Efficient Degradation of Trichloroethylene by a Hybrid Aromatic Ring Dioxygenase

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Engineering of hybrid gene clusters between the toluene metabolic tod operon and the biphenyl metabolic bph operon greatly enhanced the rate of biodegradation of trichloroethylene. Escherichia coli cells carrying a hybrid gene cluster composed of todC1 (the gene encoding the large subunit of toluene terminal dioxygenase in Pseudomonas putida F1), bphA2 (the gene encoding the small subunit of biphenyl terminal dioxygenase in Pseudomonas pseudoalcaligenes KF707), bphA3 (the gene encoding ferredoxin in KF707), and bphA4 (the gene encoding ferredoxin reductase in KF707) degraded trichloroethylene much faster than E. coli cells carrying the original toluene dioxygenase genes (todC1C2BA) or the original biphenyl dioxygenase genes (bphA1A2A3A4).

Trichloroethylene (TCE) has been recognized as one of the most significant environmental pollutants in soil and groundwater (11). TCE and related compounds have been shown to persist over time in the environment and are suspected to be carcinogenic (7). A recent study showed that TCE could be degraded by aerobic and anaerobic bacteria (1). An aerobic, methane-oxidizing bacterium that was isolated in pure culture degraded TCE by a cometary process (8). Nelson and coworkers (9) and Wackett and Gibson (12) showed that toluene dioxygenase plays a role in the degradation of TCE by using mutants of toluene-utilizing Pseudomonas putida F1. This role was confirmed with Escherichia coli carrying the structural genes ( todC1C2BA ) for toluene dioxygenase (15). Another toluene-degrading species, Pseudomonas mendocina, oxidizes toluene through toluene monoxygenase, which inserts a single atom of oxygen at the para position of toluene to form p-cresol (13). It was demonstrated that E. coli cells carrying a gene cluster coding for multicomponent toluene monooxygenase degraded TCE to carbon dioxide, chloride ion, and water-soluble metabolites. We have cloned from Pseudomonas pseudoalcaligenes KF707 bph genes coding for the catalysis of biphenyl (BP) and polychlorinated biphenyls (PCBs) to (chloro)benzoic acids (3). The bphA1A2A3A4 genes, coding for BP dioxygenase (10), are similar to the todC1C2BA genes, coding for toluene dioxygenase, in toluene-utilizing P. putida F1 (14), both in gene organization and in nucleotide sequence. The amino acid identities of the large subunit of terminal dioxygenase (BphA1 and TodC1), the small subunit of terminal dioxygenase (BphA2 and TodC2), ferredoxin (BphA3 and TodB), and ferredoxin reductase (BphA4 and TodA) are, respectively, 65, 60, 60, and 53% (10). We found previously that E. coli cells expressing BP dioxygenase (composed of BphA1A2A3A4) were totally inactive for toluene, although E. coli cells expressing toluene dioxygenase (composed of TodC1C2BA) converted BP to the dihydrodiol ( cis-2,3-dihydroxy-1-methylcyclohexa-4,6-diene ) (2). During the course of the construction of a hybrid gene cluster involving bphA1A2A3A4 and todC1C2BA, it was found that E. coli cells expressing a hybrid gene cluster containing todC1::

bphA4A3A2, todC1C2::bphA3A4, or bphA1::todC2::bphA3A4 could convert both toluene and BP to the respective dihydrodiols ( cis-2,3-dihydroxy-1-methylcyclohexa-4,6-diene from toluene and cis-2,3-dihydroxy-1-phenylcyclohexa-4,6-diene from BP), indicating that the hybrid terminal dioxygenases composed of TodC1::BphA2 and BphA1::TodC2 form a functionally active multicomponent dioxygenase associated with ferredoxin (BphA3) and ferredoxin reductase (BphA4) (2, 6). Since it has been demonstrated that toluene dioxygenase, composed of TodC1C2BA, is involved in TCE degradation (12, 15), we were interested in examining how such a hybrid aromatic ring dioxygenase could be active for TCE degradation. Here we report that the hybrid aromatic ring dioxygenase composed of TodC1::BphA2A3A4 efficiently degrades TCE much faster than the original toluene dioxygenase, composed of TodC1C2BA, and the original BP dioxygenase, composed of BphA1A2A3A4.

The plasmids used in this study are schematically represented in Fig. 1. pJHF108 is a plasmid in which the original bphA1A2A3A4 genes are introduced into pUC118; pJHF301 contains the original todC1C2BA genes inserted into pUC119; pJHF101 contains a hybrid gene cluster, todC1::bphA2A3A4, in pUC118; pJHF201 contains a hybrid gene cluster, bphA1::todC2::bphA3A4, in pUC119; and pJHF301 contains a hybrid gene cluster, todC1C2::bphA3A4, in pUC119. The construction of the above-mentioned hybrid gene clusters was described previously (6).

