Structure of Heparan Sulphate Oligosaccharides and their Degradation by Exo-enzymes

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Oligosaccharides obtained from heparan sulphate by nitrous acid degradation were shown to be degraded sequentially by β -D-glucuronidase or α -L-iduronidase followed by α -D-N-acetylglucosaminidase. Structural analysis of the tetrasaccharide fraction showed the following. (1) *N*-Acetylglucosamine is preceded by a non-sulphated uronic acid residue that can be either D-glucuronic or L-iduronic acid, but followed by a glucuronic acid residue. (2) The *N*-acetylglucosamine in the major fraction is sulphated. (3) Very few if any of the uronic acid residues are sulphated. (4) The results indicate that the area of the heparan sulphate chain where disaccharides containing *N*-acetylglucosamine and *N*-sulphated glucosamine residues alternate is higher in sulphate content than expected and that the sulphate groups are mainly located on the hexosamine units.

Heparan sulphate, the most heterogeneous of the glycosaminoglycans, occurs in a large variety of vertebrate tissues. The demonstration of its presence in cell membranes (Kraemer, 1971; Winterbourne & Mora, 1978), basement membranes (Kanwar & Farquhar, 1979) and cell adhesion size (Barrett & Culp, 1979) has added additional importance to efforts to elucidate its structure and biological role. The polysaccharide contains D-glucuronic acid, L-iduronic acid, *N*-acetyl-D-glucosamine, glucosamine *N*-sulphate, *O*-sulphate groups and α -L- and β -D-uronic and α -D-hexosaminidic linkages. Neither the detailed structure of the family of heparan sulphate nor its biological function or details of its metabolism are well known. Partial degradation by endoglycosidases has been shown to occur (Höök *et al.*, 1975). Further degradation to monosaccharide units by exo-enzymes has been inferred from studies of the mucopolysaccharidoses (Hall *et al.*, 1978; Klein *et al.*, 1978), and hydrolysis of some oligosaccharides by glucuronidases and sulphatases has been demonstrated (Klein *et al.*, 1978; Basner *et al.*, 1979). A preliminary report of the action of β -D-glucuronidase and α -L-iduronidase on tetrasaccharides has been published (Linker, 1975). The exact mechanism and optimum conditions for exo-enzyme action remain to be shown.

Structural arrangements of block-type sequences and heparin-like sequences in the heparan sulphate chains have been determined to a degree (Linker & Hovingh, 1975; Cifonelli & King, 1977), but the mixed or alternating areas are ill-defined.

The present paper addresses itself to two major problems: (1) the sequential degradation of oligosac-

charide fragments by known exo-enzymes; (2) the structural arrangement of areas of the polysaccharide chains where *N*-acetyl and *N*-sulphate groups and D-glucuronic acid and L-iduronic acid residues are alternating or occur in close proximity to each other. The distribution of charged groups, in particular, in this area is of considerable interest in relation to the pathway of biosynthesis and the interaction with other macromolecules.

Materials and Methods

Materials

Heparan sulphate isolated from a commercial 'heparin side fraction' or from ox lung had been fractionated into polymers differing in sulphate content (Linker & Hovingh, 1973). The '1.4M-salt' fractions (containing 17% sulphate) were used here to prepare oligosaccharides by nitrous acid degradation, which was carried out as described by Linker & Hovingh (1973). The degradation products were fractionated by Sephadex G-50 and ion-exchange chromatography (Linker & Hovingh, 1975) and characterized. A standard of 2,5-anhydro-D-mannitol was prepared by the method of Horton & Philips (1973), and 2,5-anhydro-D-mannitol sulphate was obtained from the disaccharide 4-*O*-(α -L-idopyranosyluronic acid)-2,5-anhydro-D-mannitol 6-sulphate (Jacobsson *et al.*, 1979) by hydrolysis with crude β -glucuronidase. D-Glucuronic acid was obtained from Pierce Chemical Co. (Rockford, IL, U.S.A.), and L-iduronic acid was prepared from dermatan sulphate as described by Fransson (1978).

