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Synthesis of vicinal diols from various arenes with a heterocyclic, amino or carboxyl group by using recombinant *Escherichia coli* cells expressing evolved biphenyl dioxygenase and dihydrodiol dehydrogenase genes

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Abstract—Various aromatic molecules, in which heterocycles are linked with a phenyl or benzyl group, were converted to their respective 2,3-diols (catechols) in the benzene ring by growing cell reactions using recombinant *Escherichia coli*, which expressed the evolved biphenyl dioxygenase [*bphA* (2072)] genes and the subsequent bacterial dihydrodiol dehydrogenase (*bphB*) gene. These vicinal diol products showed strong in vitro inhibitory activity against the lipid peroxidation induced by free radicals and strong scavenging activity towards DPPH radicals. The vicinal diols were also synthesized from ionized monocyclic aromatics incorporating an amino or carboxyl group.

1. Introduction

We intend to develop a system for the comprehensive bioconversion of a series of organic chemicals by growing cell reactions using recombinant microbes which express different combinations of a sequence of biocatalytic genes. This intention attempts to perform comprehensive 'chemical synthesis' by using the multiple biocatalytic functions of the cells to link the diversity of genes (DNA) to that of chemicals. Figure 1 suggests the concept of such living cells-based combinatorial chemistry (CellCombiChem). CellCombiChem could significantly make it practical to synthesize chemicals, which are difficult or impractical to synthesize by chemical methods. It is also important to use an enzyme with broad substrate specificity or preference to achieve comprehensive bioconversion. Directed protein evolution could be one of the most powerful methods for generating such enzymes.[1]

It is usually difficult to introduce hydroxy group(s) into an aromatic ring *regio* or *stereo*-specifically by a chemical synthesis, although the industrial need for this is strong. For attempting such a hydroxylation reaction we have started our study on CellCombiChem with the biocatalytic genes mediating biphenyl catabolism, since carrying out the first dioxygenation reaction would be difficult by the extracted enzyme, which includes ferredoxin and ferredoxin reductase and needs $NAD(P)H^+$ that must be regenerated. The selection is also based on the following reasons: during the past 35 years, the *stereo* and *regio*-specific syntheses of *cis*-dihydrodiols (*cis*-dihydrocatechols, *cis*-cyclohexadiene-diols) have mainly been performed by toluene dioxygenase-mediated microbial conversion, so as to generate two hundred structurally diverse *cis*-dihydrodiols,[2] several of which have been applied as chiral intermediates for versatile synthetic applications by synthetic chemists worldwide.[2,3] These results show that toluene dioxygenase, which is an enzyme structurally similar to biphenyl dioxygenase, desirably has very broad substrate specificity; it may therefore be feasible to introduce hydroxy group(s) into a wide range of aromatic compounds *stereo*- and *regio*-specifically with biphenyl dioxygenase, and *regio*-specifically with the subsequent dehydrogenation enzyme. Many studies on the degradation of environmental pollutants such

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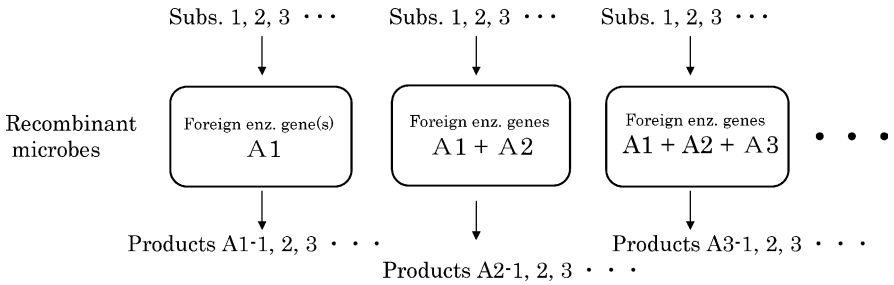

Figure 1. Concept of living cells-based combinatorial chemistry (CellCombiChem). Subs. 1, 2, 3… means a series of organic chemicals used as substrates. Foreign enz. gene(s) A1 represents foreign gene(s) coding for metabolic enzyme A1. Products A1-1, 2, 3… are generated from Subs. 1, 2, 3… through the living cells of recombinant microbes expressing the A1 gene(s). A2, which is usually a metabolic enzyme subsequent to A1, can convert A1-1, 2, 3… to A2-1, 2, 3… , which are further converted to A3-1, 2, 3… by enzyme A3.

as polychlorinated biphenyls (PCBs) have been performed by using biphenyl dioxygenases, but little work has been done on the synthesis of useful compounds by using this enzyme.[2,4]

