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REVIEW ARTICLE

Proteoglycan–fibrillar collagen interactions

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INTRODUCTION

The future of biochemistry lies in understanding how single gene products associate and function together. Interactions in connective tissues, including those between the proteoglycans and collagens are especially piquant, evolutionarily conserved and physiologically significant.

Connective tissues (e.g. skin, tendon, blood vessels, etc.) are systems of insoluble fibrils and soluble polymers which evolved to take the stresses of movement and the maintenance of shape [1]. The fibrils resist pulling forces, and the interfibrillar soluble polymers resist compressive forces, like the stuffing in a cushion. Cells communicate biochemically through the soluble polymer compartment. Within this simple framework, multicelled organisms developed [1].

It was often speculated that there were specific associations between fibrils and soluble polymers. These ideas were extremely general, lacking chemical knowledge of the participants, and methods for diagnosing interactions. The situation now tends to the other extreme. Sensitive methods discover many 'specific' liaisons – *in vitro*. Even when these are observed at physiological pH, temperature and ionic strength, caution is needed. The very large polymers characteristic of connective tissues (collagens, proteoglycans etc.) have enormous surfaces, because of their shapes, with great potential for interactions. Interactions are not functionally significant if the participants are not placed *in vivo* so that they can interact. This review is weighted towards cases where interactions were seen in tissues.

THE PARTICIPANTS

The **collagens**, comprising at least 12 different gene products [2,3], contain large amounts of hydroxyproline ($\leqslant 17\%$), glycine ($< 33\%$) and proline. They are classified simply, types I–XII. Type I is the commonest. Many tissues (e.g. tendon, sclera, bone) contain little of any other. Like types II and III it is a thread-like molecule consisting of three similar polypeptides (the α chains, $\sim 10^5$ Da), in a triple helix about 300 nm long. They aggregate in 'quarter stagger' arrays, with about 75% of each molecule in contact with its neighbours, ahead and behind (Fig. 1). This form is determined by the amino acid sequences of the α-chains, that come together to maximize hydrophobic and charge attractions between them [4]. Because of periodicity in the polypeptides, with glycine at every third residue, and a high incidence of sequences such as Gly-Pro, the fibrillar aggregates (stained with UO_2^{2+} etc.) show periodic features in electron micrographs, as repeating patterns of dark bands, designated a–e (Fig. 1). According to a simple model, the part of the fibril containing the gap between the ends of the collagen molecules (hence the 'gap zone', where bands d and e are located) alternates with the 'overlap' zone (where band b is located) with bands a and c at the 'step' to the 'gap' zone (Fig. 1).

Collagen types I, II, III, V and XI form fibrils [5]. Types I and III can coexist in the same fibril [6], and some 'minor' collagens probably associate with fibrillar collagens (e.g. type V with type I in the cornea [7], type IX with type II [8]). Type VIII collagen, in Descemet's membrane [9], may also form fibrillar or branched fibrillar structures.

Collagens are biosynthesized as procollagens, which are processed extracellularly by proteolytic cleavages at the *N*- and *C*-terminals before they can aggregate efficiently to fibrils. There are thus a number of stages at which collagens might interact with proteoglycans, from procollagen, through various aggregates, to the complete fibril. The first stages are in solution, the later interactions are surface phenomena.

The **proteoglycans**, the soluble polymers in the simple scheme of connective tissues, above, consist of a polypeptide 'core', to which is attached one or more glycan chains [10–12]. The **glycan chains** are of three types, (1) heparan, (2) keratan, and (3) chondroitin–dermatan. They consist of repeating disaccharide units, one residue of which is always hexosamine, usually with sulphate ester groups attached at the 4 or 6 positions.

(1) **Heparans** contain D-glucuronic and L-iduronic acid in varying proportions, and glucosamine, either as 2-deoxy-2-acetamido- or 2-deoxy-2-sulphamato-glucose. The glycosidic links are $1 \rightarrow 4$. The number of sulphate groups per disaccharide unit varies from < 0.5 in some heparan sulphates to > 2.5 in heparins.

(2) **Keratans** contain *N*-acetylglucosamine and D-galactose, linked $1 \rightarrow 3$ and $1 \rightarrow 4$ respectively. The degree of sulphation is typically 0.8–1.5. The molecular mass of corneal keratan sulphates is very variable, from 3×10^3 to 20×10^3 Da [13]. Mannose, fucose and sialic acid are present.

(3) **Chondroitin–dermatan** glycans contain *N*-acetylgalactosamine, with D-glucuronic and/or L-iduronic acid linked $1 \rightarrow 4$ and $1 \rightarrow 3$ respectively. Chondroitin, by definition, contains no L-iduronic acid, which is characteristic of dermatan. Iduronate-free chondroitins are present in some tissues in large amounts, but most dermatans contain glucuronic acid. Where $> 10\%$ of the uronate is iduronate, it is reasonable to call the glycan a dermatan. Sulphation is usually about 1.0/disaccharide, but in the cornea it can be < 0.2. Molecular masses are $(10–50) \times 10^3$ Da.

A fourth glycan, hyaluronan, similar in structure to the chondroitins, is found in all connective tissues. It is not attached to a peptide chain, nor is it sulphated. It contains glucuronic acid and *N*-acetylglucosamine, and is usually of molecular mass 10^5–10^7 Da [14].

The **protein cores** are diverse. It is simplest to consider three classes, determined by their morphology, i.e.

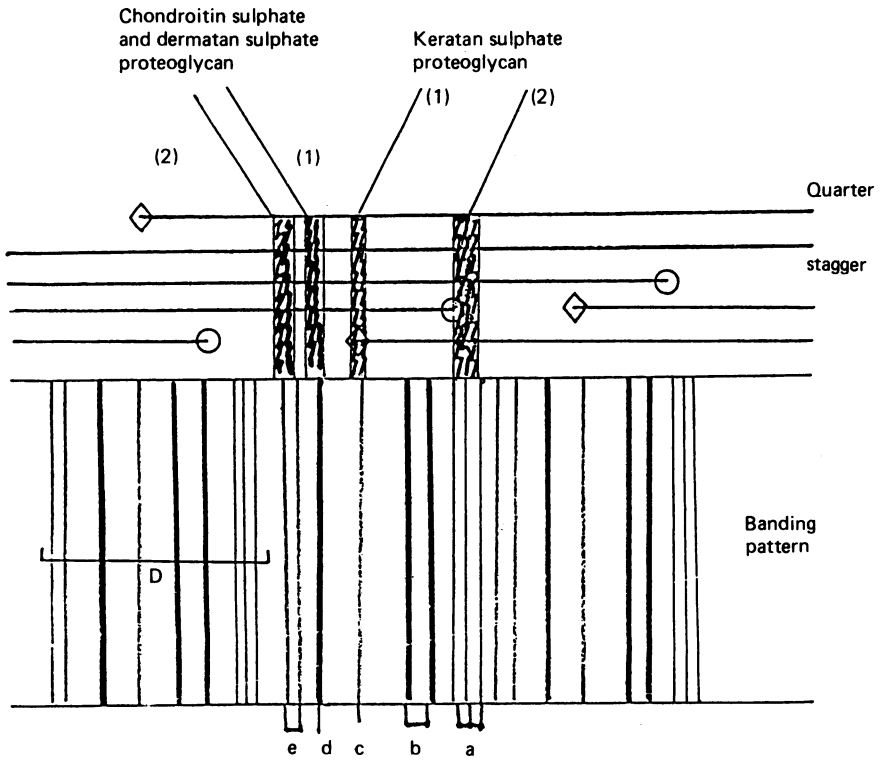

Fig. 1. Map of binding sites of proteoglycans along the collagen fibril

The a–e banding pattern within the D period of the collagen type I fibril (lower portion) is shown against the arrangement of collagen molecules in quarter-stagger (upper portion). ◇, *N*-terminal; ○, *C*-terminal. D, D period. The locations of proteoglycans are displayed across the quarter-stagger diagram, to correlate with the a, c, d and e banding pattern. The dermatan sulphate proteoglycan and keratan sulphate proteoglycan groupings are based on the staining of keratanase-, chondroitinase- (ABC and AC), or hyaluronidase-digested tissue plus biochemical morphometric analysis of tendon. Dermatan sulphate proteoglycan 1, dermatan sulphate proteoglycan 2, keratan sulphate proteoglycan 1 and keratan sulphate proteoglycan 2 are the corneal proteoglycans so designated [21]. Numbers are bracketed to show that the evidence for their location is not as definite as that for the keratan sulphate and chondroitin sulphate–dermatan sulphate groups as a whole. Staining at different salt concentrations, which indicated that the d and e band proteoglycans were different, as were the a and c band proteoglycans (J. E. Scott & M. Haigh, unpublished work), supported the attributions shown above, originally based on parallels between relative frequencies of band occupancy and tissue proteoglycan concentration [72]. The latter method is not as convincing with the keratan sulphate proteoglycans, which are present in somewhat similar amounts, as with the dermatan sulphate proteoglycans, which are present in very different concentrations [72]. The numbering of the dermatan sulphate bands was originally reversed [72] and [93,101], in error (cf. text of [72]). Results on keratan sulphate and dermatan sulphate proteoglycans were from type I collagen rich tissues (see the text), and the relevant fibrils were therefore probably of type I collagen. The chondroitin sulphate proteoglycan interactions were observed in annulus fibrosus [92] which contains much type II collagen, and it is not known whether the relevant fibrils were type I or II.

