On the poly lactose nature of chondroitin and keratan sulphates

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The recognition that keratan and chondroitin sulphates are based on the same poly lactose backbone simplifies the study of heteroduplex formation between them, suggests insights into

structure–function relationships in tissues, and permits new definitions of the linkage regions.

When the repeating units of keratan sulphate (KS) and chondroitin sulphates (CS) are written not with the HexNAc to the right, according to previous convention, but with the *D-galacto* and *D-gluco* rings aligned, their polymer backbones are seen to be

identical, consisting of identical disaccharides linked identically (Scott, 1991). In this note some consequences are briefly discussed, leading to simpler definitions of the linkage region versus the polymer chain, and to new questions about relationships

Figure 1 Primary, secondary and tertiary structures involving CS and KS

I and II, structures of KS and CS respectively. Mainly on the basis of endo-hexosaminidase degradation, which produce disaccharides of type II from CSs, the polymer repeating units were conventionally represented as in II. Repeating units of KS were analogously drawn (Ia), with the hexosamine residue on the right. However, if the alternative disaccharide Ib) is chosen instead of Ia, it is seen that the CS (II) and KS(Ib) disaccharides are both *D-gluco*, β -1,3-*D-galacto*. The choice of either Ia or Ib (and their CS analogues) as repeating units is arbitrary. The recognition that both KS and CS are poly lactoses, and that both polymers start with a complete 'lactose' unit at the reducing end, provides a formal basis for defining the repeating units of both KS and CS as 'lactose'-based (III). The last (non-reducing) sugar in the linkage sequence is Gal (CS) or GalNAc or Man (KS) (III). The non-reducing terminal sugar in the last unit (T) of CS can be either Glc- or Gal-based, i.e. the last lactose unit need not be complete. The frequently used enzymes chondroitin lyases ABC and AC and keratan hydrolase I all cleave Gal–Glc bonds (III, arrowed). IV shows the gentle waves of the lines of the sugar rings in the plane of the KS polymer (above) and at right angles to the plane of the polymer (below, no substituents; only rings and glycosidic bonds are shown). The cross-hatched areas are hydrophobic patches; X = sulphate ester anionic groups, usually present on Glc C-6, and sometimes, but not necessarily, on Gal C-4. To scale. V, Scheme of proposed antiparallel CS:KS duplex, side view, with hydrophobic patches cross-hatched. Arrows point towards the reducing ends. The waveform present in the KS and CS backbones is shown to the left, in phase with that in both components of the duplex. Sugar rings are defined by dotted lines and labelled alongside. ○, ●, Acetamido groups; □, ■, carboxylate groups.

Black symbols point out of the plane of the paper, and white symbols into the plane, respectively. Not to scale. $\text{NH} \rightarrow \text{O} \cdots \text{C}=\text{O}$ H-bonds are possible between every pair of opposing disaccharides; hydrophobic patches overlap almost completely, and the shapes of the polymers shown as in IV (upper) are complementary.

between CS and KS, their biosynthesis, enzymic degradation and evolution.

(1) The newly defined repeating unit (*D-galacto* β -1:4*D-gluco*) is *D-lacto*. KS is currently recognized as a poly lactosamine, but CS was not previously considered in this context. The recognition that CS and KS are both poly lactoses simplifies study of intermolecular interactions, since their shapes depend on identical backbones.

(2) The sugar units between the beginning (i.e. the reducing end) of the poly lactose chain and the core protein are defined as the linkage or bridging sequence. In the CS polymers, the linkage regions conventionally include a glucuronate residue. This is now seen as part of the first 'lactose' unit of the poly lactose chain (Figure 1, III). The linkage sequence is therefore Gal-Gal-Xyl-(Ser etc.). Similarly, in KS, the first GlcNAc residue begins the poly lactose chain, and the linkage sequence ends with a Man or GalNAc residue (Figure 1, III).

The non-reducing end is not as easily defined. The terminal non-reducing sugar of CS may be either GlcUA or GalNAc. In the latter case all the *D-lacto* units are complete.

One diagram (Figure 1, III) defines both KS and CS polymer backbone structures, the non-reducing ends of their linkage sequences and their non-reducing termini.

(3) The backbones of the twofold helical structures (for references see Scott, 1991) simultaneously follow two wave-like tracks, one in the plane of the tapelike molecules, due to the direction of the glycosidic bonds between sugars, and the other at right angles to the plane of the polymer, due to the angles of the bonds within the glycosidic links (Figure 1, IV). To make good intermolecular contacts involving short-range forces, the interactants must take up positions in which the two waves are complementary. It is relevant that antiparallel alignment of KS with CS, juxtaposing the Glc and Gal rings on opposing polymers, produces excellent fits in both planes, with almost complete overlapping of hydrophobic patches on the opposing polymers (Figure 1, V). In this configuration, every CS GlcUA carboxylate and KS GlcNAc acetamido NH group can form intermolecular H-bonds (i.e. between the chains). Similar H-

bonds participate (intramolecularly) in the secondary structures of CS and hyaluronan (Scott, 1991). On the basis of X-ray diffraction studies of hyaluronan fibres, Atkins et al. (1972)

postulated $\text{NH} \rightarrow \text{O} \cdots \overset{\ominus}{\text{C}} \cdots \text{O}$ H-bonds between antiparallel hyaluronan molecules, similar to those suggested here for CS and KS. It would be surprising if this outstanding example of specific fit between two glycosaminoglycan chains was without structural significance. KS:CS aggregates are seen in considerable numbers in corneal stromas (Scott, 1989), and, although nothing is known of their structure at the molecular level, anti-parallel arrangements are possible (Scott, 1992).

(4) The enzymes widely used to characterize KSs and CSs, keratanase hydrolase I and the chondroitin lyases AC and ABC, split the poly lactose backbone at precisely the same points, the Gal-Glc bonds (Figure 1, III). Since the anionic sites and hydrophobic patches are in exactly similar positions in, e.g. keratan monosulphate and chondroitin, and only the locations of the acetamido groups within the disaccharides are different (Scott, 1991), this may be crucial in determining enzyme specificity.

(5) Lactose is found in mammalian milk. Apparently it is not produced in other animal tissues or tissue fluids. The precise *raison d'être* of lactose in this context is a subject of speculation. Since lactose (but not CS or KS) is found in plants (Clamp et al., 1961), the disaccharide may be evolutionarily more ancient than the polymer. It is biosynthesized by a simpler version (in that only one of the two sugars goes through a UDP stage) of the UDP-dependent pathways that produce CS and KS.

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