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Basement membrane collagen – evidence for a novel molecular packing

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Type IV collagen is the major structural protein of basement membranes but very little is known about its molecular organisation in vivo. We have used X-ray diffraction of a thick basement membrane, bovine lens capsule, to provide information. Under constant load, lens capsule gave a collagen diffraction pattern of a similar quality to unstretched rat rail tendon. In addition there were clear meridional reflections which indexed as orders of 10 nm, and equatorial reflections at 2.1 and 3.8 nm. These results suggest the ordering of type IV collagen molecules in fibrils, with a 10 nm periodicity along the length of the fibrils.

Collagen; Basement membrane; X-ray diffraction; Lens membrane

1. INTRODUCTION

Basement membranes are ubiquitous extracellular structures which separate cell types or divide cell layers from underlying connective tissue. The major structural component of basement membranes is type IV collagen supplying 30–40% of the dry weight, and this collagen provides the strength and flexibility of the membrane. Unlike the fibrous collagens, type IV does not form banded fibres, and isolated molecules appear in the electron microscope as 400 nm long flexible rods, each with a 17 nm globular head [1]. These molecules form tetramers by antiparallel association of the short N-terminal regions [2] and the most widely accepted model [1] suggests that aggregation of the C-terminal globular regions of the tetramers leads to formation of a 'chicken-wire' network of molecules. There is little evidence to support this or any other model [3,4]. Anterior lens capsule has been widely used as a source of basement membrane collagen and in pepsin digests, the isolated collagen chains consist almost entirely of type IV collagen [5,6]. Since the organisation of type IV collagen in basement membranes is likely to be as a network, we decided to use X-ray diffraction techniques which were developed for the study of polysaccharide polymers [7]. The X-ray diffraction pattern we have obtained from this thick basement membrane suggests a fibrillar array of collagen molecules. This is the first report of a crystalline structure for any non-fibrous collagen and the results throw considerable light on the organisation of collagen within the basement membrane.

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2. MATERIALS AND METHODS

Fresh eyes were obtained from young adult cattle at slaughter and processed within 3 h. Lenses were dissected out and the anterior lens capsule peeled off as a disc. Strips of approx. 1 mm width were then cut along the vertical diameter of the lens capsule. Where possible the full length of the diameter was used, yielding specimens of 9–12 mm useful length. These were extended at

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constant load for a minimum of 24 h to produce extensions of 60–65%. X-ray diffraction patterns were then obtained using pinhole collimated cameras, either from an Elliot GX6 rotating-anode machine or a Philips fixed-tube generator, employing a specimen-to-film distance of 30–50 mm. A Rigaku-denki low-angle camera was also employed at specimen-to-film distances of 250 mm. Calcite was used as an internal calibration. For some medium-to-low-angle experiments the synchrotron X-ray source at SERC Daresbury Laboratory was used employing a pinhole collimated camera flushed with helium. For these, molybdenum sulphide was used as internal calibrant. Beam direction was normal to the capsule surface, and the beam was concentrated on the central region of the strip to ensure that there could be no contribution from fibrous collagen near the capsule perimeter. Patterns were obtained with specimens in both wet and dry states.

3. RESULTS AND DISCUSSION

Fig.1a shows a typical diffraction pattern from bovine anterior lens capsule at low relative humidity; fig.1b shows the pattern at 100% humidity. These show considerable crystallinity and good orientation along the direction of stretch, presenting collagen patterns of similar quality to unstretched rat tail tendon. The characteristic collagen wide-angle meridional reflection of the triple helix is present at 0.285 nm. In addition there is a series of meridional reflections which it has proved possible to index (table 1). These show a repeat of 10 nm along the fibre axis. In contrast to the results of Roveri et al. [8] we can find no evidence for a 67 nm repeat in our preparations.

The equatorial order is unlike that of type I collagen. There is a complex set of intense reflections centred near 1.7 nm and faint maxima at 2.1 and 3.8 nm (fig.2). This strongly suggests lateral association of the type IV molecules to form microfibrils [9]. These microfibrils may form the basis of a network and provide a strong flexible structure, as previously proposed [3,4]. Such microfibrils appear to be incompatible with the chicken-wire network proposed by Timpl et al. [1], which is a very open structure with strands only a single molecule in thickness. The spacing of the equatorial reflection at 1.7 nm (wet) is significantly greater than the 1.26 nm reflection observed from rat tail tendon (type I) collagen fibres. The wider spacing observed here clearly indicates a looser lateral packing of the molecules in the proposed type IV microfibrils than in fibrous type I and resembles that recorded for type II [10].