'small', 'large' and 'very large' (Fig. 2). Rotary shadowing electron microscopy brings out the salient features [15–17].

(1) The 'small' proteoglycans, globular proteins with one or two glycan chains, are tadpole-like.

(2) 'Large' proteoglycans have at least one globular region, with a linear polypeptide extension, to which are attached five to ten glycosaminoglycan chains.

(3) 'Very large' proteoglycans, particularly those from cartilage, have up to three globular regions attached to a long polypeptide chain, to which are attached up to 100 glycan chains.

Many small proteoglycans have similar amino acid compositions [18], tryptic peptide maps, and cross-react with antibodies [19]. Some amino acid sequences have been determined, mainly by DNA techniques (e.g. [20]).

Keratan sulphate and chondroitin sulphate are present together in some very large proteoglycans, but are attached to separate protein cores in the cornea [21]. A 'large' proteoglycan has been isolated containing both chondroitin sulphate and heparan sulphate [22].

Summary. The diversity of structures among collagens and proteoglycans implies a very large number of potential interactions. '*In vitro*' investigations have only scratched the surface of the many possibilities. One proteoglycan–collagen association is well defined: Type IX collagen contains covalently linked glycosaminoglycan [23–26].

'*IN VITRO*' MODELS

General

Most pre-1970 work on interactions between isolated collagens and proteoglycans is of historic interest only, since materials were not pure or intact. Sajdera & Hascall's work [27] on proteoglycan isolation, and Miller's on collagens other than Type I [28] were new departures.

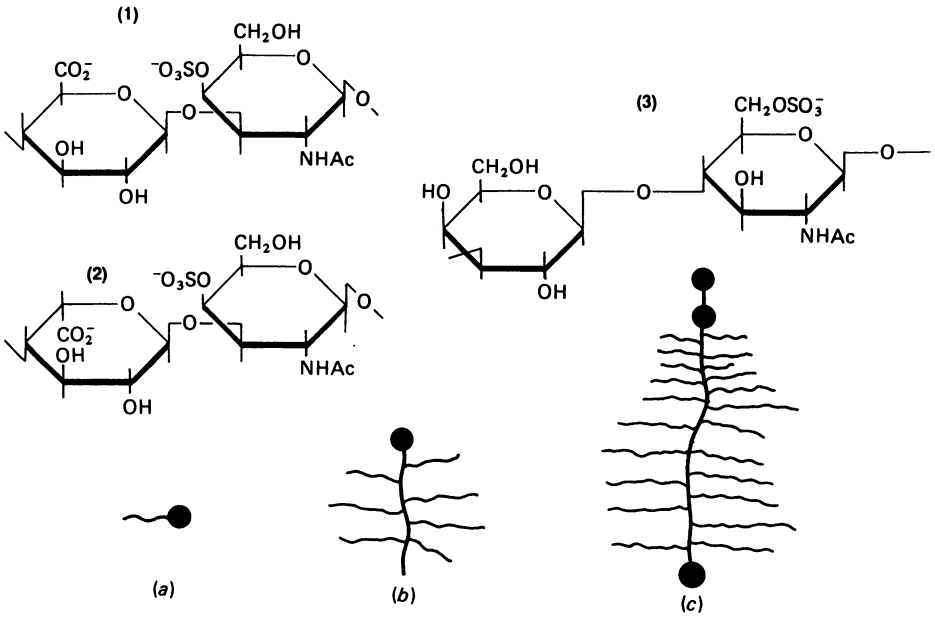

Fig. 2. Chemical structure and physical appearance of proteoglycans

(1)–(3) Structures of repeating disaccharide units of glycans in proteoglycans which associate orthogonally at specific sites along the collagen fibril: (1) chondroitin sulphate (as the 4-sulphate) (2) dermatan sulphate, and (3) keratan sulphate (as the monosulphate, but higher degrees of sulphation are likely). (*a*)–(*c*) Diagrammatic illustrations of the appearance by rotary shadowing and electron microscopy [15–17] of (*a*) the small (tadpole), (*b*) the large, and (*c*) the very large, proteoglycans. Available evidence suggests that orthogonally associated proteoglycans are of type (*a*). The status of interactions of (*b*) and (*c*) with collagen is less clear (see the text).

The state of aggregation of the collagen is significant in determining interactions. Lathyritic collagen [29] or fetal collagen, characterized physically to ensure that single tropocollagen molecules are the active species, are preferred substrates. Collagen prepared by 'standard' methods (extracted with salts, then precipitated by NaCl, ethanol etc.) is contaminated dermatan sulphate proteoglycan, which can be removed by anion-exchange [e.g. on DEAE cellulose (M. Capaldi & J. E. Scott, unpublished work)]. DNA interacts strongly with collagen [30], so proteoglycan preparations should be checked for nucleic acid content.

Polyanionic glycosaminoglycans precipitate proteins as polycations at acid pH (e.g. < pH 4.0). The general case of polyanion–polycation interactions and coacervation was exhaustively investigated by the school of Bungenberg de Jong [31]. None of the glycosaminoglycan–collagen or glycosaminoglycan–gelatin results depart from expected behaviour. Data obtained at low pH are of limited functional significance and will not be discussed ([32], reviews early work). Interactions of isolated glycosaminoglycans with collagens probably occur seldom in normal tissues, but are significant in analysing the more complex proteoglycan–collagen interaction.

Solutions of intact fibril-forming collagens at physiological pH and ionic strength are used at low temperature, to avoid spontaneous fibril formation (fibrillogenesis), which occurs quickly at 37 °C [33]. The influence of glycosaminoglycans and proteoglycans on fibrillogenesis is a complicated but functionally relevant way of detecting glycosaminoglycan–proteoglycan interactions with collagen ([34], and see below). Collagen without non-helical peptides aggregates less readily, but the data obtained are of restricted interest.

Problems of aggregation were avoided by using affinity supports with collagen, or parts thereof, as the ligand. Solutions of glycosaminoglycan, proteoglycan etc. were applied and eluted under appropriate conditions. Various supports were used; e.g. CNBr-Sepharose [35,36], agarose [37,38], or collagen itself, cross-linked with glutaraldehyde [39], as reconstituted fibrils [40], or as the insoluble residue from exhaustively extracted tissues [32]. The state of aggregation and spatial arrangement of collagen molecules in most preparations were unknown, rendering detailed interpretations difficult.

Solutions of interactants have been examined by light scattering [41] or electric birefringence [42].

Binding regions on both molecules may be recognized by electron microscopy, after rotary shadowing [43] or positive staining [44].

Results

Glycosaminoglycan–collagen interactions are electrostatic, since they are abolished by raising the salt concentration. This criterion is not as clear as often assumed; it shows only that there is an electrostatic component, and that remaining bonding(s) cannot hold the components together at ambient temperature.

The salt concentration required to prevent interactions differs according to the system. Potential confusion arises from the way results were expressed. Some [37,39] treated immobilized collagen as a gel, from which a kind of binding constant was derived, whereas others [36] saw it as an ion exchanger from which polyanion eluted at characteristic salt concentrations. The former method can demonstrate weak binding at high salt concentrations.

Hyaluronan interacted feebly or not at all with collagen at physiological pH and ionic strength [32,41]. Neither

did keratan sulphate [41]. Chondroitin sulphate and heparan sulphate did not bind to reconstituted collagen fibrils under physiological conditions [40], whereas heparin interacted strongly in all systems [39–41]. Thus, glycosaminoglycan linear change density is important. Heparan sulphate of low M_r but high sulphation was more active than one with high M_r and low sulphation [41].

Mathews [32] interpreted stronger binding of higher-M_r chondroitin sulphate in terms of multisite attachment, longer chains spanning and binding to more sites. While probably true, it is unnecessary to assume widely separated binding sites. Interactions of polyanions (chondroitin sulphate, keratan sulphate etc.) with polycations are sensitive to M_r [45].

Iduronate-rich glycosaminoglycans (dermatan sulphate and heparan sulphate) were more active at comparable size [41,46]. Such glycosaminoglycans are potentially more interactive than glucuronate analogues, because of secondary structures in the latter [47].

