STRUCTURAL STUDIES ON HEPARIN AND HEPARITIN SULFATE

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Earlier studies indicated that in heparin as well as in heparin monosulfate (heparitin sulfate) fractions the uronosyl hexosamine linkages are 1⟶4 or 1⟶6 (Cifonelli and Dorfman, 1958, 1960). On the basis of periodate oxidation, Velluz (1959) reported this linkage to be 1⟶4. Studies in this laboratory of fragments obtained by graded acid hydrolysis showed a positive Morgan-Elson reaction. Similar results have been reported by Danishefsky et al. (1960). Since 1⟶4 substitution of hexosamines blocks the Morgan-Elson reaction, it was concluded that the hexosamine moiety of heparin is substituted in position 6. However, in the application of the Morgan-Elson reaction for linkage analysis of oligosaccharides, it is observed that the use of Na_2CO_3 may produce chromogens even if the acetylated hexosamine is not in a reducing terminal position (Kuhn, et al., 1954; Aminoff, et al., 1952). The use of Na_2CO_3 on the oligosaccharide fractions from both heparin and heparin monosulfate resulted in anomalously high acetylhexosamine values, indicating that the uronic acid reducing ends were cleaved during the Morgan-Elson reaction. Interpretation of linkage position therefore may be subject to error under these conditions. Replacement of Na_2CO_3 with borate (Reissig, et al., 1955; Levvy and McAllan, 1959) gave a reagent which did not cleave uronic acid reducing groups when applied to heparin oligosaccharides containing no detectable hexosamine reducing groups.

For the study of the structure of heparin, hydrolysis with 2 N HCl was carried out for 3 hours. Oligosaccharides were isolated by adsorption of the hydrolysate on charcoal followed by elution with dilute ethanol. This frac-

tion (Hep 0), which represented 80-90% of the starting material was chromatographed on paper with ter-amyl alcohol, isopropanol, water, 4:1:1.5. Analyses of the separated fractions are shown in Table I. The fractions vary in mobility; the rapidly migrating fraction (p3) appears to be disaccharide in nature on the basis of the ratio of reducing groups to total hexosamine. Comparison of terminal reducing hexosamine to total hexosamine suggests a large portion of the mixture contains reducing terminal uronic acid.

Table I

Linkage Analysis of Heparin Oligosaccharide Fractions

Fraction	Uronic acid[a]	(Elson-Morgan) Hexosamine[b]		O.D.$\frac{540}{510}$	(Morgan-Elson) N-acetyl-hexosamine[c]	Reducing group (as glucose)[d]
		Total	Reducing end			
Hep 0	1.40	1.00	0.35	1.30	0.29	
p1	1.42	1.00	0.30	1.32	0.21	
p2	1.18	1.00	0.39	1.26	0.31	.54
p3	1.63	1.00	0.25	1.28	0.15	1.10

[a] Analyzed by carbazole method, Dische (1947).

[b] All analyses are based on molar ratios with total hexosamine arbitrarily taken as 1.00. Method described by Boas (1953) used.

[c] Determined by modification of method by Levvy and McAllan (1959).

[d] Method described by Park and Johnson (1949).

The relationship of the N-acetylhexosamine values (Morgan-Elson) to the free reducing hexosamine values (Elson-Morgan) indicates that a substantial portion of the hexosamine residues are not substituted in the 4 position. Earlier studies have ruled out 1⟶3 substitution (Cifonelli and Dorfman, 1958). The fact that the values for N-acetylhexosamine are lower than those for free reducing hexosamine suggests that 1⟶4 as well as 1⟶6 linkages occur in heparin.

Heparin monosulfate is similar to heparin (Wolfrom, et al., 1950) in rate of hydrolysis and in yield of oligosaccharides with uronic acid occupying most

of the reducing ends (Cifonelli and Dorfman, 1960). The oligosaccharides shown in Table II were isolated by the use of charcoal in the same way as for the heparin hydrolysate. Sa and Sb represent two separate heparin monosulfate preparations which differ little in composition, each having approximately 1/3

Table II

Linkage Analysis of Heparitin Sulfate Oligosaccharide Fractions

Fraction	Uronic acid[a]	(Elson-Morgan) Hexosamine[b]		O.D. $\frac{540}{510}$	(Morgan-Elson) N-acetyl-hexosamine[c]	Reducing group (as glucose)[d]
		Total	Reducing end			
Sa	1.65	1.00	0.20	1.40	0.07	0.84
Sb	1.70	1.00	0.21	1.40	0.09	0.86

[a] Analyzed by carbazole method, Dische (1947).

[b] All analyses are based on molar ratios with total hexosamine arbitrarily taken as 1.00. Method described by Boas (1953) used.

[c] Determined by modification of method by Levvy and McAllan (1959).

[d] Method described by Park and Johnson (1949).

of the hexosamine units containing N-sulfate groups. The results indicate that heparin monosulfate fractions (Table II) are similar to heparin with respect to the presence of uronosyl 1 ⟶ 6 hexosamine linkages. However, the heparin monosulfate fractions demonstrate lower acetylhexosamine values when compared to the reducing hexosamine values, indicating a larger proportion of uronosyl 1 ⟶ 4 hexosamine linkages. The results therefore suggest the possibility of branching in both mucopolysaccharides. The major linkage in heparin is uronosyl 1 ⟶ 6 hexosamine while the major linkage in heparin monosulfate is 1 ⟶ 4. This finding is consistent with the results of Linker and Sampson (1960) who have suggested branching in heparin monosulfate on the basis of enzymic studies.

Further studies were carried out to identify the uronic acid component of heparin. For this purpose heparin was desulfated with 1 N HCl for 2 hours

and then N-acetylated. This product was esterified and reduced with sodium borohydride in the cold. After a second esterification and reduction there was a two-thirds decrease in uronic acid as measured by the Dische carbazole reaction and a corresponding increase in neutral sugar. Following hydrolysis with 1 N HCl for 3 hours, paper chromatography revealed that 10-20% of the product had been hydrolyzed to a compound which migrated as free glucose. The monosaccharide was further identified as glucose by reaction with glucose oxidase. These results indicate that the uronic acid of heparin is probably glucuronic acid. These findings agree with those of Wolfrom and Rice (1946).

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