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STRUCTURAL FEATURES OF THE CONTACT ZONES FOR HEPARAN SULPHATE SELF-ASSOCIATION

LARS-ÅKE FRANSSON
Department of Physiological Chemistry 2, University of Lund, P.O. Box 750, S-220 07 Lund (Sweden)
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ABSTRACT

The self-association between heparan sulphate chains has been investigated by using heparan sulphate oligosaccharides for the competitive elution of [^{3}H]heparan sulphate from heparan sulphate–agarose. Partial or complete periodate-oxidation followed by alkali-catalysed scission afforded oligomers having the general structure GlcN–(HexA–GlcN)$_n$–R. Oligosaccharides with $n > 5$ were able to desorb bound heparan sulphate, provided that mixed or alternating arrangements of iduronate and glucuronate were present in these fragments. Longer fragments were more effective than shorter ones. The present results corroborate previous proposals that the highly copolymeric regions of heparan sulphate serve as contact zones for the chain–chain association.

INTRODUCTION

The specific self-interaction displayed by heparan sulphate chains seems to be dependent on (*a*) the overall molecular conformation and (*b*) the integrity of regions comprising both iduronate(IdoA)- and glucuronate(GlcA)-containing disaccharide units[1,2]. The structural features of the latter regions have now been further studied by using heparan sulphate oligosaccharides for competitive elution of [^{3}H]heparan sulphate from heparan sulphate–agarose.

EXPERIMENTAL

Materials. — The associating preparation HS4-A of beef-lung heparan sulphate was the same as that used in the preceding paper[2]. ^{3}H-Labelled HS4-A was prepared as described[3]. Sepharose, Sephacryl, and Sephadex gels were purchased from Pharmacia Fine Chemicals. Sepharose 4B was derivatised with heparan sulphate *via* an adipic acid dihydrazide extension, according to procedures outlined elsewhere[1,3].

Methods. — Affinity chromatography on HS4-A–agarose was conducted as described in the preceding paper[2]. Columns were eluted either with a linear gradient of guanidine HCl or with solutions of various heparan sulphate oligosaccharides.

Gel-permeation chromatography was carried out as shown in the legends to the appropriate Figures.

Effluents from column chromatography were analysed, when appropriate, for uronate by the carbazole–borate[5] or orcinol[6] methods, or for ^3H by liquid scintillation in a Packard 2650 instrument with automated quench correction.

Periodate oxidation of GlcA in $(GlcA–GlcNAc)_n$-block regions of heparan sulphate was carried out with 2 mg of glycan per mL of 0.02M $NaIO_4$/0.05M sodium formate (pH 3.0) at 4° for different time-intervals. Reactions were terminated by the addition of excess of mannitol, and oxyglycans were recovered after dialysis and freeze-drying. Alkali-catalysed scission of oxyglycans was conducted at pH 12 and room temperature for 30 min (see also refs. 1 and 3).

RESULTS

As shown in Fig. 1a, heparan sulphate HS4-A is bound to HS4-A–agarose and is eluted with a linear gradient of guanidine. Periodate oxidation of GlcA residues in $(GlcA–GlcNAc)_n$-block regions of the chain abolishes self-interaction (results not shown here, but see refs. 2 and 3). Subsequent alkaline elimination of oxyheparan sulphate produces oligosaccharides having the general structure $GlcN–(HexA–GlcN)_n–R$, where HexA may be either IdoA, IdoA-SO_4, or GlcA, and the GlcN residues may often be *N*-sulphated[4]. When such oligomers derived from preparation HS4-A were chromatographed on HS4-A–agarose, partial binding was seen (Fig. 1b and c). Fragments obtained after oxidation for 1 h showed somewhat higher affinity than did those obtained after complete oxidation (24 h).

To isolate oligosaccharide fragments that could be used in competition experiments, HS4-A heparan sulphate was oxidised with periodate for 1 h and 24 h, respectively, followed by scission in alkali. The products of the 1-h reaction were chromatographed on Sephacryl S-300 (Fig. 2). Fractions I and II, which had the same apparent size distribution as the starting material, were combined, whereas Fraction III was further chromatographed on Sephadex G-50. As seen in Fig. 3a, the products obtained after the oxidation for 24 h ranged from $n = 1$ to $n = 8$ in the general formula mentioned above. Fraction III from the 1-h oxidation comprised larger oligomers having $n = 6–8$. The various oligosaccharide fractions were combined as indicated in Fig. 3, and then re-chromatographed on a smaller column of Sephadex G-50. As seen in Fig. 4a–d, there were four oligomeric fractions: one with $n = 5–6$, two with $n = 7$, and one with $n > 8$ as their major components. These fractions were used for the competition experiments.