The following enzyme preparations were used:

bovine liver β -glucuronidase purchased from Worthington Biochemical Co. (Freehold, NJ, U.S.A.), 300 Fishman units/mg; purified β -glucuronidase from bovine liver prepared by the method of Weissman *et al.* (1967), 14000 Fishman units/mg (this enzyme does not contain α -L-iduronidase); partially purified α -L-iduronidase from bovine liver prepared by a method of Weissman & Santiago (1972) [this enzyme does contain some β -glucuronidase activity, but is enriched in iduronidase; the β -glucuronidase activity was measured by the method of Fishman & Bernfeld (1955)]; Fishman units are defined as μ g of phenolphthalein liberated/h; crude heparinase prepared from *Flavobacterium* as described by Linker & Hovingh (1972); α -N-acetylglucosaminidase prepared from bovine kidney by the method of Mersmann *et al.* (1974), 50 units/mg (this enzyme does also contain some β -N-acetylglucosaminidase). Enzyme units are defined as μ g of phenol liberated/h.

Methods

Analytical methods for uronic acid, total hexosamine, indole-reactive hexosamine, *N*-acetyl content and sulphate have been described previously (Linker & Hovingh, 1973). The ratio of D-glucuronic acid to L-iduronic acid was determined by the method of Höök *et al.* (1974), and also by ion-exchange chromatography (Spiro, 1977). Paper chromatography was carried out on Whatman no. 1 paper with the following solvent systems: A, isobutyric acid/0.5M-NH₃ (5:3, v/v); B, butanol/acetic acid/1M-NH₃ (2:3:1, by vol.). Compounds were located by spraying the chromatograms with an alkaline AgNO₃ reagent (Trevelyan *et al.*, 1950), or for radioactive compounds by cutting the paper into 0.5cm strips, placing them in Soluene and counting the radioactivity in a Packard scintillation counter. Paper electrophoresis was carried out on Whatman no. 1 paper in pyridine/acetic acid buffer (0.17M-acetic acid), pH 5.3, at 22 V/cm for 1 h.

Borohydride reduction was carried out with unlabelled NaBH₄ or with NaB³H₄ (8.2Ci/mol) purchased from New England Nuclear (Boston, MA, U.S.A.) when indicated (Lim *et al.*, 1974).

Incubations with β -glucuronidase, α -iduronidase and α -N-acetylglucosaminidase were carried out in 0.1M-sodium acetate buffer, pH 4.5, and with crude heparinase in 0.1M-sodium acetate buffer, pH 7.0, at 30°C.

Results and Discussion

Nitrous acid degradation of heparan sulphate

This method involves deaminative cleavage of the polysaccharide at *N*-sulphated hexosamine residues, *N*-acetylated residues remaining intact.

The 1.4M-salt heparan sulphate fraction (1.0g) from lung was treated with nitrous acid, and the products were chromatographed on Sephadex G-50. The material eluted at the tetrasaccharide position, about 22% of the total, was collected and further fractionated on DEAE-Sephadex (see Linker & Hovingh, 1975). Two major fractions were obtained in about equal amounts, fraction I eluted with 0.5M-NaCl, the other (fraction II) with 0.75M-NaCl.

About 10% of the degradation products recovered were eluted at the positions of hexasaccharide and higher oligosaccharides and 25% were disaccharides. Total yield based on the weights of isolated material was about 60%. This is after ion-exchange chromatography and desalting on Sephadex G-10. The disaccharide fraction is difficult to separate from salts, accounting for some of the losses; in addition, irreversible binding of a certain percentage of sulphated compounds to ion-exchangers is known to occur.

