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Iduron technical bulletin - Bacterial Heparinase Enzymes
Heparin and Heparan Sulfate Lyases from *Flavobacterium heparinum*

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Heparin and Heparan Sulfate Lyases from *Flavobacterium heparinum*

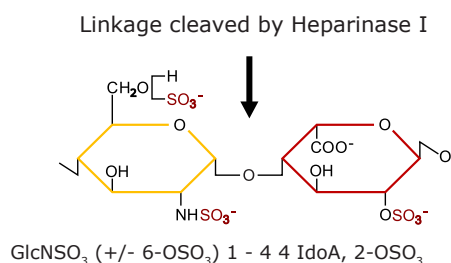
Untapped potential for structural analysis

Bacterial heparinases are eliminases that cleave the glycosidic linkage between amino sugars and uronic acids in heparin and heparan sulfate (HS). At the site of cleavage these enzymes create a C4-C5 double bond in the uronic acid that absorbs at 232nm and can be used for the detection of degradation products. When used in combination, heparinases bring about a near-complete depolymerisation of HS or heparin to constituent disaccharides. However Heparinase I and Heparinase III have restricted substrate specificities that can be exploited for more detailed characterisation of the molecular structure and intra-chain sequences in HS and heparin.

Heparinase I (Hep I) EC 4.2.2.7

Degrades heparin and the S-domains of heparan sulfate

Catalyses the eliminative scission of the glycosidic linkage between N-sulfated glucosamine (GlcNSO₃) and 2-O-sulfated iduronic acid (IdoA, 2SO₃). The IdoA, 2SO₃ residue is essential for the activity of Heparinase I while 6-O-sulfation of GlcNSO₃ enhances enzyme activity.



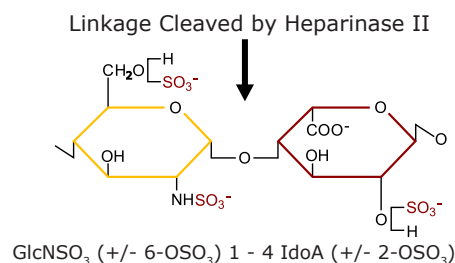
Technical Notes

The disaccharide cleaved by Heparinase I (shown above) is common in heparin but it is quite rare in HS where it is confined to the sulfated domains (S-domains - see fig. 1). Thus Heparinase I will attack HS only in the S-domains bringing about limited scission of the polymer chain. This is a very valuable and under exploited property of Heparinase I. For example Heparinase I can be used to determine the spacing between S-domains in HS. This spacing is reflected in the size of the HS fragments that resist the action of the enzyme. Heparinase I can also be used to identify biological properties of HS that depend upon the integrity of the S-domains. For example HS-activation of fibroblast growth factor 2 (FGF2) is inhibited by treatment with Heparinase I (but not by Heparinase III).

Heparinase II (Hep II) EC number not assigned

Degrades heparin and heparan sulfate

Catalyses the eliminative scission of the glycosidic linkages between N-sulfated or N-acetylated glucosamine and glucuronic or iduronic acid. This is a broad activity enzyme that tolerates O-sulfation of the uronic acid and glucosamine residues.



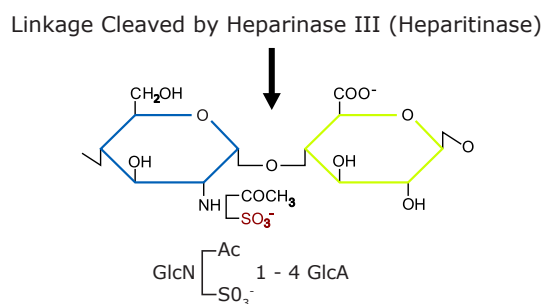
Technical Notes

This low specificity enzyme attacks most linkages in heparin and HS but it does not effect complete depolymerisation of either GAG. It is very useful for analysis of disaccharide composition but its effectiveness is enhanced when used in combination with Heparinase I and Heparinase III. Heparinase II will cleave heparin and HS at N-unsubstituted glucosamine (GlcNH₃⁺) residues; in contrast the GlcNH₃⁺-GlcA (or IdoA) linkage is resistant to Heparinase I and Heparinase III.

Heparinase III (Hep III/Heparitinase) EC 4.2.2.8

Degrades heparan sulfate (HS)

Catalyses the eliminative scission of glycosidic linkages between N-sulfated or N-acetylated glucosamine (GlcNSO₃ or GlcNAc) and glucuronic acid (GlcA)



Technical Notes

Heparinase III (Heparitinase) acts on Heparan Sulphate in regions of low sulfation and it has little activity against heparin. The preferred substrates for heparinase III are N-Acetylated or N-sulfated glucosamines linked to glucuronic acid (i.e. GlcNAc/ GlcNSO₃ 1 - 4 GlcA). In consequence it acts primarily in the non-sulfated domains (NAc domains) and transition zones of HS (see fig. 1) whereas the highly sulfated S-domains are resistant to Heparinase III. The enzyme tolerates C-6 sulfation of amino sugars. Heparinase III has weak activity with disaccharides containing Iduronic Acid (IdoA) and its action is blocked if the iduronate residue is sulfated at C-2 (IdoA, 2SO₃).



