

Heparan/Heparin Sulfate Biosynthesis: Crystal Structure of β 1,3-glucuronyltransferase 1

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Introduction: Human β 1,3-glucuronyltransferase I (GlcAT-I) is a member of the large family of Golgi-membrane enzymes that form glycosidic bonds. GlcAT-I transfers a glucuronic acid moiety from the cofactor uridine diphosphate-glucuronic acid (UDP-GlcA) to the common linkage region trisaccharide Gal β 1-3Gal β 1-4Xyl linked to Ser residue at the glycosaminoglycan (GAG) attachment site of proteoglycan. We have determined the crystal structure of GlcAT-1 to 1.9 Å in the presence of the cofactor product UDP, the catalytic Mn²⁺ ion, and the substrate analog Gal β 1-3Gal β 1-4Xyl.

Methods and Materials: The coding region of the catalytic domain of GlcAT1 was amplified from human liver cDNA by PCR. Crystals of GlcAT-1 were obtained by the vapor diffusion hanging drop method. Data for the selenomethionene GlcAT-1 crystal were collected on Beamline X9B at Brookhaven National Laboratories. Phases for the electron density map were obtained from data collected at a single wavelength = 0.97892 Å representing the peak of the anomalous dispersion.

Results and Conclusions: The GlcAT-I structure is the first ternary complex of a glycosyltransferase reported. As expected, residues involved in UDP, Mn²⁺, and substrate binding are conserved in the most closely related glucuronyltransferase GlcAT-P (Fig. The enzyme is a single alpha/beta protein with two sub-domains that constitute the co-factor and substrate-binding site. The active site resides in a cleft that extends across both sub-domains. The conserved DXD motif (D194, D195, D196) has direct interactions with the ribose of the UDP molecule as well as with the divalent cation required for catalysis. Residues E227, D252, and E281 form hydrogen bonds with the terminal Gal saccharide with E281 in position to act as a catalytic base. These key residues involved in cofactor/substrate binding and catalysis are conserved in other glycosyltransferases.