Proc. Natl. Acad. Sci. USA
Vol. 75, No. 7, pp. 3065–3069, July 1978
Biochemistry

Structure–function relationships of heparin species

(purification of highly active heparin/partial sequence of heparin/chemical composition of heparin)

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Communicated by K. M. Brinkhous, April 3, 1978

ABSTRACT **We have fractionated porcine heparin species of low molecular weight, with an average specific anticoagulant activity of 96 units/mg by affinity chromotography. Highly active and relatively inactive preparations of similar size were obtained with specific anticoagulant activities of 360 and 4 units/mg, respectively. The highly active heparin fraction possesses 1.1 additional residues of glucuronic acid and 1.5 fewer residues of *N*-sulfated glucosamine per molecule compared to the relatively inactive species. This decrease in *N*-sulfated glucosamine appears to be secondary to a corresponding increase in *N*-acetylated glucosamine. This form also contains a tetrasaccharide sequence with a *N*-sulfated glucosamine at its reducing end as well as equivalent amounts of glucuronic acid and iduronic acid. Furthermore, the internal glucosamine residue of this sequence appears to be *N*-acetylated. Sufficient amounts of this tetrasaccharide sequence are present within the highly active preparation such that each molecule may be endowed with this structure. The relatively inactive product contains a significantly decreased quantity of this tetrasaccharide sequence such that only ≃20% of these molecules may possess this structure. The mean distance between nonsulfated uronic acid residues of the highly active species is smaller than that separating similar residues of the relatively inactive product. In addition, a larger number of the nonsulfated uronic acid residues of the highly active material appears either to be present in a restricted region of the molecule separated only by glucosamine residues or to be located at penultimate positions within the polysaccharide chain.**

Heparin functions as an anticoagulant by binding to antithrombin and accelerating the rate at which this protein inactivates the serine proteases of the hemostatic mechanism (1). During the last 60 years, a number of investigators have attempted to define the chemical and physical features of heparin that are responsible for its anticoagulant action. However, the precise relationship between the structure of the mucopolysaccharide and its biologic properties has remained elusive. A communication from our laboratory provided evidence that only a small fraction (25–35%) of a given heparin preparation binds tightly to antithrombin and is responsible for 85–95% of the anticoagulant activity (2). This finding engendered the hope that, once the active fraction was carefully examined, a unique structure–function relationship for heparin would emerge. The existence of active and relatively inactive heparin species has been confirmed by Lindahl and his coworkers (3). Unfortunately, these investigators were unable to find any chemical parameters that distinguished between their two heparin fractions (3).

MATERIALS AND METHODS

Human antithrombin and human thrombin were both isolated by methods previously reported from our laboratory (4). Two porcine heparin products were utilized as starting material for affinity fractionation. The first was a commercial preparation provided by Riker Laboratories (specific activity, 142 USP units/mg). The second was obtained at an early stage in the manufacturing process from the Wilson Chemical Co., Chicago, IL, and was extensively purified by cetylpyridinium chloride precipitation (CPC heparin) (5). To obtain low molecular weight forms of heparin, 4 g of either mucopolysaccharide was filtered at flow rates of 40 ml/hr through columns of Sephadex G-100 (5 × 190 cm) equilibrated with 0.15 M NaCl/0.01 M Tris·HCl, pH 7.5. The column effluents were monitored for mucopolysaccharide by the carbazole reaction and for anticoagulant activity as described below. Fractions of molecular weight 6000–8000 (average specific activity, 96.1 units/mg) were pooled.

The mucopolysaccharide concentration of a given sample was determined by assay of uronic acid (6) or hexosamine (7). The anticoagulant activity of each fraction was established by quantitating its ability to accelerate the interaction of human antithrombin with human thrombin and comparing the extent of enzyme neutralization to that generated by a heparin standard of known USP potency. 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 (8). The total sulfate content of heparin fractions was measured as described by Dodgson and Price (9). Prior to analysis, samples were treated with Amberlite-400 (hydroxide form) to remove contaminating inorganic ions and hydrolyzed in 1 M HCl for 8 hr at 100°. The *N*-sulfate content of mucopolysaccharides was estimated by comparing the amount of 2,5-anhydro-D-mannose formed after treatment with nitrous acid (10) to the total hexosamine present (7).