Heparinase III continued...

The Heparinase III resistant S-domains in HS have a heparin-like structure being mainly composed of GlcNSO_3 1 - 4 IdoA_2SO_3 repeat units with variable O-sulfation at C-6 of the GlcNSO_3 residue. They vary in length from 3 to 9 disaccharide units. The patterns and densities of 6-O-sulfate groups along S-domains are an important determinant of their protein binding specificities.

The selectivity of Heparinase III can be exploited for the preparation of S-domains from HS. High resolution gel filtration will separate the different size classes of S-domain and these can be further subfractionated by SAX-HPLC according to sulfate content and patterning. In this way a considerable amount of information can be acquired about the structure of HS. Isolated S-domains can be used for protein binding or bioactivity studies or for structural analysis by NMR. New developments in mass spectrometry are being applied to the analysis of composition and sequence of GAG fragments including HS S-domains and heparin oligomers. These methods hold great promise for thorough structural elucidation of HS/heparin and other GAGs.

References

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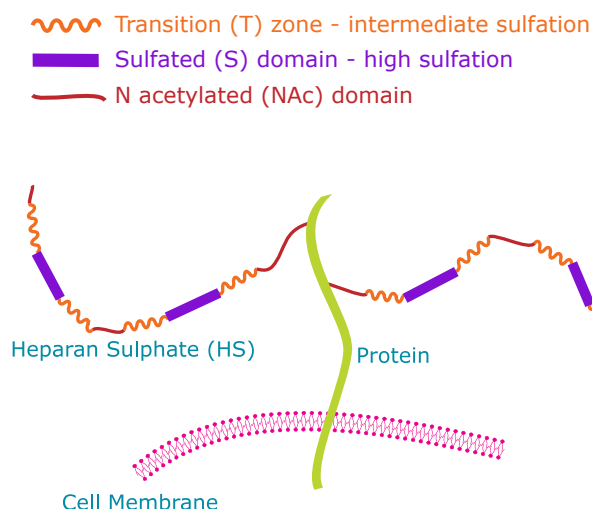
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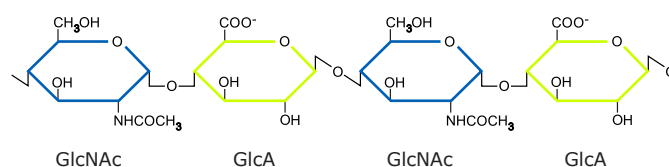
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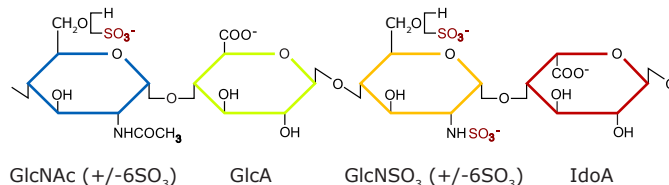
Fig. 1 Domain Structure of Heparan Sulphate



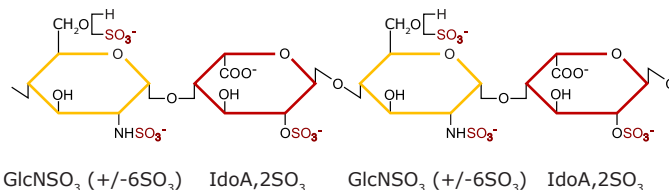
N-Acetylated Domains (NAc domains): degraded by Heparinase III



Transition Zones (T-zones): degraded by Heparinase III



Sulphated Domains (S-domains): degraded by Heparinase I



Three structural domains can be identified in heparan sulphate on the basis of their disaccharide sequence and sulphation patterns:

- N-acetylated (NAc) domains: repeating sequences of N-acetylated disaccharides, these domains are not modified by sulphation.
- Transition (T) zones: alternating sequences of N-acetylated and N-sulphated disaccharides, variable O-sulphation at C6 of the glucosamine residues, these regions are not sulphated at C2 of the iduronic acid (IdoA) residue.
- Sulphated (S-) domains: internal repeating sequences of GlcNSO_3 (+/-6SO₃) and IdoA_2SO_3 , variable O-sulphation at C6 of the N-sulphated glucosamine residues and occasional O-sulphation at C3 of glucosamine.

