Homology modeling and docking studies of *Comamonas testosteroni* B-356 biphenyl-2, 3-dioxygenase involved in degradation of polychlorinated biphenyls

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Introduction

Biphenyl dioxygenase is a microbial enzyme which catalyzes the stereospecific dioxygenation of aromatic rings of biphenyl congeners leading to their degradation. Hence, it has attracted the attention of researchers due to its ability to oxidize chlorinated biphenyls, which are one of the serious environmental contaminants. In the present study, the three-dimensional model of α-subunit of Biphenyl dioxygenase (BphA) from Comamonas testosteroni B-356 has been constructed. The resulting model was further validated and used for docking studies with a class of chlorinated biphenyls such as biphenyl, 3, 3'-dichlorobiphenyl and 4, 4'-dichlorobiphenyl. The kinetic parameters of these biphenyl compounds were well matched with the docking results in terms of conformational and distance constraints. The binding properties of these biphenyl compounds along with identification of critical active site residues could be used for further site-directed mutagenesis experiments in order to identify their role in activity and substrate specificity, ultimately leading to improved mutants for degradation of these toxic compounds.

Homology modelling of *Comamonas testosteroni* B-356 biphenyl - 2, 3-dioxygenase

The 3D model of α-subunit of BphA from Comamonas testosteroni B-356 was built by homology modeling using high-resolution crystal structures of Cumene dioxygenase (cumA1A2) from Pseudomonas fluorescens IP01 (PDB ID: 1wql) as a template. The automated homology modeling software MODELLER 7v7 on windows operating environment (**http://salilab.org**) was used to generate five 3D models of α-subunit of BphA.

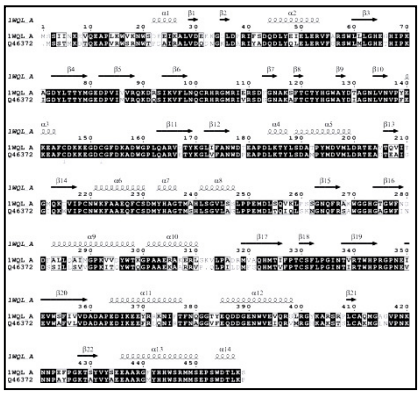

Fig.1 Sequence alignment of BphA (Q46372) with CumA1A2 (1WQL). Conserved regions are represented by black boxes. The secondary structure for CumA1A2 is demonstrated with arrows for β-sheet and spiral for α-helices.

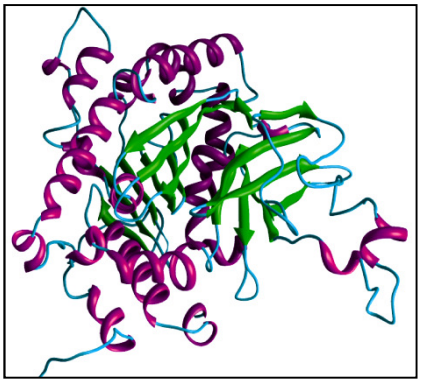

Fig.2 The 3D structure of BphA. The α-helix is represented by magenta spirals, β-sheet by green arrows.

Model validation

Backbone conformation of constructed model was evaluated by the inspection of the Psi/Phi Ramachandran plot obtained from PROCHECK analysis (**Laskowaski et al.,1993**).

The PROSA (**Sippl, 1993**) test was applied to check for energy criteria in comparison with the potential of mean force derived from a large set of known protein structures. The root mean square deviation (RMSD) between the main chain atom of model and template was calculated by structural superimposition of template (1wql).

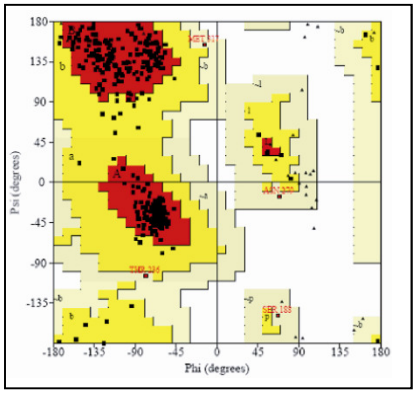

Fig.3 Ramachandran plot of BphA model built using NCBI Structural Analysis and Verification Server.