Analyses for the 'tetrasaccharides' are shown in Table 1. The data indicate that these compounds are indeed tetrasaccharides composed of two uronic acid units, one acetylglucosamine unit and one anhydromannose unit. Tetrasaccharide fraction I contains one sulphate group and fraction II contains two sulphate groups per molecule. Each gives a single spot on paper chromatography, the more highly sulphated compound moving more slowly (see Table 1). These compounds are similar to a tetrasaccharide fraction isolated by Cifonelli (1968) and also by Basner *et al.* (1979). They are not single compounds,

Table 1. Analyses of tetrasaccharides obtained from heparan sulphate
Experimental details are given in the text.

	Tetra-saccharide fraction I (0.5M-NaCl)	Tetra-saccharide fraction II (0.75M-NaCl)
<i>R_f</i> glucuronic acid (solvent B)	0.31	0.19
Uronic acid (%) (carbazole reagent)	34	31
Uronic acid (%) (orcinol reagent)	37	35
Glucuronic/iduronic acid ratio	3:1	4:1
Sulphate (%)	7	14
Hexosamine (%) (Ehrlich's reagent)	13	13
Anhydromannose (%) (indole reagent)	10	10
Acetyl (%)	5.3	6.3
Reducing sugar (equiv./mol) (hypoiodite method)	1.9	1.7

but differ in the location of sulphate groups and uronic acid units.

Material eluted from Sephadex G-50 in the hexasaccharide area was further purified by chromatography on DEAE-Sephadex. The major peak eluted by 0.75M-NaCl was desalted, isolated and analysed. The data are shown in Table 2. It should be pointed out that hexosamine values for heparan sulphate, heparin and their oligosaccharides tend to

be about 30% lower than theoretical, as noticed previously (Linker & Hovingh, 1973; Cifonelli & King, 1970; Knecht *et al.*, 1967). This is most probably due to the drastic conditions required for complete hydrolysis of these resistant polymers.

Hydrolysis of oligosaccharides by glucuronidases

Tetrasaccharide fractions I and II were incubated at 10mg/ml with crude commercial β -glucuronidase at 500 units/ml. After 24h an additional 500 units of enzyme were added and the incubation was continued for another 24h. A portion of the digest was chromatographed on paper in solvents A and B. A reproduction of the chromatogram developed with solvent B is shown in Fig. 1. Similar results were obtained with solvent A. The tetrasaccharides were also incubated with the purified bovine liver β -glucuronidase under the same conditions as above and the digests were chromatographed (see Fig. 1). The crude glucuronidase liberated both glucuronic acid and iduronic acid from the tetrasaccharides, whereas the purified glucuronidase liberated only glucuronic acid. No acetylglucosamine could be detected on the chromatograms by Ehrlich's reagent; i.e. the next step in the sequential hydrolysis, the liberation of acetylglucosamine from the trisaccharide product, did not occur under these conditions. The crude enzyme contains considerable amounts of β -N-acetylglucosaminidase but only very small amounts of α -N-acetylglucosaminidase.

Tetrasaccharide fraction I was incubated with purified β -glucuronidase as above and the remaining tetrasaccharide was re-isolated by paper chromatography, by using guide strips and eluting undegraded material with water. This tetrasaccharide was then incubated with purified iduronidase, and the products were chromatographed on paper (Fig. 2). As can be seen, iduronic acid had been liberated.

As both iduronic acid and glucuronic acid appear to be present on the non-reducing end of tetrasaccharide fractions I and II, each must contain at least two different tetrasaccharides (see Scheme 2). As the position of the sulphate group(s) was also unknown, it became important to determine whether the tetrasaccharides were completely degraded by the crude enzyme or whether some resistant compound remained. The other product of the reaction besides the uronic acid is a trisaccharide in each case. As these compounds would still contain one or two sulphate groups respectively, it is difficult to separate each trisaccharide from its parent tetrasaccharide by chromatography by using conventional detection methods. Therefore tetrasaccharide fractions I and II were reduced with NaB^3H_4 and then incubated with crude commercial β -glucuronidase. The products were chromatographed on paper, and the papers were sectioned and the radioactivities of the sections

Table 2. *Analyses of hexasaccharide obtained from heparan sulphate*

Experimental details are given in the text.

