Biochemistry and Genetics of PCB Metabolism

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Biphenyl(BP)-utilizing bacteria, which include both Gram-negative and Gram-positive strains, are ubiquitously distributed in the environment. These bacteria co-metabolically degrade a variety of polychlorinated biphenyl (PCB) congeners to the corresponding chlorobenzoic acids through 2,3-dioxygenation. Certain strains degrade even highly chlorinated PCBs through 3,4-dioxygenation. The ring meta-cleavage dioxygenase purified from *Pseudomonas pseudoalcaligenes* KF707 is a homo-octamer containing ferrous ions as the essential cofactor. Transposon mutants revealed that the *bph*-encoded enzymes possess a wide range of substrate specificity for various aromatic hydrocarbons. The *bphABCXD* gene cluster coding for the degradation of PCBs to chlorobenzoic acids was first cloned from *P. pseudoalcaligenes* KF707 and sequenced and then was cloned from a number of BP-utilizing strains and sequenced. Some strains possess a *bph* operon that is very similar, if not identical, to that of KF707. Some *bph* genes share homologies with different degrees. Deletion and shuffling of *bph* genes are also found. — *Environ Health Perspect* 103(Suppl 5):21–23 (1995)

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Polychlorinated biphenyls (PCBs) can be transformed aerobically and anaerobically by microorganisms. Aerobic PCB degraders can be isolated as biphenyl (BP)-utilizing bacteria, which are widely distributed in the environment. PCB degradation by such microbes is thus the co-metabolism by BP-metabolic enzymes. The major pathway of PCB degradation by soil bacteria proceeds via 2,3-dioxygenation, in which molecular oxygen is introduced at the nonchlorinated 2,3 site (1). This group of PCB degraders shows a rather narrow range of PCB specificities for degradation. A large number of *Pseudomonas* strains along with some strains of *Alcaligenes, Achromobacter*, *Acinetobacter*, and *Moraxella* have been isolated and characterized so far (2). Gram-positive strains such as *Arthrobacter* and *Rhodococcus* are also known to degrade PCBs through the same 2,3-dioxygenation. On the other hand, *Alcaligenes eutrophus* H850 (3) and *Pseudomonas* sp. LB400 (4) degrade PCBs primarily through 3,4-dioxygenation. The same strains also seem to possess a 2,3-dioxygenase system so that these strains show a wide range of PCB-congener specificities for the degradation. Typically, PCB degraders having 2,3-dioxygenase readily degrade 4,4'-dichlorobiphenyl (4,4'-CB), but they are almost inactive to 2,3,5,2',5'-CB. On the other hand, the PCB degraders having 3,4-dioxygenase metabolize 2,5,2',5'-CB efficiently, but metabolize 4,4'-CB rather slowly. Yeast and fungi convert PCBs mainly to monohydroxy compounds, as in the case of the mammalian system. Reductive dechlorination occurs due to anaerobic bacteria in laboratory and field experiments (5), but these anaerobic strains have not yet been purely isolated. The 3(*meta*) and 4(*para*) positions of highly chlorinated PCBs are preferentially dechlorinated by anaerobic bacteria, resulting in the formation of less chlorinated ones with chlorines at 2(ortho) positions.

The major oxidative degradation of BP/PCBs to (chloro)benzoates through 2,3-dioxygenation is presented in Figure 1. Chlorobenzoates are not metabolized any further by BP-utilizing bacteria so that these compounds usually accumulate in the reaction mixture, but catabolic intermediates such as dihydrodiols, dihydroxy compounds, ring-*meta*-cleavage compounds, and some unknown compounds were produced from commercial PCB mixtures by both Gram-negative and Gram-positive strains having 2,3-dioxygenase. The effects of these catabolic intermediates on living organisms should be evaluated from the viewpoint of environmental toxicity.