The relative proportions of iduronic acid and glucuronic acid within a heparin fraction was established by the technique of Shively and Conrad (11, 12). The amount of galactosamine and glucosamine in various samples was estimated by hydrolyzing fractions in 6 M HCl for 8 hr at 100°, degrading the respective hexosamines to their corresponding pentoses with ninhydrin, separating the various components by paper chromatography utilizing butanol/ethanol/water, 4:1:1 (vol/vol), as solvent, and detecting the resolved species by staining with silver dip reagent. Nitrous acid degradation of heparin samples and aldehyde reduction of the resultant fragments with sodium boro[^{3}H]hydride were carried out by the method of Shively and Conrad (11, 12). The various species were separated by paper chromatography with solvent system I [butanol/acetic acid/ammonium hydroxide, 2:3:2.0 (vol/vol)] or solvent system II (butanol/acetic acid/ammonium hydroxide, 2:3:1.0.). Once isolated, these components were further analyzed by high-

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Abbreviation: CPC heparin, crude heparin purified by cetylpyridinium chloride precipitation.

voltage paper electrophoresis with a pyridine/formic acid/water buffer, 1:10:4000, and a potential difference of 45 V/cm. Thereafter, uronic acid composition and the nature of the reducing end group were established for each species (11, 12). Heparin samples were also degraded at −20° for 16 hr with butyl nitrite and separated by Sephadex G-25 chromatography as described by Cifonelli and King (13, 14).

RESULTS

Fractionation of Heparin Species. Low molecular weight species isolated from a commercial heparin preparation were fractionated at 24° by a technique based upon their affinity for antithrombin. Cycle I of this process was initiated by adding the protease inhibitor to the heparin pool at a molar ratio of 0.08. The resultant mixture of ≃10 ml was filtered at flow rates of 10 ml/hr through columns of Sephadex G-100 (1.75 × 180 cm) equilibrated with 0.15 M NaCl/0.01 M Tris·HCl, pH 7.5. The column effluents were monitored for protein by absorbance at 280 nm and for mucopolysaccharide by the carbazole reaction. These measurements revealed two discrete peaks. The first peak contained protein as well as mucopolysaccharide (K_d = 0.08) and represented a stable heparin–antithrombin complex (see below). Mucopolysaccharide present in this region of the chromatogram is designated highly active heparin. The second peak consisted only of free mucopolysaccharide (K_d = 0.36). Its elution position was identical to that observed when the low molecular weight heparin fraction was gel filtered as described above but in the absence of antithrombin. Mucopolysaccharide located in this area of the chromatogram consisted of active and relatively inactive heparin species. The highly active heparin–antithrombin complex was concentrated to ≃10 ml and filtered at flow rates of 10 ml/hr through columns of Sephadex G-100 (0.9 × 190 cm) equilibrated with 3.0 M NaCl/0.01 M Tris·HCl, pH 7.5. The increase in ionic strength of the solvent disrupted the complex such that discrete peaks of protein (K_d = 0.34) as well as mucopolysaccharide (K_d = 0.65) were generated. Cycle II of the fractionation was initiated by mixing antithrombin with the pool of active and relatively inactive heparin species at a molar ratio of 1.5. The resultant solution was processed as described above to separate the active from the relatively inactive material. Further addition of antithrombin to this latter species did not result in the formation of a protein–mucopolysaccharide complex as judged by gel filtration. The relative abundance of the highly active, active, and relatively inactive forms of heparin were 7.9, 25.1, and 67.0%, respectively. Their specific anticoagulant activities were 360, 267, and 4 units/mg, respectively.