It is difficult to derive association energies from these results. In principle, the binding constants [37] could be used, but the experiments were at low salt concentrations. The data fit a plot of log K against [NaCl] (J. E. Scott, unpublished work), and lengthy extrapolation to 0.14 M-NaCl gives an estimate of 2.1 kJ/chondroitin sulphate chain, equal to only 10–20% of that of one hydrogen bond/chain. This value is of very limited validity, but if all ($\sim$ 100) chondroitin sulphate chains in the very large cartilage proteoglycan interacted as weakly, the total binding energy would be considerable. This is unrealistic, given the ease with which chondroitin sulphate proteoglycans are extracted. It is more likely that many chondroitin sulphate chains do not interact at all, as suggested by n.m.r. results (see below).

Fibrillogenesis is strongly affected by glycosaminoglycans [33,34]. Wood & Keech [34] dissected the process into a nucleation (or lag) phase and a growth phase. They demonstrated effects, originating in one phase or the other, on fibril morphology. The biological interest and attractive simplicity of these ideas led to much use of the system in diagnosing and comparing interactions of glycosaminoglycans and proteoglycans with collagens.

The terms 'precipitation' and 'fibrillogenesis' have often been used interchangeably. Precipitation (or coacervation) of most proteins with glycosaminoglycans and proteoglycans occurs at low pH and low ionic strength [48], whereas banded fibril formation is characteristic of only some collagens. In non-physiological conditions collagen precipitates in non-banded forms [49] and it is better to reserve 'fibrillogenesis' for those systems in which fibrils undoubtedly form. Fibrillogenesis is more complicated, and sensitive to as yet unidentified factors, than the early analysis suggests.

Chondroitin sulphate and keratan sulphate had little effect on nucleation or growth [33,34,49,50,51]. Hyaluronan speeded up both stages, possibly by excluded volume effects [41,49,51] that increased activity of collagen [41], but neither keratan sulphate, nor chondroitin sulphate at much higher concentrations behaved similarly [41], and the hyaluronan effect may be more subtle and specific.

Glycosaminoglycans which interacted strongly with collagen at 4 °C (dermatan sulphate, heparan sulphate [41,46] and heparin at low concentrations [41]) accelerated nucleation.

Proteoglycans did not behave according to the 'simple' glycosaminoglycan experience. Although large cartilage chondroitin sulphate proteoglycans were without effect [49,52] or decelerated [50,51,53,54] growth, they were incorporated into the type I collagen 'precipitate' [50,51,53]. The glycosaminoglycan chains chondroitin sulphate [50,51] and dermatan sulphate [51] were not, nor were they incorporated into Type II collagen fibrils [55]. Chondroitin sulphate proteoglycan was incorporated only if it was present during, but not after, fibril formation [50]. The bound proteoglycan was not in equilibrium with solution proteoglycan, and tended to be lost to the solution with time.

These results suggest that glycosaminoglycans and some proteoglycans might be 'squeezed out' of their complexes with collagens by other collagen molecules during aggregation. Such a mechanism would explain why no glycosaminoglycan chains were found in collagen precipitates, although typically two to five chains of glycosaminoglycan were associated per molecule of collagen in solution [46]. A similar mechanism may operate during growth *in vivo* (see below).

Cartilage chondroitin sulphate proteoglycans without chondroitin sulphate (by chondroitinase digestion) but with residual keratan sulphate, bound strongly to Type I collagen [38,50,56] and Swarm sarcoma proteoglycan, which contains no keratan sulphate, still bound to collagen after removal of chondroitin sulphate [50]. Digestion with papain, Pronase etc. [50,56] completely destroyed binding. These results point to a core-protein association with collagen. Nevertheless, the core-protein was unable to inhibit chondroitin sulphate proteoglycan binding [50,56] suggesting either separate binding sites for the two species, or strong binding of chondroitin sulphate proteoglycan, perhaps due to co-operation between chondroitin sulphate chains and the protein core.

It is not clear whether 'aggregated' chondroitin sulphate proteoglycan does [50] or does not [52,53] inhibit fibril formation rates.

Small dermatan sulphate proteoglycans, like the very large cartilage chondroitin sulphate proteoglycans, are incorporated into Type I collagen precipitates [44,51,57] and inhibit fibril growth *in vitro* [36,51] ([54], dermatan sulphate proteoglycan from tendon). Dermatan sulphate proteoglycan (skin) protein core associates with, and inhibits fibrillogenesis [36,54]. Antibody staining and electron microscopy demonstrated binding of the protein core to reconstituted type I collagen fibrils [58].

Results on other proteoglycans are few. A keratan sulphate proteoglycan from cornea did not bind to a type I collagen–Sepharose column [59] at 0.1 M ionic strength. A mammary epithelial cell heparan sulphate proteoglycan bound strongly to reconstituted type I collagen fibrils at higher-than-physiological salt concentrations [40]. Heparan sulphate chains did not bind under these conditions, but heparin inhibited heparan sulphate proteoglycan binding.

Collagens other than type I interact with proteoglycans and glycosaminoglycans, e.g. type II [54–56], and the $1\alpha,2\alpha,3\alpha$ collagen from cartilage [60]. The very large cartilage chondroitin sulphate proteoglycan interacted with the latter (both as fibrils and in solution) with high avidity. Heparin, dextran sulphate and chondroitin

sulphate competed in binding, but chondroitinase-digested chondroitin sulphate proteoglycan did not (cf. chondroitin sulphate proteoglycan–type I collagen interactions, above).

Type IV collagen–heparan sulphate proteoglycan interactions have been much investigated with reference to basement membrane structure (see [61] and [62] for recent reviews).

Summary and consensus of findings from model experiments

(1) Glycosaminoglycans interact electrostatically with type I collagen under physiological conditions. Additional short range bonding cannot be excluded. Linear charge density is important, as is glycosaminoglycan shape (determined by iduronate:glucuronate ratios).

(2) Strongly interacting glycosaminoglycans accelerate fibrillogenesis.

(3) Glycosaminoglycans are not incorporated into types I and II collagen fibrils.

(4) Chondroitin sulphate and dermatan sulphate proteoglycans interact strongly with type I collagen, electrostatically via glycosaminoglycan chains, and by protein–protein interactions.

(5) Chondroitin sulphate and dermatan sulphate proteoglycans are incorporated into type I collagen precipitates, if present during fibril growth.

(6) Chondroitin sulphate and dermatan sulphate proteoglycans inhibit fibrillogenesis.

Available data are insufficient to establish differences between type I and other fibrillar collagens. The influence of telopeptides on interactions is not yet clarified, and work on the morphology of fibrils produced in the presence of glycosaminoglycans or proteoglycans is incomplete.

TISSUES

General

The co-occurrence in tissues of particular glycosaminoglycans and collagens might imply interaction between them [63]. As the number of identified glycosaminoglycans, proteoglycans and collagens rises rapidly, this inference is unreliable.

The ease of extraction of proteoglycans from tissues is a rough guide to the strength of binding to collagen. Since most are extractible in 4 M-guanidinium chloride, covalent links are excluded, but interactions of lesser energy are not easily distinguished from entrappment and entanglement. Very mild methods (water, mechanical disintegration) used to extract, e.g., hyaluronan from many tissues, chondroitin sulphate proteoglycan from cartilage, etc.), imply weak or negligible bonding.

Ultrastructural methods, which inform in biochemical terms at molecular resolutions, that were developed in the last 10 years, are based on X-ray diffraction [64–66], n.m.r. [67], but mainly on electron histochemistry [68].

Whereas collagen fibrils are insoluble permanent structures, proteoglycans are soluble, invisible and swollen, in aqueous environments. Routine embedding in plastic for electron microscopy, involving dehydration through organic solvents, precipitates them randomly. In many tissues (skin, tendon, etc.) proteoglycans occur in relatively small amounts ($< 1.0\%$ w/w). It is therefore necessary to (*a*) render the proteoglycans visible (i.e. stain them) preferably specifically [69,70], (*b*) retain their molecular morphology, as far as possible [68] and (*c*) fix the proteoglycans vis-a-vis fibrils, cells and other molecules, so that the stained artefact is interpretable in terms of molecular interactions.

Staining reagents were classified as macro- and mini-, to emphasize important aspects of their application [68]. Macro-stains (mainly antibodies and derivatives) penetrate tissues poorly, often do not stain stoicheiometrically, have inferior resolution at the molecular level, but are usually specific and sensitive. Mini-stains (e.g. dyestuffs, UO_2^{2+}, etc.) penetrate tissues easily, produce images of high resolution, stain stoicheiometrically, are convenient, flexible and cheap, but lack specificity. Their high resolution has been crucially important in identifying proteoglycan binding sites (Fig. 1). Antibodies are particularly useful in staining proteoglycan protein cores, whereas mini-stains are best in demonstrating glycosaminoglycan sidechains. The two kinds of reagent are complementary, rather than alternatives [68].