Since the BP-utilizing bacteria that degrade PCBs through 2,3-dioxygenation are widely distributed in the environment, 2,3-dihydroxybiphenyl dioxygenase, the key enzyme of PCB degradation, was purified from two *Pseudomonas* strains, *P. pseudoalcaligenes* KF707 (isolated in Kyushu, Japan) and *P. paucimobilis* Q1 (isolated in Chicago, IL). Both enzymes showed similar molecular masses of 260 kDa for the native enzyme and 33 kDa for the subunit, and both contained ferrous ions as essential cofactors for enzymatic activity so that the structure of the holoenzyme is considered to be (αFeII)8. However, the antibody raised against the KF707 enzyme did not show any cross-reactivity with the Q1 enzyme, and the Q1 enzyme antibody did not cross-react with the KF707 enzyme. Sixteen PCB-degrading strains, including KF707 and Q1, were grown with BP and the cell extracts were prepared. Seven cell extracts, including KF707, among the 16 tested showed fusing precipitin bands; 5 other extracts also showed precipitin bands but formed a spur. Four cell extracts did not cross-react. On the other hand, when the Q1 enzyme...
antibody was used, no cross-reactivity was observed for any cell extracts from the other 15 PCB degraders. The ring-meta-cleavage compound hydrolase was purified from *P. pseudoalcaligenes* KF707. The molecular masses of the native enzyme and the subunits were 120 kDa and 30 kDa, respectively, indicating that this enzyme is a homo-tetramer. The antibody raised against the KF707 hydrolase cross-reacted with 10 cell extracts, including the KF707 enzyme, forming fused precipitin bands. Four other cell extracts showed precipitin bands with a spur, and two cell extracts from *P. paucimobilis* Q1 and Gram-positive *Arthrobacter* sp. M5 did not cross-react.

The gene clusters coding for the conversion of PCBs to chlorobenzoic acids were first cloned from *P. pseudoalcaligenes* KF707 (6) and then from other *Pseudomonas* strains. The organization of bph operons of *P. pseudoalcaligenes* KF707 and *P. putida* KF715 were similar, particularly in *bphA* region (~ 4 kb), which encodes biphenyl dioxygenase. The bphB [dihydriodiol dehydrogenase gene, 831 base pairs (bp) in KF707], the bphC (2,3-dihydroxybiphenyl dioxygenase gene, 894 bp in KF707 and 876 in KF715), and the bphD (hydrolyase gene, 855 bp in KF707 and 858 bp in KF715) were located downstream of bphA in this order in both operons, but the bphX region (~ 3.5 kilobases) was present between bphC and bphD in the KF707-bph operon, but the same DNA segment was missing in the KF715-bph operon. The 11.3-kb-DNA was sequenced (Figure 1), including the entire bphABCXD operon of KF707. Since biphenyl dioxygenase is a multicomponent enzyme, five open reading frames (ORF) were detected: bpha1, bpha2, bpha3, and bpha4 were assigned to be a large subunit of terminal dioxygenase, a small subunit of terminal dioxygenase, ferredoxin, and ferredoxin reductase, respectively. Another orf3 was found between bpha2 and bpha3, but its function remains unknown. The orf3-deletion mutant still retained the ability of BP oxidation (7).

The KF707 bpha1A2A3A4ABC genes are very similar to the todC1C2B1D1E genes coding for toluene/benzene metabolism in *P. putida* Fl1 (8) in terms of gene organization as well as the size and homology of the corresponding enzymes and despite their discrete substrate specificities for metabolism. Studies of the gene components responsible for substrate specificity between the bph and tod operons revealed that the large subunit of the terminal dioxygenase (encoded by bpha1 and todC1) and the hydrolase (bphaD and todF) were critical for their discrete metabolic specificities (9). Introduction of todC1C2 (coding for the large and small subunits of the terminal dioxygenase in toluene metabolism), or even of only todC1, into the bphplenyl-utilizing strain KF707 allowed it to grow on toluene/benzene. On the other hand, introduction of bphaD into toluene-utilizing *P. putida* Fl1 permitted growth on biphenyl. Thus, the other components of ferredoxins (encoded by bphaA1/1D) and ferredoxin reductases (encoded by bphaA4/1D) were complementary with one another (9). Furthermore, *Escherichia coli* cells carrying a hybrid gene cluster of todC1::bphaA3A4ABC (constructed by replacing bpha1 with todC1) converted toluene to a ring-meta-cleavage compound) indicating clearly that TodC1 formed a functionally active multicompartment dioxygenase associated with Bpha2, Bpha3, and Bpha4 (10).