Additional experiments were conducted to determine the homogeneity of highly active and active heparin species. When the molar ratio of antithrombin to mucopolysaccharide was increased to 0.15 during cycle I of affinity fractionation, the specific anticoagulant activity of the heparin that bound to antithrombin was significantly decreased compared to the highly active material. On the other hand, when the molar ratio of antithrombin to mucopolysaccharide was decreased to 0.04 during cycle I of the isolation technique, the resultant heparin bound to antithrombin was found to have a specific anticoagulant activity equivalent to that of highly active material. These critical experiments suggest that highly active heparin is homogeneous with respect to its ability to bind to and activate antithrombin. A similar analysis of the active material indicated that this fraction is composed of several heparin species with different affinities for antithrombin and different anticoagulant potencies.

Examination of low molecular weight heparin preparations isolated from crude material obtained at an early stage in the commercial manufacturing process (CPC heparin) was also undertaken. The results of these studies were similar to those described above, except that the specific anticoagulant activity of highly active heparin was 25% higher. This difference in biologic potency could be due to the slightly greater molecular size of species derived from CPC heparin. This aspect was examined by Sephadex G-100 gel chromatography utilizing columns calibrated with heparin standards of known molecular size. The data demonstrated that highly active and relatively inactive heparin derived from commercial preparations have identical masses of ≃6000 daltons. Similar components isolated from CPC heparin have masses of ≃7500 daltons.

Compositional Analysis of Heparin Species. The highly active heparin had a significantly increased content of glucuronic acid compared to the relatively inactive material (Table 1). In addition, glucosamine was the only type of hexosamine present in the two heparin species. Table 1 also reveals that the relatively inactive fraction had a slightly greater average number of total sulfate groups per disaccharide unit and a slightly lower average number of *N*-sulfate groups per disaccharide unit compared to the highly active form. At present, the magnitude of these differences remains too small to be considered meaningful. By contrast, the average number of *N*-sulfate groups per hexosamine residue was significantly higher for the relatively inactive fraction compared to the highly active species. This finding suggested that a compensatory increase in other substituents such as *N*-acetyl groups should occur within the highly active fraction. Preliminary measurements of *N*-acetyl content by high-resolution nuclear magnetic resonance have confirmed this expectation. Virtually identical results have been obtained with respect to mucopolysaccharide composition and sulfate group distribution for the two species derived from CPC heparin.

Partial Structural Analysis of Heparin Species. The ordering of *N*-sulfate groups within the highly active and relatively inactive species derived from commercial heparin was initially examined by degradation of polymers with nitrous acid (pH 1.5). Subsequently, these fragments were labeled with sodium boro[^{3}H]hydride, separated by paper chromatography in a single 40-hr descent in solvent system I, and quantitated by scintillation counting (12). Fig. 1 shows typical radiochromatograms obtained with highly active and relatively inactive heparin fragments. Table 2 gives the relative magnitudes of the various peaks as a percentage of total mucopolysaccharide mass. These techniques have been utilized by Shively and Conrad (11, 12) to probe the structure of beef lung heparin. Their detailed examination of radiochromatographic patterns similar to those depicted in Fig. 1 indicate that peak 1 is usually produced by

Table 1. Uronic acid composition and sulfate group position

Heparin	Glucuronic acid, %*	Sulfate†/ disaccharide	*N*-sulfate†/ disaccharide	*O*-sulfate‡/ disaccharide
Highly active	30.7	2.26	0.70	1.56
Relatively inactive	19.3	2.37	0.95	1.42

* Each value represents the mean derived from four separate determinations performed in triplicate. The iduronic acid value can be calculated by substracting the glucuronic acid value from 100%.

† The values of total sulfate per disaccharide are expressed as molar ratios of a given parameter to glucosamine. Each value represents the mean of two separate determinations performed in triplicate.

‡ This value was calculated by subtracting column 3 from column 2.