The specificity of mini-reagents is improved by using them in the critical electrolyte concentration mode [68–70]. This principle is fundamental to polyelectrolyte fractionation using ion exchangers. Cationic staining reagents have similar resolving power to, e.g., QAE- or DEAE-cellulose [69,71]. If the reagent is designed to have low affinity for nucleic acids (e.g. Alcian Blue, Cupromeronic Blue [68,69]) the critical electrolyte concentration approach ensures that few tissue polyanions stain. With the further help of keratanase or chondroitinase AC, most glycosaminoglycans are identifiable [72].

Staining (i.e. precipitation) with cationic reagents collapses the polyanion domain [68]. The shape and size of the staining molecule determines the extent and direction of this collapse: the reagent acts as a scaffold to the polyanion, in the stained complex. By tailoring the dye, optimum electron density was achieved compatible with retention of recognizable shapes of the tissue proteoglycans [68].

Results

Patterns of proteoglycan–collagen fibril interaction in tissues. Using bismuth at low pH, stained artefacts were sometimes seen on collagen fibrils, distributed approximately D-periodically, possibly at the a-band [73]. Later investigators, using a variety of cationic reagents on a number of tissues, claimed localization to the a band [74], d band [75] or 'between a and d bands' [76]. The apparent discrepancies were due to variations between tissues and methods [77]. Pictures improved with the use of Alcian Blue in critical electrolyte concentration methods [78,79], and again with Cuprolinic Blue and Cupromeronic Blue [70,77,80]. Using the Cupromeronic Blue/critical electrolyte concentration approach, with tungstate as an 'intensifier' [70], proteoglycans were visualized with high contrast as filaments of varying morphologies [70], easily located with respect to a–e bands stained with UO_2^{2+}.

With this technique [81] tissues rich in type I collagen were systematically studied. Very regular arrays of proteoglycan filaments, orthogonal to the collagen fibrils and spaced D-periodically, were observed in tendons [68,70,77] (Fig. 3), sclera [82] and skin [83]. Filaments

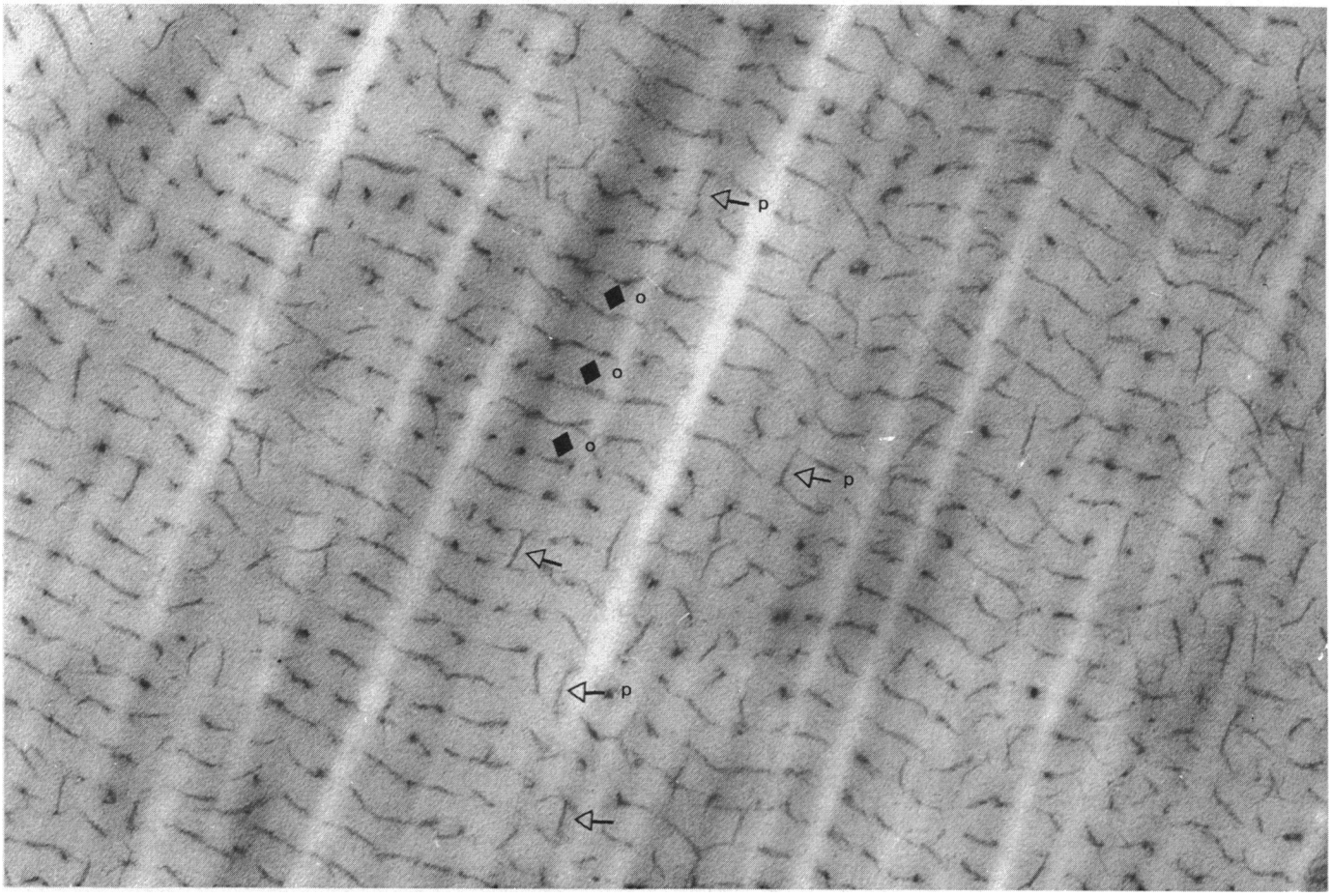

Fig. 3. Electron micrograph of fetal (45 cm long) calf flexor digitorum tendon, stained at 0.3 M-$MgCl_2$ by Cupromeronic Blue (technique described in [70])

Proteoglycans orthogonal to (O) and parallel with (P) the collagen fibrils are arrowed. The lighter bands, running from top to bottom, are collagen fibrils. The orthogonal proteoglycans (O), 1 *D* period apart, are frequently bridged by proteoglycans (P) 1*D* period long, arrayed parallel to and at the surface of the fibrils (× 110000).

were found at the d band, or less frequently at the e band, i.e. in the gap zone (Fig. 1). This distribution was not an artefact of dehydration and plastic embedding, since, using synchotron X-ray diffraction, a similar pattern was obtained from wet, stained tendon before processing for electron microscopy [65]. Other proteoglycans were seen parallel to the fibril, at its surface, exactly spanning the gap between adjacent D-spaced proteoglycan filaments [70,82] (Fig. 3). Still others, of varying length and thickness, were seen between (or on) the collagen fibrils [77,84].

Biochemical analyses and resistance to hyaluronidase digestion indicated that the orthogonal proteoglycan was dermatan sulphate-rich, in skin, sclera [83] and tendon [77]. In all three tissues, there are 'large' chondroitin sulphate proteoglycans (i.e. low in iduronate) and 'small' dermatan sulphate proteoglycans [85–88]. Thus orthogonal proteoglycans at the gap zone of type I collagen fibrils were small dermatan sulphate proteoglycans [77]. Less direct evidence suggested that interfibrillar, unspecifically located proteoglycans could be large chondroitin sulphate–dermatan sulphate proteoglycans [89]. They are more easily extracted than small dermatan sulphate proteoglycans [18]. Immunostaining of skin [90] placed the small proteoglycan at the surface of collagen fibrils (which *per se* does not prove collagen association, since the precipitated antibody complexes must come to rest on some kind of support [68]), or more specifically, in the vicinity of the gap zone [58].

Dermatan sulphate is derived from chondroitin sulphate, by epimerization at glucuronate C-5, after the glycan chain has formed. The percentage epimerized varies, from around 30% in cornea [21] and cartilage [91] to over 80% in skin and sclera. The relevant dermatan sulphate proteoglycans still locate at the gap zone. In rabbit annulus fibrosus, chondroitinase AC-sensitive proteoglycan (i.e. a chondroitin sulphate proteoglycan) appears as complete hoops about the collagen fibril at the d–e bands [92]. The collagen was not identified. It was suggested that this proteoglycan was similar to the small dermatan sulphate proteoglycans (Fig. 2), by analogy with the small chondroitin sulphate proteoglycan of cartilage [15], and that epimerization to dermatan sulphate was not initiated [92,93].

