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Highly regioselective hydroxylation of polydatin, a resveratrol glucoside, for one-step synthesis of astringin, a piceatannol glucoside, by P450 BM3

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ABSTRACT

Enzymatic conversion of natural glycosides to their corresponding hydroxylated products using cytochromes P450 has significant advantages over synthetic chemistry and even enzyme-catalyzed glycosylation of chemicals. At present, the basic strategy for making glycosides of stilbenoid compounds is to use the glycosylation activity of enzymes, such as glycosyltransferases. Here, an efficient synthesis of a valuable (*E*)-astringin, a piceatannol glucoside, was developed using CYP102A1 via the highly regioselective C-3′ hydroxylation of polydatin, a resveratrol glucoside. (*E*)-astringin is a high added value compound found in plants and wine. Benzylic hydroxylation of polydatin provides an attractive route to (*E*)-astringin, a catechol product. Thus far, chemical and enzymatic methods of producing (*E*)-astringin have not been developed. In the present study, a set of CYP102A1 mutants from *Bacillus megaterium* was found to catalyze regioselective hydroxylation of polydatin at the C-3′ position to generate an (*E*)-astringin, a piceatannol glucoside.

1. Introduction

Stilbenes are an important family of natural products from various plants. Grapes and related products are the most important sources of stilbenes [1]. Moderate wine consumption seems to be related to the decrease in cardiovascular diseases. Phenolic compounds, including stilbenes with potent antioxidant properties in wine, are considered to be associated with the French Paradox [2].

Among the polyphenolic stilbenes, resveratrol (3,4′,5-trihydroxystilbene) is the most popular and widely studied. Resveratrol (Fig. 1A), a natural phytoalexin, has been shown to have tremendously beneficial pharmacological activities against cardiovascular diseases, inflammatory diseases, cancer, obesity, diabetes, neurodegenerative diseases, aging, and reproductive system diseases [3–6]. Recently, piceatannol (3,5,3′,4′-tetrahydroxystilbene) (Fig. 1B), a hydroxylated resveratrol, has also been noticed due to its potential beneficial effects on cardiovascular diseases, such as the prevention of hypercholesterolemia, arrhythmia, atherosclerosis, and angiogenesis [7,8]. It is a human metabolite of resveratrol and produced as a major metabolic product of the hydroxylation reaction by human cytochromes P450 (P450 or CYP) 1B1, 1A1, and 1A2 [9,10]. Piceatannol can be directly produced from resveratrol via regioselective hydroxylation catalyzed by P450 BM3 (CYP102A1) from *Bacillus megaterium* [11], a non-heme monooxygenase (HpaBC) from *Escherichia coli* [12], and secreted tyrosinase from *Streptomyces avermitilis* [13]. Despite its high potential benefits for human health, the pharmaceutical applications of piceatannol are somewhat limited by low bioavailability due to its poor water solubility. Therefore, its glucoside may represent a means of improving its solubility, stability, and functionality.

The most abundant form of resveratrol in nature is *trans*-polydatin (3,4′,5-trihydroxystilbene-3-β-D-glucoside; *E*-polydatin; piceid), a glucoside of resveratrol (Fig. 1C) [14,15] that is mainly isolated from the plant *Polygonum cuspidatum* Sieb. et Zucc. (Polygonaceae). The polydatin concentration exceeds that

Abbreviations: NADPH, β-nicotinamide adenine dinucleotide phosphate (reduced form); p-NP, p-nitrophenol; P450 or CYP, cytochromes P450; TTN, total turnover number; WT, wild-type.