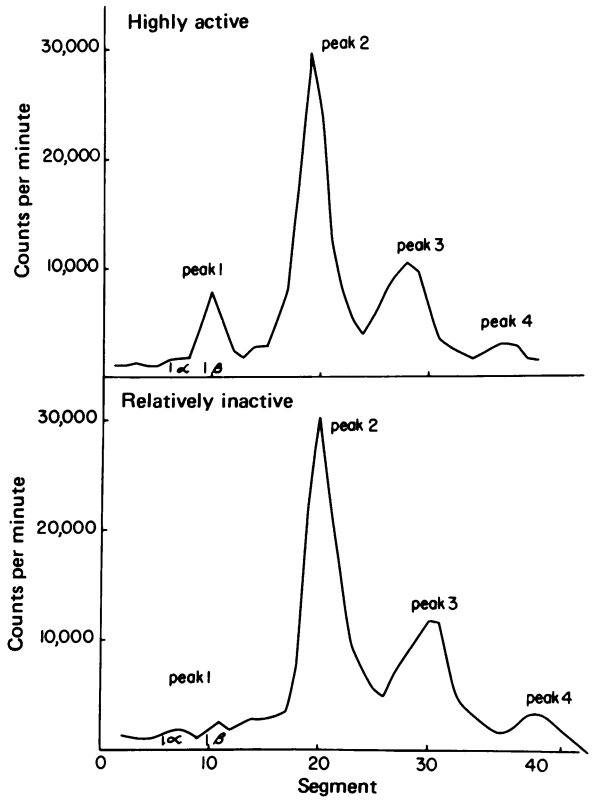

FIG. 1. Nitrous acid degradation patterns for highly active (*Upper*) and relatively inactive (*Lower*) heparin species.

tetrasaccharides, peak 2 is customarily generated by disulfated disaccharides, and peak 3 is largely due to monosulfated disaccharides.

We confirmed these assignments for our porcine heparin fragments and defined the structure of species within several of these radiochromatographic peaks. To this end, components in specific regions of multiple chromatograms were eluted quantitatively (>90% total recovery), pooled, and subsequently separated by paper chromatography with a second descent for 90–120 hr in solvent system II. Species fractionated by this approach were also examined by high-voltage electrophoresis. Once a component was shown to be relatively homogeneous by these two techniques, the nature of its reducing end group was determined, its uronic acid composition was established, and the ratio of uronic acid residues to reducing end-group moieties was obtained. Peak 1 obtained from highly active material was composed of one major and one minor component. The major species, termed 1β, was a tetrasaccharide. It contained equivalent amounts of iduronic acid and glucuronic acid as well as anhydromannitol at the reducing end (Table 2). The minor species, termed 1α, most probably was a tetrasaccharide although its ratio of uronic acid residues to the reducing end group was somewhat higher than expected. Furthermore, this oligosaccharide had iduronic acid as the predominant uronic acid and anhydromannitol at the reducing end. The small amount of glucuronic acid present in 1α is most likely secondary to contamination with 1β. Peak 1 derived from relatively inactive material contained two major and one minor components. Compositional analysis of the 1α and 1β species suggests that they correspond to the tetrasaccharides previously isolated from the highly active heparin.

The 1β tetrasaccharides derived from both forms of heparin were further examined in order to determine whether their internal glucosamine residues possessed a free amino group or

Table 2. Nitrous acid degradation data

Peak	Amount, % of mass	Uronic acid composition* % IduA	% GlcUA	Uronic acid per end groups
	Highly active heparin			
Peak 1†	20	—	—	—
1α	1.0	88	12	2.4
1β	19.0	48	52	2.2
1γ	Trace	—	—	—
Peak 2	53.00	100	0	1.1
Peak 3	27.0	50	50*	1.1
	Relatively inactive heparin			
Peak 1†	8.4	—	—	—
1α	2.9	90	10	2.4
1β	3.8	46	54	2.1
1γ	0.7	—	—	—
Peak 2	60.2	100	0	1.0
Peak 3	32.4	56	44*	1.0

* See footnote * in text. IduA, iduronic acid; GlcUA, glucuronic acid. The glucuronic acid content of the two heparin species may be estimated by summing contributions from peaks 1 and 3. However, these values must be adjusted upward by ~2–3% due to the glucuronic acid present in peak 4 (not shown). These adjusted estimates remain at some variance with data obtained by compositional analysis of intact mucopolysaccharides. The residual differences, which range from 1 to 4%, may be attributable to small systematic errors in quantitating peak abundance or to the different sequences of radiolabeling and degradation utilized in the two methods.

