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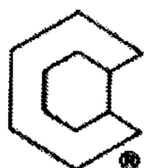

HEPARAN SULFATES

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Heparan sulfate, otherwise known as heparitin sulfate or heparin monosulfate, is a generic terr describing polysaccharides which are linear and consist of N-acetylated [->4)alpha-D-GlcNpA (1->4)°-D-GlcAp(1->] and N-sulfated disaccharides [->4)alpha-D-GlcNpS-(1->4)-°-D-GlcAp alpha-L-IdoAp(1->] that are arranged mainly in a segregated manner.
The sulfate-rich fractions (Heparan HQ and Heparan HR) in heparan sulfate (Heparan HS) are heparin-like, though they rarely possess the sulfate density found in heparin.
Approximately 25% of the total polymer is initially formed by alternating arrangements of the tw disaccharide units, ->4)alpha-D-GlcNpS (1->4)UAp(1->4)alpha-D-GlcNpAc(1->4)UAp (1->4)-alpha-D-GlcAp(1->. The polymer is formed as a repeating ->4)alpha-D-GlcNpAc(1->4)-°-D-GlcAp(1-> disaccharide sequence that is attached to a serine residue of a core protein througl a tetrasaccharide, glucuronosyl->galactosyl->galactosyl->xylosyl, linkage region. It then undergoes partial N-deacetylation followed by N-sulfation of the newly exposed amino groups, partial C-5 epimerization of D-GlcAp to L-IdoAp and O-sulfation. O-sulfates are always found proximity to N-sulfates which enhances the clustering of the sulfate residues and the heterogeneity in chemical composition and charge density of heparan sulfate.

Specifications

	Heparan HS	*Heparan HO*	*Heparan HT*	*Heparan HQ*	*Heparan HI*
Cationic Counterion	Sodium	Sodium	Sodium	Sodium	Sodium
Specific Rotation, °	+66	> +70	> +65	> +60	> +60
Mean MW, kD	10	15 ± 2	9 ± 1	7 ± 1	7 ± 1
Total Sulfur, %	8.8	4.5 - 6.5	6.0 - 8.5	7.5 - 9.5	8.5 - 10.5
Nitrogen, %	2.2	1.7 - 2.5	1.9 - 2.3	1.7 - 2.1	1.7 - 2.1
Anti-Xa, u/mg	< 30	< 10	< 30	> 30	> 100
Anti-IIa, u/mg	< 30	< 10	< 30	> 30	> 100

Ordering Information

Unit Measure	*Heparan HS*	*Heparan HO*	*Heparan HT*	*Heparan HQ*	*Heparan HI*
10 mg	HS-03011	HO-03101	HT-03201	HQ-03301	HR-03401
100 mg	HS-03012	HO-03102	HT-03202	HQ-03302	HR-03402
1 gram	HS-03013	HO-03103	HT-03203	HQ-03303	HR-03403
10 grams	HS-03014	HO-03104	HT-03204	HQ-03304	HR-03404
100 grams	HS-03015	HO-03105			HR-03405
bulk	HS-3015	HO-3105			HR-3405

References

1. Linhardt RJ, Hileman RE, Griffin CC, Brown SE, Schubert RL, Van Gorp CL. Characterization of a heparan sulfate fraction (CL-03105). Thromb Haemost 1995; 73

(6):1352.
2. Griffin CC, Linhardt RJ, Van Gorp CL, Toida T, Hileman RE, Schubert RL, Brown SE. Isolation and characterization of heparan sulfate from crude porcine intestinal mucosa peptidoglycan. Carbohydr Res 1995; 276:183-197.
3. Schwartz D, Yang L, Ghigliotti G, Griffin C, Van Gorp C, Abenschein D, Eisenberg P. Efficacy of Heparan Sulfate in Inhibiting Vascular Wall Procoagulant Activity after Arteria Injury and Neointimal Thickening. AHA, submitted.

