

## Enzymes Related to Polychlorinated Biphenyl and Enzyme Kinetics

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Polychlorinated biphenyls (PCBs) are now one of the most serious recalcitrant pollutants, the use of microorganisms is expected to be an effective tool for remediation of PCB-polluted environments. In the most PCB degrader, the conversion of biphenyl to a benzoic and other 2-hydroxypenta-2,4-dienoate is catalyzed by a series of enzymes: biphenyl dioxygenase (BphA), dihydrodiol dehydroxygenase (BphB), 2,3-dihydroxybiphenyl dioxygenase (BphC) and 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid hydrolase (BphD). Biphenyl 2,3-dioxygenase catalyzes the oxygenation of two vicinal ortho-meta carbons of the biphenyl ring to yield a 2,3-dihydrodihydroxybiphenyl (DDBP). The dihydrodiol intermediate is then substrate for BphB, an NAD<sup>+</sup> dependent dehydrogenase that produces 2,3-dihydroxybiphenyl (DHBP) for BphC, a ring fission dioxygenase. Following ring fission, hydrolytic cleavage by BphD produces benzoate and 2-hydroxypenta-2,4-dienoate.

In *Rhodococcus* sp. strain R04, genes (*bphBCA1A2A3A4D*) encoding for biphenyl 2,3-dioxygenase (*BphA1A2A3A4*), cis-2,3-dihydro-2,3-dihydroxybiphenyl dehydrogenase (BphB), 2,3-dihydroxybiphenyl 1,2-dioxygenase (BphC) and 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase (HOPDA hydrolase, BphD), played an important role in degradation of biphenyl, and organized in an operon. The *bph* genes in strain R04 are preceded by *bphB*, and followed by *bphC*, *bphA1A2A3A4* and *bphD*. The gene encoding the  $\alpha$ -subunit of biphenyl

dioxygenase (BphA1) separated from *bphC* by an *orf2* of unknown function. Furthermore, a 330 bp and a 450 bp fragments are localized between *bphA2* and *bphA3*, *bphA3* and *bphA4*, respectively. A more detailed analysis revealed that *bph* genes organization in strain R04 varied from other PCB degrader.

We have characterized two enzymes BphC and BphD in the *bph* pathway, and proved them to be thermostable. Pre-steady-state analysis was carried out on wild-type BphD and the data simulated by curve-fitting. The results shows that Ser-110 plays a dominant role in the course of C–C cleavage; His-265 is responsible for ketonisation of the substrate and participates C–C cleavage along with Ser-110 and Trp-266; in the course of C–C cleavage catalyzed by the BphD, Trp-266 also plays a very important role in this enzyme catalyzed reaction expect for catalytic triad (Ser-110, Asp-237, His-265).

Our objective is to provide the reader with some view of the 2,3-dihydroxybiphenyl 1,2-dioxygenase and biphenyl hydrolase, including (a) the regulation of PCB metabolism to several 2,3-dihydroxybiphenyl 1,2-dioxygenase, (b) the directed evolution of biphenyl hydrolase, (c) pre-steady-state kinetics of biphenyl hydrolase and its mutants. We hope that the result of this endeavour is a repository of information that will be useful not only for the specialist to delve the wishes into the complexities of a specific area but also for those more casual readers just wanting to dip their toes in the water.

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