Affinity gels derivatised with HS4-A heparan sulphate were charged with [^3H]heparan sulphate of the same kind, and elution was performed with solutions of oligosaccharides followed by 2M guanidine HCl. As shown in Fig. 5a–c, the oligomer $n = 5–6$ obtained after a 24-h oxidation was able to displace [^3H]heparan sulphate at concentrations of 2 mg/mL or higher. At 4 mg/mL, ~85% of the bound material was eluted. The oligomer $n = 7$ obtained after a 24-h oxidation desorbed ~65%

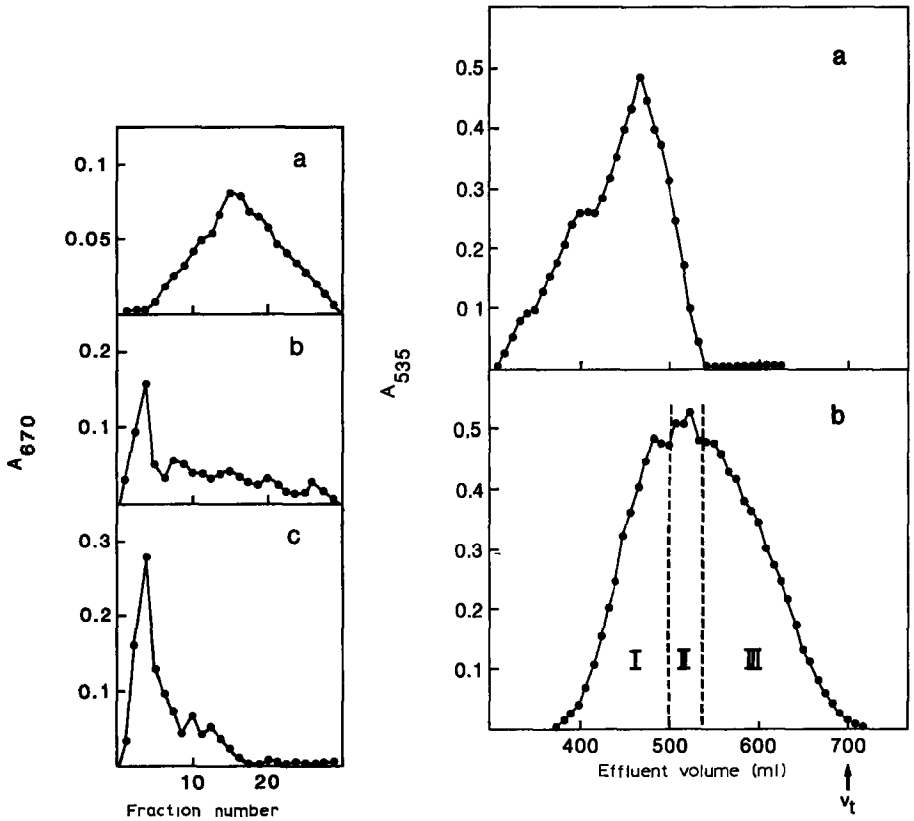

Fig. 1. Affinity chromatography on HS4-A-agarose of heparan sulphate HS4-A before treatment (a), after 1-h periodate oxidation–alkaline elimination (b), and after 24-h periodate oxidation–alkaline elimination (c). For preparation of affinity matrix, application of sample, and operation of column, see Experimental. Elution, linear gradient of guanidine HCl (up to 1.5M); analysis, orcinol reaction for uronate.

