

Crystal Structure of SULT2A3, Human Hydroxysteroid Sulfotransferase

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Beamline(s): **X9B**

Introduction: Sulfation plays a role in the metabolism, transport, and synthesis of steroids. Dehydroepiandrosterone (DHEA) is produced in adrenal and secreted as a DHEA sulfate (DHEA-S). DHEA sulfation is catalyzed by hydroxysteroid sulfotransferase, an enzyme that transfers the sulfonyl group of the co-factor 3'-phosphate 5'-phosphoadenosine sulfate (PAPS) to the 3-hydroxyl of DHEA. The crystal structure of SULT2A3 human hydroxysteroid sulfotransferase has been solved at 2.4 Å resolution in the presence of 3'-phosphoadenosine 5'-phosphate (PAP).

Methods and Materials: Crystals were grown by mixing equal volumes of SULT2A3 protein at 25 mg/ml in 25 mM HEPES pH 7.5 and 4 mM PAP with 0.8 M citrate and 80 mM cacodylate, titrated to pH of 5.75. These crystals (0.4 mm x 0.4 mm x 0.6 mm in size) belong to space group $P2_12_12_1$ with unit cell dimensions of 73.19 Å x 96.82 Å x 127.38 Å and diffracted to 2.4 Å. Data were collected at BNL on beam line x9B on crystals which had been transferred to 0.9M citrate, 90mM cacodylate, 4mM PAP, saturated DHEA, and 10% ethylene glycol. Phases for the structure factors were calculated using molecular replacement with the model built from the mouse estrogen sulfotransferase coordinates.

Results and Conclusions: The overall structure of SULT2A3 is comprised of a single α/β domain with a five-stranded parallel β sheet that constitutes the PAPS-binding site and catalytic center. A strand-loop-helix motif that contains the PSB-loop (41-TYPKSGT-47) forms specific hydrogen bond interactions with the 5'-phosphate of the PAP molecule. The 3'-phosphate binding site is comprised of interactions from residues along β -strand 8 and α -helix 6 (Arg121 and Ser129, respectively) as well as backbone interactions with the residues Lys248 and Gly249 near the carboxy terminus. SULT2A3 appears to catalyze an in-line sulfonyl transfer reaction that has been suggested for other SULT enzymes. His99 and Lys44 of SULT2A3 are in positions to assist in the deprotonation of the acceptor group and the dissociation of the sulfonyl group from PAPS, as has been proposed for other SULT enzymes. Thus, these structural features and reaction mechanism appear to be well conserved in all SULTs.