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Chondroitin sulphate and keratan sulphate are almost isosteric

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Keratan sulphate and chondroitin sulphate (KS and CS) in the 2-fold helical configurations that are prevalent in solution are of very similar tacticity. The chiral centres, anionic sites and hydrophobic patches are in identical conformations. Only the position of the acetamido group varies from CS to KS, but part of its intramolecular H-bonding potential in CS is retained in KS. The formation of tertiary aggregates, observed *in vitro* and in tissues, is explicable on these bases. The proposal that KS may be a functional substitute for CS [Scott & Haigh (1988) J. Anat. **158**, 95–108] under low-O_2 conditions is relevant.

THEORY

The structures of the repeating units of the glycosaminoglycans from connective tissue are conventionally written as in Fig. 1 (structures **I** and **II**), with the hexosamine to the right [1–4]. This system was used by Karl Meyer to show parallels between hyaluronan (HA) and the chondroitin sulphates (CS) [1]. Moreover, the structures reflect the smallest hydrolysis products produced by the hyaluronidases, i.e. they cleave the β-1:4 hexosaminidic bond, as do the chondroitinases. When the structure of keratan sulphate (KS) was established, with Karl Meyer's group again prominently involved, similarities with HA and CS were expressed in the same way (Fig. 1, **I**). However, this is only an analogy; Fig. 1 (**I**) does not represent breakdown products produced by keratanases, which, on the contrary, cleave the 1:4 galactosidic bond. The very widespread use of Fig. 1 (**I**) led to implied or stated assumptions that galactose is a substitute for, i.e. 'instead of', the hexuronic acid in the glycosaminoglycuronans (e.g. [2]). Although true in the metabolic sense, the present paper points out that it is not true in a structural sense. The convention of Fig. 1 (**I**) obscures very fundamental resemblances between CS and KS, which are configurationally identical and almost isosteric.

Simply writing the KS disaccharide as in Fig. 1 (**III**) stresses that both polymer backbones are β-1:3, β-1:4 linked and that the sugar rings have alternately the D-*gluco* and the D-*galacto* configuration, with some anionic groups in equivalent positions, at 'Glc' C-6. The similarities are most striking when the secondary structures, recently acquired, are compared.

Secondary structures

Secondary structures of HA and CS in solution, based on periodate kinetics [5], n.m.r. [6] and computer simulation [7], are 2-fold helixes with extended H-bonding systems down both sides of the tape-like structures (Fig. 1, **IV**). Important corollaries are (*a*) an extended hydrophobic patch, of eight or nine CH units, occurs on alternate sides of the molecule [8], and (*b*) both sides of the polymer backbone are identical [9]. These properties account for the very efficient meshwork-forming behaviour of HA [9].

The secondary structure of 'over-sulphated' KS oligosaccharides, based on n.m.r. and X-ray-diffraction studies [10], has an equivalent β-1:4, β-1:3 2-fold helix (Fig. 1, **V**), and the presence of hydrophobic patches on alternate sides of the molecule was noted [10]. It follows (*a*) that both sides of the polymer backbone must be identical, as for HA and CS, assuming (as in [10]) that sulphation patterns are regular, and (*b*) that the hydrophobic patches are identical in size, shape and position with those of CS (Fig. 1, **IV** and **V**).

How do the substituents (anionic and acetamido groups) affect this picture?

Sulphation

The resemblance between chondroitin and monosulphated KS has been noted above. The negative charge at C-6 of the D-*gluco* ring of CS is obligatory, and it is relevant that the corresponding position in KS is almost always sulphated, whereas C-6 in the galactose ring is sulphated only in oversulphated KS. The latter position corresponds exactly to that in chondroitin 6-sulphate (CS6) (Fig. 1, **IV**). Thus increasing sulphation of KS increases the resemblance to CS6.

Acetamido

The position of this group on the D-*galacto* rather than the D-*gluco* ring is the important difference between CS and KS. However, examination of the secondary structures (Fig. 1, **IV** and **V**) shows that the 2-OH and acetamido groups have simply exchanged positions across the glycosidic bond, and the original potential to interact (–OH → O=C) remains. In KS, –NH does not interact with O–C–O, as it does in CS [11].

Certain corollaries follow.

(1) Aggregation via the hydrophobic patches, as in the case of HA [9], is possible for CS and KS, somewhat modified by greater electrostatic repulsion between CS molecules, owing to their higher charge (J. E. Scott, A. Brass & Y. Chen, unpublished work). A low-sulphated KS was shown by gel chromatography to dimerize reversibly [12]. Hetero-duplex formation should also be possible, subject to electrostatic constraints, and indeed, KS–CS aggregates were seen by electron microscopy in cornea [13]. The KS and CS in these complexes were of low sulphation [14] and consequently less mutually repellant than more highly charged KS and CS, which did not participate in similar aggregates.

