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Biochemistry

Correlation between structure and function of heparin

(mucopolysaccharide/anticoagulant function)

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ABSTRACT **We have fractionated crude porcine heparin to obtain highly active as well as relatively inactive species of molecular weight ≈7000 with specific anticoagulant activities of 360 and 12 units/mg, respectively. Nitrous acid degradation of both of these polymers yielded a tetrasaccharide fraction, 1β, that contained equimolar amounts of iduronic and glucuronic acids, possessed an internal *N*-acetylated glucosamine, and carried anhydromannitol at the reducing end position. The 1β tetrasaccharide derived from the highly active heparin, 1βa, was recovered in a yield of 1.1 mol/7000 daltons. Our analyses indicate that at least 95% of the 1βa is a single structure that consists of the following unique monosaccharide sequence: L-iduronic acid → *N*-acetylated D-glucosamine-6-sulfate → D-glucuronic acid → *N*-sulfate D-glucosamine-6-sulfate. The 1β tetrasaccharide fraction from relatively inactive mucopolysaccharide, 1βi, was recovered in a yield of 0.3 mol/7000 daltons and was a mixture of several components. Only 8.5% of the 1βi tetrasaccharide fraction exhibited the same uronic acid placement and sulfate group position found in 1βa. Thus, 2.6% of relatively inactive mucopolysaccharide molecules contain the unique tetrasaccharide sequence found within each molecule of highly active heparin. Given the correlation between abundance of this unique 1βa tetrasaccharide sequence and biologic potency, we suggest that this structure represents the critical site responsible for anticoagulant activity.**

Data from our laboratory provided the first evidence that only a small fraction of a given heparin preparation binds tightly to antithrombin and is responsible for the bulk of the anticoagulant activity (1). Subsequently, we showed that this product is itself heterogeneous and described methods for isolating highly active and relatively inactive heparin species of molecular weight ≈7000 (2). Chemical analysis revealed that these two components differ in uronic acid composition as well as in sulfate group placement. In particular, highly active heparin possesses a tetrasaccharide structure, termed 1βa, that correlates to some extent with anticoagulant activity. However, significant amounts of a similar species, termed 1βi, were found in relatively inactive mucopolysaccharide. On this basis, we suspected that these two components might be subtly different in structure (2). In this communication, we demonstrate that this is the case and delineate a unique tetrasaccharide sequence that may represent the structural basis of heparin's anticoagulant activity.

MATERIALS AND METHODS

The mucopolysaccharide concentration of samples was determined by assay of uronic acid (3) or hexosamine (4). The anticoagulant activity of fractions was established by quantitating their ability to accelerate the interaction of antithrombin with thrombin as compared to a heparin standard of known USP potency (1). The enzymatic activity of β-glucuronidase was quantitated by a spectrofluorometric method using umbelliferyl glucuronide (Koch-Light Laboratories, Colnbrook, England) as substrate (5). The activity of α-iduronidase was measured by a spectrophotometric technique utilizing phenyl iduronide (Calbiochem) as substrate (6). The level of iduronate sulfatase activity was estimated by a high-voltage electrophoretic method using iduronsyl-2-sulfate-[H^3]anhydromannitol-6-sulfate as substrate (7).

The molecular weights of heparin samples were determined by gel chromatography on a Sephadex G-100 column (0.6 × 190 cm) equilibrated with 0.5 M NaCl/0.01 M Tris·HCl, pH 7.5. The column was calibrated with heparin standards whose molecular weights had been established by analytic ultracentrifugation and viscometry (2). Human antithrombin and human thrombin were isolated by reported techniques (1). The β-glucuronidase from human placenta was generously provided by William Sly (St. Louis, MO) (8). This enzyme was homogeneous when analyzed by immunodiffusion and contained no detectable α-iduronidase or iduronate sulfatase activity. A crude mixture of β-glucuronidase, α-iduronidase, and iduronate sulfatase was prepared from bovine liver (9). This extract contained appreciable quantities of all three enzymes as quantitated by the assays described above. Partially purified α-iduronidase was isolated from this crude enzyme mixture by heparin-Sepharose affinity chromatography (10). The final product contained no measurable iduronate sulfatase activity and only barely detectable levels of β-glucuronidase activity.