† Estimates of peak 1 were obtained by multiplying total counts by 2. This correction factor is required because there is only one end group labeled per four monosaccharide units in peak 1 compared to one end group labeled per two monosaccharide units in peaks 2 and 3.

were *N*-acetylated. This analysis was conducted by treating the two aldehyde-labeled components with nitrous acid at pH 4.2 and subsequently fractionating the resultant species by paper chromatography in solvent system I. The 1β tetrasaccharide obtained from highly active material was uneffected by this procedure. However, the 1β tetrasaccharide isolated from the relatively inactive preparation was cleaved to a small extent ($\simeq$20%).

Peak 2 isolated from highly active and relatively inactive material migrated as a single component on paper chromatography in system II and on high-voltage electrophoresis. Data in Table 2 indicate that this component is a disaccharide that contains iduronic acid. End-group determinations revealed that anhydromannitol was at the reducing end of this molecule. Furthermore, this species comigrated, on paper chromatography and high-voltage electrophoresis, with the disaccharide iduronsyl-2-SO_4 anhydromannitol-6-SO_4 that has been isolated from beef lung heparin.

Peak 3 derived from highly active and relatively inactive heparins migrated on paper chromatography in system I as single species. Data summarized in Table 2 provide the uronic acid composition of these products and suggests that species present in these fractions are predominantly disaccharides. Further analysis revealed that the reducing end group of peak 3 fragments derived from highly active as well as from relatively inactive heparins consisted of 13.0% hexitol* and 87.0% anhydromannitol. The above data indicate that peak 3 fractions are heterogeneous. This surmise was substantiated by partial separation of several distinct components by paper chromatography with a 90–120 hr descent in solvent system II. Preliminary experiments suggest that the hexitol end groups

* It is assumed that uronic acid residues at the reducing end of heparin molecules are predominantly glucuronic acid. This is in accord with the findings of Jansson *et al.* (15)

present in peak 3 most probably are due either to sulfated iduronic acid or to trisaccharide species released from the nonreducing or reducing end. The anhydromannitol end groups of peak 3 are associated with three types of structural elements. These include sulfated anhydromannitol liberated in equivalent amounts from the nonreducing ends of both types of heparin molecules as well as monosulfated disaccharides containing iduronic acid and glucuronic acid. As expected, the monosulfated disaccharides of peak 3 migrated more slowly than the disulfated disaccharides of peak 2 on high-voltage electrophoresis. After nitrous acid degradation of either type of heparin, a rapidly migrating species was observed (Fig. 2, peak 4). This material represented free aldonic acid but constituted only 2–3% of the total mass of these mucopolysaccharides.* This species has not been included in Table 2 because of its relatively minor quantitative contribution. We also degraded the highly active heparin with butyl nitrite at −20° and separated the resultant fragments on Sephadex G-25. The amount of tetrasaccharide derived from the highly active species was ~2.5 times greater than the amount generated from the relatively inactive fraction. This is in excellent agreement with the data presented in Table 2.

Because the highly active fraction of heparin contains a greater number of glucuronic acid residues then the relatively inactive material, the position of these groups within the mucopolysaccharide chain may be of critical importance to anticoagulant activity. In two separate paired experiments, we used periodate oxidation and Smith degradation to cleave heparin species at nonsulfated uronic acid residues and gel filtration on Sephadex G-50 to separate the resultant components. In each instance, complete destruction of glucuronic acid residues was established by measurement of uronic acid composition. The results of a representative experiment are depicted in Fig. 2. The fragments derived from the relatively inactive material were larger than those liberated from the highly active species. In addition, we also noted that the amount of glucosamine liberated near the bed volume of the gel filtration column was considerably greater for the highly active heparin species than for the relatively inactive material (not shown).

