PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Physiological Function and Chemical Composition of Pericellular Proteoglycan (an Evolutionary View)

J. E. Scott

Phil. Trans. R. Soc. Lond. B 1975 **271**, 235-242
doi: 10.1098/rstb.1975.0047

Phil. Trans. R. Soc. Lond. B. **271**, 235–242 (1975) [235]
Printed in Great Britain

COMPOSITION AND STRUCTURE OF THE PERICELLULAR ENVIRONMENT

Physiological function and chemical composition of pericellular proteoglycan (an evolutionary view)

By J. E. Scott
M.R.C. Rheumatism Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berkshire

Connective tissue cells exist in a meshwork of insoluble fibres, the interstices of which are filled with soluble, high molecular mass, anionic material of a predominantly carbohydrate nature. The interactions of fibres with the interfibrillar material are central to the discussion of connective tissue physiology.

As with all soluble polymers, the interfibrillar polyanion tends to 'swell' and the tangled mass of chains offers considerable resistance to penetration by the large insoluble fibres. The consequent pressure to 'inflate' the fibrous network is important in giving elasticity to cartilage, transparency to cornea, etc. Branched structures (of proteoglycans) and straight-chain forms (of hyaluronate) are compared for their ability to fulfil these functions.

Apart from their physical ('non-specific') roles proteoglycans and glycosaminoglycans are able to interact physicochemically with, for example, collagen in ways which show considerable specificity, and which presumably are important in the laying down of the fibrous network as well as in maintaining its mechanical integrity.

It is proposed that the role played by radiation, particularly as mediated via the hydrated electron (e^-_{aq}) was dominant in the pre- and post-biotic evolution of pericellular environments.

A very large number of facts are known about connective tissue. I intend to view this mass of data from the standpoint of one or two simple ideas, which tend to show that the *physiology* of connective tissue is an integrated study, and in so doing, to emphasize the evolutionary aspect.

Soluble polymer–fibre interactions in the pericellular environment

The evolution of single cells into multi-celled organisms and thence into higher animals is also the process of evolution of connective tissue. Initially, the requirements of cells living in groups must have been simple. Most important would be that the connecting medium did not prevent the inflow and outflow of materials essential to life, while offering a measure of protection from the outside world. This is the first step towards a controlled environment in which the division of labour of cell specialization can occur. The connecting medium then adapted to mechanical stresses associated with active movement and with the maintenance of shape. The framework in which much of evolution has taken place is remarkably simple (figure 1).

In the animal kingdom the fibres are usually proteins (collagen or elastin) but in some tunicates they are of cellulose (for a review, see Hunt 1970). The interfibrillar soluble polymer seems always to be predominantly carbohydrate, although of very varying composition (especially in the invertebrates). This pattern, of a fibre-reinforced composite material, has great versatility. By changing the ratio of fibre to soluble polymer, or by altering the spatial

relations between the two, quite different situations can be coped with, without altering the chemical composition of the participants. For instance, collagen fibres and hyaluronic acid are the major constituents of the matrices of both the vitreous humour of the eye (Balazs 1962) and the tumescent sex skin of the female baboon (Szirmai 1966). The cornea of the eye, and inter-costal cartilage have *very* similar fibres (collagen) and carbohydrate polymers (chondroitin sulphate and keratan sulphate).

