PURIFICATION AND CLONING OF A 2-O-SULFOTRANSFERASE THAT RECOGNIZES THE NON-TRIVIAL DISSACHARIDE GlcNAc-IdoA.

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Sulfated proteoglycans are implicated in numerous pathophysiological phenomena in vertebrates and invertebrates. During biosynthesis of Heparin (Hep) and heparan sulfate (HS), sugar chains are polymerized by the action of glycosyltransferases and further modified by the action of N-deacetylases, N-sulfotransferases, C-5 epimerases and 2-, 6-, and 3-O sulfotransferases. These modifications occur throughout the chains producing regions of sulfation with modified and unmodified domains. Specific sulfation patterns are critical for the binding of HS chains to proteins. In this work, we report partial purification and molecular cloning of the membrane bound 2-O-sulfotransferase from the mollusk *Achatina fulica*. The structure of the main glycosaminoglycan in this invertebrate cannot be explained by the current biosynthetic theories ([alpha]-D-GlcNAc(1-4)-[alpha]-L-IdoA2S). Degenerated oligonucleotides from previous cloned sulfotransferases were used to amplify cDNA clones from *A. fulica* and *A. brasiliana* libraries. One clone with high similarity with described 2-O-sulfotransferases was found in *A. brasiliana*. No consistent cDNA clones were obtained from *A. fulica*,. Thus the enzyme, was purified using heparin-Sepharose and PAP-Agarose affinity chromatographies. The activity was assayed using 2-O-desulfated heparin and acharan sulfate and PAP[^{35}S] as donor. Peptide sequencing were obtained by mass spectrometry and used to design oligonucleotides. The understanding of how this sulfotransferase recognizes its substrate should shed light on the regulatory patterns in the biosynthesis of complex glycosaminoglycans. (FAPESP, CNPq, CAPES).