Porcine heparin was obtained from the Wilson Chemical Co. (Chicago) at an early stage in the manufacturing process and was purified by cetylpyridinium chloride precipitation (11). Low molecular weight species were prepared by filtering 4 g of this material at flow rates of 40 ml/hr through a column of Sephadex G-100 (5 × 190 cm) equilibrated with 0.15 M NaCl/0.01 M Tris·HCl, pH 7.5, and pooling fractions of molecular weight 6000–8000. This product was subsequently subjected to affinity chromatography utilizing antithrombin, in order to isolate highly active as well as relatively inactive forms of the mucopolysaccharide (2). The highly active and relatively inactive heparin species were treated with nitrous acid at pH 1.5, and the newly generated fragments were reduced with sodium boro[H^3]hydride (12, 13). The resultant species were separated by descending chromatography for 90 hr on Whatman 3 MM paper in solvent system I or II (13). In these systems, peak 1 is known to contain tetrasaccharide moieties (12, 13). Therefore, it was quantitatively eluted (>90%), pooled, and fractionated by multiple descents as described above.

The relative proportions of iduronic and glucuronic acids, the nature of the reducing end groups, and the ratios of uronic acid to anhydromannitol were established by hydrolyzing oligosaccharides or heparin fractions in 0.5 M H_2SO_4 for 6 hr at 95°C (complete acid hydrolysis) and subsequently analyzing the monosaccharide moieties by descending chromatography on DEAE-cellulose paper in solvent system II. The *N*-sulfate content of mucopolysaccharides was estimated by comparing

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Table 1. Chemical and functional characteristics of heparin species

Heparin	Specific activity, USP units/mg	Molecular size, daltons	Glucuronic acid, %*	N-Sulfate disaccharide	$1\beta a/1\beta i$, % of mass
Highly active	360	≈7000	32.9	0.75	20.3
Relatively inactive	12	≈7000	26.0	0.85	5.7

* The iduronic acid value can be calculated by subtracting the glucuronic acid value from 100%.

the amount of 2,5-anhydro-D-mannose formed after treatment with nitrous acid to the total hexosamine present (2).

The following solvent systems were utilized for paper chromatography and high-voltage electrophoresis: I, 1-butanol/acetic acid/1 M ammonium hydroxide, 2:3:2 (vol/vol); II, 1-butanol/acetic acid/1 M ammonium hydroxide, 2:3:1 (vol/vol); III, ethyl acetate/acetic acid/formic acid/water, 18:3:1:4 (vol/vol); IV, pyridine/acetic acid/water, 1:10:400 (vol/vol); V, pyridine/acetic acid/water, 26:10.6:4000 (vol/vol); VI, ethyl acetate/pyridine/5 mM boric acid, 3:2:1 (vol/vol).

RESULTS

Highly active and relatively inactive heparin species of low molecular weight were prepared from crude porcine tissue extracts. Table 1 gives the molecular weights and specific anticoagulant potencies of the two fractions. Chemical analysis of these two species revealed significant differences in uronic acid composition and sulfate group placement. The highly active heparin had 0.75 additional residue of glucuronic acid and 1.1 fewer residues of N-sulfated glucosamine per molecule compared to the relatively inactive mucopolysaccharide. Preliminary evidence obtained by high-resolution NMR suggests that this decrease in N-sulfated glucosamine is accompanied by a parallel increase in N-acetylated glucosamine.

We suspected that the additional glucuronic acid and N-acetylglucosamine residues might be located at adjacent sites on the highly active heparin polymer. (In this case, the sequence would be insensitive to nitrous acid degradation and should be captured within a tetrasaccharide fragment after exposure to this reagent.) For this reason, we individually subjected the two species to nitrous acid degradation at pH 1.5, radiolabeled the newly formed reducing ends with sodium boro[H^3]hydride, separated the various components by paper chromatography via a single 40-hr descent in solvent system I, and quantitated the respective peaks by scintillation counting (2). Analyses of numerous chromatograms indicated that larger amounts of a specific oligosaccharide are found within the tetrasaccharide zone of highly active heparin compared to relatively inactive mucopolysaccharide. These components are termed $1\beta a$ and $1\beta i$, respectively. There were 1.1 $1\beta a$ sequences per molecule in highly active heparin and 0.3 $1\beta i$ structure per molecule in the relatively inactive mucopolysaccharide.*

