Cis-2′, 3′-Dihydrodiol production on flavone B-Ring by Biphenyl Dioxygenase from Pseudomonas pseudoalcaligenes KF707 expressed in Escherichia coli

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Abstract

Escherichia coli JM109 strains expressing either toluene dioxygenase from Pseudomonas putida F1 or biphenyl dioxygenase from Pseudomonas pseudoalcaligenes KF707 were examined for their ability to catalyze flavones. Biphenyl dioxygenase produced metabolites from flavone and 5,7-dihydroxyflavone which were not found in the control experiments. The absorption maxima of UV-visible spectra for the metabolites from flavone and 5,7-dihydroxyflavone were found at 337 and 348 nm respectively by using a photodiode array detector in the HPLC. Liquid chromatography/mass spectroscopy (LC/MS) showed molecular weights 256 and 288 for the metabolites, respectively. The metabolite of flavone, which was isolated and purified from the bacterial culture, was further subjected to analysis by H and C nuclear magnetic resonance (NMR) spectroscopy. Based on the LC/MS and NMR results, biphenyl dioxygenase inserted oxygen at C2' and C3' on the B-ring of flavone, resulting in the formation of flavone cis-2′, 3′-dihydrodiol (2-[3,4-dihydroxy-1,5-cyclohexadienyl]-4H-chromen-4-one). Since this product is not found in Chemical Abstracts, this compound is considered a novel one. In addition, biotransformation of flavones by biphenyl dioxygenase suggested a potential role of bacterial dioxygenase to synthesize novel compounds from plant secondary metabolites.

Introduction

Flavones are one of the major constituents of plant secondary metabolites, and are synthesized using chalcone via so called a phenylpropanoid pathway (Weisshaar and Jenkins 1998; Winkel-Shirley 2001). Due to the diverse modification pathways found in the plants, several functional groups such as hydroxyl, methoxyl, glycoside, etc. have been found on varying positions of its basic chemical backbone structure (Birt et al. 1998). This structural diversity of flavone chemicals affects characteristic interactions of each plant with the surrounding environments and living organisms. The typical example for the interaction is (iso)flavone-induced nodule formation by nitrogen fixing bacteria on their host plants which release (iso)flavones to the rhizosphere (Rolfe 1988; Hartwig et al. 1990). In addition to the effects of flavones on the environment, flavones have shown pharmaceutical effects on human beings, such as protection against certain forms of cancer and cardiovascular disease and development of estrogenesis (Dixon and Steele 1999; Formica and Regelson 1995; Hollman and Katan 1999; Kellis and Vickery 1984; Middleton
These biological effects through the free radical-scavenging antioxidative and metal ion-chelating activities have been considered to be positively related with the presence of the functional groups, a catechol structure on the B-ring, a C2-C3 double bond together with a C4-keton group, and C3 and C5-hydroxyl groups on C- and A-rings, respectively.

The structure-based pharmaceutical activation of flavones has led to extensive researches for biotransformation by microorganisms from either mammalian intestines or environmental samples (Hosny et al. 2001; Hur and Rafii 2000; Ibrahim and Abul-Hajj 1990; Klus and Barz 1998; Winter et al. 1989). The reactions of flavones catalyzed by microorganisms are oxidation, reduction, conjugation, and deglycosylation. Recently, Hur et al. (Hur and Rafii 2000; Hur et al. 2000, 2002) reported demethylation of isoflavones by Eubacterium limosum, and C2-C3 double bond reduction and C-ring cleavage by pure cultures from a human intestine under anaerobic condition. (Hosney et al. 2001) also found that Streptomyces griseus catalyzed hydroxylation and methylation reactions of quercetin, fisetin, and catechin. A wide range of functional modification capacity of microorganisms for (iso)flavones has led us to apply toluene dioxygenase and biphenyl dioxygenase from Pseudomonas putida F1 (Gibson et al. 1970b) and Pseudomonas pseudoalcaligenes KF707 (Furukawa and Miyazaki 1986), respectively. These two enzymes, which appear to have a similar evolution experience, initially integrate molecular oxygen into the aromatic compounds, resulting in the formation of stereochemically pure cis-dihydrodiol compounds (Furukawa and Miyazaki 1986; Gibson et al. 1970a, b; Maeda et al. 2001), which cannot be easily synthesized by conventional chemical synthetic methods. Since (Ley et al. 1987) reported the chemical synthesis of racemic pinotol from cis-cyclohexadienediol, there have been extensive researches in terms of finding new substrates for novel dioxygenases from bacteria and versatile applications of the cis-dihydrodiol for making pharmaceutical reagents and polymers (Hudlicky et al. 1999).

For the purpose of expanding the substrate spectrum to flavones and making a novel (iso)flavone derivative from flavones to toluene dioxygenase and biphenyl dioxygenase, both enzymes were expressed in E. coli incubated with synthetic flavones. Comparing the structures of flavones (Figure 1) with that of biphenyl, the physiological substrate for biphenyl dioxygenase, the enzyme may initiate oxygen insertion into either the A- or B-ring of flavone, making corresponding flavone cis-dihydrodiols. Biphenyl dioxygenase alone produced cis-2’, 3’- dihydrodiols on the B-ring of certain flavones. Here, we report biotransformation of flavones and positional effects of hydroxyl group(s) on the biotransformation of flavones by biphenyl dioxygenase from P. pseudoalcaligenes KF707. (The data presented was previously reported in the ASM Conference on Biodegradation, Biotransformation, and Biocatalysis (B3), Poster # 74, which was held at San Juan, Puerto Rico from October 2 – 6, 2001).

Materials and methods

Materials

Flavone, 5,7-dihydroxyflavone, 5,4’-dihydroxyflavone, baicalein, 2’,3’-dihydroxyflavone, 3’,4’-dihydroxyflavone, 6,7-dihydroxyflavone, 7,8-dihydroxyflavone, 5-hydroxy-7-methoxyflavone and 8-hydroxy-7-methoxyflavone were purchased from Indofine Chemical Company (Somerville, NJ). The organic solvents were obtained from Duksan Co. (Ansan, Korea).

Bacterial strains and analytical-scale culture conditions

Escherichia coli JM109 (pJHF108) (Furukawa et al. 1994) carrying a biphenyl dioxygenase gene from P. pseudoalcaligenes KF707 and E. coli JM109