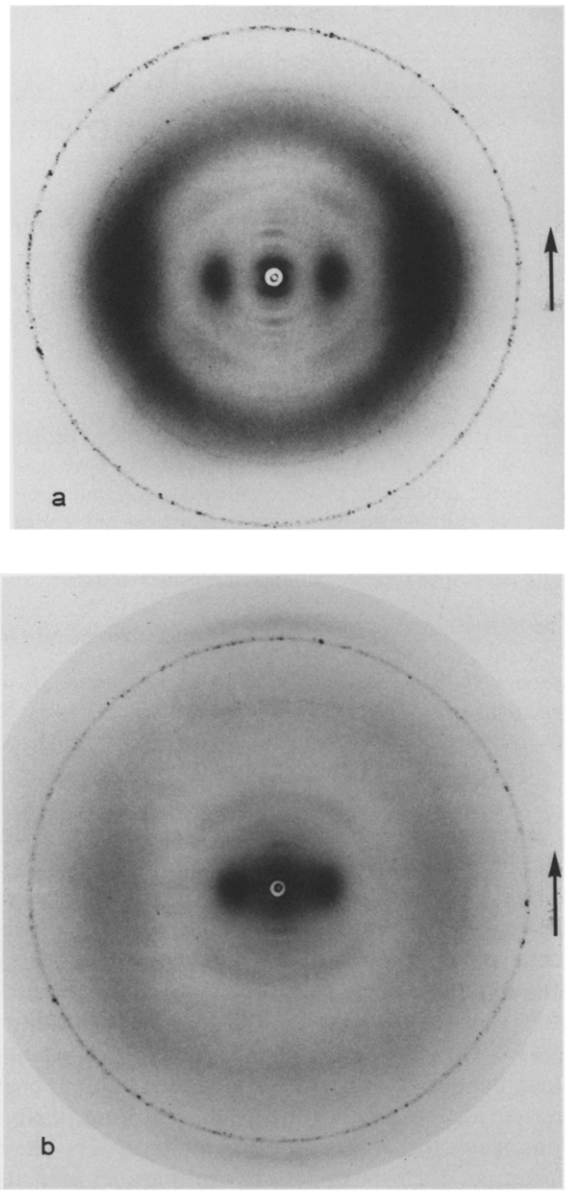

Fig.1. X-ray diffraction patterns of bovine lens capsule: (a) dry, (b) wet. The direction of stretch is indicated by the arrows. Calcite is included as an internal standard.

The meridional repeat at 10 nm can be inter-

Table 1

Meridional pattern of bovine lens capsule

Vacuum dried				Water vapour saturated			
I_o	$d_{obs.}$ (nm)	n	$d_{calc.}$ (nm)	I_o	$d_{obs.}$ (nm)	n	$d_{calc.}$ (nm)
ms	9.0[a]	1	9.44				
vs	4.55	2	4.75	m	5.1	2	5.1
s	3.19	3	3.16	m	3.55	3	3.40
mw	2.49	4	2.36	m	2.57	4	2.55
vs	1.66	6	1.57	s	1.72	6	1.70
s	1.39	7	1.35	ms	1.41	7	1.46
mw	1.15	8	1.18				
mw	0.80	12	0.79	w	0.83	12	0.83
w	0.70	13	0.73				
w	0.63	14	0.67				

[a] Detected in medium–low-angle synchrotron diffraction patterns; approximate value

The observed intensities (I_o) are estimated as very strong (vs), strong (s), moderately strong (ms), medium (m), moderately weak (mw) and weak (w). The observed spacings ($d_{obs.}$) are compared with the calculated spacings ($d_{calc.}$) corresponding to orders of 9.44 nm (dry) and 10.2 nm (wet)

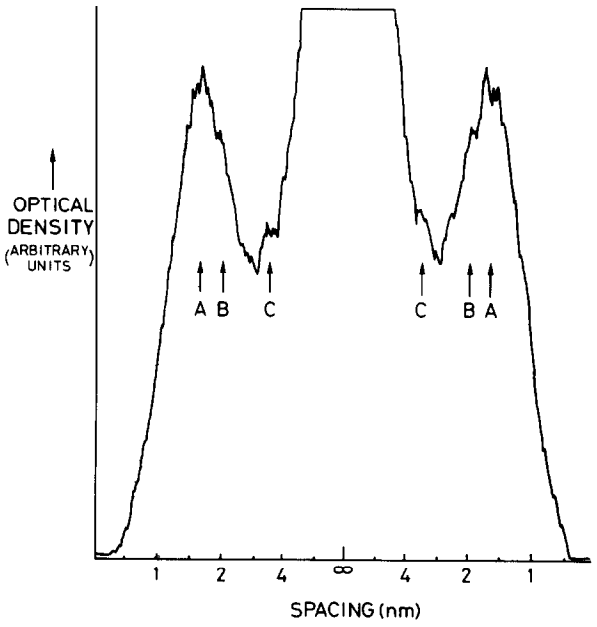

Fig.2. Densitometer trace of equatorial reflections of wet lens capsule as in fig.1b. (A) Complex set of maxima centred near 1.7 nm; (B) weak reflection at 2.1 nm; (C) weak reflection at 3.8 nm. This may easily be compared with published traces for tendon type I [18].

preted in several ways. The repeat could result from supramolecular ordering of the collagen molecules themselves, such as a twisting of the bundle structure into a super-helix with a pitch of 10 nm. Estimates for the dimensions of the globular NC1 region of type IV range from 17.5 nm [11,12] to 12–14 nm [13]. The repeat dimensions found here approximately fit Weber's estimated diameter of the NC1 domain [13], so it is possible that the 10 nm repeat may be due to staggered clusters of NC1 domains. Some other component of the basement membrane may exist in a fibrillar form with an axial repeat of 10 nm, or other components could be regularly associated with the collagen, giving a repeat distance of 10 nm. So far no such highly ordered components have been reported. Basement membranes do contain large amounts of the protein laminin [14], but there is no evidence that laminin and collagen associate in such a regular fashion [15]. Pre-extraction of samples with 1 M NaCl or 1 M guanidine hydrochloride failed to alter the diffraction patterns obtained.

A fibrillar ultrastructure has already been observed in lens capsule, and superhelices proposed on the basis of mechanical properties [16,17]. That this texture may be related to type IV collagen now appears likely. This fibrillar organisation is probably present in all basement membranes and its destruction may be an important step in the basement membrane damage which initiates many pathological conditions.

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