Additional studies of the $1\beta a$ and $1\beta i$ oligosaccharides demonstrated that both contained equimolar amounts of iduronic and glucuronic acids, had anhydromannitol at the reducing end position, and exhibited ratios of uronic acid to anhydromannitol appropriate for tetrasaccharides (see below). In addition, complete destruction of all uronic acid residues resulted when $1\beta a$ was dissolved in 0.1 M sodium acetate (pH 3.9) and titrated with 0.1 M sodium periodate for 48 hr at 5°C. Furthermore, the internal glucosamine residue of both $1\beta a$ and $1\beta i$ appeared to be N-acetylated because the two components were completely resistant to cleavage with nitrous acid at pH 4.2 (12, 13). Given the known alternating glucosamine/uronic acid structure of heparin, these experiments indicate that $1\beta a$ and $1\beta i$ components are tetrasaccharides with a monosaccharide sequence of uronic acid → N-acetylated glucosamine → uronic acid → [^{3}H]anhydromannitol. These findings are similar to those previously reported for heparin species isolated from commercially purified mucopolysaccharide (2). Of course, $1\beta a$ and $1\beta i$ may be subtly different in structure. For this reason, we examined these moieties by high-voltage electrophoresis at pH 3.6 and 5.3 with monosulfated disaccharide, disulfated disaccharide, and tetrasulfated tetrasaccharide markers. $1\beta a$ and $1\beta i$ migrated slightly faster than the monosulfated disaccharide marker at pH 3.6 (Fig. 1). These findings suggest that each component contains two sulfated monosaccharide moieties. The patterns also indicate that $1\beta i$ is considerably more heterogeneous than $1\beta a$.

To elucidate the possible structural differences between $1\beta a$ and $1\beta i$, we determined the type of uronic acid residue present at the nonreducing end of these species as well as their degree of sulfation. This was accomplished by using exoglycosidases that remove single uronic acid residues from the nonreducing end of these oligosaccharides. The reaction mixtures were subsequently filtered on column of P2-polyacrylamide (0.6 × 200 cm) equilibrated with 0.5 M NaCl to determine the extent of enzymatic cleavage. In this system, tetrasaccharides, disulfated disaccharides, and sulfated monosaccharides elute at fixed

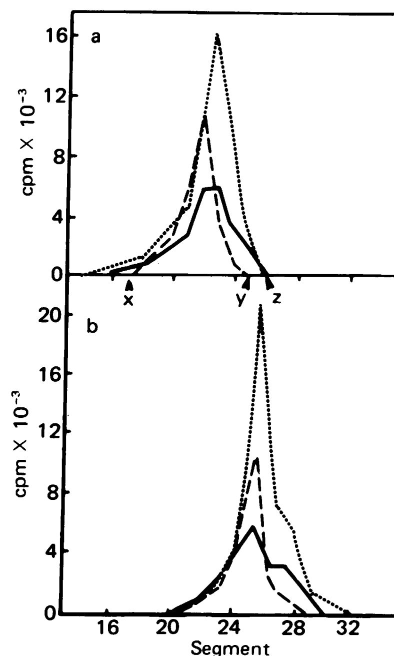

FIG. 1. Electrophoretic examination of $1\beta a$ and $1\beta i$ tetrasaccharides in solvent system IV at pH 3.6 (*a*) or V at pH 5.3 (*b*) at 35 V/cm for 2 hr. The samples were $1\beta a$ tetrasaccharide derived from highly active heparin (- - -), $1\beta i$ tetrasaccharide isolated from relatively inactive mucopolysaccharide (—), and an equimolar mixture of the two species (·····). The radiolabeled markers utilized were monosulfated disaccharide (x), disulfated disaccharide (y), and tetrasulfated tetrasaccharide (z) prepared as outlined by Shively and Conrad (12, 13).

* The calculated ratios are based on an average of 22 monosaccharide residues per molecule. This value is suggested by the molecular sizes of the two fractions (Table 1).

