The term 'proteoglycan' was introduced in 1967 (see Balazs, 1970) to describe the family of molecules in which glycosaminoglycan chains are linked to protein. However, their rate of diffusion into textbooks of biochemistry has been rather slow, and I will therefore begin this presentation with a general introduction, which I hope will set the basis for their structural identity and indicate how their properties may be of importance in a broad biological context, before describing in more detail studies with proteoglycans from cartilage.

Glycosaminoglycans are characteristic components of the extracellular space of vertebrate tissues. They are long unbranched polysaccharide chains which contain many acidic, carboxylate and/or sulphate groups. They do not normally occur as free chains in vivo, but as proteoglycans, in which many like chains are linked by the reducing end of the terminal sugar residue to a protein. A whole technology was developed for the isolation, identification and quantification of glycosaminoglycan chains before their attachment to proteins was clearly established (Roden et al., 1972). Proteoglycan research thus began as a specialized area of carbohydrate chemistry. It is now apparent that in understanding the biological role of these macromolecules, the structure and interactions of the proteoglycans cannot be assessed by the analysis of the glycosaminoglycan chains alone. However, there are many difficulties in isolating and purifying intact proteoglycans, and it is only in recent years that a beginning has been made in exploring the full diversity of proteoglycan structure.

There are seven types of glycosaminoglycan, on the basis of different repeating disaccharide units, which occur in mammalian tissues (Table 1) (see Muir & Hardingham, 1975). With the exception of hyaluronate, they have all been reported to occur linked to protein in different types of proteoglycan. The linkage to protein is via a neutral trisaccharide (Gal-Gal-Xyl) at the reducing end of the glycosaminoglycan chain, and xylose forms an O-glycosidic bond with a serine residue in the protein. Keratan sulphate contains no neutral trisaccharide, but is linked via an O-glycosidic bond between N-acetylgalactosamine to

Table 1. Composition of mammalian glycosaminoglycans

<table>
<thead>
<tr>
<th>Disaccharide repeating unit</th>
<th>Hexuronic acid</th>
<th>Hexosamine*</th>
<th>Sulphate</th>
<th>Linkage to protein</th>
</tr>
</thead>
</table>
| Hyaluronic acid            | d-Glucuronic acid | d-Glucosamine | O-Sulphate | -Gal-Gal-Xyl-Ser-
| Chondroitin 4-sulphate     | d-Glucuronic acid | d-Galactosamine | O-Sulphate | -Gal-Gal-Xyl-Ser-
| Chondroitin 6-sulphate     | d-Glucuronic acid | d-Galactosamine | O-Sulphate | -Gal-Gal-Xyl-Ser-
| Dermatan sulphate          | l-Iduronic acid | d-Galactosamine | O-Sulphate | -Gal-Gal-Xyl-Ser-
| Keratan sulphate           | d-Galactose | d-Glucosamine | O-Sulphate | (a) skeletal: -GalNAc Ser(Thr) / AcNeu-Gal-
|                            |                |             |          | (b) corneal: -GalNAc Asn / -Gal-Gal-Xyl-Ser-
| Heparan sulphate           | d-Glucuronic acid or l-Iduronic acid | d-Glucosamine | O-Sulphate and N-sulphate | -Gal-Gal-Xyl Ser-
| Heparin                    | d-Glucuronic acid or l-Iduronic acid | d-Glucosamine | O-Sulphate and N-sulphate | -Gal-Gal-Xyl Ser-

* Always N-acetylated, except when N-sulphated.
serine or threonine in skeletal keratan sulphate or via an N-glycosylamine linkage from N-acetylgalactosamine to asparagine in corneal keratan sulphate. The structure of a proteoglycan is thus that of a specialised glycoprotein, and the information available on the biosynthesis suggests that they are synthesized along pathways in common with other secreted glycoproteins. For hyaluronate no clear linkage to protein has been established, and it does not appear to be a multichain proteoglycan.

The structure of proteoglycans gives them distinct physical properties. The glycosaminoglycan chains are hydrophilic, and the branched 'bottle-brush' structure and high charge density make the proteoglycans highly expanded molecules that occupy a large domain in solution which is excluded to other macromolecules, but freely permeable to small molecules.

