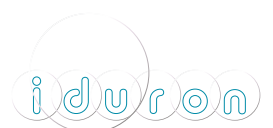


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## Iduron technical bulletin - K5 Heparan Lyase

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Not for clinical, diagnostic or therapeutic applications.

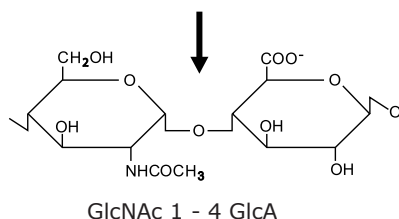


### K5 Heparan Lyase:

- Unique substrate specificity, novel applications.
- Acts exclusively in the non-sulfated domains of heparan sulfate.
- Enables excision of entire sulfated regions in heparan sulfate.
- Highly stable enzyme.

K5 Heparan Lyase catalyses the eliminative scission of the glycosidic linkage between N-acetylated glucosamine (GlcNAc) and glucuronic acid (GlcA). N-sulfation of the glucosamine residue blocks the activity of the enzyme.

Linkage cleaved by K5 Heparan Lyase



This enzyme was first identified in the tail region of the bacteriophage K5. It plays an important role in phage infectivity by degrading the capsular polysaccharide coat of its host *E. coli* K5. This polysaccharide coat is a glycosaminoglycan consisting of **GlcNAc 1-4 GlcA 1-4** disaccharide repeat units, a structure that is identical to the non-sulfated domains (NAC domains) of heparan sulfate (HS); these domains can extend for up to 10-12 disaccharide units in length.

K5 Heparan Lyase scissions HS only in the NAC domains. This unique substrate specificity of the enzyme can be exploited for the excision of the entire sulfated regions of HS (S-domains and transition (T)-zones, see figure 1 and 2). The sulfated regions of HS are areas of intense biological activity but investigations of their specific properties have been hampered by the lack of a suitable method of isolation. K5 Heparan Lyase meets this need and it should find many applications in structure-function studies of HS.

The strict substrate specificity of K5 Heparan Lyase distinguishes it from heparinase III (heparitinase) which acts on HS in both the NAC domains and in T-zones. S-domains are the only sections of the GAG chain that resist heparinase III.

In common with the bacterial heparinases, K5 Heparan Lyase generates a C4-C5 double bond in the uronic acid at the site of cleavage which absorbs strongly at 232nm. This can be used to monitor progress of degradation of heparan sulfate, and of K5 polysaccharide, the latter being the natural substrate for the enzyme.

### K5 Lyase generated oligosaccharides separated on a Superdex Peptide column; substrate K5 Polysaccharide

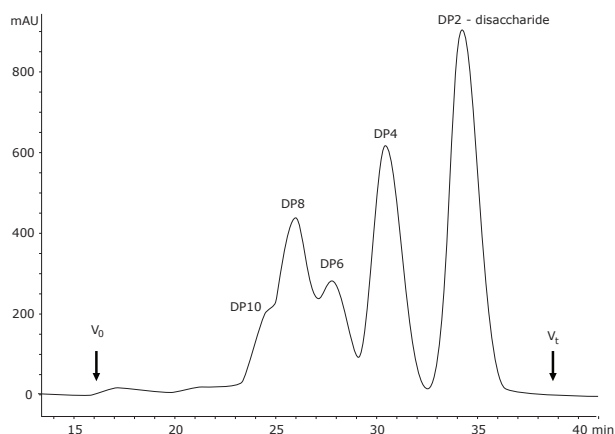
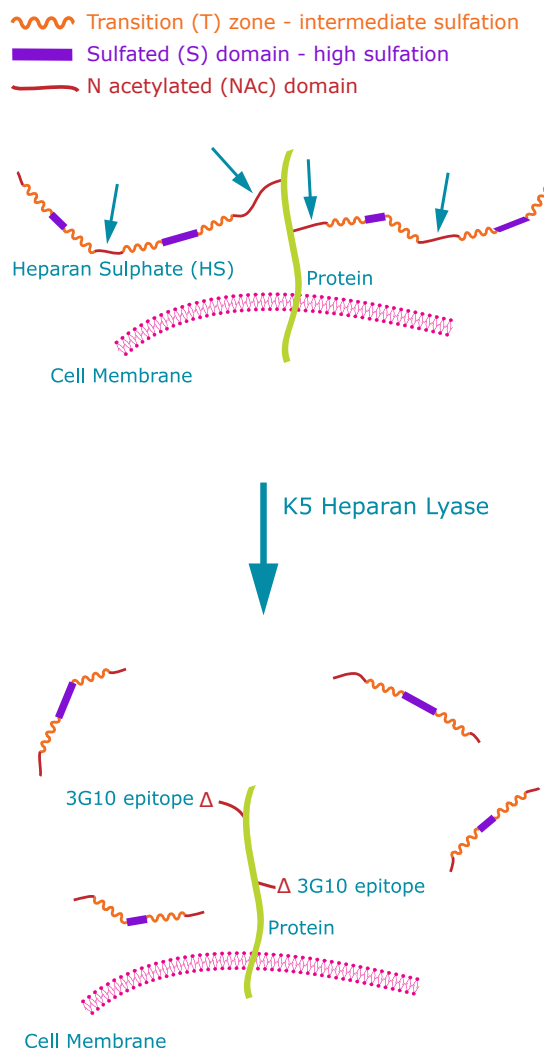