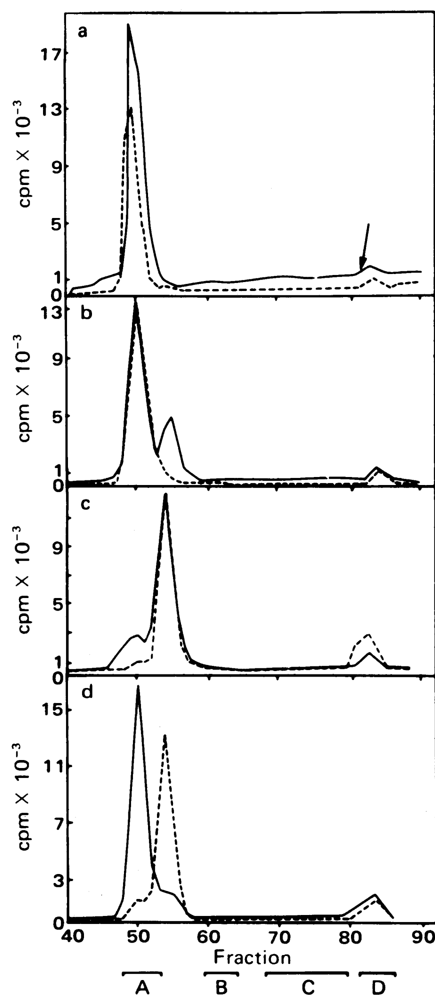

FIG. 2. Enzymatic degradation of 1*βa* and 1*βi*. The 1*βa* (---), derived from highly active heparin, and 1*βi* (—), from a relatively inactive heparin were incubated in 0.05 M sodium acetate (pH 5.0) for 18 hr with various exoglycosidases and subsequently analyzed by gel filtration on P2-polyacrylamide. (*a*) Before addition of exoglycosidases. Arrow, [^{14}C]glucose. (*b*) After exposure to 6000 units of *β*-glucuronidase. (*c*) The *β*-glucuronidase-resistant material was exposed to a crude enzyme mixture containing *β*-glucuronidase, *α*-iduronidase, and iduronate sulfatase. (*d*) The *β*-glucuronidase-resistant material was exposed to 0.05 unit of *α*-iduronidase. The column markers, prepared as described in Fig. 1, were: A, tetrasaccharide; B, disulfated disaccharide; C, sulfated monosaccharide; D, monosaccharide. Fraction size was 0.5 ml.

positions relative to [^{14}C]glucose utilized as a column marker (Fig. 2). The data cited below are based on an average of three experiments conducted with each of the 1*βa* and 1*βi* species.

The 1*βa* migrated within the tetrasaccharide zone prior to treatment with enzyme. The uronic acid composition of this species was 51% iduronic acid and 49% glucuronic acid. The ratio of uronic acid to anhydromannitol was 2.1. After enzymatic degradation with *β*-glucuronidase, virtually 100% of this component continued to migrate within the tetrasaccharide region. The uronic acid composition of this species and the ratio of uronic acid to anhydromannitol were unchanged. When the same component was exposed to a crude enzyme mixture containing *α*-iduronidase, iduronate sulfatase, and *β*-glucuronidase, ≈95% of the material shifted its elution position into a region of the chromatogram located between the tetrasaccharide and disaccharide zones. Analysis of the uronic acid composition of this material revealed that it contained only glucuronic acid. Furthermore, the newly generated species exhibited a ratio of uronic acid to anhydromannitol of 1.1. The residue liberated from the 1*βa* migrated within the sulfated monosaccharide region. This product was identified as iduronic acid after conversion to iditol and subsequent chromatography on DEAE-cellulose paper utilizing solvent system VI with appropriate standards (not shown). To determine whether the iduronic acid moiety present at the nonreducing end of the 1*βa* tetrasaccharide had an ester sulfate substituent, we subjected this component to degradation with *α*-iduronidase free of iduronate sulfatase. This treatment shifted the elution position of 95% of the tetrasaccharide mass into the trisaccharide zone. Thus, virtually all of 1*βa* has a nonsulfated iduronic acid at the nonreducing end.