Fig. 2. Gel-permeation chromatography of heparan sulphate HS4-A before treatment (a), and after 1-h periodate oxidation–alkaline elimination (b). The heparan sulphate (250 mg) was degraded as described in the Experimental, and chromatographed on a column (22 × 2100 mm) of Sephacryl S-300 which was eluted with 0.5M sodium acetate (pH 7.0). The effluent was analysed for hexuronate by the carbazole–borate reaction (A_{535}) and fractions were combined as indicated by vertical dashed lines. Fractions I and II were recovered by ethanol precipitation, whereas fraction III was subjected to further gel chromatography (see Fig. 3b). V_0 = 250 mL.

of bound [^{3}H]heparan sulphate at only 1 mg/mL (Fig. 5d). A similar oligosaccharide (n = 7) obtained after oxidation for 1 h was unable to compete with bound chains at the same concentration (Fig. 6a). However, longer fragments obtained after oxidation for 1 h, *i.e.*, $n > 8$ (Fig. 6b) or the polymeric Fraction I (Fig. 6c), displaced 60% and 75%, respectively, of [^{3}H]heparan sulphate.

As shown elsewhere[4], oligosaccharides obtained after periodate oxidation–alkaline elimination may be susceptible to re-oxidation under the same conditions

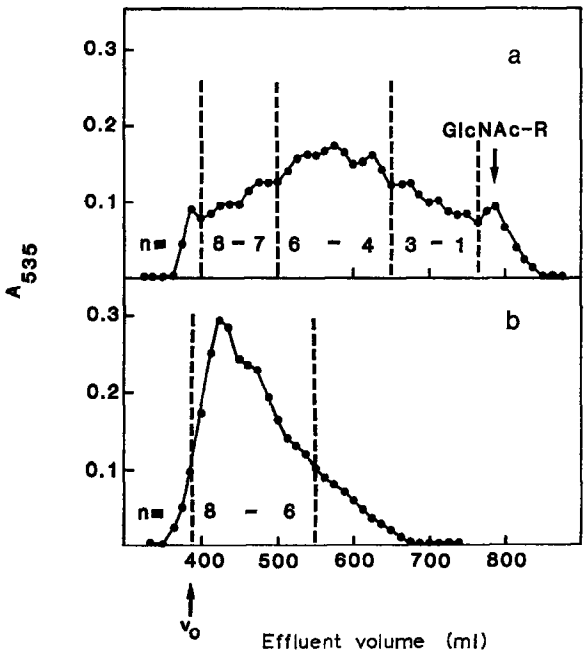

Fig. 3. Gel-permeation chromatography of heparan sulphate HS4-A after 24-h periodate oxidation–alkaline elimination (a), and fraction III obtained after 1-h periodate oxidation–alkaline elimination (b). The heparan sulphate (250 mg) was degraded as described in the Experimental, and, in one case (the 1-h oxidation), first chromatographed on Sephacryl S-300 (Fig. 2b) where fraction III was collected. The two samples were fractionated on a column (22 × 2430 mm) of Sephadex G-50 (superfine) which was eluted with 0.2M pyridine acetate (pH 5.0). The effluent was analysed for hexuronate by the carbazole–borate reaction (A_{535}) and fractions were combined as indicated by vertical dashed lines. The column was calibrated with oligomers of the general structure GlcN–(HexA–GlcN)$_n$–R which have been characterised previously (ref. 4). GlcNAc–R (*i.e.*, $n = 0$) is the compound derived from segments composed of continuous runs of –GlcA–GlcNAc– repeats. The fractions were designated according to the approximate size ($n = 1$–8) of the oligosaccharide fragments.

(see Scheme 1 in ref. 2); the GlcA residues are particularly vulnerable to this re-oxidation. By this treatment, the oligomers are degraded to smaller fragments of the same general formula, but with IdoA or IdoA-SO_4 as their HexA constituents. Hence, the fragmentation pattern provides information about the distribution of susceptible GlcA residues. When such degradations were performed, the results shown in Fig. 4e–h were obtained. The shortest oligomer used for competition experiments, *i.e.*, $n = 4$–6 (Fig. 4a) was degraded to fragments of $n = 0$–4 (Fig. 4e). The proportion of $n = 0$ that is derived from at least two consecutive GlcA-containing disaccharides cannot be adequately assessed, but it is probably small, as fragments of $n = 3$ or 4 comprise a major portion of the products. The latter could be generated after cleavage of only one GlcA residue at various positions in the oligosaccharide. The somewhat larger oligosaccharide $n = 7$ (Fig. 4b), which was also obtained after a 24-h oxidation, yielded a similar degradation pattern upon re-oxidation (Fig. 4f). In this case, it is possible to accommodate two GlcA–GlcN repeats in an alternating