Fig 1 Cell Surface Heparan Sulfate: domain structure and cleavage sites for K5 Heparan Lyase



Heparan sulfate is attached to transmembrane or glycolipid-anchored membrane proteins (syndecans and glypicans respectively). The N- and O-sulfated disaccharides are grouped together in a series of domains or clusters in which two sub-regions can be identified. The most highly sulfated sections of the HS chains are the heparin-like S-domains that are flanked by sequences of intermediate sulfation called transition zones (T-zones). The sulfated regions of HS are separated by non-sulfated domains composed of N-acetylated disaccharides (NAC domains). The molecular structures of the domains in heparan sulphate are shown in Fig 2.

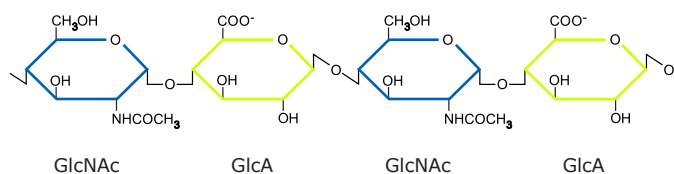
The NAC domains (non-sulfated) are sensitive to scission by K5 Heparan Lyase. Enzyme activity is restricted to these sites and leads to the excision of entire sulfated regions (i.e. S-domains plus T-zones).

K5 heparan lyase can efficiently remove heparan sulfate from proteoglycans because an NAC domain is present in close proximity to proteoglycan core proteins. The enzyme creates an unsaturated epitope in the stub region of heparan sulfate that remains attached to enzyme treated proteoglycans. The 3G10 antibody, which recognises this epitope, can be used in conjunction with FACS analysis to monitor loss of HS chains from cell surfaces.

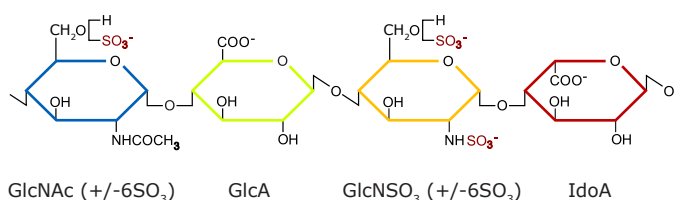


Fig. 2 Typical sequences of the structural domains in heparan sulphate.

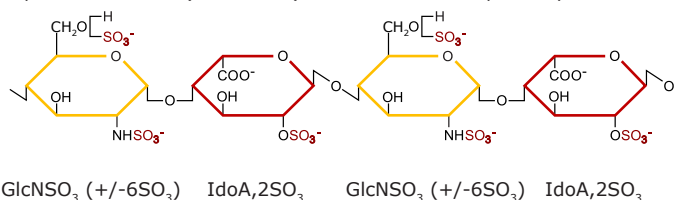
N-Acetylated Domains (NAc domains): degraded by K5 Heparan Lyase



Transition Zones (T-zones): resistant to K5 Heparan Lyase



Sulphated Domains (S-domains): resistant to K5 Heparan Lyase



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<sub>3</sub> (+/-6SO<sub>3</sub>) and IdoA,2SO<sub>3</sub>, variable O-sulphation at C6 of the N-sulphated glucosamine residues and occasional O-sulphation at C3 of glucosamine.