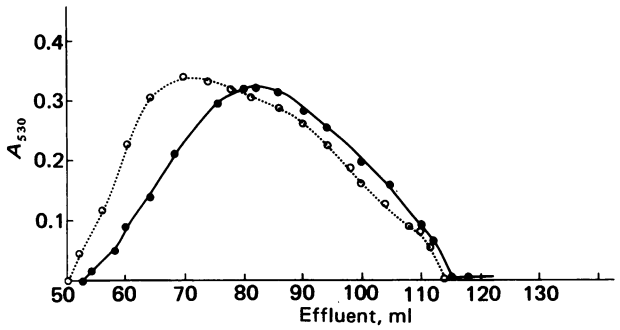

FIG. 2. Gel filtration patterns of highly active (●) and relatively inactive (○) heparin subjected to periodate oxidation and Smith degradation. Each form of the mucopolysaccharide (4 mg) was treated as described by Cifonelli and King (14) and then applied as a 1-ml sample to a Sephadex G-50 column (1 × 189 cm) equilibrated with 0.3 M NaCl. Fractions (1 ml) were collected and assayed for uronic acid by the carbazole reaction.

DISCUSSION

In this communication, we outline a highly discriminating affinity technique for fractionating active forms of heparin and obtaining species that are homogeneous with respect to their interaction with antithrombin. We have chosen to study low molecular weight forms in order to maximize the relative percentage of the mucopolysaccharide that is directly involved in binding to antithrombin. This is essential because the affinity fractionation process should be most efficient in distinguishing between molecules that have different primary sequences within this critical area. In addition, the structural characteristics of this "contact" region are more easily defined with heparin species of low molecular weight. We designate the upper 8% of this material as highly active and the lower 67% of the preparation as relatively inactive. Because this separation procedure can distinguish between fine gradations in heparin's affinity for antithrombin, our inability to subfractionate the highly active species provides the best currently available evidence for relative homogeneity.

On the basis of molecular weight analysis, monosaccharide composition, and sulfate group distribution, we estimate that the highly active form has ~1.1 additional residues of glucuronic acid per molecule as well as ~1.5 fewer residues of *N*-sulfated glucosamine per molecule compared to the relatively inactive species. The reduction in *N*-sulfated glucosamine appears to be secondary to a concomitant increase in *N*-acetylated glucosamine.

The structural differences between the two heparin species were further defined by nitrous acid degradation of the respective polymers and subsequent analysis of the resultant fragments. The peak 1 fraction derived from either mucopolysaccharide form contained two species. One was a component (termed 1β) that has equimolar amounts of iduronic acid and glucuronic acid and has anhydromannitol at its reducing end. The internal glucosamine residue is most probably *N*-acetylated because neither low pH nor high pH nitrous acid treatment cleaved this species to any great extent (11, 12). Within the highly active fraction, there is sufficient 1β tetrasaccharide so that each molecule may contain this structure. In the relatively inactive preparation only enough of this fragment is present for one-fifth of these molecules to possess the 1β sequence. The other species (termed 1α) was obtained in small amounts and has iduronic acid as the predominant uronic acid and anhydromannitol at its reducing end. We suspect that the generation of a 1α tetrasaccharide represents a minor side reaction of the nitrous acid degradation (11, 12).

Peaks 2 and 3 isolated from both heparin species were composed of disulfated iduronsyl disaccharide, monosulfated iduronsyl and glucuronsyl disaccharides, and sulfated anhydromannitol as well as components that contain hexitol as an end group. Both forms of the mucopolysaccharide contained equivalent amounts of the latter two species (not shown). The relatively inactive heparin fraction exhibited a 7% increase in the level of disulfated iduronsyl disaccharide (peak 2) and a 5% increase in the amount of monosulfated disaccharides (peak 3) compared to highly active species. Furthermore, peak 3 derived from the relatively inactive material contained an increased amount of iduronic acid. This latter finding may be attributable either to the occurrence of additional monosulfated iduronsyl disaccharide or to an alteration in the composition of the other species present within this peak.