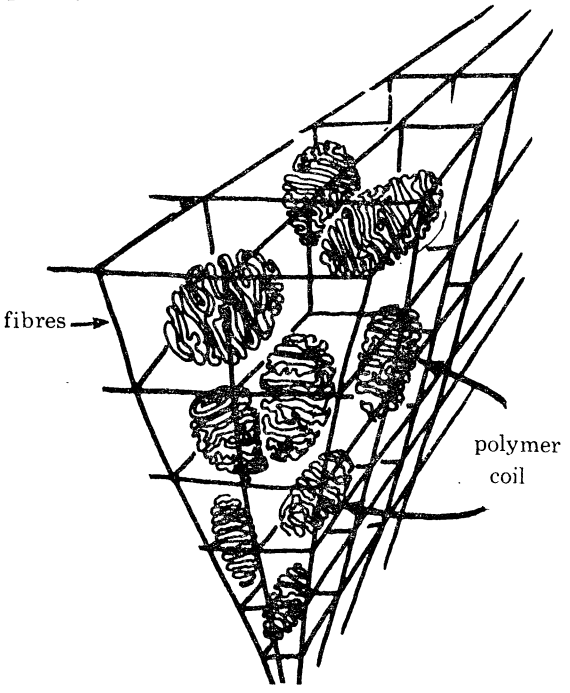

FIGURE 1. Diagrammatic illustration of connective tissue matrix, taken and adapted from Balazs (1962).

Of the two elements, the fibres have tensile strength, i.e. resistance to forces which would extend them, whereas the interfibrillar polymer 'inflates' the meshwork, and hence provides resistance to compressive forces. Movement of small molecules can take place more or less freely in the polymer compartment, to sustain life in the cells of the matrix. To have been able to fulfil a great variety of functions on such a simple basic plan, with such economy of biosynthetic means, must have been an important factor in the development of pericellular environments during evolution.

The role of the fibres in determining the shape and maximum size of the matrix is obvious, but the function of the soluble polymer requires further examination.

Polymers expand in solution to occupy 'domains', into which large particles move with difficulty, i.e. a part of the solution is excluded to other molecules, depending on their size (Ogston 1958). Hedbys (1961) showed that this effect is responsible for keeping apart the collagen fibres of the cornea, which is vital to the maintenance of transparency. The reversible elasticity of articular cartilage also depends on this effect, as may be inferred from an experiment by Sokoloff (1963). In physiological saline, articular cartilage recovered completely from the deformation induced by a load, whereas, in the presence of 0.085 M lanthanum chloride (or similar 3–1 electrolytes), deformation was much greater and recovery was very incomplete. Cartilage proteoglycan is completely precipitated from solution by La^{3+} (Mason & Mayes 1973) – in which form it would be unable to inflate the fibre meshwork. Pressure could then

disperse the aqueous phase, which, without the natural tendency of the soluble polymer to 'reflate' (or increase in entropy) would lead to a permanent change in the shape of the matrix (Scott 1973).

The extent to which the sizes and shapes of the polymer molecules correspond to those of the 'holes' in the fibre meshwork is clearly important in determining the properties of the tissue. 'Small' or very flexible polymers would be able to move bodily through the meshwork, resulting in an easily deformable, inelastic matrix. In this context, it is interesting that hyaluronate, a linear polymer which behaves as a flexible random coil in solution, is the major glycosaminoglycan in several soft tissues (e.g. vitreous humour, Balazs 1962), in which hardness or rigidity is not needed. On the other hand, a tightly packed, 'inflexible' polymer would be displaced with great difficulty. In this respect, the interfibrillar polymer of cartilage is a highly

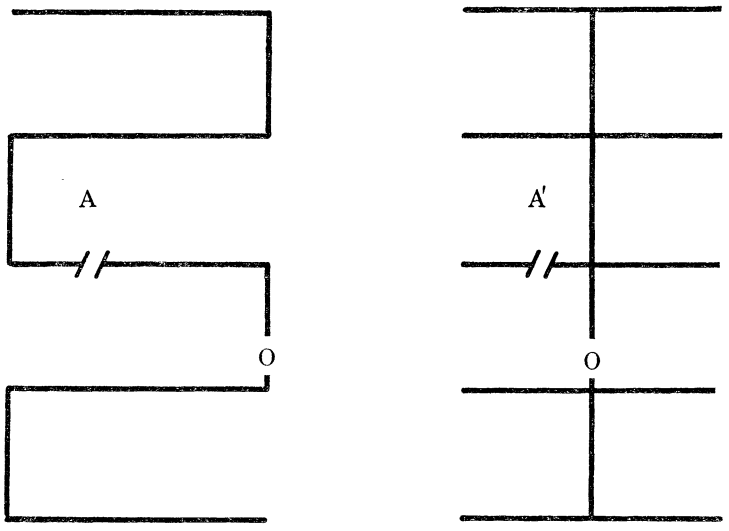

FIGURE 2. Comparison of single chain (left) and branched chain (right) molecules. The total length of polymer is the same in both. The configuration of the single chain polymer is drawn to show that it can fill the same space as the branched chain molecule. Scission at points O causes serious degradation of a similar extent to both polymers, whereas at points A damage to the branched molecule is minor. Thus, if the likelihood of scission is proportional to the number of monomers in the molecule, there is considerably lower risk of serious damage to the branched structure.