In the past ten years, there has been much increased interest in the structure and organization of components in the extracellular matrix and in the influence they have on cell behaviour and on the interactions of a cell with its neighbours. The extracellular matrix has a dual role to play in all tissues. It contains the fibrous elements collagen and elastin that provide the structural framework of the tissue, but it also forms a fluid compartment that gives channels for the flow of nutrients and waste products to and from the cell and also access for the uptake or release of chemical messengers and hormones. Water is thus a very important component of the extracellular matrix, and the structure and hydrophilic properties of proteoglycans give them the potential for influencing both the organization of the fibrous elements and the maintenance of the water compartment and its ionic environment.

The broad range of physical properties encountered in different tissues is determined by variations in the 'blend' of fibrous (collagen and elastin) and non-fibrous components (particularly proteoglycan). The extent of the development of the extracellular matrix is related to the function of the tissue, and it is particularly large and well developed in cartilage, skin or blood-vessel wall, where there is a mechanical function to perform. The fibrous elements provide the tensile properties and the overall shape of the tissue, whereas the proteoglycans extend the fibrous network by drawing water in and thereby give the tissue resilience.

**Cartilage proteoglycans**

Cartilage is a tissue with a specialized biomechanical function, where the extracellular matrix is particularly large and accounts for more than 90% of the volume of the tissue. It is composed of a dense meshwork of fine collagen fibres (type II) embedded in a highly concentrated solution (up to 100 mg/ml) of aggregated proteoglycan molecules. The extracellular components thus account for most of the tissue dry weight, 40–70% is collagen and 10–30% glycosaminoglycan (Stockwell, 1979). It is because of this high tissue content that the proteoglycans of cartilage have been studied in much more detail than those of other tissues.

The proteoglycans found in cartilage are of high molecular weight, in the range $0.5 \times 10^6$–$4.0 \times 10^6$ (Hascall & Sajdera, 1970). They contain many chondroitin 4- or 6-sulphate chains, fewer keratan sulphate chains and many short oligosaccharides, attached to a protein core of about $2 \times 10^6$–$3 \times 10^6$ mol wt. (Fig. 1). The composition of a typical hyaline cartilage proteoglycan is shown in Table 2.

They are not only of high molecular weight, but also form large aggregates in which many proteoglycans bind to a chain of hyaluronate (Fig. 2). The native aggregate also contains a specific link protein that further stabilizes the proteoglycan–hyaluronate bond. Calculation shows that up to 200 proteoglycans can bind to a single hyaluronate chain of mol wt. $1.6 \times 10^6$, producing an aggregate 4 µm long and of particle weight $350 \times 10^6$ (Hardingham et al., 1981).

This aggregating type of proteoglycan appears to be a specific

---

**Fig. 1. Structural model of the major proteoglycan from vertebrate cartilage**

**Fig. 2. Aggregation of cartilage proteoglycans**

Hyaluronate generally occurs in the molecular-weight range $2 \times 10^4$–$2 \times 10^6$, and each extended chain is $0.5–5.0 \mu m$ long. Proteoglycans bind to hyaluronate with a minimum spacing of 25–50 nm, each aggregate may thus contain 20–250 proteoglycans (Hardingham et al., 1981).
component of the cartilage matrix. Chick embryonic menenchymal cells do not produce the cartilage type of proteoglycans before differentiation into chondrocytes (De Luca et al., 1977), and together with type II collagen, cartilage proteoglycans have been taken as a marker for chondrogenic expression. Although aggregating proteoglycans of the 'cartilage type' are characteristically produced by chondrocytes, they may also be expressed by other cell types, since mononuclear cells, synoviocytes, and macrophages contain some aggregating proteoglycans (Oegema et al., 1979) and proteoglycans in the lamina intima of aorta cross-react with antibodies raised against cartilage proteoglycans (Gardell et al., 1980). Gial cells in culture also produce proteoglycans which aggregate with hyaluronate (Norling et al., 1978).

**Elucidation of structure**

The primary structure of cartilage proteoglycan has not yet been entirely resolved, and current work assumes a model of structure that remains open to further revision. The slowness to elucidate even the basic structure of cartilage proteoglycans can largely be attributed to the general features of proteoglycans that make them difficult to study. The protein accounts for only a small proportion of the molecular weight (7–12%; Table 2), and the high carbohydrate density (one chain attached per four to six amino acid residues over the greater part of the molecule) creates major problems in characterizing the protein because it limits the application of normal amino acid-sequencing techniques and has made more difficult the isolation of specific cleavage products. Because of their expanded structure in solution and very high molecular weight, proteoglycans behave non-ideally even in quite dilute solutions (Comper & Laurent, 1978). This makes the interpretation of their molecular weight and shape from their physical properties much more difficult. This difficulty is also compounded by the proteoglycans being both polydisperse and heterogeneous. Each preparation is a family of closely related molecules rather than a single molecular species.