Gap-zone-associated proteoglycans were seen in human cartilage [94]. Neither the collagen nor the proteoglycan was identified. Small chondroitin sulphate [95] and dermatan sulphate proteoglycans [91] as well as types I and II collagen [96] are found in cartilage. Hyaluronidase digestion completely removed the ortho-

gonal, gap-zone-associated proteoglycans (J. E. Scott & M. Haigh, unpublished work).

Corneal stroma is rich in type I collagen, and in dermatan sulphate and keratan sulphate, which are present in separate proteoglycans [21]. After keratanase digestion, dermatan sulphate proteoglycan was seen at the d and e bands, as in skin, etc. [83]. Removal of dermatan sulphate by chondroitinase ABC digestion left keratan sulphate proteoglycan, located at the a and c bands. Keratan sulphate was readily isolable from bovine, rabbit and rat corneas, but not from mouse cornea [97,98]. Accordingly, mouse corneal stroma proteoglycans were not seen at the a/c bands, but only at the d/e bands. These proteoglycans were keratanase-resistant and chondroitinase ABC-sensitive, i.e. they were chondroitin sulphate–dermatan sulphate containing [97,98].

Corneal proteoglycans are important in keeping the fibrils apart, at regular spacings, for transparency [99,100]. Nevertheless, mouse cornea is fully functional in the absence of keratan sulphate [98].

A map of proteoglycan binding sites on the collagen fibril, based on these results [83,93,101] (Fig. 1) embodies two important concepts: (1) that each proteoglycan has its specific binding site, and (2) that in the absence of this proteoglycan, the site is not occupied.

The richest source of type I collagen is bone. Electron microscopical study of **bone organic matrix** requires prior removal of the electron-dense mineral. Demineralization in organic solvents retained the proteoglycan [102], which was seen as an integral part of the matrix, but distributed parallel to the fibrils [83]. The major bone proteoglycans contain chondroitin sulphate, rather than dermatan sulphate [83,103] in small proteoglycans similar to those discussed above [103].

The absence of gap-zone-associated proteoglycan in bone, and its presence in non-mineralizing tissue, suggested an inhibitory role for some small tadpole-shaped dermatan sulphate and chondroitin sulphate proteoglycans during calcification [77,83,93]. The gap zone was claimed to be the site of nucleation of calcification [104]. The powerful Ca^{2+}-binding properties of dermatan sulphate (or chondroitin sulphate) [105] at this site could hardly be without effect on nucleation. In calcifying turkey tendon the orientation of gap-zone-associated proteoglycan filaments changed from orthogonal to axially parallel, as the calcification front moved along the tissue (J. E. Scott & M. Haigh, unpublished work). These phenomena may be secondary to the calcification process, but it is relevant that of two osteoblast cell lines, isolated from the same tissue, one which made calcified matrix could not express small chondroitin sulphate proteoglycan, while the other, which did not calcify, could [106].

In spite of strong indications from model experiments (see above) that it can interact with collagen, the very large chondroitin sulphate proteoglycan of cartilage has not been seen in structures similar to those mapped in Fig. 1 [68,107]. This may be because of the huge scale of the proteoglycan molecule, which, coupled with the thinness of the ambient collagen fibrils, requires that many clusters of chondroitin sulphate chains be located against a poorly defined frame of reference. The situation in cartilage is complicated by the presence of small proteoglycans, which may be responsible for observed orthogonal arrays (see above).

^{13}C-n.m.r. spectra of chondroitin sulphate were obtained from pieces of cartilage [67], implying that chondroitin sulphate chains were freely mobile, since if they were tied down by interactions to collagen, n.m.r. spectra could not have been observed.

The specific associations summarized in Fig. 1 were at the fibril surface. The ratio of tissue concentrations of regularly placed fibril-surface associated species X to collagen, [X]/[Coll], should be proportional to d^{-1} where d is the average diameter of the cylindrical collagen fibrils [89], providing all of X were at the fibril surface, i.e. $[X] = [X_S]$. Dermatan sulphate in developing tendons from chick, rat and cow obeyed this relationship, whereas chondroitin sulphate and hyaluronan in the same tissues did not [89,108]. The combined inaccuracies of measurement of the three parameters was such that significant amounts of glycosaminoglycan might not be so distributed. More complicated distribution patterns than those of the simple model are conceivable, which nevertheless produce the same relationships. {e.g. if X_S were in equilibrium with interfibrillar X (X_I) so that $[X_S] = k\,[X_I]$}.

Not all a, c, d, e bands were occupied. Considerable amounts of unassociated proteoglycan lay between the collagen fibrils, in proteoglycan-rich tissue such as young tendon or cornea [70]. There may thus be an equilibrium between bound and free proteoglycans [101]. Other explanations are conceivable until equivalence or otherwise of the bound and free proteoglycan is established.

Notwithstanding, the biochemical morphometric conclusions were compatible with those from electron histochemistry [89].

Intrafibrillar artefacts, very probably proteoglycans, were stained in ultrathin plastic sections [109] or with Cupromeronic Blue before embedding ([101], Fig. 4). With a critical electrolyte concentration of 0.3 M-$MgCl_2$, the latter must be sulphated proteoglycan. They were of uniform length (about 0.5–0.7 D, 30–50 nm long) and spanned the gap region. They may be surface proteoglycans which were incorporated into the fibril, e.g. by overlaying of collagen molecules, or by fusion of fibrils [84]. Alternatively, they might be type IX collagen (which has attached glycosaminoglycan [23–26]), an integral part of the fibril structure. They were seen in tendon, demineralized rat femur, human and rabbit intercostal cartilage [101] (Fig. 4).

MECHANISMS OF INTERACTION IN THE TISSUES

Specifically located proteoglycans are usually intimately associated with collagen fibrils. Thus, other molecules (fibronectin, etc.) do not necessarily mediate the interaction, although model systems suggest the possibility [110].

There are at least two modes of association: (1) orthogonal and (2) axially parallel.

(1) Orthogonal interactions. Each binding site might involve several collagen molecules, since proteoglycan filaments often circle the entire fibril, with upwards of 30 collagen molecules aligned in parallel in a surface band. Precisely how many proteoglycan molecules are in each filament or loop is unknown, but rough calculations [111] indicate that a single dermatan sulphate proteo-