The single chain molecule is representative of the class including, for example, hyaluronate, and the branched molecule typifies the proteoglycan structure.

branched and aggregated molecule with a considerable cross-sectional area, seen from any aspect (Rosenberg, Hellman & Kleinschmidt 1970), which should greatly hinder free movement through meshworks. In fact, it seems that extraction in high yield of the cartilage proteoglycans is possible only if the meshwork is disrupted by mechanical means, or else the aggregates are broken down in high concentrations of guanidinium chloride (Sajdera & Hascall 1969). A high concentration of such a material, firmly trapped in a meshwork, is well adapted to maintaining the shape of a matrix and elastically resisting deformation, as in the case of cartilage.

The branched structure of the proteoglycan molecule may contribute to the preservation of the macromolecular properties in the face of damage due to physical or chemical means resulting in chain scission (figure 2). Scission at A′ leads to a minor loss of a portion of a side-chain from the branched polymer, whereas at the 'corresponding point' A, there would be drastic degradation of the linear polymer. Only scission at corresponding points O causes

severe damage to both polymers, and there are many fewer such points in the branched molecule. The theoretical improvement in the durability of the branched molecule may have been of practical importance in the evolution of *extracellular* molecules, subject to severe wear and tear, and, once excreted, beyond the reach of cellular repair mechanisms.

In addition to the 'mechanical' interactions between fibres and soluble polymer, there are also specific interactions depending on the detailed chemical structures of each. Öbrink (1973*a*) demonstrated electrostatic interactions between cationic groups of collagen and the anionic interfibrillar proteoglycans and polysaccharides, which, under 'physiological' conditions were strikingly specific. The interactions are fairly weak, and their significance in moment-to-moment functioning of connective tissues remains to be worked out. As discussed by Öbrink (1973*b*), they may influence the laying down of collagen fibres *in vivo* (a process necessarily carried out in the presence of proteoglycan) and hence the microscopic anatomy of the tissue.

TABLE 1. AGGLUTINATING TITRE (μg/WELL) OF POLYANION AGAINST LATHYRITIC CHICK CARTILAGE COLLAGEN

	native collagen	denatured collagen	light-scattering results by Öbrink (lathyritic rat skin collagen)
hyaluronate	100	.	−
CS 'A'	0.24	NA	+
CS 'C'	7.81	.	.
CS 'D'	62.5	.	.
dermatan sulphate	0.24	NA	+
keratan sulphate	50.0	NA	−
cartilage proteoglycan	1.17	.	+

NA, no agglutination; −, no interaction; +, positive interaction.

Öbrink's data were obtained by rigorous physicochemical methods, which have as their sole disadvantage that few biologists can use them. We have borrowed a simple technique from the immunologists, which demonstrates these interactions, and which uses very little time or material. Tanned red cells from sheep, coated with collagen, show the phenomenon of passive agglutination in the presence of certain connective tissue polysaccharides (Conochie, Scott, Faulk & Bailey, unpublished). The 'strength' of the agglutinating power is assessed by serially diluting the polysaccharide solution to an 'endpoint' at which the collagen coated cells do not agglutinate. The results (table 1) are entirely compatible with those of Öbrink (1973*a*). Hyaluronate and keratan sulphate do not cause agglutination, whereas chondroitin sulphate A and dermatan sulphate are highly active, as is the cartilage proteoglycan containing predominantly chondroitin sulphate A. The triple helix of collagen appears essential for the interaction. It is interesting that keratan sulphate, chondroitin sulphate C and oversulphated chondroitin sulphate (D) are relatively inactive, since these glycosaminoglycans accumulate in considerable amounts in ageing cartilage, and are frequently almost absent from young cartilage (Kaplan & Meyer 1959).