The 1*βi* tetrasaccharide isolated from relatively inactive heparin was examined in a similar fashion. This material migrated within the tetrasaccharide zone prior to treatment with *β*-glucuronidase. Its uronic acid content was 51% iduronic acid and 49% glucuronic acid. The ratio of uronic acid to anhydromannitol was 2.1. After degradation with *β*-glucuronidase, the elution position of 29% of the 1*βi* mass shifted into the trisaccharide zone. The material that remained within the tetrasaccharide region exhibited no alteration in uronic acid composition or in the ratio of uronic acid to anhydromannitol. Material that migrated within the trisaccharide zone showed an iduronic acid content of >95% and a ratio of uronic acid to anhydromannitol of 1.1. Thus, 29% of the 1*βi* contained a glucuronic acid residue at the nonreducing end. To demonstrate that the remaining 71% had an iduronic acid residue at the nonreducing position, we degraded material that was resistant to *β*-glucuronidase with the crude enzyme mixture. As expected, the elution position of 87% of this tetrasaccharide shifted to the trisaccharide zone. The material that migrated as a trisaccharide contained only glucuronic acid and a ratio of uronic acid to anhydromannitol of 1.1. To determine whether the iduronic acid liberated from the 1*βi* had an ester sulfate substituent when present on the parent molecule, we treated species resistant to the *β*-glucuronidase with *α*-iduronidase free of iduronate sulfatase. Fig. 2 reveals that 88% of this 1*βi* mass was unaffected by exposure to this enzyme. Thus, only 12% of this component contains a nonsulfated iduronic acid at the nonreducing position.

We also utilized an alternate method to quantitate directly the degree of sulfation of the iduronic acid at the nonreducing end of the two tetrasaccharides. This approach is based on the observation by Shively and Conrad (12, 13) that carboxy reduction of iduronsyl-2-sulfate-anhydromannitol-6-sulfate will permit scissioning of the glycosidic bond of this disaccharide with dilute HCl without producing complete desulfation of the liberated monosaccharides. These authors have identified the resultant cleavage fragments by paper chromatography and rigorously confirmed the assigned structures by mass spectroscopy (12, 13). We subjected idose-2-sulfate-anhydromannitol 6-sulfate radiolabeled at either the idose or anhydromannitol residue to hydrolysis in 0.1 M HCl at 95°C for 1.5–2.5 hr (partial acid hydrolysis). The reaction mixtures were subsequently analyzed by paper chromatography with solvent system III. This allowed us to establish the relative mobilities of various well-characterized fragments in our chromatographic system. Furthermore, results obtained with this model disaccharide could be used as a control when estimating the extent to which sulfate substituents initially present on the idose residue are retained by this hexose moiety upon release by partial acid hydrolysis.

The nonsulfated monosaccharides derived from idose and anhydromannitol were distinctly separated after a 16-hr descent