The current model takes into account many of these factors (Fig. 1). The protein backbone is composed of a globular region with intramolecular disulphide bridges, is of low carbohydrate content, and forms a specific site for binding to hyaluronate and to link protein (see below). An adjacent region of extended polypeptide has a high proportion of the keratan sulphate chains attached to it. The largest portion is a further extended polypeptide which contains the majority of the keratan sulphate chains (see Hascall, 1977). The keratan sulphate attachment region is proposed to be of variable length (Fig. 1), and this accounts for the polydispersity and the changing composition within each proteoglycan preparation, which shows the largest molecules to be of highest keratan sulphate content and lowest protein content. Electron micrographs of proteoglycans in amorphous monolayer show the protein core of proteoglycans attached to aggregates to be of variable length (Rosenberg et al., 1975; Kimura et al., 1978). Further evidence for the structural model has also been obtained by specific enzymic 'dissection' of the molecule (Fig. 3). The hyaluronate-binding region is degraded by proteolytic enzyme action on fully aggregating proteoglycan monomer, but it appears to be protected when it is part of the aggregate structure with link protein and hyaluronate. Thus digestion of proteoglycan aggregates with chondroitinase ABC and trypsin (Heinegård & Hascall, 1974) yields a hyaluronate-binding region fragment of 60 000–80 000 mol.wt., and a keratan sulphate-rich peptide of about 30 000 mol.wt. (Hascall, 1977). Digestion of monomer with papain releases single keratan sulphate chains attached to peptide, but trypsin and many other proteolytic enzymes release groups of chains. The determination of the size of the products of digestion with papain, trypsin and chondroitinase ABC (Fig. 3b) is thus a useful technique for characterizing proteoglycans. Recent evidence has shown that there are also many smaller oligosaccharides attached to the proteoglycan. These are both O-glycosidically linked oligosaccharides that occur throughout the molecule and N-glycosylamine-linked oligosaccharides that are mainly on the hyaluronate-binding region (Thornar & Sweet, 1979; De Luca et al., 1980; Lohmander et al., 1980).

Although the present model of proteoglycan structure provides a rational framework that accounts for many features, it cannot be taken as complete or final. For example, the separation of proteoglycan monomer and keratan sulphate-rich peptide into two or three discrete components on electrophoresis (Stanesu et al., 1973, 1977; Roughley & Mason, 1976) is not predicted from the model, and the explanation for it is unclear, but it may indicate that there are additional features that have yet to be described. Small proportions of non-aggregating proteoglycan that appear to be structurally unrelated to the aggregating type have also been reported (Heinegård & Hascall, 1979). More recent evidence on the cell-free translation of proteoglycan mRNA has also provided evidence for a single high-molecular-weight protein core (mol.wt. 300000) both from chick cartilage (Upholt et al., 1979) and from calf articular cartilage (Treadwell et al., 1980) with no evidence of polydispersity. This is a challenge to the concept of a variable chondroitin sulphate region, and it remains to be re-established how far the polydispersity within different proteoglycan preparations is related to a variation in length of the chondroitin sulphate attachment region and, if present, how far this is of biosynthetic origin or is produced as a result of degradation in the matrix.

A major feature of the model is that it permits the interpretation of some of the variations in proteoglycan size and composition among different cartilaginous tissues and the changes that occur with aging on the basis of a single composite structure (Fig. 4). The proteoglycans of different cartilages may be based on polypeptides to which variable numbers and sizes of glycosaminoglycan chains and oligosaccharides have been attached (Stevens et al., 1979). For example, some of the shorter O-glycosidically linked oligosaccharides may reflect abortive keratan sulphate chains and may be the sites at which full-length keratan sulphate chains are synthesized on the proteoglycans of older tissues.