Uronic acid (%) (carbazole reagent)	36
Uronic acid (%) (orcinol reagent)	38
Sulphate (%)	7.7
Hexosamine (%) (Ehrlich's reagent)	23
Anhydromannose (%) (indole reagent)	10
Glucuronic/iduronic acid ratio	3:1

Fig. 1. Tracing of paper chromatogram showing the degradation by glucuronidases of tetrasaccharide fractions I and II obtained from heparan sulphate

Experimental details are given in the text. The chromatography was performed with solvent B. Key: S, standards of D-glucuronic (slower spot) and L-iduronic acid; A, tetrasaccharide fraction I or II; B, crude β -glucuronidase digest of tetrasaccharide fraction I or II; C, purified β -glucuronidase digest of tetrasaccharide fraction I or II.

Fig. 2. Tracing of paper chromatogram showing the degradation of tetrasaccharide fraction I, obtained from heparan sulphate, digested first with purified β -glucuronidase and then with iduronidase

Experimental details are given in the text. The chromatography was performed with solvent B. Key: A, residual tetrasaccharide after β -glucuronidase digestion (only a very faint spot could be detected by the AgNO_3 reagent); B, digest of the residual tetrasaccharide by purified iduronidase; S, standards of D-glucuronic (slower spot) and L-iduronic acid.

counted (see Fig. 3). As can be seen, tetrasaccharide fraction I was completely degraded by exhaustive digestion. Although this appeared to be true for tetrasaccharide fraction II also, the presence of two sulphate groups per molecule in the parent material as well as the product makes separation very difficult. Therefore, as an alternative method, the total uronic acid liberated was measured by ion-exchange chromatography (Spiro, 1977). Of the total uronic acid, 45% was recovered as monosaccharide with a ratio of iduronic acid to glucuronic acid of 1:2 for tetrasaccharide fraction I, indicating that hydrolysis was fairly complete. For tetrasaccharide fraction II, 35% of the total uronic acid was recovered in the monosaccharide fraction with a ratio of iduronic acid to glucuronic acid of 1:2. These data show that the uronic acids on the non-reducing end of the tetrasaccharides are essentially non-sulphated, the iduronic acid as well as the glucuronic acid, as the glyco-

Fig. 3. Paper chromatography of a digest by crude β -glucuronidase of labelled tetrasaccharide fraction I, obtained from heparan sulphate

Experimental details are given in the text. The chromatography was performed with solvent A. Then the papers were sectioned and the radioactivities of the sections were counted in a scintillation counter. Less radioactive material was put on the chromatogram for the 8 h digest, to get better separation. (a) Undigested tetrasaccharide fraction I (control); (b) 8 h digest of tetrasaccharide fraction I; (c) exhaustively digested tetrasaccharide fraction I.

uronidases do not act on sulphated uronic acids (Basner *et al.*, 1979). No sulphatase acting on uronic acid sulphates could be detected in this crude glucuronidase preparation under the conditions used, as shown by the lack of activity on disulphated disaccharide from heparin (Lim *et al.*, 1974).

To check the degradation of the tetrasaccharides further, the trisaccharide product obtained after exhaustive digestion with crude glucuronidase was isolated as follows. Tetrasaccharide fractions I and II (10 mg of each) were incubated with 500 units of

Table 3. Analyses of trisaccharides obtained from heparan sulphate

Experimental details are given in the text. The values shown in parentheses are molar proportions expressed as mol/mol of hexosamine.

	Trisaccharide I	Trisaccharide II
Uronic acid (%) (carbazole reagent)	25 (1.3)	23 (1.4)
Hexosamine (%) (Ehrlich's reagent)	17.5 (1.0)	15 (1.0)
Sulphate (%)	10.5 (1.1)	16.5 (2.1)
Glucuronic/iduronic acid ratio	>10:1	9:1

crude enzyme. After 24 h, 500 units were added again and incubation was continued for 24 h. The products were chromatographed on Sephadex G-25, and material corresponding to trisaccharides (separated from free uronic acid) was collected and analysed. The analyses are shown in Table 3.

