Poster Presentation

MS29.P12

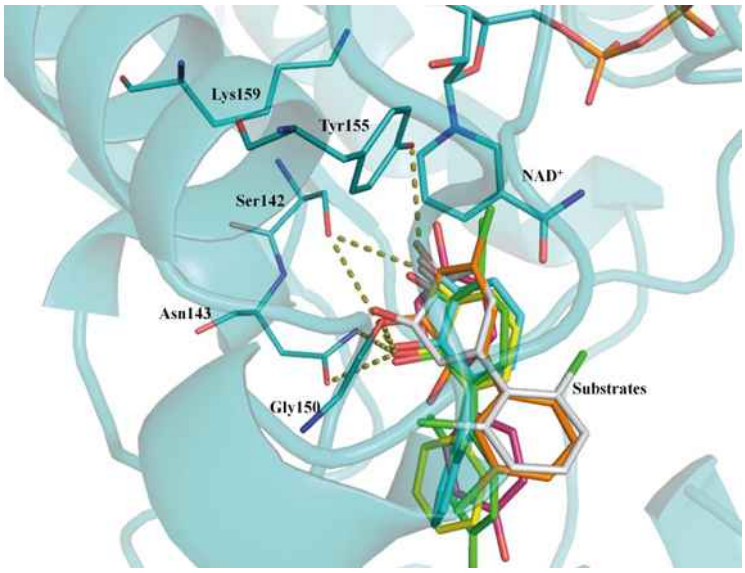
Structural insights into broad substrate range of biphenyl dioxygenases &dehydrogenase

S. Dhindwal[1], M. Sylvestre[2], P. Kumar[1]

[1]*Indian Institute of Technology Roorkee, Department of Biotechnology, Roorkee, India,* [2]*Institut National de Recherche Scientifique, Institut Armand-Frappier, Laval, QC, Canada*

Biphenyl dioxygenase(BphAE) & biphenyl dehydrogenase(BphB) catalyze first two steps of aerobic degradation of biphenyl & various polychlorinated biphenyls,PCBs. These enzymes are of interest for their potential to oxidize toxic pollutants & manufacture of fine chemicals. We have determined the crystal structures of two variants of BphAE from B. xenovorans LB400, viz BphAERR41 & BphAEp4, evolved by site directed mutagenesis. We have compared the structures of wild type BphAELB400 & BphAEp4. Biochemical properties of BphAELB400 variants with single substitutions, T335A or F336M shows that residue 336 contacts biphenyl & influences regiospecificity of the reaction, but do not enhance enzyme's reactivity toward 2,6-dichlorobiphenyl. However, residue 335 does not contact biphenyl but contributes significantly to expand enzyme's substrate range. Crystal structure of variant BphAERR41 explains the transformation of dibenzofuran & 2-chlorodibenzofuran. This study provides structural insight for expanded substrate range of Rieske-type oxygenases through mutations that enhance the plasticity and/or mobility of enzyme segments lining the catalytic site. Enzyme, BphB catalyze 2nd step of PCB degradation pathway. We have determined the crystal structure of apoenzyme, binary complex with NAD+ & ternary complexes with NAD+2,3-dihydroxybiphenyl from P. pnomenusa B356. A crystal structure representing an intermediate state of the enzyme was also obtained where the substrate binding loop was ordered in comparison with apo & binary forms but was displaced significantly w.r.t ternary structure. These structures reveal that the substrate binding loop is highly mobile & changes conformation during ligand binding, starting from a disorganized loop in apo state to a well organized loop in ligand-bound form. Conformational changes are induced during ligand binding; forming a well defined cavity to accommodate wide range of substrates. For the 1st time, a combination of structural, biochemical, & molecular docking studies of BphBB356 elucidate the unique ability of enzyme to transform the cis-dihydrodiols of double meta-, para-, & ortho-substituted chlorobiphenyls.

[1] *P. Kumar, M. Mohammadi, J. Viger et al., Journal of Molecular Biology, 2011, 405.2, 531-547,* ***[2]*** *S. Dhindwal, D.N. Patil, M. Mohammadi et al., Journal of Biological Chemistry, 2011, 286.2, 37011-37022,* ***[3]*** *P. Kumar, M. Mohammadi, S. Dhindwal et al., Biochemical and Biophysical Research Communications, 2012, 421.4, 757-762*



Keywords: Enzyme mechanism, Bioremediation, Enzyme complex