**Preparation of proteoglycans**

The advances in our understanding of structure owe much to the development of efficient, non-degradative methods for extracting and purifying proteoglycans. This has helped resolve the conflicting evidence of structure, size and polydispersity which arose when methods of extraction gave variable yields, and a wide variety of fractionation methods lead to the isolation of different samples of the proteoglycan population. Proteoglycans can also be degraded by high shear forces (Sajdera & Hascall, 1969) and by proteolysis during extraction (Oegema et al., 1975). This can be difficult to detect, as purified proteoglycans appear to be both polydisperse in size and hetero-
Fig. 3. Enzymic dissection of cartilage proteoglycans

(a) Digestion of proteoglycan aggregate with chondroitinase ABC (CSase ABC) and trypsin to release the binding region of proteoglycan and link protein. Also released are keratan sulphate-rich peptides (KS-P), the stubs of chondroitin sulphate chains attached to peptide (CS-P) and chondroitinase-digested disaccharides (ΔDi-CS) (Heinegård & Hascall, 1974). The elution profile is shown of the digest from pig laryngeal-cartilage proteoglycan aggregates on Sepharose 6BCL in 0.5 M-sodium acetate, pH 6.8 (T. E. Hardingham & D. G. Dunham, unpublished work). (b) Digestion of proteoglycan monomer: (i) chondroitinase ABC to remove chondroitin sulphate chains (80–90%); (ii) trypsin and chymotrypsin to release peptides bearing several chondroitin sulphate chains; (iii) papain to release peptides bearing single chondroitin sulphate chains. Abbreviations: $V_o$, void volume; $V_t$, total volume.

Fig. 4. Examples of variation in structure of cartilage proteoglycans synthesized on homologous protein cores (based on the model in Fig. 1)

(a) Model of the major hyaline-cartilage proteoglycan. (b) Shorter chondroitin sulphate chains. (c) Fewer chondroitin sulphate chains. (d) Longer keratan sulphate chains.

The work of Sajdera & Hascall (1969) established that a high proportion of proteoglycans could be extracted from cartilage in 4 M-guanidinium chloride without the use of high-speed homogenization or other disruptive treatment. They also developed the use of equilibrium density-gradient centrifugation in CsCl solutions for the purification of proteoglycans. This had the benefit of being a mild procedure of high capacity which permitted the purification of proteoglycans of high buoyant density without bringing them out of solution at any stage. They showed that proteoglycans reversibly aggregated and were dissociated in 4 M-guanidinium chloride, but remained aggregated in concentrated CsCl (Hascall & Sajdera, 1969). This permitted the isolation of aggregated proteoglycan from a CsCl gradient with low guanidinium chloride concentration (0.5 M) or of disaggregated proteoglycan from a gradient with high concentration of guanidinium chloride (4.0 M) (Fig. 5). Aggregates were shown to be of high sedimentation coefficient in the ultracentrifuge (50–60S) compared with the monomer sedimentation coefficient of 24S. Aggregate and monomer were also shown to be separated by gel chromatography on Sepharose 2B (see Fig. 9). In the CsCl gradient in the presence of 4 M-guanidinium chloride, not only are the proteoglycans largely separated from hyaluronate and link protein, but there is also some fractionation of the proteoglycan population such that molecules of higher protein content occur in fractions of lower buoyant density. Analysis of the proteoglycan fractions of different buoyant density revealed a change in composition and in size (Tsiganos et al., 1971).
Investigation of the components of aggregation led to the identification of hyaluronate among the density-gradient fractions (Hardingham & Muir, 1973a). It accounted for about 1% of the glycosaminoglycan in cartilage and was shown to bind to proteoglycan. This interaction, involving two large polyanions, was unexpected, but was shown to be highly specific, and the stoichiometry suggested that many proteoglycans were binding to each hyaluronate chain (Hardingham & Muir, 1972b). In addition to hyaluronate, a glycoprotein component was released from the aggregate in the density gradient. This protein component was shown to bind to the aggregate and stabilize the proteoglycan–hyaluronate bond, as described below.