As can be seen, trisaccharide I (from tetrasaccharide fraction I) contains 1 mol.prop. of uronic acid, 1 mol.prop. of hexosamine and 1 mol.prop. of sulphate; the third unit is the anhydromannose at the reducing end; very little, if any, iduronic acid is present. Trisaccharide II (from tetrasaccharide fraction II) has the same structural arrangement except that it contains 2 mol.prop. of sulphate groups, and a small amount of iduronic acid may be present. The analytical values are somewhat low, due in part to low values obtained in the hexosamine reaction, mentioned above, and partly due to impurities derived from the enzyme reaction mixture. The ratios are the most significant part of the data.

At this point, the location of the sulphate groups remained to be determined.

When the hexasaccharide fraction (Table 2) was incubated with crude β -glucuronidase, and the products were chromatographed on paper, a chromatogram similar to the one shown in Fig. 1 was obtained, i.e. both glucuronic acid and iduronic acid were liberated in about the same proportion as in the tetrasaccharides, indicating that the hexasaccharide fraction also terminates in non-sulphated uronic acids.

Hydrolysis of oligosaccharides by α -N-acetylglucosaminidase

To investigate the sequential degradation of tetrasaccharides by exo-enzymes and also to determine the position of sulphate groups, tetrasaccharide fraction I was first exhaustively digested with crude β -glucuronidase. To this digestion mixture 50 units of α -N-acetylglucosaminidase were then added. After 24 h the products were chromatographed on paper.

Fig. 4. Paper chromatography of α -hexosaminidase digests of tetrasaccharides, obtained from heparan sulphate, first treated with crude β -glucuronidase

Experimental details are given in the text. Labelled tetrasaccharide fraction I was first treated exhaustively with crude β -glucuronidase, and the product of this reaction (a trisaccharide) was then hydrolysed by α -N-acetylglucosaminidase. The chromatography was performed with solvent B. Then the papers were sectioned and the radioactivities of the sections were counted in a scintillation counter. (a) Undigested tetrasaccharides (control); (b) α -N-acetylglucosaminidase digest of the trisaccharide resulting from β -glucuronidase action. The large slower component corresponds to undegraded trisaccharide (see Fig. 3c), and the fast component migrates like sulphated disaccharide.

A spot for N-acetylglucosamine was detected in both solvents A and B by spraying the chromatograms with Ehrlich's reagent. To obtain more quantitative information the tetrasaccharide fraction that had been reduced with NaB^3H_4 was incubated as above and the products were chromatographed. As shown in Fig. 4, about 20% of the labelled material migrated in the area where sulphated disaccharides are expected to move. Further addition of the α -N-

acetylglucosaminidase did not increase the amount of disaccharide produced. This indicated that a sulphate group is present on the acetylglucosamine unit of the major trisaccharide fraction, as the hexosaminidases will not act on sulphated hexosamines (Basner *et al.*, 1979).

The hexasaccharide fraction was also exhaustively digested with crude β -glucuronidase. When this was followed by hydrolysis with α -N-acetylglucosaminidase, as above, a substantial amount of N-acetylglucosamine was liberated, as shown by paper chromatography.

Hydrolysis of tetrasaccharides by crude Flavobacterium enzyme

As a first step, the crude heparinase degrades tetrasaccharides to disaccharides. As the enzyme acts as an eliminase (Linker & Hovingh, 1972), the disaccharide from the reducing end of the tetrasaccharide contains an $\alpha\beta$ -unsaturated uronic acid. This disaccharide is degraded to monosaccharides (see Scheme 1) by an unusual glycuronidase also present (Hovingh & Linker, 1977). The disaccharide originally at the non-reducing end of the tetrasaccharide remains intact, as the enzyme does not act on saturated uronides (see Scheme 1).

