are only 3 or 4 peptides produced by digestion of the desmosine-containing insoluble preparation. (Lane 2, Figure 2). They are faster migrating than the major digestion products of type VIII collagen. More importantly, there is very little similarity in size between the peptides obtained from the digestion of each of the preparations.

When the antiserum to α-elastin was incubated with oxalic acid digests of type VIII collagen and the PAW-insoluble residue in an ELISA, the elastin content of the former was approximately 2.7 μg per mg protein whilst that from the insoluble residue was much higher at 22.2 μg per mg protein.

The perichondrium of cartilage shows strong immunofluorescent staining with type VIII collagen-specific antibodies and so may be rich in type VIII collagen (5,7).In this study the perichondrium was carefully dissected from the trachea so as to eliminate elastic tissue as completely as possible. The residue remaining after PAW extraction of this material showed very little evidence of collagen (Column 5, Table 1). There was negligible hydroxylysine, low hydroxyproline, low levels of acidic residues, high alanine and valine. In fact it was similar to an elastin preparation.

DISCUSSION

The first tissue that type VIII was isolated from was bovine Descemet's membrane (4). Immunofluorescence has shown that it is concentrated in this

membrane. Extraction of connective tissues with PAW solubilizes collagen but not elastin (8). Treatment of this membrane with this solvent produced a small quantity of insoluble protein. This residue contained the characteristic elastin crosslinks, desmosine and isodesmosine, but a number of other compositional features suggested that it was rather collagen-like (9).

Because of the presence of type VIII collagen in Descemet's membrane and also of the unique crosslinked material in the same tissue, it has been suggested that the two may be related (4,10). Investigation of this relationship by conventional protein analytical procedures has been hampered by the fact that the PAW extraction product is insoluble. However the results obtained in this study suggest that they are not closely related. If the PAW residue was the tissue form of type VIII collagen, it would be expected that a number of the peptides produced by cyanogen bromide digestion would be similar, although the former might be expected to have a larger number. That there was nothing in common suggests that the type VIII collagen is not the pepsin fragment of the PAW material. The cyanogen bromide peptide map of the ovine collagen is similar to that of the bovine protein (4).

The oxalic acid digest of the PAW material cross-reacted with an antibody raised against an oxalic acid digest of elastin whereas the oxalic acid digest of type VIII collagen showed negligible reaction. As well as highlighting the lack of similarity this result also suggests that the desmosine-containing insoluble fraction is elastin-like. It is possible that the PAW residue is a mixture of elastin-like and collagen-like proteins.

Immunofluorescence suggests that the perichondrium of trachea is rich in type VIII collagen (5,7). If the PAW insoluble material was the tissue form of this collagen, then it would be expected to be present in this tissue also. Extraction of carefully dissected perichondrium produced a very elastin-like residue with little evidence of collagen characteristics.

These experiments suggest that the PAW insoluble protein with elastin crosslinks but a collagen-like amino acid composition does not contain the tissue form of type VIII collagen. The insolubility of it prevents the use of antibodies to type VIII collagen for determining any cross-reactivity. As well cyanogen bromide digests of either preparation reacted weakly with type VIII collagen antibodies.

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