Link-free aggregates

The binding of proteoglycan to form link-free aggregates has been characterized in some detail (Hardingham & Muir, 1972b, 1974; Hascall & Heinegård, 1974; Christner et al., 1977, 1979; Nieduszynski et al., 1980). Aggregates rapidly form when proteoglycan and hyaluronate solutions are mixed in physiological saline (0.9% NaCl) or many other buffers at neutral pH. The aggregate formed is completely dissociated at acid pH and in high concentrations of guanidinium chloride, CaCl₂, MgCl₂, or in 6M-urea or 0.1% (w/v) sodium dodecyl sulphate but not in high concentrations of NaCl or CsCl. It is also reversibly dissociated on heating to 60°C. The binding site is very specific for hyaluronate (HA) and has a high affinity for decasaccharide or larger fragments (K₂₅, 10⁻⁶–5 x 10⁻⁸ M; Christner et al., 1978; Nieduszynski et al., 1980), but lower affinity for HA₆₅ or smaller fragments (Hardingham & Muir, 1973b; Hascall & Heinegård, 1974). Chemical modification of hyaluronate suggest that at least four carboxylic acid groups on a decasaccharide unit are necessary for strong binding, as it was greatly decreased by other bulky substitutions in their place or by their reduction to the alcohol (Christner et al., 1977, 1979). The lack of binding of desulphated chondroitin sulphate (chondroitin) suggests considerable specificity on the hexosamine moiety, as this only differs from hyaluronate at the hydroxyl position at C₂ (Hascall & Heinegård, 1974).

Investigation of the low-angle neutron-scattering properties of the isolated binding region prepared from pig laryngeal proteoglycan aggregates after trypsin digestion suggested a globular, but elongated, shape (Perkins et al., 1981). Overall dimensions, when modelled as an ellipsoid, were 15 nm x 3 nm x 3 nm. This compares with the observed length of the protein core of 350 nm (Kimura et al., 1978). It is compatible with a binding site for an extended hyaluronate segment (HA₁₀₅) 5 nm long, but only if the axis of the binding site is approximately parallel to the long axis of the binding region. The binding region was thus suggested to lie along the hyaluronate chain and largely perpendicular to the rest of the proteoglycan molecule (Fig. 6).

Link-stable aggregates

The nature of the interaction of proteoglycan with link protein has not yet been characterized in detail, and much of the evidence of the way in which link protein functions has come

![Fig. 5. Preparation of cartilage proteoglycans](image)

Fresh sliced cartilage was extracted for 24 h at 4°C in 10 vol. of 4 M-guanidinium chloride buffered at pH 5.8 containing 0.1 M-6-aminohexanoic acid, 0.01 M-disodium EDTA, 5 mMN-benzenemethoxide hydrochloride and 1 mM-phenylephrine sulphonyl fluoride (inhibitors of proteinases). The clarified extract was dialysed to 0.5 M-guanidinium chloride in the presence of the proteinase inhibitors to re-form proteoglycan aggregates. Associative gradient: CsCl was added to a density of 1.5–1.6 g/ml and a gradient was established at 100 000 g, for 48 h at 10°C in an angle or swinging-out rotor. Dissociative gradient: purified proteoglycan aggregate from the bottom of the associative gradient was dissociated in 4 M-guanidinium chloride and separated into components in a second CsCl gradient (starting density 1.6 g/ml) run under similar conditions to the first but in the presence of 4 M-guanidinium chloride.

![Fig. 6. Structure of the binding region isolated from pig laryngeal cartilage proteoglycan](image)

The results of low-angle neutron scattering of binding region in 0.2 M-NaCl; pH 6.5, at 6°C fitted a prolact-eellipsoid model of major axes 15 nm x 3 nm x 3 nm. The carbohydrate (30% of the total weight) contained keratan sulphate and probably N-linked oligosaccharides and was evenly distributed about the protein, which was estimated to be of mol. wt. 55 000. Abbreviation: HA, hyaluronate.
from more indirect observations (Hardingham, 1979; Tang et al, 1979). In the absence of link protein, proteoglycans bind reversibly to hyaluronate, and this is open to competition by oligosaccharides, but with link-stable aggregates there is no longer competitive binding with oligosaccharides, and no dissociation is evident (Hardingham, 1979) (Fig. 7). The attachment of proteoglycan to hyaluronate is therefore much stronger, and the link protein thus effectively locks proteoglycan on to the hyaluronate chain. In vitro the stabilizing effect of link protein is evident under physiological conditions of pH and ionic strength and also on heating up to 50°C in 0.5 M guanidinium chloride and even up to 60°C in 0.15 M NaCl buffered at pH 7.4 (Hardingham, 1979). The link-stable aggregate is dissociated similarly to the link-free aggregate in high concentrations of guanidinium chloride, CaCl₂, MgCl₂ and sodium dodecyl sulphate, but it is not dissociated in 4 M urea. Even when proteoglycans are dissociated from hyaluronate, however, there may be some residual binding of link protein to proteoglycan or hyaluronate.