These observations seem to provide a new departure point from which to investigate the changing properties of cartilage (and other connective tissues) during ageing.

The chemical evolution of the pericellular environment

The immediate environment of connective tissue (and countless other) cells is an envelope of soluble polymer (usually polysaccharide) (figure 3). Not only is this pattern encountered

everywhere in the plant and animal kingdoms, but identical polysaccharides are to be found in the most unrelated species. For instance, hyaluronic acid is produced by mammals, and also by haemolytic streptococci (Lowther & Rogers 1956). Chondroitin sulphates are produced by sponges, crabs and mammals (see Hunt 1970). The simplest assumption is that an evolutionary precursor conformed to this *pattern*, which has therefore had survival value for several thousand million years.

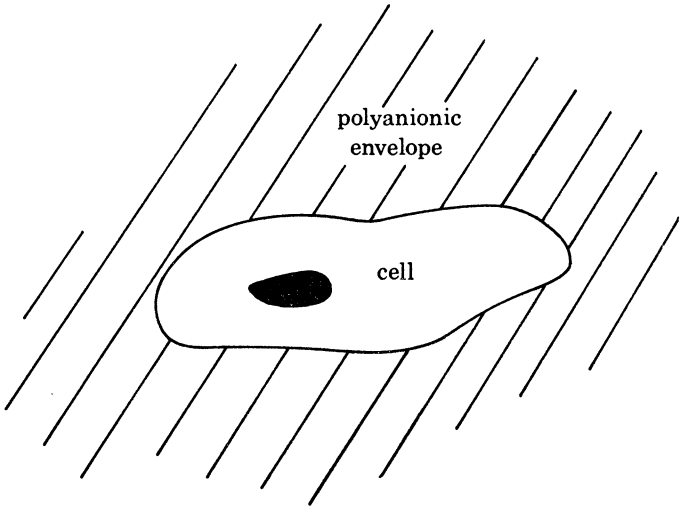

FIGURE 3. Diagrammatic illustration of the fundamental pattern of the pericellular environment. The envelope may vary in size from the large and diffuse, as in the case of slime-dwelling organisms to the small and compact (e.g. a glycocalyx).

It is not surprising that anionic polymers should be quantitatively and qualitatively dominant among biopolyelectrolytes, since *in an oxygen rich environment*, the oxidized (or acid) forms of readily oxidizable elements such as carbon, phosphorus and sulphur are the stable forms. One thinks of the enormous amounts of pectins, gums, alginates, mucins, nucleic acids, etc, and on the contrary, of the relative paucity of cationic polymers such as the histones. Given this overwhelming preponderance of negatively charged colloid, the competitive position of cationic colloidal forms is poor, and independent survival would be difficult. Indeed, polycations are *intracellular* constituents, predominantly.

Atmospheric oxidizing power may be a sufficient explanation for the successful *evolution* of the fundamental cell pattern (as well as a good reason for its continuing existence) but this would assume that there was sufficient free oxygen in the atmosphere in the very distant past, which is far from sure (see, for example, Calvin 1969).

We have to look for an agent which was available and active before the appearance of oxygen in the atmosphere. One which seems to satisfy requirements, and to offer further insights, is the hydrated electron (e^-_{aq}), which is a highly reactive species produced, for example, by the action of ionizing radiation on water, or by the hydration of electrons emitted during radioactive decay, etc. First demonstrated in 1960, its properties as a universally reactive agent have been elucidated since then (Hart & Anbar 1970). It has a half life in water measured in microseconds, and it reacts even more rapidly with almost everything else. e^-_{aq} easily gives rise to free radicals, (particularly $H^{\cdot}$) which, in their own way, are just as highly reactive.