Thus, the significant decrease in the 1β tetrasaccharide sequence within the relatively inactive heparin is largely compensated for by a proportional increase in monosulfated and disulfated disaccharides. A simple structural change could account for most of the differences between the two heparin species. This would consist of the replacement of $\simeq$0.8 residue of glucuronic acid and an adjacent *N*-acetylglucosamine in the 1β tetrasaccharide sequence of the highly active heparin by iduronic acid and *N*-sulfated glucosamine in the relatively inactive material. In this case, the relatively inactive mucopolysaccharide would exhibit an increase in its content of iduronic

acid and *N*-sulfated glucosamine as compared to the highly active species. This is in reasonable accord with data in Table 1. Furthermore, nitrous acid degradation of the relatively inactive heparin should result in the liberation of additional monosulfated and disulfated iduronsyl disaccharides. Whereas data in Table 2 show the expected increase in disulfated iduronsyl disaccharide, they do not demonstrate that the increased levels of monosulfated disaccharide are attributable to the iduronsyl disaccharide component. The development of chromatographic systems capable of resolving all of the species present within peak 3 would be required to settle this issue.

The two heparin species were further examined by Smith degradation of the respective polymers and subsequent gel filtration of the resultant fragments. These studies indicate that the mean distance between nonsulfated uronic acid residues of the highly active fraction is smaller than that separating similar residues of the relatively inactive species. Furthermore, a larger number of nonsulfated uronic acid residues of the highly active material appear either to be present in a restricted region of the molecule separated only by glucosamine groups or to be located at penultimate positions within the polysaccharide chain. At present, we cannot be certain that glucuronic acid is the only uronic acid being cleaved during the degradative process because occasional iduronic acid residues may be nonsulfated. Thus, the different patterns obtained for the two heparin species may either reflect the increased number and differing distribution of glucuronic acid residues with the highly active fraction, or they may represent a similar alteration in total nonsulfated uronic acid residues.

In summary, our studies would suggest that the 1β tetrasaccharide sequence represents a critical structural element required for anticoagulant activity. It should be emphasized that either the presence of the *N*-acetyl group or the alteration of the two uronic acid types may be of importance. However, $\simeq$20% of the molecules within the relatively inactive fraction have the 1β tetrasaccharide sequence and yet this preparation exhibits only $\simeq$1% of the anticoagulant potency of the highly active species. These observations indicate that the presence or absence of some additional structural feature may be required for biologic activity and that this feature is either included in or missing from the small number of relatively inactive molecules that appear to have the 1β tetrasaccharide sequence.

In this regard, three possible models come to mind. First, the 1β tetrasaccharide of the relatively inactive species may be subtly different from that of the highly active fraction. Alterations in bond configuration, inversions in uronic acid placement, changes in substituent position, etc. would probably escape detection by the methods used. Second, the position of the 1β tetrasaccharide within the polysaccharide chain may be of critical importance. Sufficient numbers of trisulfated iduronic acid disaccharide sequences on either side of the 1β tetrasaccharide may be required for biologic activity. If such is the case, the 1β tetrasaccharide sequence within the relatively inactive fraction may be located too close to one or the other end of the molecule. Third, an additional group on the polysaccharide chain may be positioned at a specific distance from the 1β tetrasaccharide. This structural feature might either endow the 1β tetrasaccharide with greater biologic activity or severely diminish its expression. Elements that might act in this fashion would include glucuronic acid, nonsulfated iduronic acid (if it exists), or monosulfated disaccharides. Indeed, the nitrous acid as well as the Smith degradation studies clearly indicate that the highly active and relatively inactive heparin species differ with respect to relative abundance of monosulfated disaccharides and distribution pattern of nonsulfated uronic acids.

Major portions of the primary structure of highly active heparin must be determined in order to establish which of these alternate models is correct. This level of analysis has not been attempted with other mucopolysaccharides because of their inherent heterogeneity. Given the relative homogeneity of our highly active material, it may be possible to attain this objective. However, this undertaking will probably require the development of new techniques analogous to those currently available for sequencing proteins and nucleic acids.

The authors thank Professors S. Stivala and S. Lasker for providing heparins of known molecular weights. This work was supported by grants from the National Institutes of Health (IIL-19131) as well as from Riker Laboratories. R.D.R. is a recipient of an Established Investigatorship from the American Heart Association.

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