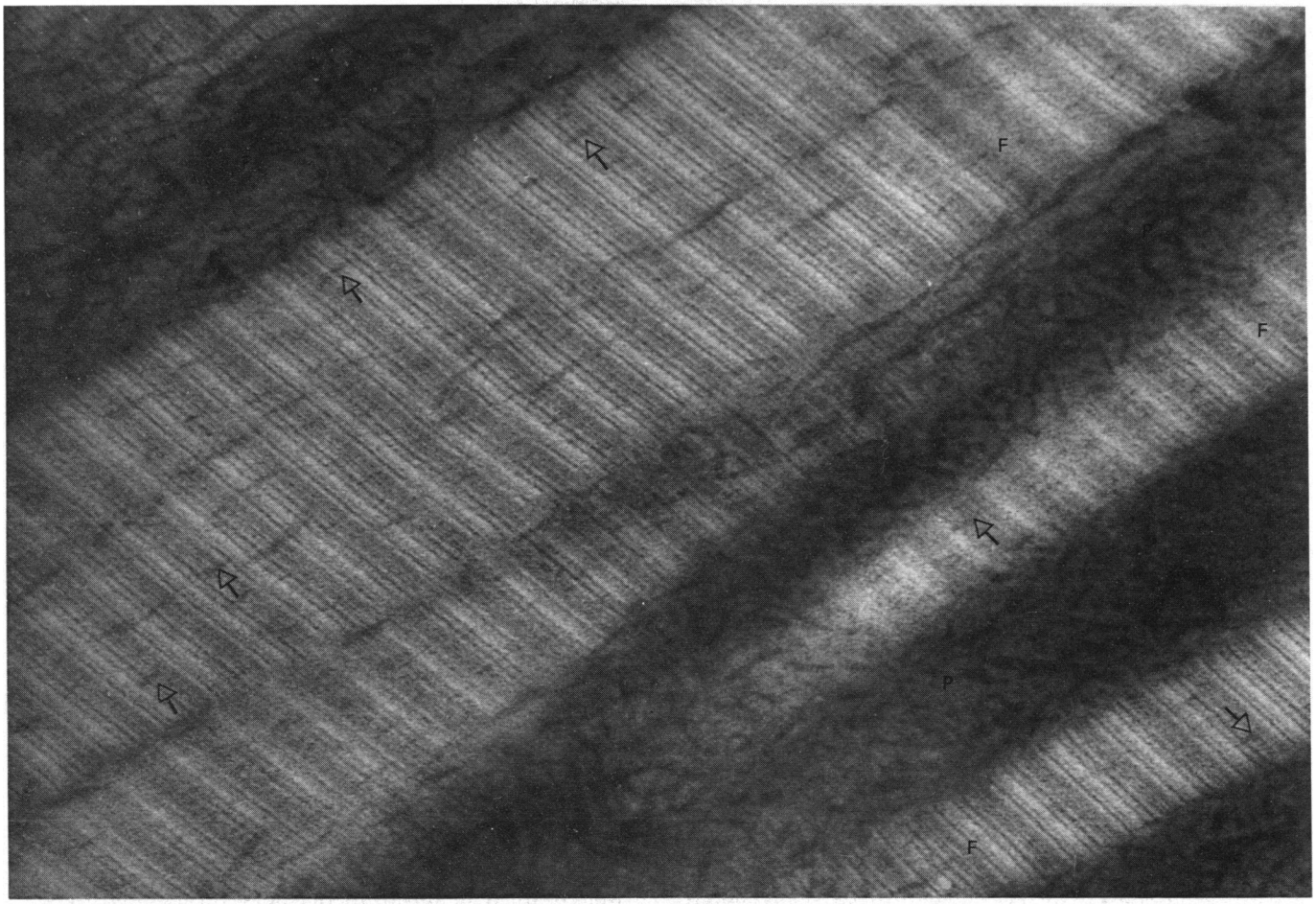

Fig. 4. 70-year-old human intercostal cartilage, stained at 0.3 M-$MgCl_2$ with Cupromeronic Blue to show sulphated proteoglycans, and with UO_2^{2+} to show collagen banding pattern

Thick collagen fibrils (F) are seen in longitudinal section, with densely packed proteoglycans (P) between them. Intrafibrillar proteoglycans, arrowed, are of various lengths, but most are about 0.6 *D* long, traversing the c–b region, containing the gap zone (×138000).

glycan molecule could interact with one to four collagen molecules, each of 1.5 nm diameter.

(2) Axially parallel interactions in many tissues (see above) need involve only one collagen molecule per interaction. Often, only a part of the length of a collagen molecule is engaged. In tendons [70] (Fig. 3) and annulus fibrosus [92] peripheral axial proteoglycans are commonly 1 *D* unit long, i.e. about 25% of the length of a collagen molecule.

Further interpretation of the pictures is not possible, since the detailed relationship of the electron microscopic image to the proteoglycan molecule has not been worked out. The stain must be associated electrostatically with the glycosaminoglycan chain, but the contribution of the protein core to the image is unclear. Subject to this proviso, pictures of an *in vitro* model [44] suggest that the dermatan sulphate chain may not be involved in the interaction, except at one end, which may be the protein core region.

Additional arguments for a proteoglycan-protein–collagen interaction are:

(*a*) the d/e bands are not cationic [76], and hence would not be preferred as dermatan sulphate binding sites [77];

(*b*) despite considerable change in the dermatan sulphate glucuronate:iduronate ratios the relevant proteoglycans still bind to d/e bands (Fig. 1);

(*c*) if dermatan sulphate was responsible for the specificity of association, the d and e bands should be occupied equally, and not, as is usual, mainly the d band [77,83];

(*d*) similarly, unequal occupancy of a/c bands by keratan sulphate proteoglycans [72,98] would be unexpected, if only keratan sulphate was responsible for binding;

(*e*) in the rabbit cornea, the tissue concentration of each kind of dermatan sulphate proteoglycan correlates with the percentage occupancy of d and e bands [72,98];

(*f*) similarly, there is a correlation between percentage occupancy of the a/c bands, and tissue concentrations of keratan sulphate proteoglycans, which differ in their protein cores [72,98] (but see legend to Fig. 1).

FIBRIL RADIAL GROWTH

In embryonic tissues, collagen fibrils grow in an environment rich in hyaluronan and/or proteoglycans [84,112]. Correlations between fibril nucleation *in vivo*

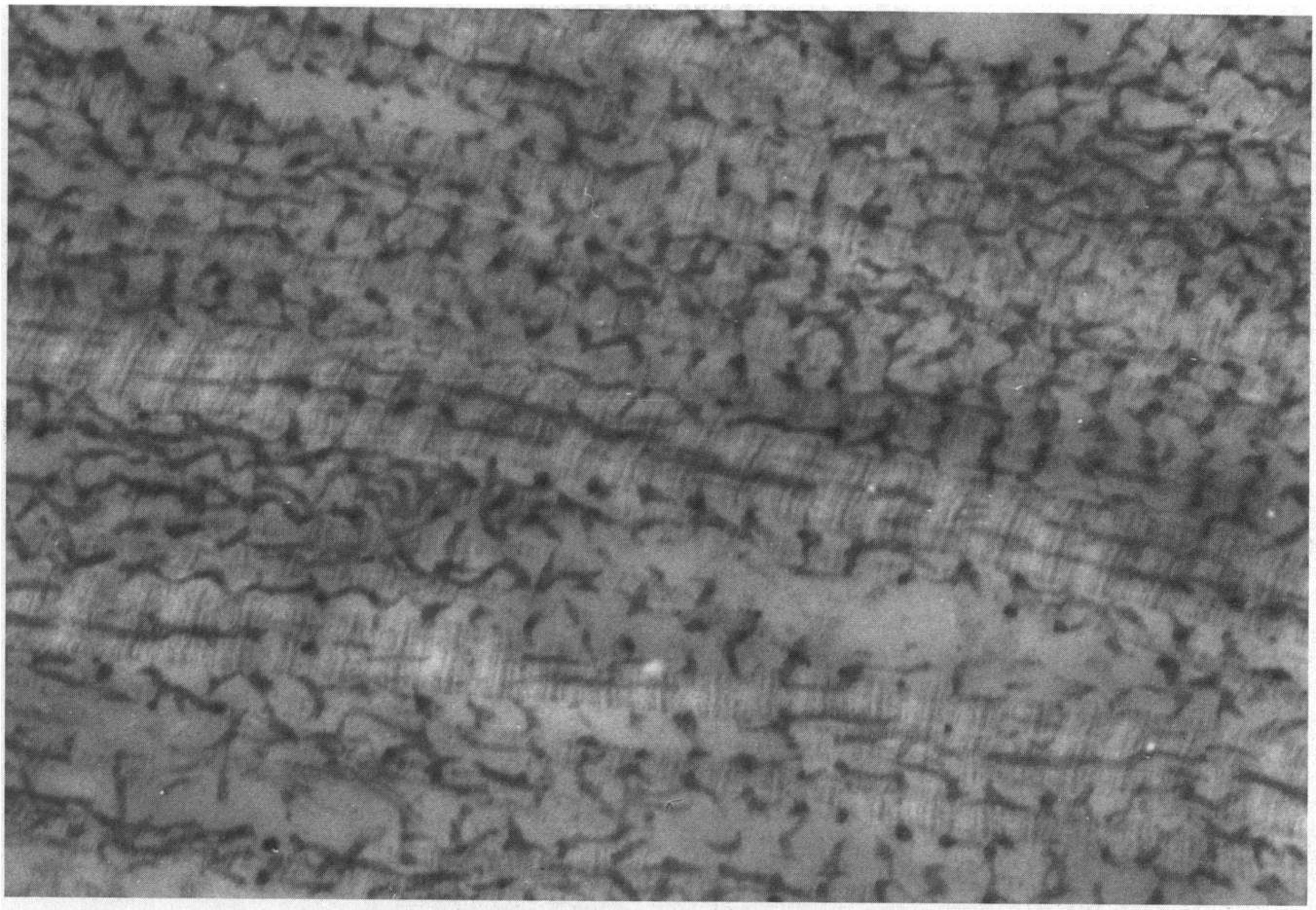

Fig. 5. Sea cucumber (holothurian, *Stichopus japonicus*) ventral body wall, stained with Cupromeronic Blue at 1.0 M-$MgCl_2$ to show proteoglycans, and counter-stained with UO_2^{2+} to demonstrate collagen fibril banding pattern

The high critical electrolyte concentration demonstrates high sulphation of the proteoglycans (H. Z. Fan & J. F. Kennedy, unpublished work). The fibril banding pattern is similar to, but not identical with, that of mammalian types I–III fibrillar collagens. Markedly periodic orthogonal proteoglycan association occurs at the heavily stained banding region (analogous to the gap zone complex of c, d, e, a), rather than to the lightly stained region, which compares with the overlap zone in Fig. 1. To this extent, the proteoglycan–collagen fibril interaction is very similar to that mapped in Fig. 1 (×150000).

and proteoglycan–hyaluronan interactions, although possible, are not proved.

