

## **Search for the heparin antithrombin III-binding site precursor**

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The last step of heparin biosynthesis is thought to involve the action of 3-O-sulfotransferase resulting in the formation of an antithrombin III (ATIII) binding site required for heparin's anticoagulant activity. The isolation of a significant fraction of heparin chains without antithrombin III-binding sites and having low affinity for ATIII suggests the presence of a precursor site, lacking the 3-O-sulfate group. Porcine mucosal heparin was depolymerized into a mixture of oligosaccharides using heparin lyase. One of these oligosaccharides was derived from heparin's ATIII-binding site. In an effort to find the ATIII-binding site precursor, the structures of several minor oligosaccharides were determined. A greater than 90% recovery of oligosaccharides (on a mole and weight basis) was obtained for both unfractionated and affinity-fractionated heparins. An oligosaccharide arising from the ATIII-binding site precursor was found that comprised only 0.8 mol % of the oligosaccharide product mixture. This oligosaccharide was only slightly enriched in heparin having a low affinity for ATIII and only slightly disenriched in high affinity heparin. The small number of these ATIII-binding site precursors, found in unfractionated and fractionated heparins, suggests the existence of a low ATIII affinity heparin may not simply be the result of the incomplete action of 3-O-sulfotransferase in the final step in heparin biosynthesis. Rather these data suggest that some earlier step, involved in the formation of placement of these precursor sites, may be primarily responsible for high and low ATIII affinity heparins.