It occurred to me that e^-_{aq} (as other anions) should, in the presence of polyelectrolytes, be subject to a kind of Donnan equilibrium, and experiments performed at the Paterson

Laboratories in Manchester showed that this was so (Scott, Davies & Ebert 1975). e^-_{aq} is therefore strongly excluded from the domains of polyanions, whereas it concentrates within the domains of polycations (figure 4). The negative charge of the polyanion 'protects' it against the ravages of e^-_{aq}, and as a corollary, minimizes the destructive actions of H$^\cdot$ radicals, etc., which might be produced by e^-_{aq}. On the other hand, polycations are especially vulnerable to the attack of e^-_{aq} and free radicals produced from it after its arrival within the polymer domain.

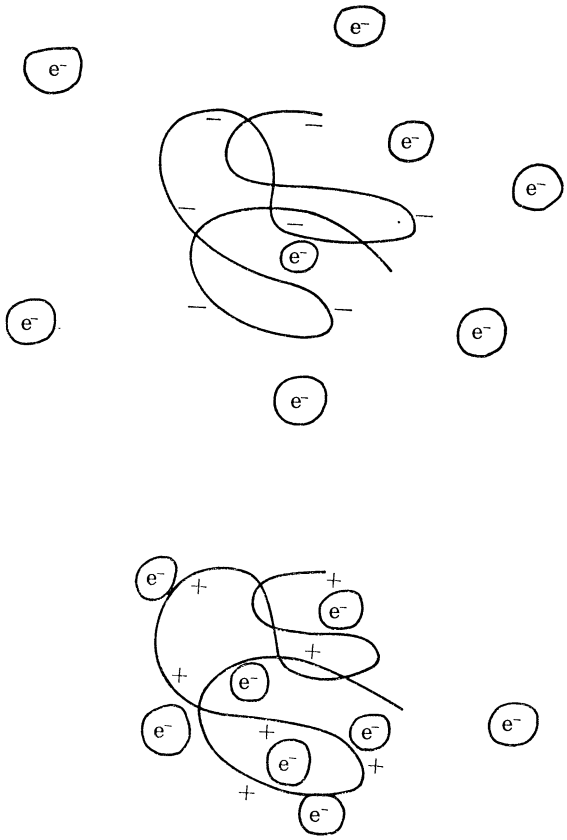

FIGURE 4. The distribution of hydrated electrons (e^-_{aq}) in the electrostatic fields of polyanions (top) and polycations (lower), in aqueous salt free solutions (Scott *et al.* 1974). For simplicity, polyelectrolyte counterions are not shown, and the charges associated with the polymer are 'net'.

TABLE 2. BIMOLECULAR RATE CONSTANTS FOR THE REACTION OF e^-_{aq} WITH SELECTED SUBSTRATES

	pH	rate constant / $M^{-1}\,s^{-1}$
water	8.4	1.6×10^1
methanol	—	$< 10^4$
glucose	—	$\leqslant 10^6$
Gly-Gly-Gly	6.0	9×10^8
haemoglobin	—	2.6×10^{10}
adenine	7.7	6×10^9
thymine	6.0	1.7×10^{10}

Taken from Anbar & Hart (1970).

It is worth pointing out that conditions thought to exist in the pre-biotic era (and subsequently), i.e. oxygen free atmosphere, slightly alkaline seas and high levels of radiation, could sustain e^-_{aq} activity at much higher levels than are found now, which must have greatly influenced the evolution of polyelectrolytes in the 'primordial soup'.

On comparing various classes of chemical compounds, it is interesting that alcohols and carbohydrates are among the least reactive towards e^-_{aq} (table 2), while susceptibilities towards $OH^\cdot$ and $H^\cdot$ are much less variable. Thus, a carbohydrate polyanion would be a durable and effective shield against e^-_{aq}.

It might be suggested, therefore, that during both the pre-biotic and subsequent eras, the survival value of a polyanionic carbohydrate shield against e^-_{aq} was considerable, and lead to the 'selection' of cells, organelles or co-acervates which possessed it.

Interestingly, experiments which confirm the possibility of such protection (table 3) have been in the literature since 1968 (Balazs *et al.*) but have not been seen in this light.