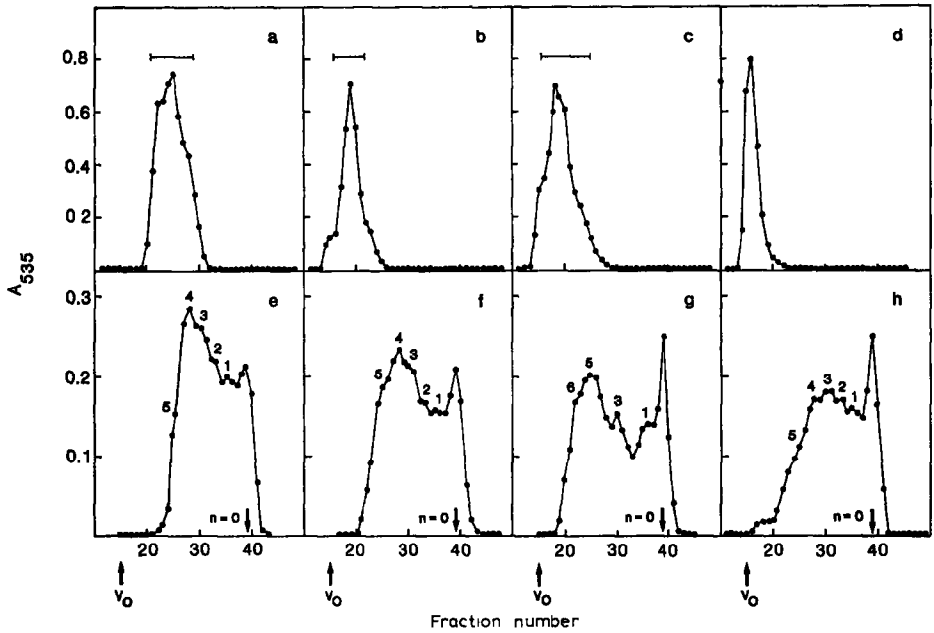

Fig. 4. Gel-permeation chromatography of heparan sulphate oligosaccharides before (a–d) and after (e–h) re-oxidation with periodate followed by scission in alkali. The samples chromatographed were a 24-h oxidation product with $n = 4$–6 (a and e), a 24-h oxidation product with $n = 7$–8 (b and f), a 1-h oxidation product with $n = 6$–8 (c and g), which were all obtained as shown in Fig. 3, and the 1-h oxidation product fraction II (d and h) obtained as shown in Fig. 2b. The samples were fractionated on a column (8 × 1500 mm) of Sephadex G-50 (superfine) which was eluted with 0.2M pyridine acetate (pH 5.0). The effluent was analysed for hexuronate by the carbazole–borate rection (A_{535}) and, in a–c, material was recovered as indicated by the horizontal bars. The numbers indicate the approximate size of the oligomers of the general structure GlcN–(HexA–GlcN)$_n$–R; $n = 0$, the position of GlcNAc–R, which gives some non-specific colour in the carbazole rection.

arrangement with IdoA–GlcN repeats. A similar degradation pattern was also produced from the $n > 8$ fragment obtained after a 1-h oxidation (Fig. 4d and h). As this fragment was longer, there should be even more opportunities for alternating or highly mixed arrangements of the IdoA- and GlcA-containing units. One oligomer was different from the others, *i.e.*, $n = 7$ from a 1-h oxidation (Fig. 4c and g), inasmuch as it afforded mainly very large fragments, $n = 5$–6. The GlcA residues susceptible to re-oxidation must have been located largely in the terminal regions of the oligomer or, occasionally, in the middle (yielding two $n = 3$ fragments). Hence, alternating or mixed sequences must be very rare in this oligosaccharide fragment.

DISCUSSION

It is concluded from the results of the present study that segments of the heparan sulphate chain that comprise both IdoA- and GlcA-containing repeats serve as contact zones for self-interaction. These segments may be released, wholly or in

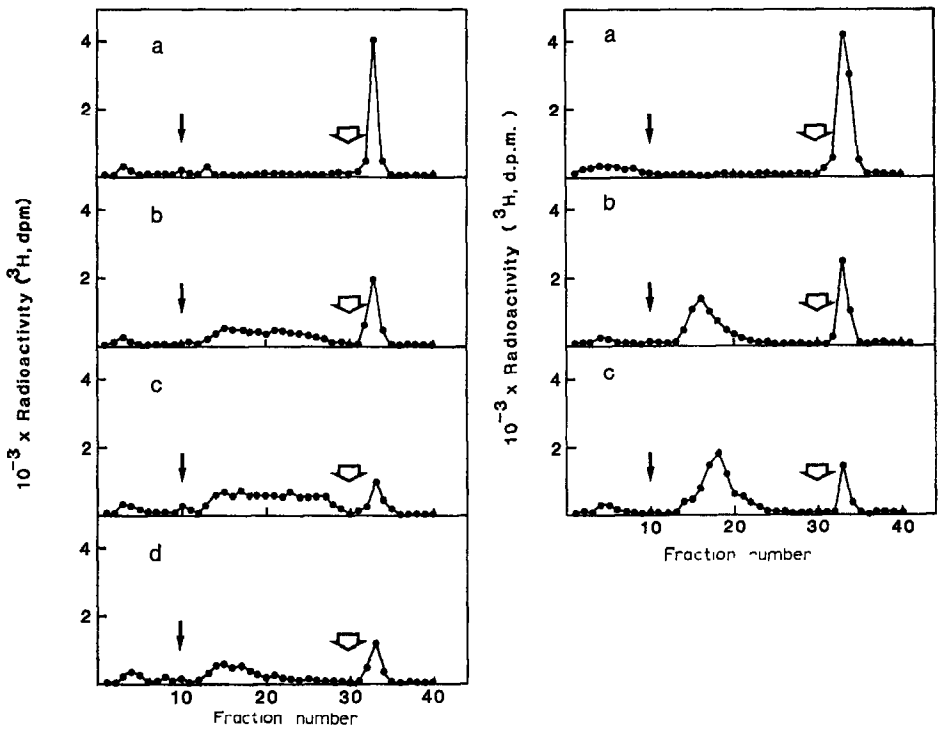

Fig. 5. Affinity chromatography of [^{3}H]heparan sulphate HS4-A on HS4-A–agarose which was eluted with solutions of heparan sulphate oligosaccharides obtained after 24-h periodate oxidation, *i.e.*, fragment n = 4–6 at 0.5 mg/mL of 0.15M NaCl (a), 2 mg/mL (b), 4 mg/mL (c), and fragment n = 7–8 at 1 mg/mL (d). Samples were applied in 0.15M NaCl, the columns were washed with the same solvent, and then solutions containing the indicated oligomers were passed through the columns (↓). The oligosaccharides were those shown in Fig. 3a. Finally, the gels were washed with 2M guanidine HCl (⇩). Analysis, ^{3}H-radioactivity.

Fig. 6. Affinity chromatography of [^{3}H]heparan sulphate HS4-A on HS4-A-agarose which was eluted with solutions of oligomers obtained after 1-h periodate oxidation, *i.e.*, fragment n = 6–8 (a), fraction II (b), and fraction I (c) all at 1 mg/mL of 0.15M NaCl. The heparan sulphate fragments used were those shown in Fig. 3b (n = 6–8) and Fig. 2b (I and II). For further details, see the legend to Fig. 6.

part, by periodate oxidation–alkaline elimination. Periodate oxidation alone precludes chain–chain interaction, probably because of steric reasons; the oxyglycan chain should be "kinked" at the point of oxidation because of the formation of a hemialdal between the two CHO groups, yielding a seven-membered ring structure. As discussed in the preceding paper[2], the integrity of the overall molecular conformation is important, as both carboxyl reduction and *N*-desulphation/*N*-acetylation abolish self-association.

Oligosaccharide fragments showed greater ability to compete for binding with increasing length, which may be ascribed to a co-operative effect. It is not known which groups and which types of bonds are involved in chain–chain interaction, but

oligomers having the ability to desorb bound heparan sulphate all have highly mixed or alternating arrangements of IdoA–GlcN and GlcA–GlcN repeats. Such regions may assume secondary structures that are different from those formed by continuous runs of either IdoA–GlcN or GlcA–GlcN repeats. The shapes of the alternating regions may favour direct intermolecular contacts, or indirect ones *via* solvent molecules.

Further examination of the self-interaction requires further sub-fractionation of heparan sulphates and fragments thereof to obtain species that are chemically more homogeneous. It may then be possible to investigate whether complementary sequences are required for appropriate chain–chain contact.

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