As the products obtained would give considerable information about the structures of the tetrasaccharides, the latter were incubated with crude heparinase. Unreduced and NaB³H₄-reduced fractions were used.

The degradation products obtained from the radiolabelled tetrasaccharides are shown in Fig. 5. Only the split product from the reducing end can be

seen. Tetrasaccharide fraction I gives a major peak migrating in the position of anhydromannitol. Material from this peak when eluted from paper behaved on paper electrophoresis as a neutral compound. The second peak, which migrated in the position of anhydromannitol sulphate, behaved as a monosulphated compound on paper electrophoresis. Only one major product was obtained from tetrasaccharide fraction II. This migrated in the position of anhydromannitol sulphate on the paper chromatogram and behaved as a monosulphated compound on electrophoresis. These findings indicated that tetrasaccharide fraction I, which contains one sulphate group per molecule, has a major component that has no sulphate on the reducing end [compound (a) or (c) in Scheme 2] (about 70% of the total) and a minor component that contains anhydromannose sulphate [compound (b) in Scheme 2] (about 30% of the total). In tetrasaccharide fraction II, which contains two sulphate groups per molecule, the reducing-end anhydromannose is always sulphated. The position of the second sulphate group in tetrasaccharide fraction II and of the sulphate group in the major fraction of tetrasaccharide fraction I remained to be shown.

Therefore the disaccharide from the non-reducing end of both tetrasaccharides was examined further. Reduced (unlabelled) tetrasaccharides (12mg) were incubated with crude *Flavobacterium* enzyme, and a portion of the digestion mixture was reduced with NaB³H₄ to make detection easier. The total digest was then chromatographed on columns of Sephadex G-25 and elution followed by analysis for uronic acid and scintillation counting of radioactivity. The

Scheme 1. Degradation of 'tetrasaccharide' by crude *Flavobacterium* enzyme

Abbreviations: (SO₄) indicates sulphate may or may not be at this position; UA, uronic acid; GlcNAc, N-acetyl-D-glucosamine; AnMan, anhydromannose.

Fig. 5. Paper chromatography of crude heparinase digests of tetrasaccharides obtained from heparan sulphate. Experimental details are given in the text. The chromatography was performed with solvent A. Then the papers were sectioned and the radioactivities of sections were counted in a scintillation counter. (a) Undigested tetrasaccharides (control); (b) digested tetrasaccharide fraction I; (c) digested tetrasaccharide fraction II. Abbreviation: AnMan, anhydromannose.

disaccharide fractions (separated from the monosaccharides) were desalted on Sephadex G-10 and isolated by freeze-drying. The labelled disaccharides were then chromatographed on paper and run on paper electrophoresis to determine sulphate content. It appeared that about 75% of the disaccharide fractions were monosulphated and the rest non-sulphated.

As the data on tetrasaccharide degradation had indicated that the uronic acids on the non-reducing end were non-sulphated, and, as the disaccharides are therefore potential substrates for β -glucuronidase and α -iduronidase, the disaccharide fractions were incubated with crude β -glucuronidase and the

Fig. 6. Paper chromatography of crude β -glucuronidase digests of disaccharides obtained from heparan sulphate.

Experimental details are given in the text. Tetrasaccharide fractions I and II were treated with crude heparinase and the residual disaccharides were isolated by chromatography with solvent B and then incubated with crude β -glucuronidase. (a) AgNO_3 spray reagent; (b) Ehrlich's spray reagent. Key: A, disaccharide from tetrasaccharide fraction I or II; B, enzyme digest of disaccharide; C, standards of glucuronic acid (slower spot) and iduronic acid; D, standard of monosulphated disaccharide from heparan sulphate (Hovingh & Linker, 1974); E, *N*-acetylglucosamine 6-sulphate obtained from the monosulphated disaccharide used for D by glucuronidase action (Hovingh & Linker, 1977); F, *N*-acetylglucosamine standard.

