Structure and Function of Human Cytochrome P450 8B1

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Human Cytochrome P450 8B1 (CYP8B1) is involved in the conversion of cholesterol to bile acids. It specifically hydroxylates the steroid ring system at C12 to produce the bile acid cholic acid. As such, CYP8B1 activity controls the ratio of cholic acid over another bile acid called chenodeoxycholic acid. The ratio of these two bile acids controls the overall hydrophobicity of the bile pool and the signaling through the farnesoid X receptor (FXR). Recent studies knocking out CYP8B1 implicated this enzyme as a good drug target for nonalcoholic fatty liver disease and type 2 diabetes. However, there are no selective inhibitors known for this enzyme, which restricts the further study of this enzyme and potential disease treatment. There are also no structures of CYP8B1 to guide the development of such inhibitors. Herein the CYP8B1 protein was generated recombinantly in E. coli from a synthetic engineered gene, purified to homogeneity, and characterized. The resulting protein was catalytically active and displayed normal spectral features. Changes in the absorbance spectra were used to evaluate the binding of multiple ligands. The tight-binding non-selective CYP8B1 inhibitor tioconazole was identified and then used to solve the first available X-ray structure of CYP8B1 (Figure 1 A). This structure reveals the *(S)*-tioconazole imidazole nitrogen forming a coordinate covalent bond to the CYP8B1 heme iron, consistent with enzyme inhibition (Figure 1B). The ligand is close towards the B' helix due to the spatial hinderance of W281 and N286 on the opposite side of the active site (Figure 1B). The availability of the CYP8B1 active site architecture should aid structure-based design of inhibitors that selectively bind CYP8B1 vs. other closely related P450 enzymes. Selective inhibitors should promote a better understanding of the role of CYP8B1 inhibition in these normal physiology and disease states and provide a possible treatment for nonalcoholic fatty liver disease and type 2 diabetes.

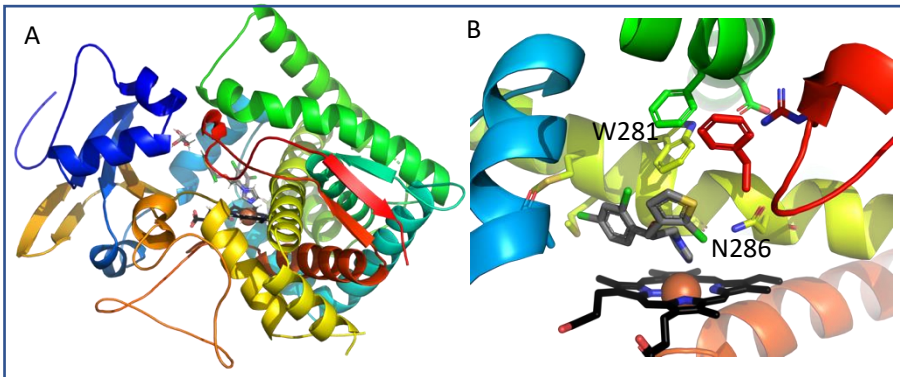

Figure 1. Structure of CYP8B1 with its non-selective inhibitor. A) Overall structure of CYP8B1 rainbow-colored from blue N terminus to the red C terminus. B) (S)-tioconazole binding.