A Bacterial BVMO Capable of Turning Over Human Flavin Containing Monooxygenase 3 (hFMO3) Substrates

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Baeyer-Villiger monooxygenases (BVMOs) are flavin-containing enzymes that mediate specific oxidations on the carbonyl moiety of substrates. These proteins have a great value in the conversion of ketones into the corresponding esters and lactones, bioremediation purposes and green chemistry applications such as the synthesis of chiral intermediates without the use of strong chemical oxidants as peracids. A novel BVMO gene from *Acinetobacter radioresistens* S13 was cloned in the expression vector pT7, successfully expressed in *E.coli* BL21 cells and purified in high yields (20mg/L culture) using anion exchange and Nickel affinity chromatography. The characterization showed a 57 kDa soluble protein with a tightly but non-covalently bound FAD that can be reversibly reduced by NADPH in anaerobiosis. Due to the large substrate specificity of BVMOs, a 3D homology model of this enzyme was generated in order to select its putative substrates. For this purpose, docking experiments were carried out using the HIC UP and Drug Bank databases (more than 10000 proteins' co-crystallized ligands). According to the energy output of the docking experiments, a selection of putative substrates of biotechnological interest (drugs and environmental pollutants) was screened by high-pressure liquid chromatography. The purified BVMO enzyme was observed to catalyze S- and N-oxidation of human flavin containing monooxygenase 3 substrates including ethionamide (antituberculosis drug), danusertib and tozasertib (anticancer drugs), with Km values in the micromolar range. This work reports on the various catalytic activities of this novel enzyme.