products were chromatographed on paper. As shown in Fig. 6, a strong spot of glucuronic acid and a weak spot for iduronic acid are obtained. When Ehrlich's spray reagent was used, a strong spot at the R_F of *N*-acetylglucosamine 6-sulphate was obtained (no colour was obtained with the disaccharide itself owing to the 4-linkage) and a very weak spot at the R_F of *N*-acetylglucosamine. These findings indicate that the major disaccharide in these fractions (derived from tetrasaccharide fraction I or II) consists of glucuronic acid and *N*-acetylglucosamine *O*-sulphate (most probably 6-sulphate; Basner *et al.*, 1979). A non-sulphated disaccharide appears to be a minor component of the fractions (this agrees with the data on the heparinase degradation products of the labelled tetrasaccharide) (see Scheme 1). The β -glucuronidase, which does not act on the β -(1 $\rightarrow$ 3)-linked disaccharide from hyaluronic acid (Linker *et*

Tetrasaccharide units

Fig. 7. Structures of tetrasaccharide repeating units of a section of heparan sulphate chains

The most likely structures on the basis of the present data are shown as they would occur in the original polymer before nitrous acid degradation. Other minor structures with L-iduronic acid or O-sulphate groups in different positions are possible, but are not shown.

was treated with nitrous acid a much lower yield of tetrasaccharides was obtained (Linker & Hovingh, 1975). Hence the structures shown here represent a smaller portion of the lower-sulphated polysaccharide chains. It should therefore be emphasized again that variations in sulphate content can be due to multiple structural features, i.e. variations in content and distribution of glucosamine N-sulphate, glucosamine O-sulphate, iduronic acid sulphate and N-acetylglucosamine sulphate

Since heparan sulphates appear to have low anti-coagulant activity, it is noteworthy that tetra-

saccharide (e) (Fig. 7) has the same structure as the tetrasaccharide unit proposed for the 'active site' of heparin chains by Rosenberg & Lam (1979).

Enzymic digestion of heparan sulphate

The degradation of glycosaminoglycans presents an interesting biological problem. Along with the heterogeneity in structures of this group of related anionic polymers goes considerable variety in the mechanism of breakdown. The chondroitin sulphates and hyaluronic acid appear to be mainly degraded by the action of endo-hexosaminidases (hyaluronidases) to small fragments. These fragments can be further degraded by exo-enzymes to mono- and di-saccharides (Linker *et al.*, 1955; Weissman & Santiago, 1975), but it is not certain that this is indeed a major biological pathway. Heparin, heparan sulphate and dermatan sulphate, on the other hand, seem to be poorly degraded by endo-enzymes, yielding relatively large fragments (Höök *et al.*, 1975; Klein & von Figura, 1976) that seem to require further degradation by exo-enzymes and sulphatases. Evidence for this was first demonstrated by studies on the mucopolysaccharidoses, where lack of individual enzymes (Hall *et al.*, 1978) leads to the accumulation of heparan sulphate and dermatan sulphate fragments of about 3000–4000 molecular weight.

Homogenates of skin fibroblasts were shown to degrade a trisaccharide obtained from heparan sulphate to monosaccharides (Basner *et al.*, 1979). The present data show that well-known and characterized enzymes such as β -D-glucuronidase, α -L-iduronidase and α -D-N-acetylglucosaminidase will degrade oligosaccharides from heparan sulphate by sequential removal of monosaccharide units from the non-reducing end. As no sulphatases seem to be present in my preparations, the reaction stops at a sulphated position. It is noteworthy that a disaccharide from heparan sulphate can be hydrolysed to monosaccharides by β -D-glucuronidase, whereas the isomeric disaccharide from hyaluronic acid [linked β -(1 $\rightarrow$ 3) rather than β -(1 $\rightarrow$ 4)] cannot (Linker *et al.*, 1955). I could not demonstrate sequential degradation of heparan sulphate itself; even after desulphation only the terminal uronic acid was removed (Linker, 1975). This may be due to lack of sensitivity of the methods. A similar problem had been encountered with hyaluronic acid (Bach & Geiger, 1978).