E. coli JM109 cells carrying recombinant plasmids were grown with shaking at 37°C in mineral salts basal medium (5) supplemented with 20 mM glucose, 1 mM thiamine, and 100 μg of ampicillin per ml. When the culture attained an optical density at 600 nm of 0.8 to 1.0, isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1 mM and cell growth was continued for 1 h. The optical density of the culture reached 1.6 to 2.0 under these conditions. Cells were harvested by centrifugation at 3,900 × g for 10 min at 4°C and suspended in fresh mineral salts basal medium supplemented with 20 mM glucose. Ten milliliters of the cell suspension was added to a glass vial (125 ml), which was sealed with a rubber septum and an aluminum crimp seal. TCE (dissolved in N,N'-dimethylformamide) was added to triplicate sets of vials for each experiment. The vials were incubated at 37°C on a rotary shaker at 200 rpm. Samples (100 μl) of the gas phase contained in each sealed vial were removed every hour and injected into a
Shimadzu 9A gas chromatograph equipped with a flame ionization detector, fitted with a silicone DC-550 column (GL Science Inc., Kyoto, Japan), and operated at 100°C with a nitrogen gas flow of 30 ml/min.

The formation of a cis-dihydriodiol from BP or toluene was measured by use of recombinant E. coli JM109 resting cells carrying various plasmids (Fig. 1). Resting cells were prepared to yield an optical density at 600 nm of 1.0 and incubated with BP or toluene at a final concentration of 0.5 mM. The formation of dihydriodiol compounds was monitored measuring the A_{303} for BP or the A_{265} for toluene. The dihydriodiol products thus obtained were extracted with ethyl acetate, and their identities were confirmed by gas chromatography-mass spectrometry (JEOL model JMS D-300) as described previously (4). Cells expressing the original BP dioxygenase (encoded by bphA1A2A3A4) quickly produced cis-dihydriodiol from BP, but the same cells did not attack toluene at all (Fig. 2). On the other hand, cells expressing the original toluene dioxygenase (encoded by todC1C2B4) quickly converted toluene to cis-dihydriodiol, and the same cells also converted BP to cis-dihydriodiol. Cells carrying pJHF101, containing hybrid todC1::bphA2A3A4, converted both BP and toluene to the respective dihydriodiols at rates similar to that of the original toluene dioxygenase-producing cells (pJHF3051). This was also the case for cells carrying pJHF301, containing hybrid todC1C2::bphA3A4. On the other hand, cells carrying pJHF201, containing hybrid bphA1::todC2::bphA3A4, converted both BP and toluene, but converted BP poorly, compared with pJHF101- or pJHF301-carrying cells. Thus, recombinant E. coli cells carrying bph::tod hybrid genes all expressed functionally active aromatic ring dioxygenases.

E. coli (pJHF108), expressing the original BP dioxygenase, did not attack TCE at all (Fig. 3). E. coli (pJHF3051), expressing the original toluene dioxygenase, degraded TCE at an initial degradation rate of 0.6 μg/ml/h, and the degradation rate decreased over time. To our surprise, cells carrying pJHF101, expressing the hybrid enzyme composed of TodC1::BphA2A3A4, rapidly degraded TCE at an initial degradation rate of 1.8 μg/ml/h. E. coli (pJHF301), expressing the hybrid enzyme composed of TodC1C2::BphA3A4, also showed significant TCE-degrading activity, but it was slower than that of E. coli (pJHF101) and faster than that of E. coli (pJHF3051). In contrast, E. coli (pJHF201), expressing the hybrid enzyme composed of BphA1::TodC2::BphA3A4, did not show any TCE-degrading activity, as was the case for E. coli (pJHF108), expressing the original BP dioxygenase.

Although toluene dioxygenase and some hybrid dioxygenases are thus certainly involved in the degradation of TCE, the
enzymatic activities for aromatic compounds and TCE are not correlated. The reason remains to be elucidated. Native polyacrylamide gel electrophoresis revealed that TodC1 and BphA2 formed heterodimers as well as heterotetramers, but TodC1 and TodC2 as well as BphA1 and TodC2 formed only heterotetramers (14). The purification of Bph::Tod hybrid dioxygenases is currently being undertaken. It should be interesting to know whether such dimeric structures enhance the affinity between an enzyme and TCE or cause efficient electron flow from NADH.

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