Reference Abstracts

1. Linhardt RJ, Hileman RE, Griffin CC, Brown SE, Schubert RL, Van Gorp CL. Characterization of a heparan sulfate fraction (CL-03105). Thromb Haemost 1995; 73 (6):1352.
   Heparan Sulfate (CL-03105) is obtained by charge fractionation of heparin and dermatan sulfate - depleted porcine intestinal mucosal peptidoglycan heparin. It has an average molecular weight of approx. 15,500 Daltons, an optical rotation of +72ﾕ, and as compared heparin, has no significant ATIII-mediated anti-Factor IIa or anti-Factor Xa activity. CL-03105 was characterized as a heparan sulfate by disaccharide analysis (digestion wit heparin lyases I, II and III; analysis by capillary electrophoresis) and by NMR. Electrogram: clearly demonstrate that CL-03105 affords primarily UA->GlcNAc product and is resistaı to degradation by heparin lyase I, consistent with its being a heparan sulfate. The disaccharide analysis showed UA->GlcNAc to be the major disaccharide present (75.7%) followed by UA-> GlcNS (14.1%) and UA->GlcNAc6S (4.4%). Polyacrylamide gel electrophoresis (PAGE) demonstrates that treatment of CL-03105 with heparin lyase I and III is an effective tool to distinguish a heparan sulfate from heparin and to determine its degree of purity.

2. Griffin CC, Linhardt RJ, Van Gorp CL, Toida T, Hileman RE, Schubert RL, Brown SE. Isolation and characterization of heparan sulfate from crude porcine intestinal mucosa peptidoglycan. Carbohydr Res 1995; 276:183-197.
   A method for the preparation of heparan sulfate from peptidoglycan heparin is described. The objective of this research was to provide a basis for the development and validation an industrial process to support the preclinical development of heparan sulfate and/or heparan sulfate derivatives. In the preparation of heparan sulfate, heparin was recovered by alcohol fractionation and dermatan sulfate was isolated by selective precipitation. The remaining crude heparan sulfate was fractionated by anion-exchange chromatograpł into five subfractions. The biological activities of these subfractions were examined by anticoagulant and amidolytic assays. Molecular weight and molecular size were determine using capillary viscosimetry and polyacrylamide gel electrophoresis.
   Charge density and degree of sulfation were determined by cellulose acetate electrophoresis and elemental analysis. Oligosaccharide and disaccharide analysis relied (enzymatic depolymerization using heparin lyases followed by polyacrylamide gel and capillary electrophoresis. Proton NMR analysis provided detailed structural information oı each subfraction.

3. Schwartz D, Yang L, Ghigliotti G, Griffin C, Van Gorp C, Abenschein D, Eisenberg P. Efficacy of Heparan Sulfate in Inhibiting Vascular Wall Procoagulant Activity after Arteria Injury and Neointimal Thickening. AHA, submitted. The heparan sulfate, CL-03405 (HR) has been shown to have antiproliferative activity in cultured smooth muscle cells and antithrombin-dependent anticoagulant activity.

   Method:

   We characterized the anticoagulant and antiproliferative activity of 1 mg/kg HR iv and then sc every 12 hours for 14 days after balloon overinflation injury to the aorta in New

Zealand White rabbits. Bound thrombin (IIa) and Xa/Va activity was characterized at 4 or 24 hours after injury in two subgroups (n=6). IIa activity was characterized by increases i fibrinopeptide A (FPA) induced by incubating isolated washed injured aortic segments wit plasma depleted of vitamin K-dependent factors by barium adsorption (BaPlasma). Xa/Va activity was determined by measuring additional increases in FPA after addition of 0.9オM prothrombin to the BaPlasma. Verhoff's van Geisson stained aortic sections were digitall analyzed at 14 days (n=5) and the ratios of intimal to medial (I:M) area calculated and compared with injury alone.

Result:

FPA (ng/ml/cm sq, mean+SD)[*p<0.01 vs with injury alone]

	IIa@4 h	*XA/Va@4 h*	*IIa@24 h*	*XA/Va@24 h*
Injury Alone	306 ± 139	147 ± 67	268 ± 367	211 ± 135
Heparan HR	65 ± 24*	106 ± 65	15 ± 20	26 ± 35*

Mean I:M ratios were 0.3 ± 0.1 injury alone vs 0.2 + 0.1 HR ($p < 0.02$)

Conclusion:

Thus, HS inhibits bound IIa activity early and both IIa and Xa/Va by 24 hours. HR also inhibits neointimal thickening by 30%.

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