The bphX region (3.5 kb) between bphC and bphD in the KF707 bph operon has been sequenced, and at least three ORFs (BphX1, BphX2, and BphX3) were found. The amino acid sequences of BphX1 showed approximately 70% identity with DmpE or XylJ, which is a 2-hydroxypenta-2,4-dienoate hydratase in *phenol/3,4-dimethylphenol* catabolism of *Pseudomonas* sp. strain CF600 (11) and the meta-cleavage pathway in the xyl operon of *P. putida* (12). Similarly, BphX2 showed approximately 56% identity with DmpF and XylQ of acetdehyde dehydrogenase (acylating), and BphX3 showed approximately 57% identity with DmpG and XylK (4-hydroxy-2-oxovalerate aldolase). Since 2-hydroxypenta-2,4-dienoate is formed as a counterpart product of benzoic acid in the hydrolysis of BP-meta-cleavage compound, it is likely that the bphX region could be involved in the metabolism of 2-hydroxy-penta-2,4-dienoate to acetyl coenzyme A via 4-hydroxy-2-oxovalerate and acetaldehyde (Figure 1).

The molecular relationships of bph genes in various PCB degraders were investigated using bphABC gen of *P. pseudoalcaligenes* KF707 as the DNA probe (2). Among 15 strains tested, five *Pseudomonas* strains and one *Alcaligenes* strain possessed the bphABC gene cluster on a *XhoI* 6.8-kb fragment corresponding to that of *P. pseudoalcaligenes* KF707. The restriction profiles of these *XhoI* 6.8-kb fragments containing bphABC genes were almost identical, despite the dissimilarity of the flanking chromosomal regions. Three other strains also possessed bphABC genes homologous to those of KF707, but the restriction profiles among them were different. Five other strains showed weak or no significant homology with the KF707 bphABC genes. On the other hand, the bphC gene of *P. paucimobilis* Q1 lacked genetic homology with any of the other 15 PCB degraders. These data corresponded well with the immunological cross-reactivity of 2,3-dihydroxybiphenyl dioxygenase (2). The existence of the nearly identical chromosomal genes among various strains suggests that the bph-DNA segment of this group has a mechanism for transferring the
genes from one strain to another. It is surprising to note that the organization as well as the nucleotide sequences of the \textit{bph} operon of \textit{P. putida} LB400 (12), which converts PCBs through 3,4-dioxygenase, is almost identical to those of \textit{P. pseudoalcaligenes} KF707, having 2,3-dioxygenase. The \textit{bph} operon of \textit{Pseudomonas} sp. strain KKS102 has been recently sequenced (13). The \textit{bph} genes are greatly shuffled, compared with those of \textit{P. pseudoalcaligenes} KF707. In the KKS \textit{bph} operon, \textit{bphA1A2A3BCD} is organized in this order but \textit{bphA4} is located downstream of \textit{bphD}, and the \textit{bphX} region is located upstream of \textit{bphA1} (Figure 1).

A transposon, Tn5-21 (Tc''), was gene-specifically inserted into the \textit{bph} operon of \textit{P. pseudoalcaligenes} KF707 (14). First, the cloned \textit{bphA}, \textit{bphB}, and \textit{bphC} genes were mutagenized by random insertion of Tn5-21. The mutagenized \textit{bphABC} DNA, carried by a suicide plasmid, was then introduced back into the parent strain KF707, resulting in the appearance of mutants in which Tn5-21 was gene-specifically inserted in the chromosomal \textit{bph} operon. The \textit{bphA::Tn5-21} mutant thus obtained did not attack 4-chlorobiphenyl at all; the \textit{bphB::Tn5-21} mutant accumulated dihydrodiol, and the \textit{bphC::Tn5-21} mutant accumulated dihydroxy compound. The transposon mutants revealed that the \textit{bph}-encoded enzymes possessed very relaxed substrate specificities for a variety of biphenyl-related compounds. \textit{P. pseudoalcaligenes} KF707 \textit{bph} enzymes convert biphenyl with various substituents, such as halogen, hydroxyl, methyl, and nitro, and also biphenyl-related compounds, including biphenylmethane, dibenzyl, diphenylether, and benzaacetophenone. However, the KF707 enzyme system is almost inactive to benzene and its derivatives. Another \textit{Pseudomonas} sp., KF712, produced Bph enzymes that showed much wider substrate ranges, compared with the KF707 enzymes. The \textit{bphC::Tn5-21} mutant of KF712 converted many benzene derivatives, as well as various BP derivatives and BP-related compounds, to the corresponding dihydroxy compounds. The relaxed substrate ranges of Bph enzymes, along with the fact that BP/PCB degraders are widely distributed in the environment, indicate that BP/PCB-degrading bacteria might be primarily involved in the degradation of plant lignin at the final stage, since plant lignin is massively distributed in the environment and is the source of various aromatic compounds, which include many benzene and biphenyl derivatives.

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