The degradation of biphenyl and PCBs is enzymatically initiated by the action of biphenyl dioxygenase, as has been elucidated for the biphenyl-degrading bacteria, *Pseudomonas pseudoalcaligenes* KF707,[4,5] *Burkholderia* sp. strain LB400,[6] and *Sphingomonas yanoikuyae* strain B1.[2] Biphenyl dioxygenase is a multi-component enzyme consisting of BphA1, BphA2 [large α and small β subunits of iron–sulfur protein, respectively], BphA3 (ferredoxin), and BphA4 (ferredoxin reductase), as shown in Figure 2.[2,4,7–9] This enzyme (BphA) is responsible for the conversion of a biphenyl to its *cis*-dihydrodiol [(1*S*,2*R*)-dihydroxy-3-phenylcyclohexa-3,5-diene].[2,8] This compound is further converted to 3-phenylbenzene-1,2-diol by the subsequent desaturation reaction, which is catalyzed by BphB (biphenyl *cis*-dihydrodiol dehydrogenase).[7,9] The substrate-binding sites of biphenyl dioxygenase are considered to be present in its α-subunit (BphA1). This consideration is supported by the results of an X-ray structural analysis of naphthalene dioxygenase,[10] which is an enzyme with a topology slightly different from that of biphenyl dioxygenase, and by data obtained from DNA shuffling and in vitro mutagenesis experiments.[4,11] Modified *bphA1* genes were generated by DNA shuffling, using the *bphA1* genes derived from *P. pseudoalcaligenes* KF707 and *Burkholderia* sp. LB400.[11a] One of the shuffled genes, *bphA1* (2072), has been shown to mediate with broad substrate specificity, when expressed in combination with *bphA2A3A4* from *P. pseudoalcaligenes*.[8] We have shown for the first time that various molecular species, in which heterocyclic aromatics are linked with phenyl or benzyl groups, can be converted with high efficiency to the corresponding *cis*-dihydrodiols (*cis*-dihydrocatechols) by recombinant *Escherichia coli* or *Streptomyces lividans* strains carrying the evolved biphenyl dioxygenase genes [*bphA1*(2072)*A2A3A4*: *bphA* (2072)] (Fig. 3).[8,12] The *cis*-dihydrodiols generated may be capable of being used as substrates for BphB to synthesize their respective diols (catechols).

We report here the formation of vicinal diols (catechols) from various aromatic molecules, in which heterocycles including heteroaromatics are linked with phenyl or benzyl groups, through the growing cells of *E. coli* that express the *P. pseudoalcaligenes bphB* gene in addition to the *bphA* (2072) genes (Fig. 3). The antioxidative activity of these vicinal diols is examined. The vicinal diol formation from ionized monocyclic aromatics incorporating an amino or carboxyl group is also described.

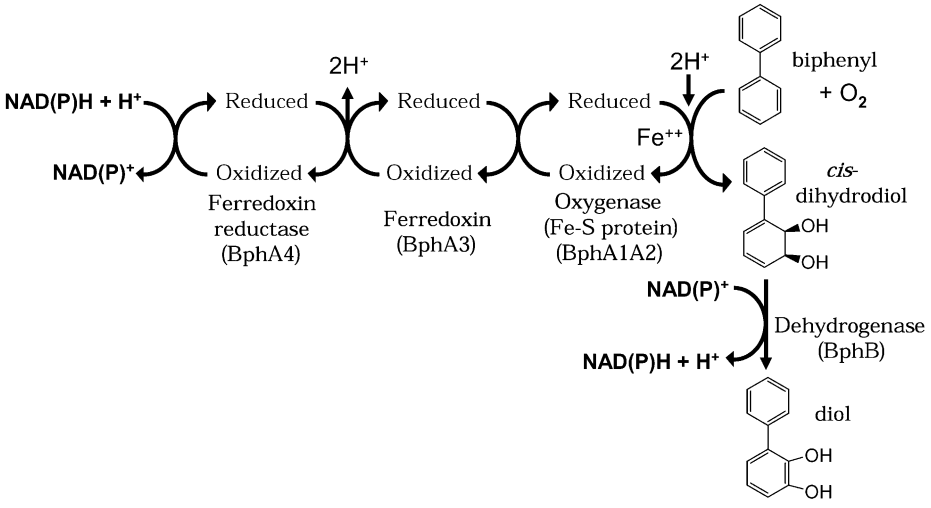

Figure 2. Catabolic pathway from biphenyl to diol (3-phenylbenzene-1,2-diol) via *cis*-dihydrodiol [(1*S*,2*R*)-dihydroxy-3-phenylcyclohexa-3,5-diene] for the biphenyl-degrading bacterium, *Pseudomonas pseudoalcaligenes* KF707. *Sphingomonas yanoikuyae* strain B1 seems to have the same pathway.[2]