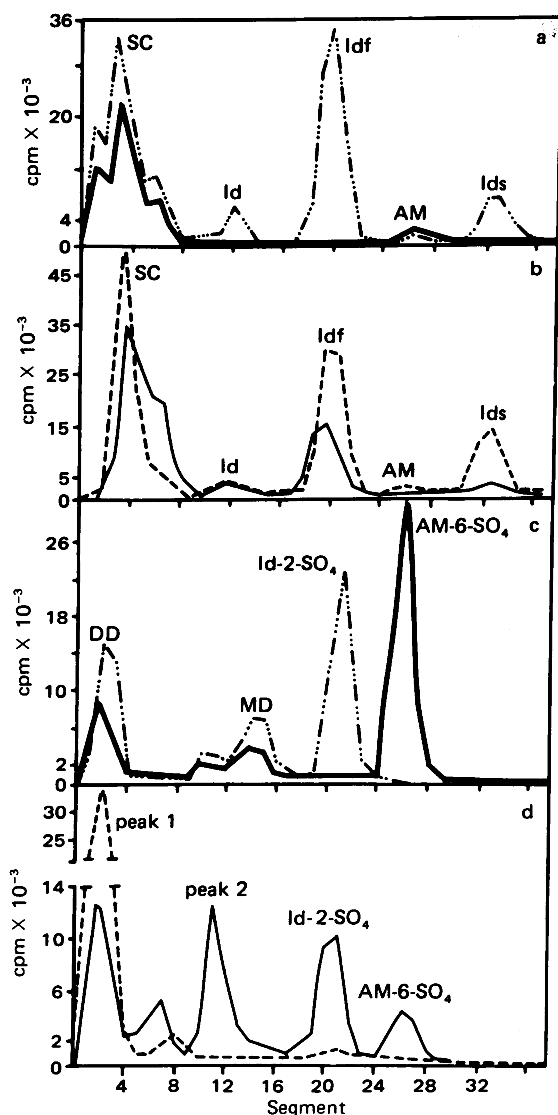

FIG. 3. Analysis of the uronic acid present at the nonreducing terminal of 1βa and 1βi species. (a) Chromatogram (16 hr) of partial acid hydrolysates of idose-2-sulfate-anhydromannitol-6-sulfate radiolabeled at the idose (- • • • -) or the anhydromannitol (—) residue. This model disaccharide was prepared as described by Shively and Conrad (12, 13). (b) Chromatogram (16 hr) of partial acid hydrolysates of carboxy reduced 1βa derived from highly active heparin (- - -) or 1βi (—) isolated from relatively inactive mucopolysaccharide but resistant to β-glucuronidase. After carboxy reduction, these 1βa and 1βi moieties were re-isolated by high-voltage electrophoresis at pH 3.6. (c) Chromatogram (90 hr) of the model compound as described above. (d) Chromatogram (90 hr) of the 1βa and 1βi tetrasaccharides as in b. Abbreviations: SC, sulfated components; Id, idose; Idf, idofuranose; AM, anhydromannitol; Ids, idosan; DD, disulfated disaccharide; MD, monosulfated disaccharides.

whereas sulfated components of various sizes remained at the origin (Fig. 3a). Components derived from anhydromannitol or the reduced uronic acid moiety were evident only when disaccharide radiolabeled at the appropriate residue was examined. The mobilities of these various fragments relative to an added [^{14}C]glucose marker were in excellent agreement with previously reported data (12, 13). After a single 90-hr descent in an identical chromatographic system, the sulfated components were resolved and the nonsulfated moieties had migrated off the matrix (Fig 3c). The first and second peaks represent residual model disaccharide and a monosulfated disaccharide derivative, respectively. This identification is based on the observation that both components had radiolabel derived from idose as well as anhydromannitol and the demonstration that the two species comigrated with carboxy reduced disulfated or monosulfated disaccharides, respectively (not shown). The third and fourth peaks are produced by idose-2-sulfate and anhydromannitol-6-sulfate, respectively. This is suggested by Fig. 3c which reveals that the third peak originated from the idose residue whereas the fourth peak was derived from the anhydromannitol group.

These conclusions are strengthened by additional experiments showing that both species comigrated on high-voltage electrophoresis at pH 3.6 with monosulfated moieties (not shown). The degree of idose desulfation that occurred under the conditions of partial acid hydrolysis can be estimated by dividing the amount of idose-2-sulfate formed by the total level of idose derivatives generated. Based upon these data, we calculate that 37% of the idose moieties present within the model disaccharide retained their ester sulfate substituents upon release by dilute acid (12, 13).

We conducted identical studies with 1βa and 1βi tetrasaccharide species that were resistant to treatment with β-glucuronidase. Initially, these species were aldehyde reduced with sodium boro[H^3]hydride after nitrous acid cleavage of heparin polymers such that one ^{3}H was incorporated into each anhydromannitol residue. Subsequently, these components were also carboxy reduced and radiolabeled with sodium boro[H^3]hydride such that two ^{3}H were introduced into each hexose formed from uronic acid. The carboxy reduced species were then subjected to partial acid hydrolysis under conditions identical to those utilized with the model disaccharide. The resultant fragments were analyzed by paper chromatography as described above (Fig. 3). The data cited below are based on an average of three experiments in which the time of hydrolysis was varied from 1.5 to 2.5 hr. These experiments revealed that the release of idose or its derivatives accounts for 43% of the counts initially present in the two tetrasaccharides. Given that 40% of the ^{3}H theoretically had been incorporated into the nonreducing uronic acid moiety, we estimate that complete hydrolysis of the idose-N-acetylglucosamine bond was achieved with both components. In the case of 1βa, only 2.0% of the total counts liberated as idose derivatives were released as idose-2-sulfate. When this estimate is normalized by comparison with the recovery of the same moiety from the model disaccharide, we calculate that 5.4% of iduronic acid residues present at the nonreducing position of 1βa are sulfated. With respect to the 1βi resistant to β-glucuronidase, 33% of the total counts present as idose derivatives were liberated as idose-2-sulfate. When this value is normalized to the maximal level of idose-2-sulfate attained during partial acid hydrolysis, we estimate that 89% of the iduronic acid residues located at the nonreducing end of this tetrasaccharide must bear ester sulfate substituents.