(2) Two different metabolic routes, based on glucuronate and galactose respectively, lead to two different polymers of very similar tacticity. Each can perform the same functions (e.g. in swelling the corneal stroma, or endowing cartilage with elasticity [13]). The mechanisms that decide which one is made are beginning to be investigated. One parameter appears to be the

Abbreviations used: CS, chondroitin sulphate; KS, keratan sulphate; HA, hyaluronan; CS6, chondroitin 6-sulphate.

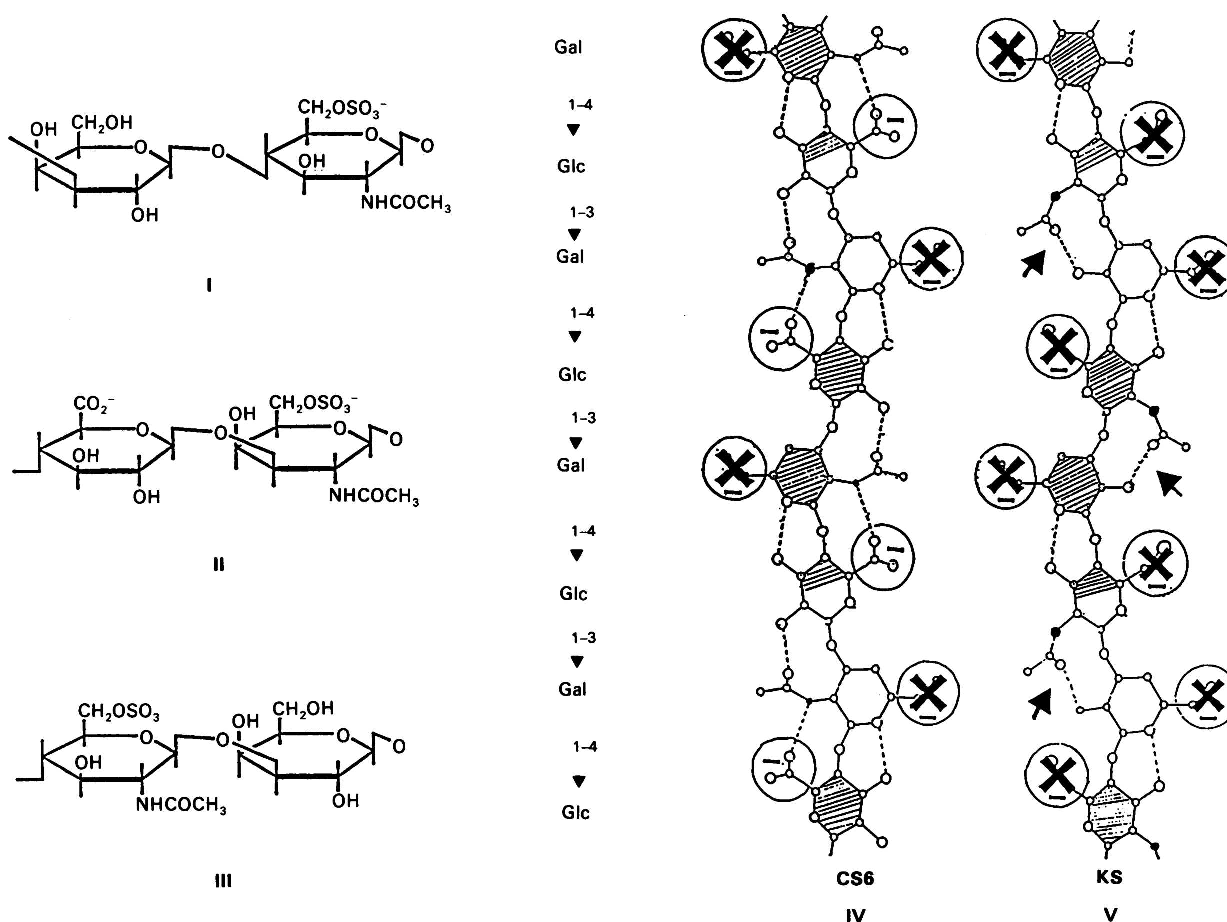

Fig. 1. Formulae and secondary structures of CS6 and KS

I, II and III, formulae of repeating disaccharide units from KS (I and III) and CS6 (II). IV and V, drawings to scale of the 2-fold helical structures, present in aqueous solution of CS6 (IV) [16] and 'oversulphated' KS (V) [10]. Both are β-1:4, β-1:3-linked chains of alternating D-*gluco*, D-*galacto*, with anionic (ester sulphate and/or carboxylate) and neutral (acetamido) substituents. The positions of the anionic sites are ringed, those with an **X** are sulphate ester groups, the others (in CS6) are carboxylates. The anionic sites are at precisely equivalent locations in both CS-6 and KS, as are the extended hydrophobic patches (cross-hatched, ▨). The positions of the acetamido and 2-OH groups are switched in KS as compared with CS, but the interaction between them still persists (arrowed). Hyaluronan adopts a similar configuration to that of CS, with an extra H-bond from the equatorial C-4 hydroxy group of acetamidoglucose to the ring oxygen atom of glucuronate [8].

state of oxygenation of the tissue which, when low, favours KS rather than CS production [15].

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