Link protein has been shown to exist in two molecular-weight forms in several cartilaginous tissues. Evidence suggests that the two forms are structurally related, with the smaller form lacking a glycopeptide present on the larger form (Baker & Caterson, 1979). Both forms appear to be functionally active, and there is one mol of link per mol of proteoglycan (Kimura et al., 1980).

Link protein can be prepared from proteoglycan aggregates by isolating the fraction resistant to trypsin digestion that contains both the hyaluronate-binding region and the link protein (Heinegard & Hascall, 1974). In this preparation, the link is entirely of low molecular weight and may be even smaller than the naturally occurring small form, but it still retains its function (Hardingham et al., 1980). The significance of the two forms is therefore not clear, and, although it appears likely that the smaller may be derived from the latter by proteolytic cleavage, examination of newly synthesized and secreted radioactive link protein in cultures of chondrosarcoma cells showed only the smaller form, which suggests that if any selective cleavage occurs, it must be intracellular and before secretion (Kimura et al., 1980).

Secretion and assembly of proteoglycan aggregates

Proteoglycans are synthesized by chondrocytes and secreted into the matrix. Radioautography of sections of cartilage pulse-labelled with [³⁵S]sulphate suggests that movement of proteoglycans out of chondrocytes and into the matrix is fairly rapid (Hardingham & Muir, 1972). The assembly of aggregates appears to provide a mechanism for immobilizing proteoglycans within the collagenous matrix of cartilage. The binding of link protein provides a potentially irreversible step in aggregate formation. It is therefore important to determine whether aggregation occurs intracellularly or after secretion of the proteoglycans into the cartilage matrix. Evidence for the extracellular assembly of proteoglycan aggregates was obtained with cultures of chondrocytes from a chondrosarcoma (Kimura et al., 1979). Examination of the proteoglycans secreted into the medium in pulse-chase experiments (Fig. 8) showed that at early times (10-20 min) proteoglycan monomers were present, but they were steadily incorporated into aggregates over 2 h. By adding an excess of non-radioactive carrier proteoglycan to the radioactive samples, it was possible to distinguish between link-free and link-stable aggregates, as the carrier could displace radioactive proteoglycans from link-free aggregates, but not from those already stabilized by link protein. It was therefore possible to follow link-stabilization in the medium (Fig. 9). Oligosaccharides of the proteoglycan derived by digestion with testicular hyaluronidase were also used as probes of stable aggregate formation. HA₄ oligosaccharides were found to delay stable aggregate formation in the medium and to increase the flow of proteoglycan from the pericellular matrix into the medium. With larger

![Fig. 7. Stabilizing effect of link protein on proteoglycan aggregates](image)

Hyaluronate oligosaccharides (averaging 38 monosaccharides) were added to solutions of link-free and link-stabilized proteoglycan aggregate in 0.5 M guanidinium chloride/0.05 M sodium acetate, pH 5.8, at 30°C. The viscosity was followed with time. The solution of link-free aggregate (a) rapidly came to a new equilibrium at a lower viscosity, as proteoglycans were displaced from hyaluronate. The link-stabilized aggregate showed no fall in viscosity with (●) or without (O) oligosaccharides. The oligosaccharides could not compete with binding to proteoglycan when link protein was in the structure. [Redrawn from Hardingham (1979).]

![Fig. 8. Pulse-chase incorporation of [³⁵S]sulphate into proteoglycans in cultures of chondrocytes](image)

Cultures were labelled with [³⁵S]sulphate for 5 min and then chased for the times indicated. Incorporated radioactivity excluded from Sephadex G-50 was determined in the culture medium (O), 4 M guanidinium chloride extract of the cell layer (C) and the papain-digested cell residue (●). Reproduced with permission from Kimura et al. (1979).]
Fig. 9. Formation of link-stabilized aggregate in the medium of chondrocyte cultures

Cultures were pulse-labelled with $^{35}$S sulphate for 5 min. At various times of chase, samples of medium were mixed with excess carrier proteoglycan monomer (AID1) and chromatographed on Sepharose 2B. The shaded areas indicate the proportion of $^{35}$S proteoglycan in link-stabilized aggregates. The broken line shows the hexuronic acid elution profile of the carrier proteoglycan. Abbreviations: $V_o$, and volume; $V_t$, total volume. [Reproduced with permission from Kimura et al. (1979).]