Two additional differences in cleavage pattern are evident in Fig 3d. First, hydrolysates of 1βi exhibit equal quantities of peak 1 and peak 2 as well as significant levels of anhydromannitol-6-sulfate. Second, similarly treated samples of 1βa contain large amounts of peak 1 but minimal quantities of peak 2 and anhydromannitol-6-sulfate. These results suggest that the glucose-anhydromannitol bond has been scissioned more readily in 1βi than in 1βa. This phenomenon may be secondary to alterations in sulfate group placement. Furthermore, the data also indicate that partial cleavage of 1βi may result in the generation of large amounts of monosulfated oligosaccharide species (peak 2) that migrate close to the monosulfated disaccharide marker. This component is most probably a monosulfated trisaccharide that is formed when idose-2-sulfate is released from the parent tetrasaccharide. A similar scission of 1βa would be expected to

result in the formation of a disulfated trisaccharide (peak 1) that would migrate close to the disulfated disaccharide marker.

DISCUSSION

Highly active and relatively inactive heparin of approximate molecular weight 7000 were prepared from crude tissue extracts. This starting material was utilized in order to avoid polymer modification, by oxidizing agents, that may occur during commercial purification of mucopolysaccharide. The highly active fraction appears to be a relatively homogeneous entity because it cannot be subfractionated by affinity chromatography with antithrombin (2). The limited ability of the relatively inactive species to bind to and accelerate inhibitor action appears to be secondary to trace contamination with active heparin of intermediate potency (2, 14). Highly active heparin possesses 0.75 additional residue of glucuronic acid per molecule and 1.0 additional residue of *N*-acetylglucosamine per molecule compared to the relatively inactive mucopolysaccharide. These differences in uronic acid and glucosamine composition are consistent with the relative amounts of $1\beta a$ and $1\beta i$ tetrasaccharides found within the two heparin fractions. Highly active heparin contains 1.1 $1\beta a$ tetrasaccharide structures per molecule. Careful examination of this $1\beta a$ fragment by chemical as well as enzymatic degradation techniques has revealed that 95% of the nonreducing end group consists of nonsulfated iduronic acid and 5% may bear an ester sulfate substituent. This minor degree of heterogeneity may be due to trace contamination of our sample with oligosaccharides such as 1α tetrasaccharide that migrate close to $1\beta a$ and contain sulfated iduronic acid as the predominant uronic acid residue (2).

If the above data are used in conjunction with the uronic acid composition, electrophoretic mobility, periodate sensitivtiy, and high-pH nitrous acid resistance of the $1\beta a$ tetrasaccharide, it is possible to propose a structure for this component. On the basis of the available evidence, we conclude that the $1\beta a$ tetrasaccharide should be represented by the following monosaccharide sequence at the polymer level: L-iduronic acid → *N*-acetylated D-glucosamine-6-sulfate → D-glucuronic acid → *N*-sulfated D-glucosamine-6-sulfate.

The relatively inactive mucopolysaccharide has 0.3 $1\beta i$ sequence per molecule. Treatment of this species with purified β-glucuronidase indicates that only 71% of this component has an iduronic acid at the nonreducing position. Furthermore, chemical as well as enzymatic degradation techniques demonstrate that 88% of the $1\beta i$ fraction resistant to degradation with β-glucuronidase contains an ester sulfate substituent on the iduronic acid residue. Thus, only 2.6% of the molecules present within the relatively inactive mucopolysaccharide can have a $1\beta i$ sequence identical to the unique $1\beta a$ tetrasaccharide structure found within each molecule of highly active heparin. It should be noted that the preparations of relatively inactive mucopolysaccharide utilized in this study are not completely depleted of active species. Indeed, we estimate that these products contain 2–3% of heparin components that exhibit anticoagulant activity (2, 14). The observed correlation between the relative abundance of the unique $1\beta a$ tetrasaccharide structure and the anticoagulant potency of these preparations strongly suggests that this critical sequence must represent a binding site on porcine heparin that is recognized by antithrombin.

Our conclusion concerning the importance of this sequence is considerably strengthened by the invariant nature of this structure within the highly active heparin as well as the presence of two relatively rare nonsulfated uronic acid moieties in close proximity. It is of particular interest that a non sulfated iduronic acid residue has been found within the critical tetrasaccharide because this moiety can assume unique conformations within the heparin polymer (15). We believe that our data provide an example of a rigidly defined structure–function relationship for a mucopolysaccharide. Our findings suggest that this class of biologic macromolecules may prove to be similar in this regard to proteins and nucleic acids.

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