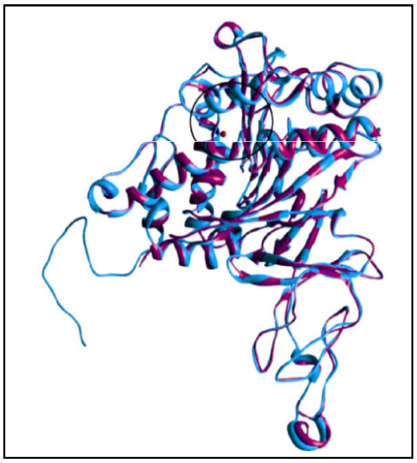

Fig.4 Superimposed backbones of BphA model (cyan) and the template CumA1A2 (Purple). Active site residues have been encircled

Active site of *C.testosteroni* biphenyl - 2, 3-dioxygenase

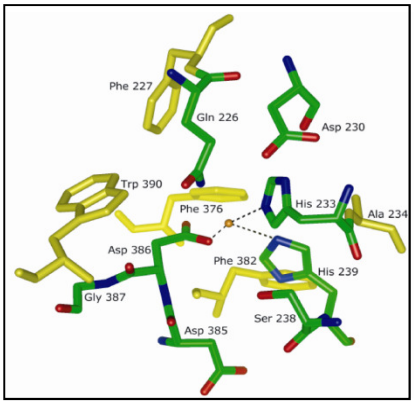

Fig. 5 Stick representation displaying active site residues (atom color) located around the Fe++ ion. Hydrophobic residues are shown in yellow color.

Docking studies

Docking of α-subunit of BphA with the CBs was carried out with version 4.0 of the program AutoDock (**http://autodock.scripps.edu/**). This program combines a rapid energy evaluation through pre-calculated grids of affinity potentials with a variety of search algorithms to find suitable binding positions for a ligand on a given protein. The program allows torsional flexibility in the ligand.

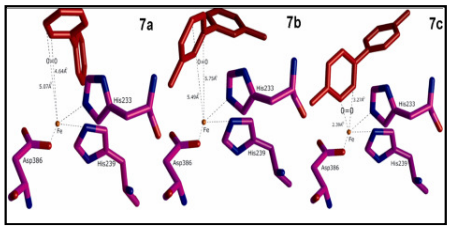

Fig.6 Docked conformation of (a) biphenyl (b) 3, 3 dicholorobiphenyl (c) 4, 4 dichlorobiphenyl into the active site of BphA (atom color).

Table 1.

Coordination geometry of Fe-ion along with the oxygen and different PCB congeners in each docking experiment along with their dock score.

S. No.	Compounds	Km(O_2) (**Nathalie et al,2000**)	Distance of Fe ion from PCB congeners (A°)	
			C2-Fe	C3-Fe
1.	Biphenyl	28	4.64	5.07
2.	3,3'-dichlorobiphenyl	190	5.75	5.49
3.	4,4'-dichlorobiphenyl	NR	2.39	3.27

NR: Not reported

*[a] In presence of 4,4'-dichlorobiphenyl,the initial rate of O_2 uptake increased linearly with the concentration of O_2 (**Nathalie et al,2000**).*

Conclusion

In the present work the 3D model of BphA was constructed in order to accomplish its molecular modeling and docking studies. The model was validated and further used for docking analysis with some well known PCBs. The resulting docking solutions were analyzed for binding pattern and conformational analysis. It was observed that the proximity of the C2 and C3 position of the docked PCBs were determinant of their oxygen utilization and ultimate catalysis. Hence, this work could be further useful for the mutagenesis studies where mutations created at various positions of the binding site may alter the substrate specificity and potency. Further work is still going on in our laboratory for the same.

References

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