Fig. 10. Effect of hyaluronate oligosaccharides, $HA_{16}$ and $HA_{40(19)}$, on the formation of link-stabilized proteoglycan aggregates in the medium of cultured chondrocytes

The cultures were pulse-labelled for 5 min with $^{35}$S sulphate and chased with and without $HA_{16}$ and $HA_{40(19)}$ in separate experiments. The formation of link-stabilized aggregate was determined in the medium as described in Fig. 8. [From the results of Kimura et al. (1979).]

Fig. 11. Scheme showing effects of hyaluronate oligosaccharides on the formation of stabilized aggregates in chondrocyte cultures

(1) Reversible binding of proteoglycan to hyaluronate. (2) Irreversible link stabilization of aggregate. (3) Reversible binding of proteoglycan to $HA_{16}$. (4) Reversible binding of proteoglycan to $HA_{40(19)}$. (5) Irreversible link stabilization of proteoglycan–$HA_{40(19)}$ complex. [Reproduced with permission from Kimura et al. (1979).]

oligosaccharides ($HA_{10}$), which were able to bind to both proteoglycan and link protein simultaneously, the formation of stable aggregates was blocked completely (Fig. 10). The results showed that the formation of link-stable aggregate was an extracellular event, as the oligosaccharides were only able to compete with hyaluronate in binding to proteoglycan before link stabilization (Fig. 11). The oligosaccharides were also only effective if present at a time when proteoglycans were newly secreted from the cells. Although the cells used for this study were from a chondrosarcoma, similar displacement effects with oligosaccharides were also observed with normal chick chon-

drocytes, suggesting that proteoglycans are also secreted before link stabilization in this system (Solursh et al., 1980).

Although the results indicate that aggregate stabilization was only after secretion, it is not clear what mechanism delays it. All
three components of aggregate are synthesized by the chondrocytes, and if they are present within the same compartment within the cell, why should they not interact? Experiments using exogenous proteoglycans suggest that link protein and proteoglycan may be bound together before secretion (Kimura et al., 1980), although in the chondrosarcoma cells link protein appears to be synthesized in excess over the amount of proteoglycan. The limiting factor in aggregate formation might therefore be the availability of hyaluronate. The site of synthesis of hyaluronate in the cell is not known, and it may well be separate from the normal routes of synthesis of proteoglycan. Furthermore, other mechanisms involving the conversion of link protein or proteoglycan from inactive into active binding forms during intracellular processing could not be ruled out. This was of particular interest, as isolated link protein is difficult to keep in solution in associative solvents such as 0.15 m-NaCl (Tang et al., 1979) and might therefore be synthesized and secreted in a more soluble form. However, experiments in vitro mimicking the aggregation observed in vivo, demonstrated the high specificity of its binding in aggregates and its lack of association with serum proteins. Link protein isolated from cartilage behaved in the same way as link protein newly synthesized and secreted by chondrocytes.

The role of proteoglycans in cartilage is dependent on their providing a very high concentration of fixed negative charge in the tissue. Aggregation can be seen as a way of immobilizing the proteoglycans within the network of collagen fibres. The extracellular assembly of aggregates and the stabilization with link protein is thus analogous to the secretion and assembly of collagen fibres, although the mechanisms involved are entirely different.

The origin and significance of the large variation in cartilage proteoglycan structure and the polydispersity remain unclear, but it is evident that there is immense scope for modulation of the final structure of proteoglycans may thus interact with each other and with other connective-tissue components, including different molecular types of collagen, fibronectin and cell-surface components, through multiple glycosaminoglycan chain associations or through ordered protein interactions, and these will not be predicted from the properties of free glycosaminoglycan chains. For the majority of proteoglycans, it is these possibilities that have yet to be explored.

I acknowledge all the help given to me by my colleagues at the Kennedy Institute, and I am particularly indebted to Dr. Helen Muir, F.R.S., and Professor Charles Phipps for their continued support and encouragement.


Vol. 9