The very thin fibrils of fetal tendons showed D-periodic proteoglycan attachment [70], and dermatan sulphate proteoglycan was present [84]. There was a striking transition from a chondroitin sulphate/hyaluronan-rich tendon to one containing predominantly dermatan sulphate, when the collagen fibrils began to expand rapidly, at the time of first muscle loading [84,108]. Before this the collagen fibrils were of thin uniform diameter and very regularly spaced. Cross sections of immature tendon fibres showed remarkable similarity to mature cornea. Correlations between thin collagen fibrils and hyaluronan and/or chondroitin sulphate proteoglycan abundance were made [84,113] and extended to other tissues.

The presence of high concentrations of hyaluronan/proteoglycan may control the radial expansion, and possibly the fusion, of collagen fibrils [84,113,114].

Fibrils grow radially *in vivo*, even though proteoglycan is present at the fibril surface, during normal development, implying that collagen molecules compete with and displace proteoglycan molecules from the surface. Fusion of fibrils may take place, with displacement of surface-bound proteoglycan [84]. The model experiments (above) hint at how this could occur. Proteoglycans were incorporated into fibrils *in vitro*, during growth, and inhibited the rate of growth. There was a tendency to lose (chondroitin sulphate) proteoglycan from the complex, and once the fibril formed, proteoglycan binding was limited [50]. A picture similar in some respects to those from tissues (Fig. 3), with D-periodically associated (dermatan sulphate) proteoglycan at the fibril surface, was obtained *in vitro* [44].

Fibril radial growth may cease (apart from fusion) at the end of development because the supply of collagen monomers ceases [115].

EVOLUTIONARY ASPECTS

The association between collagen and proteoglycans has been evolutionarily conserved, as implied by the constancy of dermatan sulphate–collagen relationships in three species [89].

Very primitive animals contain collagens and proteoglycans [116] similar to those present in man, birds,

etc. Fig. 5 shows a proteoglycan–collagen binding pattern in holothurian (sea cucumber) tissue like that in rat tail tendon [70], rabbit skin [83] etc. The glycosaminoglycan is based on chondroitin sulphate, but with sulphated fucose residues attached to the glucuronic acid (H. Z. Fan & J. F. Kennedy, unpublished work). A second polysaccharide, polyfucose sulphate, is also present in the tissue [117,118].

Thus, in spite of major differences in the glycans, and probably the collagens, the regular association of proteoglycan with collagen fibrils remains, confirming the fundamental importance of these proteoglycan–collagen interactions throughout evolution.

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REFERENCES

1. Scott, J. E. (1974) Philos. Trans. R. Soc. London Ser. B **271**, 235–242
2. Fleischmayer, R., Olsen, B. R. & Kuhn, K. (eds.) (1985) Biology, Chemistry and Pathology of Collagen, Ann. NY Acad. Sci. **460**
3. Mayne, R. & Burgeson, R. E. (eds.) (1987) Structure and Function of Collagen Types, Academic Press, New York
4. Hulmes, D. J. S., Miller, A., Parry, D. A. D., Piez, K. A. & Woodhead-Galloway, J. (1973) J. Mol. Biol. **79**, 137–148
5. Miller, E. (1985) Ann. NY Acad. Sci. **460**, 1–13
6. Henkel, W., Rauterberg, J. & Glanville, R. W. (1979) Eur. J. Biochem. **96**, 249–256
7. Birk, D. E., Fitch, J. M. & Linsenmeyer, T. F. (1986) Invest. Ophthalmol. Vis. Sci. **27**, 34–41
8. Vaughan, L., Mendler, M., Huber, S., Bruckner, P., Winterhalter, K. H., Irwin, M. & Mayne, R. (1988) J. Cell. Biol., in the press
9. Kapoor, R., Bornstein, P. & Page, H. (1986) Biochemistry **25**, 3930–3937
10. Poole, R. A. (1986) Biochem. J. **236**, 1–14
11. Evered, D. & Whelan, J. (eds.) (1986) Functions of the Proteoglycans, Ciba Found. Symp. **124**, John Wiley and sons, Chichester
12. Wight, T. N. & Mecham, R. P. (eds.) (1987) Biology of Proteoglycans, Academic Press, New York
13. Anseth, A. & Laurent, T. C. (1961) Exp. Eye Res. **1**, 25–38
14. Laurent, T. C. (1986) Ciba Found. Symp. **124**, 9–29
15. Wiedmann, H., Paulsson, M., Timpl, R., Engel, J. & Heinegård, D. (1984) Biochem. J. **224**, 331–333
16. Ward, N. P., Scott, J. E. & Cöster, L. (1987) Biochem. J. **242**, 761–766
17. Paulsson, M., Mörgelin, M., Wiedmann, H., Beardmore-Gray, M., Hardingham, T., Heinegård, D., Timpl, R. & Engel, J. (1987) Biochem. J. **245**, 763–772
18. Pearson, C. H. & Gibson, G. J. (1982) Biochem. J. **201**, 27–37
19. Heinegård, D., Bjorne-Persson, A., Cöster, L., Franzen, A., Gardell, S., Mamström, A., Paulsson, M., Sandfalk, R. & Vogel, K. (1985) Biochem. J. **230**, 181–194
20. Bowdon, M. A., Oldberg, A., Pierschbacher, M. & Ruoslahti, E. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 1321–1325
21. Gregory, J. D., Cöster, L. & Damle, S. J. (1982) J. Biol. Chem. **257**, 6965–6970
22. Kato, M., Koike, Y., Suzuki, S. & Kimata, K. (1987) J. Biol. Chem. **262**, 7180–7188
23. Noro, A., Kimata, K., Oike, Y., Shinomura, T., Maeda, N., Yano, S., Takahashi, N. & Suzuki, S. (1983) J. Biol. Chem. **258**, 9323–9331
24. Van der Rest, M., Mayne, R., Ninomiya, Y., Seidah, N. G., Chretien, M. & Olsen, B. R. (1985) J. Biol. Chem. **260**, 220–225
25. Vaughan, L., Winterhalter, K. H. & Bruckner, P. (1985) J. Biol. Chem. **260**, 4758–4763
26. Bruckner, P., Vaughan, L. & Winterhalter, K. H. (1987) in Structure and Function of Collagen Types (Mayne, R. & Burgeson, R. E., eds.), pp. 397–398, Academic Press, New York
27. Sajdera, S. W. & Hascall, V. C. (1969) J. Biol. Chem. **244**, 77–87
28. Miller, E. J. (1971) Biochemistry **10**, 1652–1659
29. Öbrink, B. (1972) Eur. J. Biochem. **25**, 563–572
30. Conochie, L., Scott, J. E. & Faulk, W. P. (1975) J. Immunol. Methods **7**, 393–398
31. Bungenberg de Jong, H. G. (1949) in Colloid Science (Kruyt, H. R., ed), vol. 2, pp. 276–295, Elsevier, Amsterdam
32. Mathews, M. B. (1970) in Chemistry and Molecular Biology of the Intercellular Matrix (Balazs, E. A., ed.), vol. 2, pp. 1155–1169, Academic Press, London and New York
33. Gross, J. & Kirk, D. (1958) J. Biol. Chem. **233**, 355–360
34. Wood, G. C. & Keech, M. K. (1960) Biochem. J. **75**, 588–598
35. Valli, M., Tira, M. E. & Balduini, C. (1982) Ital. J. Biochem. **31**, 183–197
36. Scott, P. G. & Pearson, C. H. (1987) in Glycoconjugates (Montreuil, J., Verbert, A., Spik, G. & Fournet, B., eds.), A. Lerouge, Tourcoing
37. Öbrink, B., Laurent, T. C. & Carlsson, B. (1975) FEBS Lett. **56**, 166–169
38. Greenwald, R. A., Schwartz, C. E. & Cantor, J. O. (1975) Biochem. J. **145**, 601–605
39. Öbrink, B. & Wasteson, A. (1971) Biochem. J. **121**, 227–233
40. Koda, J. & Bernfeld, M. (1984) J. Biol. Chem. **259**, 11763–11770
41. Öbrink, B. (1973) Eur. J. Biochem. **33**, 387–400
42. Kikuchi, K. & Jennings, B. R. (1981) Int. J. Biol. Macromol. **3**, 207–208
43. Laurie, G. W., Bing, J. T., Kleinman, H. K., Hassell, J. R., Aumailley, M., Martin, G. & Feldmann, R. J. (1986) J. Mol. Biol. **189**, 205–216
44. Scott, P. G., Winterbottom, N., Dodd, C. M., Edwards, E. & Pearson, C. H. (1986) Biochem. Biophys. Res. Commun. **138**, 1348–1354
45. Laurent, T. C. & Scott, J. E. (1964) Nature (London) **202**, 661–662
46. Öbrink, B. (1973) Eur. J. Biochem. **37**, 226–232
47. Scott, J. E., Heatley, F., Jones, M. R. N., Wilkinson, A. & Olavesen, A. H. (1983) Eur. J. Biochem. **130**, 491–495
48. Scott, J. E. (1967) in Solution Properties of Natural Polymers, Special Publication No. 23., pp. 263–272, Chemical Society, London
49. Snowden, J. M. & Swann, D. A. (1980) Biopolymers **19**, 767–780
50. Oegema, T. R., Laidlaw, J., Hascall, V. C. & Dziewiatknowski, D. D. (1975) Arch. Biochem. Biophys. **170**, 698–709
51. Öbrink, B. (1973) Eur. J. Biochem. **34**, 129–137
52. Chandrasekhar, S., Kleinman, H. K., Hassell, J. R., Martin, G., Termine, J. & Trelstad, R. L. (1984) Collagen Relat. Res. **4**, 323–328
53. Lowther, D. A. & Natarajan, M. (1972) Biochem. J. **127**, 607–608