TABLE 3. FIRST-ORDER RATE CONSTANTS FOR THE REACTION OF e^-_{aq} WITH METHYLENE BLUE OR POLYLYSINE, ALONE OR IN THE PRESENCE OF HEPARIN

	$10^{-4}k_1/s^{-1}$		
	alone	+	heparin, 10^{-3} M
methylene blue 10^{-5} M	26.0		3.1
polylysine hydrobromide 10^{-4} M	43.0		3.6

Data from Balazs *et al.* (1968*a*).

This approach throws light on the absolute lack of cationic polysaccharides in our present biosphere. Clearly, a positively charged carbohydrate polymer would have been worse than useless in this context. Whatever the continuing role of the hexosamines (e.g. in chitin, etc.) no use whatever is made of their potentially cationic amino group, which is always acetylated, etc. The machinery for producing a cationic monomer exists, but invaraibly the product is deflected, at considerable expense of energy and material, from being incorporated into a polymer.

The appearance of oxygen in the atmosphere probably greatly reduced the role of e^-_{aq}. The development of an ozone layer would have lessened the amounts of radiation reaching ground level, and oxygen is an efficient scavenger of e^-_{aq}.

In summary, therefore, I have presented a picture of the pericellular environment in which the characteristic feature is the presence of large or small amounts of carbohydrate polyanions. Evolution, with or without fibrous components, occurred according to the demands of the larger environment of the biosphere, in which first radiation, then oxygen, and finally mechanical stress, played parts.

My thanks are due to Professor R. Harkness for helpful discussions.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

References (Scott)

Balazs, E. A. 1962 In: *Symposium on structure of the eye* (ed. G. K. Smelser), pp. 293–310. New York: Academic Press.

Balazs, E. A., Davies, J. V., Philips, G. O. & Scheufele, D. 1968*a* *J. chem. Soc.* (*C*) 1424–1429.

Balazs, E. A., Davies, J. V., Philips, G. O. & Scheufele, D. 1968*b* *J. chem. Soc.* (*C*) 1429–1433.

Calvin, M. 1969 *Chemical evolution*. Oxford: Clarendon Press.

Conochie, L., Scott, J. E., Faulk, W. P. & Bailey, A. 1975 *Ann. Rheum. Dis.* (*Suppl*). In press.

Hart, E. J. & Anbar, M. 1970 *The hydrated electron*. New York: Wiley-Interscience.

Hedbys, B. O. 1961 *Exp. Eye Res.* **1**, 81–91.

Hunt, S. 1970 *Polysaccharide–protein complexes in invertebrates*. London: Academic Press.

Kaplan, D. & Meyer, K. 1959 *Nature, Lond.* **183**, 1267–1268.

Lowther, D. A. & Rogers, H. J. 1956 *Biochem. J.* **62**, 304–314.

Mason, R. M. & Mayes, R. W. 1973 *Biochem. J.* **131**, 535–540.

Öbrink, B. 1973*a* *Eur. J. Biochem.* **33**, 387–400.

Öbrink, B. 1973*b* *Eur. J. Biochem.* **34**, 129–137.

Ogston, A. G. 1958 *Trans. Farad. Soc.* **54**, 1754.

Rosenberg, L., Hellman, W. & Kleinschmidt, A. K. 1970 *J. biol. Chem.* **245**, 4123–4130.

Sajdera, S. W. & Hascall, V. S. 1969 *J. biol. Chem.* **244**, 77–87.

Scott, J. E. 1973 In: *Current developments in rheumatology* (eds. O. Lövgren, H. Boström, B. Olhagen & R. Sannerstedt), pp. 10–29. Mölndal: A. Lindgren.

Scott, J. E., Davies, J. V. & Ebert, M. 1975 *Trans. Biochem. Soc.* In press.

Sokoloff, L. 1963 *Science, N.Y.* **141**, 1055–1057.

Szirmai, J. A. 1966 In: *The amino surgars* (eds. E. A. Balazs & R. W. Jeanloz), Vol. 11B, pp. 129–154. New York: Academic Press.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES