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THE SUBSTRATE SPECIFICITY OF HEPARAN SULPHATE LYASE AND HEPARIN LYASE FROM *Flavobacterium heparinum*

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ABSTRACT

The substrate specificity of heparan sulphate lyase and heparin lyase has been examined by using various oligosaccharides produced by deaminative cleavage or periodate oxidation–base-catalysed elimination of heparan sulphate. The saccharides were separated by gel and ion-exchange chromatography into fractions having high proportions of the following linkages: 2-acetamido-2-deoxy-D-glucosyl→D-glucuronic acid (type I), 2-deoxy-2-sulphoamino-D-glucosyl→D-glucuronic acid (type II), 2-deoxy-2-sulphoamino-D-glucosyl→L-iduronic acid (type III), or 2-deoxy-2-sulphoamino-D-glucosyl→2-*O*-sulpho-L-iduronic acid (type IV). Heparan sulphate lyase cleaved heparan sulphate preparations containing high proportions of type I linkages. In contrast, oligosaccharides obtained after deaminative cleavage and rich in the type I linkage were poor substrates; longer saccharides were better substrates than shorter ones. Saccharides generated *via* periodate oxidation were cleaved when the type I linkage was present but resistant when the linkages were of types II–IV. The presence of ester sulphate on the 2-acetamido-2-deoxy-D-glucose residue in a type I linkage seems to hinder the enzyme. Heparan sulphate lyase was able to degrade intact chains to the same extent as did two cycles of periodate oxidation–base-catalysed elimination, suggesting that all the regular type I linkages were accessible to the enzyme. The heparin lyase could cleave saccharides which contained type IV linkages. When type II and III linkages were the only ones present, the saccharides were resistant. This enzyme also appears to require ester-sulphation of the 2-deoxy-2-sulphoamino-D-glucose residue.

INTRODUCTION

Most animal cells synthesise proteoheparan sulphate, which is mainly associated with the cell surface. In some cases, perhaps most, the core protein is intercalated into the plasma membrane via a non-glycosylated, hydrophobic peptide portion[1–4], whereas the peripheral part of the molecule carries the heparan sulphate side-chains[4]. The latter can be removed *in situ* by degradation with bacterial heparan sulphate lyases (heparitinases)[5]. All heparan sulphates (and heparin)

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TABLE I

MONOSACCHARIDE COMPOSITION OF HEPARAN SULPHATE

GlypA	*GlcpN*
D-GlcA	D-GlcNAc
L-IdoA	D-GlcNSO$_3^-$
L-IdoA2SO$_3^-$	D-GlcNSO$_3^-$(6SO$_3^-$)

have a common carbohydrate backbone composed of repeating disaccharides of the general structure -(1→4)-α-L/β-D-Glyp*A*-(1→4)-α-D-Glc*p*N-.

As shown in Table I, the monosaccharide constituents are one hexuronic acid (GlyA) and one 2-amino-2-deoxyglucose (GlcN), and there are three different types of each. Any one of the GlyA residues can be (1→4)-linked to any one of the GlcN residues. Hence, there are nine possible hexosiduronic linkages in heparan sulphate. Owing to restrictions during biosynthesis[6], there are only seven possible hexosaminidic linkages. A GlcA that is preceded by a (1→4)-linked GlcNAc cannot be epimerised to IdoA.

Hexosaminidic linkages in heparan sulphate and heparin may be cleaved by bacterial lyases (heparitinase or heparinase) which are obtained from heparin-induced *Flavobacterium heparinum*[7]. It has been proposed[8] that heparan sulphate lyase (heparitinase) can cleave bonds between GlcNAc/GlcNSO$_3$ and GlcA, whereas heparin lyase (heparinase) seems to require GlcNSO$_3$ and IdoA-OSO$_3$. Dietrich's group[9] found evidence for two heparan sulphate lyases which could possibly distinguish between GlcNSO$_3$→GlcA and GlcNSO$_3$→IdoA bonds. Recently, the separation and purification of two heparan sulphate lyases was accomplished by affinity chromatography on heparin-coated heparin–agarose[10]. Studies of the substrate specificity of these two enzymes[11] suggested that one was specific for GlcNAc/GlcNSO$_3$→GlcA bonds. The latter heparan sulphate lyase as well as heparin lyase are now commercially available. We have investigated their substrate specificity in more detail by using various heparan sulphate oligosaccharides that contain high proportions of the linkages GlcNAc→GlcA, GlcNSO$_3$→GlcA, GlcNSO$_3$→IdoA, or GlcNSO$_3$→IdoA-OSO$_3$, respectively.

EXPERIMENTAL

Materials. — Heparin from pig mucosa and heparin by-products from beef lung were supplied by Dr. W. E. Lewis (Glaxo Operations Ltd., Runcorn, U.K.). Heparan sulphate was prepared from the by-products essentially as described previously[12,13]. Analyses of the gross chemical composition of these preparations have been listed elsewhere[14]. The structural features of these heparan sulphate fractions are summarised in Table II.

[^{3}H and ^{35}S]Heparan sulphate was isolated from fibroblast cultures after in-

TABLE II

COMPOSITION[a] OF HEPARAN SULPHATE FRACTIONS AND HEPARIN, AND THEIR SENSITIVITY[b] TO THE HEPARAN SULPHATE AND HEPARIN LYASES

Heparan sulphate fraction[c]	*Structural features*						*Sensitivity to*	
	-NAc	*-NSO_3*	*-OSO_3*	*GlcA*	*IdoA*	*IdoA-OSO_3*	*Heparan sulphate lyase (ΔA_{232})*	*Heparin lyase (ΔA_{232})*
	(mol/mol of GlcN)			*(% of total GlyA)*				
HS1	0 79	0 21	0.20	75	20	5	0.40	n.d.
HS2-A	0.80	0.21	0.25	70	20	10	0.35	n.d.
HS3-A	0.65	0.35	0.35	65	20	15	0.25	n.d.
HS4-A	0.60	0.40	0.40	60	20	20	0.15	0 10
HS5	0.28	0.72	0.90	50	5	45	n.d.	0.25
Heparin	0.27	0.73	1.70	20	5	70	n.d.	0.50

[a]The analyses were performed by methods listed elsewhere[12]. [b]Samples (100 μg) in 3mM $Ca(OAc)_2$ (pH 7.0, 1 mL) were treated with 6 munits of enzyme, and A_{232} was recorded automatically using a Cary 210 spectrophotometer until further addition of enzyme produced no increase in absorbance; n.d. = not determined. [c]The total pool of heparin by-products was fractionated according to charge density by step-wise precipitation with cetylpyridinium chloride in the presence of decreasing concentrations of NaCl. The following fractions were obtained: HS1 (0.2–0.4M NaCl), HS2 (0.4–0.6M NaCl), HS3 (0.6–0.8M NaCl), HS4 (0.8–1.0M NaCl), and HS5 (1.0–1.2M NaCl) As shown elsewhere[13], fractions HS2, HS3, and HS4 may be separated into more or less association-prone variants by gel chromatography under associative conditions. This was also performed here, and the self-associating sub-species HS2-A, HS3-A, and HS4-A were obtained.

corporation with [^{3}H]GlcN and $^{35}SO_4^{2-}$, as described elsewhere[15].

The heparan sulphate and heparin lyases were products of Seikagaku Kogyo Co. Ltd. (Tokyo, Japan). One international unit of enzyme releases 1 μmol of unsaturated hexuronate/min.

Heparan sulphate oligosaccharides. — Deaminative cleavage of heparan sulphate was performed at pH 1.5 by the method of Shively and Conrad[16]. The oligosaccharide products [general formula GlyA–GlcNAc–(GlcA–GlcNAc)$_n$–GlcA–anMan] were resolved by gel chromatography on Ultrogel AcA 202 (LKB) as shown in Fig. 1. Heparan sulphate chains were also degraded by selective periodate oxidation of GlcA in (GlcA–GlcNAc)$_n$ regions (pH 3.0; 4°, 24 h) followed by scission in alkali (pH 12; 20°, 30 min) to produce fragments of the general structure GlcN–(GlyA–GlcN)$_n$–R (ref. 12), where GlyA is IdoA, IdoA-OSO_3, or GlcA, and the GlcN residues are largely *N*-sulphated; R is the remnant of an oxidised and degraded GlcA residue[17]. Thus, some GlcA residues associated with $GlcNSO_3$ resist the initial oxidation. Such oligosaccharide fragments were produced from heparan sulphate HS4-A and fractionated by gel and ion-exchange chromatography essentially as described previously[12]. The structural features of the various oligosaccharide fractions are summarised in Table III.

Methods. — Heparan sulphate chains or oligosaccharides were digested with heparan sulphate lyase or heparin lyase in 3mM $Ca(OAc)_2$ (pH 7.0, 1 mL) at 37°

TABLE III

COMPOSITION[a] OF HEPARAN SULPHATE OLIGOSACCHARIDES[b] AND SENSITIVITY[c] TO THE HEPARAN SULPHATE AND HEPARIN LYASES

Oligosaccharide fraction from		*Structural features*[d]								*Sensitivity to*	
G-50	*DE-52*	*Backbone*	NH_3^+	*NAc*	NSO_3	OSO_3	*IdoA*	*GlcA*	*IdoA-OSO₃*	*Heparan sulphate lyase*	*Heparin lyase*
1	A	GlcN–GlcA–GlcN–R	1	1	0	0	0	1	0	–	n.d.
1	B	GlcN–GlcA–GlcN–R	1	1	0	1	0	1	0	–	n.d.
1	C	GlcN–GlcA–GlcN–R	0	2	0	1	0	1	0	+	n.d.
2	C	GlcN–(GlcA–GlcN)$_2$–R	1	2	0	2	0	2	0	+	n.d.
2	D	GlcN–(GlcA–GlcN)$_2$–R	0	2	1	2	0	2	0	–	n.d.
3	C	GlcN–(GlyA–GlcN)$_3$–R	0	2	2	1	1	2	0	+	–
3	D	GlcN–(GlyA–GlcN)$_3$–R	0	1	3	1	0	2	1	–	–
4	C	GlcN–(GlyA–GlcN)$_4$–R	0	2	3	2	0	3	1	n.d.	+
4	D	GlcN–(GlyA–GlcN)$_4$–R	0	1	4	2	0	3	1	n.d	–
5	C	GlcN–(GlyA–GlcN)$_5$–R	0	2	4	5	1	2	2	n.d.	+
5	D	GlcN–(GlyA–GlcN)$_5$–R	0	2	4	6	2	1	2	n.d	+

[a]The data were obtained as described elsewhere[12] and expressed as approx. number of residues per molecule. [b]The oligosaccharides were first fractionated according to size on Sephadex G-50 (fractions 1–5) and then each pool was sub-fractionated according to charge on DE-52 DEAE-cellulose[12] (fractions A–D). [c]The data in Figs. 2 and 3 are summarised; n.d. = not determined. [d]OSO_3 is total ester sulphate, *i.e.*, both on GlcN and IdoA.

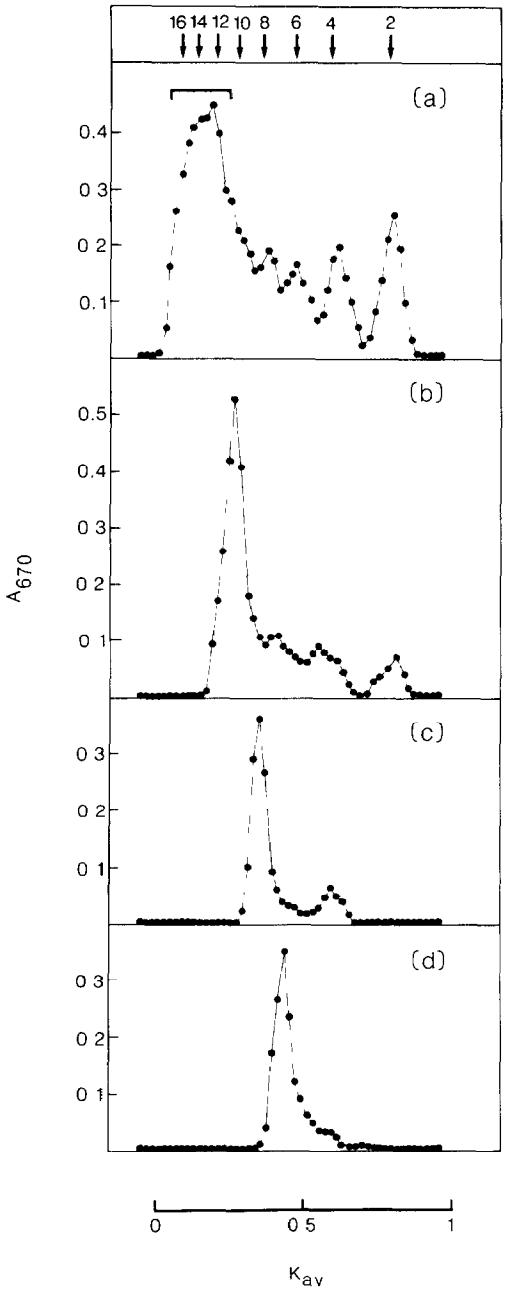

Fig. 1. Gel chromatography of heparan sulphate oligosaccharides (obtained after deaminative cleavage) after digestion with heparan sulphate lyase. The samples were (*a*) large oligosaccharides, mainly hexadecasaccharides; (*b*) decasaccharide; (*c*) octasaccharide; and (*d*) hexasaccharide. The saccharides were treated with 3 munits/mg of substrate. Before treatment, the various saccharides were chromatographed as follows. The large oligosaccharides (n = 12–16) were eluted in the position indicated by a bar in (*a*). The decasaccharide, octasaccharide, and hexasaccharide fractions emerged as symmetrical peaks in positions n = 10, 8, and 6, respectively, in (*b–d*). Column: Ultrogel AcA-202; size, 16 × 850 mm; eluant, 0.5M NH_4HCO_3; elution rate, 8 mL/h.

overnight. The proportion of substrate and enzyme used is given in the appropriate legends to the Figures. Hexuronate was quantitated by using the carbazole[18] or orcinol[19] methods. Radioactivity was determined by liquid scintillation counting.

RESULTS AND DISCUSSION

Specificity of heparan sulphate lyase. — This eliminase is considered to cleave GlcNAc→GlcA bonds. Accordingly, there was an increased sensitivity with increasing proportions of GlcNAc and GlcA in the heparan sulphate fractions (Table II). Heparan sulphate oligosaccharides, which should contain extended runs of GlcA–GlcNAc repeating-units, were prepared by deaminative cleavage followed by gel filtration. Surprisingly, such oligomers were poorly degraded by the heparan sulphate lyase (Fig. 1). Longer saccharides (Fig. 1*a* and *b*) were better substrates than shorter ones (Fig. 1*c* and *d*). These saccharides have the general structure GlyA–GlcNAc–$(GlcA–GlcNAc)_n$–GlcA–anMan, where GlyA may be GlcA, IdoA, or IdoA–OSO_3. It is possible that the two terminal sugars inhibit the enzyme so that the two peripheral repeating-disaccharides (GlyA–GlcNAc and GlcA–anMan, respectively) cannot be released. In keeping with this notion, the hexasaccharide (n = 1 in the above-mentioned structure) was essentially resistant (Fig. 1*d*), the octasaccharide (n = 2) afforded tetrasaccharide but no hexa- and disaccharide (Fig. 1*c*), the decasaccharide (n = 3) yielded di-, tetra-, and hexasaccharide but no octasaccharide (Fig. 1*b*), and so on. The undegraded oligosaccharides could be degraded on re-digestion with the enzyme (results not shown). However, even prolonged digestion did not result in complete degradation.

A series of oligosaccharide fractions produced by periodate oxidation–base-catalysed elimination of heparan sulphate[12] was tested as substrates for heparan sulphate lyase (Table III). The saccharides in fractions 1A and 1B were resistant to the enzyme (Fig. 2*h* and *i*) despite the presence of both GlcNAc and GlcA residues. However, saccharides of fraction 1C were cleaved to a large extent by the enzyme (Fig. 2*j*). It is possible that free amino groups generated during degradation[12], and present in 1A and 1B, prevent access of the enzyme to the glycosidic bond. Accordingly, saccharides of fraction 2C were partially susceptible to the lyase (Fig. 2*k*). Among these saccharides, there are three possible sequences, namely, GlcNAc–GlcA–GlcNAc–GlcA–$GlcNH_3^+$–R, GlcNAc–GlcA–$GlcNH_3^+$–GlcA–GlcNAc–R, and $GlcNH_3^+$–GlcA–GlcNAc–GlcA–GlcNAc–R. Presumably, only the first was susceptible to heparan sulphate lyase. The next saccharide fraction (2D) was completely resistant to the enzyme (Fig. 2*l*), whereas the material in fraction 3C was partially susceptible (Fig. 2*m*). The oligomers of fractions 2D and 3C should contain $GlcNSO_3$→GlyA bonds (Table III), which appear to be resistant to the enzyme. In addition, the presence of ester sulphate on GlcNAc (which could occur in 1B, 1C, 2C, 2D, and 3C) may preclude attack on the adjacent hexosaminidic bond. The composition of fraction 3C (Table III) allows for the presence of such sequences as GlcNAc±GlcA–$GlcNSO_3$–GlcA–$GlcNSO_3$–IdoA–GlcNAc–R or

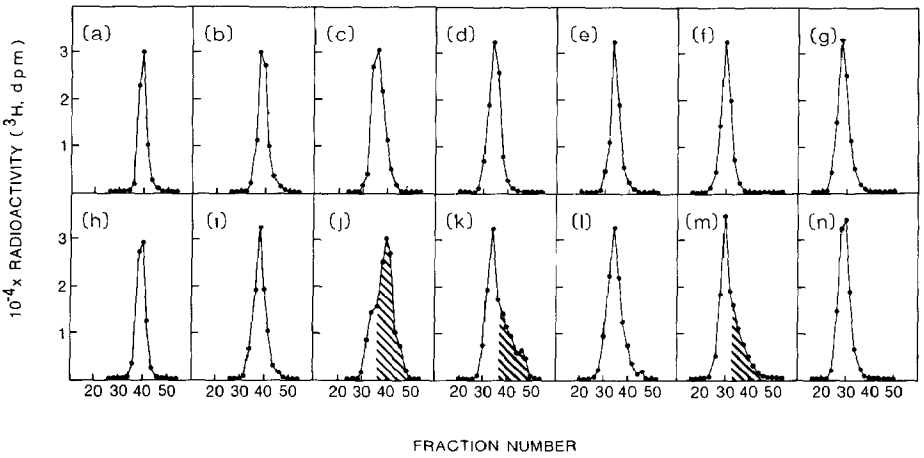

Fig. 2. Gel chromatography of heparan sulphate oligosaccharides (obtained after periodate oxidation) before (*a–g*) and after (*h–n*) digestion with heparan sulphate lyase. The samples were (*a,h*) saccharide fraction 1A, (*b,i*) 1B, (*c,j*) 1C, (*d,k*) 2C, (*e,l*) 2D, (*f,m*) 3C, and (*g,n*) 3D; for further details, see Table III. The saccharides were treated with 3 munits/mg of substrate. Column: Sephadex G-25, superfine; size, 6 × 1450 mm; eluant, 0.5M NH_4HCO_3; elution rate, 4 mL/h. The shaded areas indicate the positions of degradation products.

$GlcNSO_3$–GlcA–GlcNAc↓GlcA–$GlcNSO_3$–IdoA–GlcNAc–R both of which contain a putative cleavage-point (see arrow). Furthermore, the -OSO_3 group is probably attached to a $GlcNSO_3$ residue. These results and the complete insensitivity of fraction 3D strongly suggest that heparan sulphate lyase only cleaves linkages between GlcNAc–GlcA, whereas $GlcNSO_3$–GlcA and $GlcNSO_3$–IdoA bonds are insensitive.

As a further test of the specificity of heparan sulphate lyase, the enzymic breakdown products were compared with the degradation profiles obtained after periodate oxidation–base-catalysed elimination of the same samples. When beef-lung heparan sulphate HS4-A was degraded *via* periodate oxidation–base-catalysed elimination (Fig. 3*a*), oligosaccharides of the general structure GlcNAc/$GlcNSO_3$–$(GlyA–GlcNSO_3/GlcNAc)_n$–R, with *n* varying from 0 to 8, were obtained. As shown previously[17], the GlyA residues may be either IdoA-OSO_3, IdoA, or GlcA. Hence, a few GlcA residues joined to GlcNAc at C-4 and to $GlcNSO_3$ at C-1 are resistant to the initial periodate-oxidation. However, on re-oxidation of these saccharides (see bar in Fig. 3*a*) followed by scission in alkali, the residual GlcA residues were cleaved[17] and smaller saccharides, largely of the general structure $GlcNSO_3$–$[IdoA(-OSO_3)/IdoA–GlcNSO_3]_n$–R, were obtained (Fig. 3*b*). Although, the amounts of smaller saccharides could not be evaluated with certainty, it is clear that the GlcA-free saccharides were smaller (peak position at $n = 2$ and 3) than those obtained after the first oxidation step (peak position at $n = 4$ and 5). The oligosaccharides obtained (Fig. 3*c*) after treatment of the same sample with heparan sulphate lyase were similar in size to those produced after two cycles of periodate oxidation and

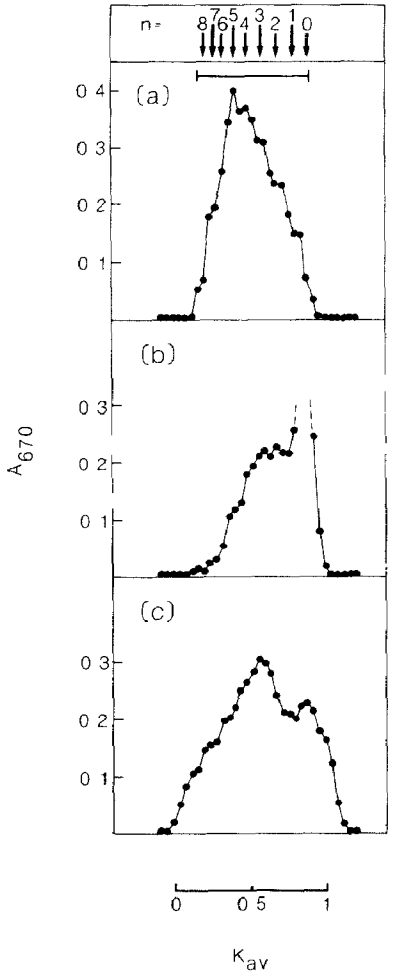

Fig. 3. Gel chromatography of heparan sulphate HS4-A after (*a*) periodate oxidation–base-catalysed elimination, (*b*) re-oxidation of material pooled from (*a*) (see bar) followed by treatment with base, and (*c*) digestion with heparan sulphate lyase (3 munits/mg). Column: Sephadex G-50, superfine; size, 6 × 1500 mm; eluant, 0.5M NH_4HCO_3; elution rate, 6 mL/h. The panel at the top indicates the tentative positions of saccharides with the general structure $GlcNSO_3$/GlcNAc–(GlyA–$GlcNSO_3$/GlcNAc)$_n$–R, where R is the remnant of an oxidised and degraded GlcA residue. As the dialysis step was omitted in (*b*), the low-molecular-weight reaction products (which were eluted in the most retarded fractions) interfere with the uronic acid estimations

alkaline treatment. The peak position was at $n = 3$ of the general structure ΔGlyA–($GlcNSO_3$–GlyA)$_n$–GlcNAc. Similar results were obtained with a [^{3}H and ^{35}S]heparan sulphate from fibroblasts. Whereas the first oxidation–elimination step afforded saccharides in the range $n = 0$–8 (Fig. 4*a*), re-oxidation and elimination produced mainly saccharides with $n = 3$ and $n = 1$ (Fig. 4*b*). The oligosaccharide profile obtained after treatment with heparan sulphate lyase (Fig. 4*c*) was very similar to that generated by the second oxidation–elimination step (Fig. 4*b*). From these re-

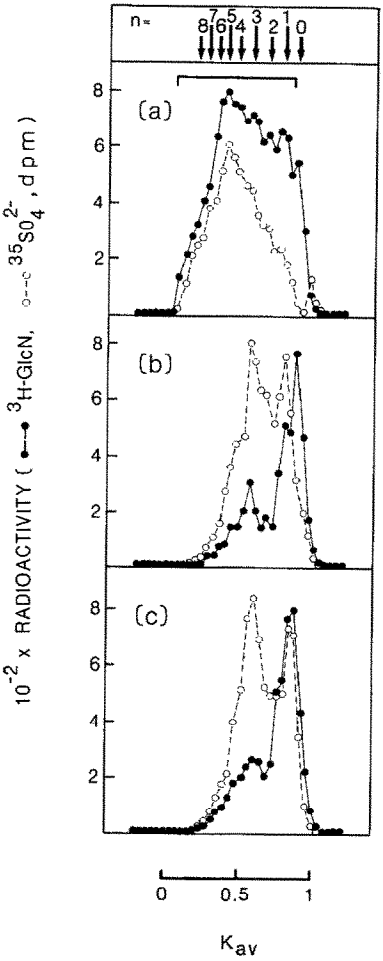

Fig. 4. Gel chromatography on Sephadex G-50 of [^{3}H and ^{35}S]heparan sulphate after (*a*) periodate oxidation–base-catalysed elimination, (*b*) re-oxidation of material pooled from (*a*) (see bar) followed by treatment, with base, and (*c*) digestion with heparan sulphate lyase (3 munits). For further details, see legend to Fig. 3. Large saccharides have a higher $^{35}S/^{3}H$ ratio than small ones, because the former are more fully sulphated.

sults, it is concluded that heparan sulphate lyase will cleave most, if not all, GlcNAc–GlcA bonds in heparan sulphate, even those that involve GlcA residues that are insensitive to the initial periodate oxidation. Moreover, these results are consistent with the notion that $GlcNSO_3$→GlcA/IdoA bonds are insensitive to the enzyme. The results shown in Figs. 3*c* and 4*c* further suggest a certain block structure in heparan sulphate, *i.e.*, GlcA–GlcNAc repeats often occur in runs of at least two units with separate block structures of IdoA/GlcA–$GlcNSO_3$ repeats which are generally larger (n = 3). Strictly alternating sequences, for example, -GlcNAc$^{\downarrow}$–GlcA–$GlcNSO_3$–IdoA/GlcA–GlcNAc$^{\downarrow}$–GlcA–$GlcNSO_3$-, seem to be less

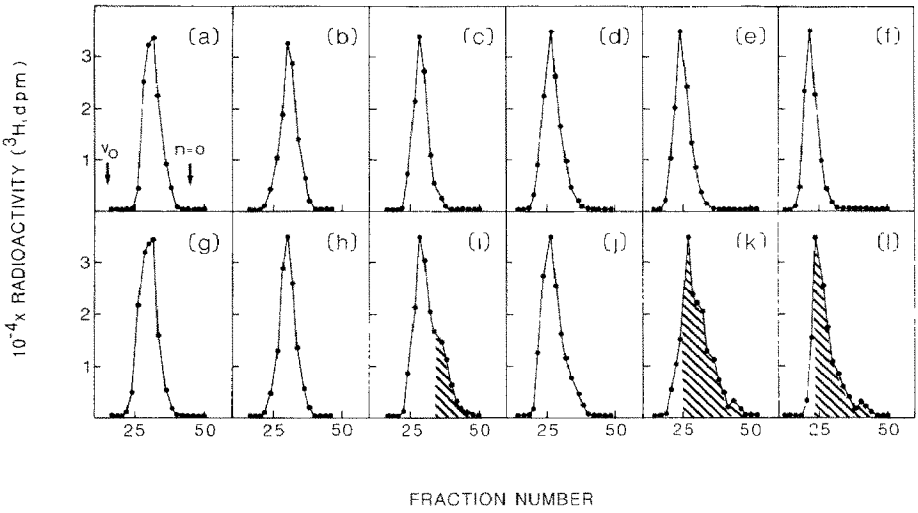

Fig. 5. Gel chromatography on Sephadex G-50 of heparan sulphate oligosaccharides (obtained after periodate oxidation) before (*a–f*) and after (*g–l*) digestion with heparin lyase. The samples were (*a,g*) saccharide fraction 3C, (*b,h*) 3D, (*c,i*) 4C, (*d,j*) 4D, (*e,k*) 5C, and (*f,l*) 5D; for further details, see Table III. The saccharides were treated with 6 munits/mg of substrate. For further details, see legends to Figs 2 and 3.

frequent, as they would have generated fragments with $n = 2$ (see arrow) upon treatment with heparan sulphate lyase.

Specificity of heparin lyase. — This eliminase will cleave $GlcNSO_3 \rightarrow IdoA$ bonds. Presumably, both sugars have to carry ester-sulphate groups, but this has not been unequivocally established. As shown in Table II, heparan sulphate HS4-A contains some linkages that are sensitive to the heparin lyase. To ascertain whether $GlcNSO_3 \rightarrow IdoA$ bonds that were joining sugars without ester-sulphate groups could also be sensitive, some of the oligosaccharides listed in Table III were used as substrates. The first two saccharides tested (fractions 3C and 3D) were resistant (Fig. 5*g* and *h*), presumably because the first contained no $IdoA\text{-}OSO_3$ and the second had no $GlcNSO_3(\text{-}OSO_3) \rightarrow IdoA\text{-}OSO_3$ linkage. The latter saccharide had only one $\text{-}OSO_3$ which was obviously on the IdoA. The next saccharide fraction (4C), which had two $\text{-}OSO_3$ groups and one $IdoA\text{-}OSO_3$ residue, was partially sensitive to heparin lyase (Fig. 5*i*). Clearly, a $GlcNSO_3(\text{-}OSO_3) \rightarrow IdoA\text{-}OSO_3$ bond could be present in this material. The same combination may be missing in saccharide 4D, as this material was not appreciably degraded by the enzyme (Fig. 5*j*). In all of the aforementioned saccharides, $GlcNSO_3$ and GlcA were the main monosaccharide constituents. The results obtained strongly suggest that $GlcNSO_3 \rightarrow GlcA$ bonds are not cleaved by heparin lyase. The last two saccharides tested (fractions 5C and 5D) were degraded by the enzyme (Fig. 5*k* and 5*l*) to an extent comparable with the occurrence of $GlcNSO_3(\text{-}OSO_3)$ (4 residues) and $IdoA\text{-}OSO_3$ (2 residues). The results thus suggest that $GlcNSO_3(\text{-}OSO_3) \rightarrow IdoA$ bonds are also resistant to

TABLE IV

LINKAGES OR RESIDUES IN HEPARAN SULPHATE THAT CAN BE CLEAVED SPECIFICALLY BY ENZYMIC OR CHEMICAL METHODS

Method	*Linkage/residue*[a,b]
Endo-β-glucuronidase[c]	GlcA→GlcNAc
Heparan sulphate lyase[d]	GlcNAc→GlcA
Periodate oxidation (pH 3; 4°)	GlcNAc– [GlcA] –GlcNAc
Periodate oxidation (pH 7; 37°)	GlcNAc / $GlcNSO_3$ – [GlcA / IdoA] – GlcNAc / $GlcNSO_3$
Nitrous acid	$GlcNSO_3$→ GlcA / IdoA / IdoA-OSO_3
Heparin lyase	OSO_3 | $GlcNSO_3$→IdoA | OSO_3

[a]Linkages cleaved are indicated by arrows. [b]Residues that are degraded are placed in boxes. [c]This enzyme appears to require large stretches of $(GlcA–GlcNAc)_n$, perhaps with $n > 3–4$. [d]This enzyme may not cleave the unusual combination GlcNAc(-OSO_3)→GlcA.

the enzyme. These findings are in agreement with those reported by Linker and Hovingh[20].

General comments. — The foregoing results support the concept that the heparan sulphate lyase and heparin lyase are each specific for one type of hexosaminidic bond. The heparan sulphate lyase prepared by the Japanese scientists should be specific for GlcNAc→GlcA bonds, whereas the heparin lyase should be specific for $GlcNSO_3$(-OSO_3)→IdoA-OSO_3 bonds. Each batch of enzyme is analysed for contaminating carbohydrases by the manufacturers and the data sheet is appended. Enzymes that could have degraded the oligosaccharides used in this study would be the exo-enzymes 6-sulphatase and α-*N*-acetylhexosaminidase. Neither of these enzymes is known to be present in the preparations used. The crude *Flavobacterium heparinum* extracts undoubtedly contain further types of heparan sulphate lyases, possibly specific for $GlcNSO_3$→GlcA and $GlcNSO_3$→IdoA bonds, as indicated by studies cited above[7–11]. Accordingly, digestion of heparan sulphate with crude *Flavobacterium* enzyme usually depolymerises the substrate much more extensively than would a combination of the purified heparan sulphate and heparin lyases[5]. When using the heparan sulphate lyase for degradation of small oligosaccharides obtained by chemical depolymerisation methods, certain GlcNAc→GlcA bonds may be insensitive to the enzyme. As shown above, terminal residues generated by the chemical procedure, or natural ones exposed in unusual positions, may hinder the enzyme.

Heparan sulphate is a complex polymer of great structural diversity, comprising, at least, six different sugar residues and 16 different glycosidic linkages (Table I). For complete sequence determination of this glycan, a large number of specific degradation methods are needed. The methods available at present are listed in Table IV. An endo-β-glucuronidase specific for glucosiduronic bonds has been demonstrated and/or isolated from platelets[21], liver[22], spleen[23], and placenta[24]. The heparan sulphate and heparin lyases are dealt with in this report. Of the two chemical methods, periodate oxidation and deaminative cleavage, the latter is widely used despite its low specificity. The most limited degradation of heparan sulphate is probably achieved by using either endo-β-glucuronidase or heparin lyase, or periodate oxidation at low pH and temperature.

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REFERENCES

1 L. KJELLÉN, I PETTERSSON, AND M. HOÖK, *Proc. Natl. Acad. Sci. U.S A.*, 78 (1981) 5371–5375.
2 B. NORLING, B. GLIMELIUS, AND Å. WASTESON, *Biochem. Biophys. Res Commun*, 103 (1981) 1265–1272
3 A. C. RAPRAEGER AND M. BERNFIELD, *J Biol. Chem.*, 258 (1983) 3632–3636.
4 I CARLSTEDT, L. COSTER, A. MALMSTROM, AND L.-Å. FRANSSON, *J. Biol. Chem*, 258 (1983) 11629–11635.
5 P J. GILL, J. ADLER, C. K. SILBERT, AND J. E. SILBERT, *Biochem. J.*, 194 (1981) 299–307.
6 I. JACOBSSON AND U. LINDAHL, *J Biol. Chem.*, 255 (1980) 5094–5100
7 P HOVINGH AND A. LINKER, *J. Biol Chem*, 245 (1970) 6170–6175
8 P HOVINGH AND A. LINKER, *Carbohydr. Res.*, 37 (1974) 181–192.
9 M. E SILVA, C P DIETRICH, AND H B. NADER, *Biochim Biophys. Acta*, 437 (1976) 129–141
10 N OTOTANI, M KIKUCHI, AND Z. YOSIZAWA, *Carbohydr. Res.*, 88 (1981) 291–303.
11 N OTOTANI AND Z. YOSIZAWA, *Proc. Int. Symp. Glyconconjugates, VIth*, 1981, p. 141.
12 L.-Å. FRANSSON, I. SJOBERG AND B HAVSMARK, *Eur J Biochem*, 106 (1980) 59–69
13 L.-Å FRANSSON, I. A. NIEDUSZYNSKI, AND J K. SHEEHAN, *Biochim Biophys. Acta*, 630 (1980) 287–300.
14 L -Å FRANSSON, B. HAVSMARK, AND J. K SHEEHAN, *J. Biol Chem*, 256 (1981) 13039–13043
15 I. SJOBERG AND L.-Å. FRANSSON, *Biochem J*, 167 (1977) 383–392
16 J E. SHIVELY AND H E CONRAD, *Biochemistry*, 15 (1976) 3932–3942.
17 L -Å FRANSSON, A MALMSTROM, I. SJOBERG, AND T. H. HUCKERBY, *Carbohydr Res*, 80 (1980) 131–145.
18 D HEINEGÅRD, *Chem. Scripta*, 4 (1973) 199–201.
19 A. H. BROWN, *Arch. Biochem. Biophys*, 11 (1946) 269–275
20 A. LINKER AND P HOVINGH, *Carbohydr. Res.*, 127 (1984) 75–94.
21 G. M OOSTA, L. V. FAVREAU, D L BEELER, AND R D ROSENBERG, *J Biol. Chem*, 257 (1982) 11249–11255.
22 M. HOOK, Å WASTESON, AND Å. OLDBERG, *Biochem Biophys. Res. Commun*, 67 (1975) 1422–1425
23 M HOOK, I. PETTERSSON, AND S. OGREN, *Thromb. Res.*, 10 (1977) 857–861.
24 U KLEIN AND K VON FIGURA, *Hoppe-Seyler's Z Physiol Chem*, 360 (1979) 1465–1471.