54. Vogel, K. G., Paulsson, M. & Heinegård, D. (1984) Biochem. J. **223**, 587–597
55. Kuijer, R., van de Stadt, R. J., de Koning, M. H. M. T. & van der Korst (1985) Collagen Relat. Res. **5**, 379–391
56. Toole, B. P. (1976) J. Biol. Chem. **251**, 895–897
57. Toole, B. P. & Lowther, D. A. (1968) Arch. Biochem. Biophys. **128**, 567–578
58. Pringle, G. A. (1985) Ph.D. Thesis, University of Alberta, Canada
59. Speziale, P., Bardoni, A. & Balduini, C. (1980) Biochem. J. **187**, 655–659
60. Smith, G. N., Williams, J. M. & Brandt, K. D. (1985) J. Biol. Chem. **260**, 10761–10767
61. Gallagher, J. T., Lyon, M. & Steward, W. P. (1986) Biochem. J. **236**, 313–325
62. Reale, E. (1984) in Ultrastructure of the Connective Tissue Matrix (Ruggeri, A. & Motta, P. M., eds.), pp. 192–211, Martinus Nijhoff Publishers, Boston
63. Junqueira, L. C. U. & Montes, G. S. (1983) Arch. Histol. Jpn. **46**, 589–629
64. Ronziere, M.-C., Berthet-Colominas, C. & Herbage, D. (1985) Biochim. Biophys. Acta **842**, 170–175
65. Meek, K. M., Scott, J. E. & Nave, C. (1985) J. Microsc. **139**, 205–219
66. Meek, K. M., Elliott, G. F. & Nave, C. (1986) Collagen Relat. Res. **6**, 203–218
67. Schaefer, J., Stejskal, E. O., Brewer, C. F., Keiser, H. D. & Sternlicht, H. (1978) Arch. Biochem. Biophys. **190**, 657–661
68. Scott, J. E. (1985) Collagen Relat. Res. **5**, 541–575
69. Scott, J. E. (1974) Biochem. Soc. Trans. **1**, 787–806
70. Scott, J. E. (1980) Biochem. J. **187**, 887–891
71. Scott, J. E. & Hughes, E. W. (1982) J. Microscop. **129**, 209–219
72. Scott, J. E. & Haigh, M. (1985) Biosci. Rep. **5**, 765–774
73. Smith, J. W. & Frame, H. (1969) J. Cell Sci. **4**, 421–436
74. Myers, D. B. (1976) Histochem. J. **8**, 191–199
75. Nakao, K. & Bushey, R. I. (1972) Exp. Mol. Pathol. **17**, 6–13
76. Doyle, B. B., Hukins, D. L., Hulmes, D. J. S., Miller, A. & Woodhead-Galloway, J. (1975) J. Mol. Biol. **91**, 79–100
77. Scott, J. E. & Orford, C. R. (1981) Biochem. J. **197**, 213–216
78. Ruggeri, A., Dell'Orbo, C. & Quacci, D. (1975) Histochem. J. **7**, 187–197
79. Schofield, B. H., Williams, B. R. & Doty, S. B. (1975) Histochem. J. **7**, 139–149
80. Scott, J. E., Jones, C. & Kyffin, T. W. (1977) Upsala J. Med. Sci. **82**, 152
81. Haigh, M. & Scott, J. E. (1986) Bas. Appl. Histochem. **30**, 479–486
82. Young, R. D. (1985) J. Cell. Sci. **74**, 95–104
83. Scott, J. E. & Haigh, M. (1985) Biosci. Rep. **5**, 71–81
84. Scott, J. E., Orford, C. R. & Hughes, E. W. (1981) Biochem. J. **195**, 573–581
85. Damle, S. P., Cöster, L. & Gregory, J. D. (1982) J. Biol. Chem. **257**, 5523–5527
86. Cöster, L. & Frånsson, L.-Å. (1981) Biochem. J. **193**, 143–153
87. Anderson, J. C. (1975) Biochim. Biophys. Acta **379**, 444–455
88. Vogel, K. G. & Heinegård, D. (1985) J. Biol. Chem. **260**, 9298–9306
89. Scott, J. E. (1984) Biochem. J. **218**, 229–233
90. Longas, M. O. & Fleischmajer, R. (1985) Connect. Tissue Res. **13**, 117–120
91. Rosenberg, L. C., Choi, H. U., Tang, L.-H., Johnson, T. L., Pal, S., Webber, C., Reiner, A. & Poole, A. R. (1985) J. Biol. Chem. **260**, 6304–6313
92. Scott, J. E. & Haigh, M. (1986) Biosci. Rep. **6**, 879–888
93. Scott, J. E. (1987) Trends Biochem. Sci. **12**, 318–321
94. Orford, C. R. & Gardner, D. (1984) Connect. Tissue Res. **12**, 345–348
95. Heinegård, D., Sommarin, Y., Hedbom, E., Wieslander, J. & Larsson, B. (1985) Anal. Biochem. **151**, 41–48
96. Stanescu, V., Stanescu, R. & Maroteaux, P. (1976) C.R. Hebd. Seances Acad. Sci. Ler. D. **283**, 279–282
97. Haigh, M., Gibson, S. & Scott, J. E. (1987) Biochem. Soc. Trans. **15**, 711–712
98. Scott, J. E. & Haigh, M. (1988) J. Anat., in the press
99. Maurice, D. (1957) J. Physiol. (London) **136**, 262–286
100. Hedbys, B. O. (1961) Exp. Eye Res. **1**, 81–91
101. Scott, J. E. (1986) Ciba Found. Symp. **124**, 104–124
102. Scott, J. E. & Kyffin, T. W. (1978) Biochem. J. **169**, 697–701
103. Franzen, A. & Heinegård, D. (1984) Biochem. J. **224**, 59–66
104. Fitton-Jackson, S. (1957) Proc. R. Soc. London Ser. B **146**, 270–281
105. Buddecke, E. & Drzeniek, R. (1962) Hoppe-Seyler's Z. Physiol. Chem. **327**, 49–64
106. Dedhar, S., Stallcup, W. B., Mitchell, M. & Piersbacher, M. D. (1988) J. Cell. Biol., in the press
107. Scott, J. E. (1980) in Biology of the Articular Cartilage in Health and Disease (Gastpar, H., ed.), pp. 49–53, F. K. Schattauer Verlag, Stuttgart
108. Scott, J. E. & Hughes, E. W. (1986) Connect. Tissue Res. **14**, 267–278
109. Castellani, P. P., Franchi, M., Raspanti, M. & Ruggeri, A. (1987) Arch. It. Anat. e Embriol., in the press
110. Oldberg, A. & Ruoslahti, E. (1982) J. Biol. Chem. **257**, 4859–4863
111. Scott, J. E., Hughes, E. W., Marcyniuk, B. & Scott, J. P. (1983) Biochem. Soc. Trans. **11**, 770–771
112. Toole, B. P. (1982) Connect. Tissue Res. **10**, 93–100
113. Parry, D. A. D., Flint, M. H., Gillard, G. C. & Craig, A. S. (1982) FEBS Lett. **149**, 1–7
114. Kuhn, K. & von der Mark, K. (1978) in Collagen–Platelet Interaction (Gastpar, H., Kuhn, K. & Marx, R., eds.), pp. 123–126, F. K. Schattauer Verlag, Stuttgart
115. Scott, J. E., Haigh, M., Neo, G.-E. & Gibson, S. (1987) Clin. Sci. **72**, 359–363
116. Mathews, M. B. (1975) Connective Tissue: Macromolecular Structure and Evolution, Springer Verlag, Berlin
117. Fan, H. Z. & Chen, J. D. (1982) Bull. Chinese Materia Medica **7**, 27–29
118. Fan, H. Z., Chen, J. D. & Li, H. D. (1983) Acta Pharmaceut. Sinica **18**, 203–208