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References

- Bach, G. & Geiger, B. (1978) *Arch. Biochem. Biophys.* **189**, 37–43

- Barrett, J. R. & Culp, L. A. (1979) *Biochemistry* **18**, 141–148
- Basner, R., Kresse, H. & von Figura, K. (1979) *J. Biol. Chem.* **254**, 1151–1158
- Cifonelli, J. A. (1968) *Carbohydr. Res.* **8**, 233–242
- Cifonelli, J. A. & King, J. A. (1970) *Biochim. Biophys. Acta* **215**, 273–279
- Cifonelli, J. A. & King, J. A. (1977) *Biochemistry* **16**, 2137–2141
- Fishman, W. H. & Bernfeld, P. (1955) *Methods Enzymol.* **1**, 262–269
- Fransson, L.-A. (1978) *Carbohydr. Res.* **62**, 235–244
- Hall, W. C., Liebaers, I., Di Natale, P. & Neufeld, E. F. (1978) *Methods Enzymol.* **50**, 439–456
- Höök, M., Lindahl, U. & Iverius, P.-H. (1974) *Biochem. J.* **137**, 33–43
- Höök, M., Wasteson, A. & Oldberg, A. (1975) *Biochem. Biophys. Res. Commun.* **67**, 1422–1428
- Horton, D. & Philips, K. D. (1973) *Carbohydr. Res.* **30**, 367–374
- Hovingh, P. & Linker, A. (1974) *Carbohydr. Res.* **37**, 181–192
- Hovingh, P. & Linker, A. (1977) *Biochem. J.* **165**, 287–293
- Jacobsson, I., Höök, M., Pettersson, I., Lindahl, U., Larm, O., Wirén, E. & von Figura, K. (1979) *Biochem. J.* **179**, 77–87
- Kanwar, Y. S. & Farquhar, M. G. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1303–1307
- Klein, U. & von Figura, K. (1976) *Biochem. Biophys. Res. Commun.* **73**, 569–576
- Klein, U., Kresse, H. & von Figura, K. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5185–5189
- Knecht, J., Cifonelli, J. A. & Dorfman, A. (1967) *J. Biol. Chem.* **242**, 4652–4661
- Kraemer, P. M. (1971) *Biochemistry* **10**, 1437–1445
- Lim, T. W., Leder, I. G., Bach, G. & Neufeld, E. F. (1974) *Carbohydr. Res.* **37**, 103–109
- Lindahl, U., Bäckström, G., Höök, M., Thunberg, L., Fransson, L. A. & Linker, A. (1979) *Proc. Natl. Acad. Sci. U.S.A.* in the press
- Linker, A. (1975) *Connect. Tissue Res.* **3**, 33–37
- Linker, A. & Hovingh, P. (1972) *Methods Enzymol.* **28**, 902–911
- Linker, A. & Hovingh, P. (1973) *Carbohydr. Res.* **29**, 41–62
- Linker, A. & Hovingh, P. (1975) *Biochim. Biophys. Acta* **384**, 324–333
- Linker, A., Meyer, K. & Weissman, B. (1955) *J. Biol. Chem.* **213**, 237–248
- Mersmann, G., von Figura, K. & Buddecke, E. (1974) *Biochim. Biophys. Acta* **364**, 88–96
- Rosenberg, D. R. & Lam, L. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1218–1222
- Spiro, M. J. (1977) *Anal. Biochem.* **82**, 348–352
- Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950) *Nature (London)* **166**, 444–445
- Weissman, B. & Santiago, R. (1972) *Biochem. Biophys. Res. Commun.* **46**, 1430–1433
- Weissman, B. & Santiago, R. (1975) *Connect. Tissue Res.* **3**, 7–15
- Weissman, B., Rowin, G., Marshall, J. & Friederici, D. (1967) *Biochemistry* **6**, 207–214
- Winterbourne, D. J. & Mora, P. T. (1978) *J. Biol. Chem.* **253**, 5109–5120