Proc. KSAM International Symposium
June 20-22, 2001, Chonan, Korea

Potential Application of Aromatic Oxygenases in the Synthesis of Useful Synthons

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Aromatic dioxygenase, the initial enzyme of the aromatic degradative pathway, is normally a three-component enzyme system, which consists of a flavoprotein reductase, a ferredoxin containing a Rieske-type [2Fe-2S] center, and an iron sulfur protein. It has been known that reductase and ferredoxin components form a short electron transport chain that supplies electrons to an iron sulfur protein component (terminal dioxygenase), which adds both atoms of molecular oxygen to one of the aromatic rings (1). Currently, several hundred vicinal aromatic *cis*-dihydrodiols are known to be produced during the bacterial oxidation of aromatic hydrocarbons (2). During the past decade extensive research has been made to develop environmentally-friendly biotransformation procedures for the synthesis of value-added fine chemicals using aromatic dioxygenases (3). The main reason for this effort is that the enzymatic dihydroxylation of aromatics can produce various enantiopure aromatic *cis*-dihydrodiols, which are subsequently used as synthons for manufacturing new compounds of chemical and pharmaceutical importance. In this paper, we present the identification of some novel reactions catalyzed by two different aromatic dioxygenases, which apparently provide more sources of new enantiopure aromatic *cis*-dihydrodiols.

A gram positive bacterial strain DK17 was isolated for the ability to grow on *o*-xylene as sole carbon and energy sources from crude oil-contaminated soil, and identified to belong to the genus *Rhodococcus* through determination and analysis of its 16S rRNA sequence. *Rhodococcus* sp. DK17 is also able to utilize toluene or benzene as a growth substrate, but not *m*- or *p*-xylene isomers. One UV-generated mutant strain DK176 lost simultaneously the ability to grow on *o*-xylene, toluene, or benzene although it can still grow on other putative metabolic intermediates including *m*- and *p*-cresols and phenol. Also, the mutant strain is unable to produce indigo from indole (blue colonies) when cells are exposed to indole after growth in the presence of *o*-xylene. This observation indicates that the same oxygenase may be involved in the initial oxidation of the BTX compounds tested. However, the other mutant strain DK180 retains the ability to grow on benzene although it is unable to grow on *o*-xylene and toluene due to the loss of the *meta*-cleavage activity for (methyl)catechols. Furthermore, DK180 was found to possess the *ortho*-cleavage pathway, which is specifically by benzene, but not by *o*-xylene, suggesting the presence of a substrate-dependent induction mechanism. These results indicate that DK17 possesses at least two different pathways for the degradation of (alkyl)benzenes although the initial oxidation reactions may be catalyzed by a common oxygenase. A major metabolite in the degradation of *o*-xylene by DK180 was identified as 3,4-dimethylcatechol by gas-chromatographic/mass-spectrometric, and 1H

nuclear magnetic resonance spectral techniques. In addition, a trace intermediate was identified as 3,4-dimethylphenol.

Sphingomonas yanoikuyae strain B1 is able to metabolize a wide variety of monocyclic and polycyclic aromatic hydrocarbons. Recently, it was found that B1 is also able to mineralize C4 to C16 alkanes including butane, *n*-hexane, *n*-octane, dodecane, and hexadecane. Interestingly, a deletional mutant strain EK537, which is unable to grow on the aromatics listed above due to the deletion of approximately 21 kb genomic DNA region containing many of the aromatic degradative genes, is also unable to grow on the alkanes tested. This means that the same aromatic degradative genes are implicated in alkane degradation by *S. yanoikuyae* strain B1. In the beginning it is suspected that *xylM* would be involved in alkane metabolism. But insertional mutant strain SY1 (*xylM*::Km) unfortunately has no phenotype: ability to grow on alkanes tested as well as m-xylene. On the other hand it is observed that an insertional mutant strains EK385 (*bphC*::Km) and MB1 (*bphA4*::Cm), which lost BphA3 and BphA4 activitis, are also unable to grow on all of the alkanes tested. This observation indicates that the same ferredoxin and reductase components are associated even with the aliphatic-oxidizing oxygenase. The most interesting fact is that iron sulfur protein large and small subunits of biphenyl dioxygenase encoded by *bphA1dA2d* play a role as alkane oxygenase in the first step of alkane degradation. Both mutant strains EK533 (*xylX*::Km), which lost bphA1dA2d activity due to polar effect, and MB2 (*bphA1d*) is unable to grow on alkanes. Also, *S. yanoikuyae* strains EK533 and MB2 were complemented on all of the substrates by recombinant plasmids containing only *bphA1dA2d* (pKEB1522) gene, respectively.

References

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