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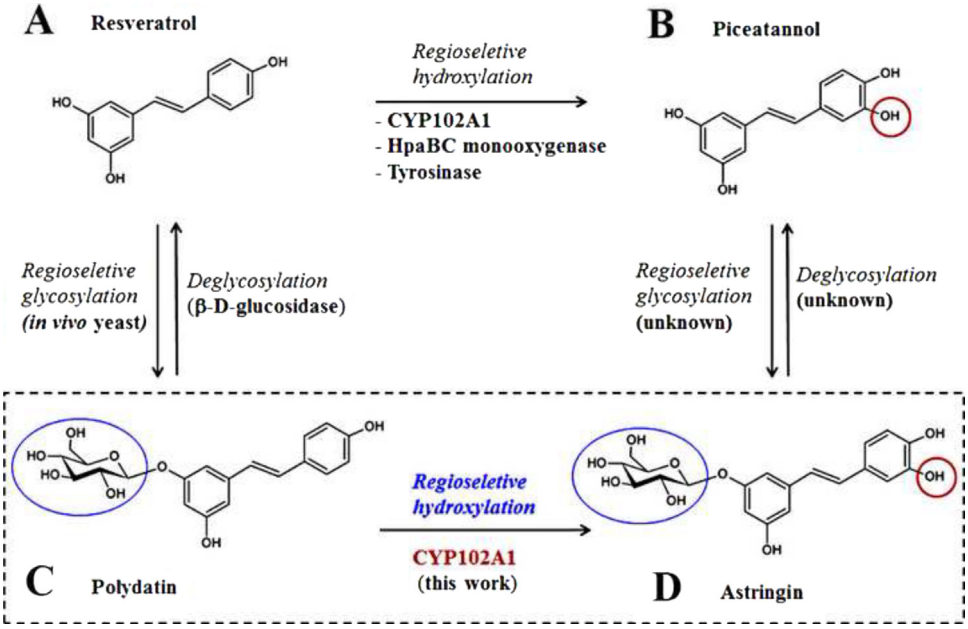

Fig. 1. P450 catalyzed regioselective hydroxylation of polydatin to produce (*E*)-astringin. A scheme of currently known enzymatic interconversion reactions of resveratrol (A), piceatannol (B), and polydatin (C) is also shown. In this study, enzymatic conversion from polydatin to (*E*)-astringin (D) was studied using CYP102A1 in the presence of NADPH (dashed box).

of resveratrol by 5- to 10-fold in *P. cuspidatum* [16]. It is also detected in many daily foods, such as grapes, peanuts, hop cones, red wine, hop pellets, cocoa-containing products, and chocolate products. Resveratrol can be produced from polydatin fermented by *Aspergillus oryzae* [17], which is a species that produces a piceid-β-D-glucosidase [18], and by stilbene glucoside-specific β-glucosidase from *Lactobacillus kimchi* [19]. Polydatin is also known to have many biomedical properties, such as anti-platelet aggregation, antioxidative activity, cardioprotective activity, and anti-inflammatory and immune-regulating functions [20]. Although polydatin is the most abundant form of resveratrol in plants and red wine, hydrolysis of polydatin can occur in the small intestine and liver, which would enhance the amount of the biologically active resveratrol [21]. After oral administration of polydatin in rats, polydatin undergoes extensive deglycosylation to form resveratrol [22]. Polydatin was found to be the main substance in serum after intragastric administration of polydatin or resveratrol, and the mutual transformation between polydatin and resveratrol maintains a balance; both of them have antioxidative effects *in vivo*, and polydatin has a better effect than resveratrol [23].

(*E*)-astringin (3,5,3′,4′-tetrahydroxystilbene-3-β-D-glucoside) (Fig. 1D) is a natural glycoside found in the bark of *Picea sitchensis* and *Picea abies* (Norway spruce) [24], in *Vitis vinifera* cell cultures [25], and in wine [26]. It is a stilbene that is piceatannol substituted at position C-3′ with a β-D-glucosyl residue. Although its pharmacological and physiological activities have not been extensively studied yet, it was found to be more potent than polydatin as an antioxidant [27]. Furthermore, (*E*)-astringin itself was reported to have potential cancer-chemopreventive activity [28].

At present, (*E*)-astringin is directly produced from natural sources, mainly plants, by solvent extraction methods [24,27]. Although it is produced in *Vitis vinifera* cell cultures, its abundance is too low for it to be isolated [25]. Chemical synthesis of (*E*)-astringin has not been reported yet. Biotransformation using enzymes usually requires mild conditions, simple procedures, and lower cost, and it results in less pollution [29]. There are two possible enzymatic pathways to make (*E*)-astringin. First, piceatannol can be used as an aglycone for regioselective glycosylation at its C-3 position to produce (*E*)-astringin. However, the regioselective glycosylation of piceatannol may be a challenge as it has four hydroxyl groups. Cultured *Phytolacca americana* cells glucosylate piceatannol to its 4′-glucoside but not 3-glucoside ((*E*)-astringin) [30]. Second, polydatin can be used as a substrate to make (*E*)-astringin. If it is regioselectively hydroxylated to produce (*E*)-astringin, polydatin has superior benefits over piceatannol. The cost of polydatin is much lower than that of piceatannol. Enzymatic regioselective hydroxylation of polydatin to make (*E*)-astringin would constitute a highly favorable synthetic procedure. However, such a procedure is not currently available.

The ability of P450 to catalyze regio- and stereo-selective C—H hydroxylation of non-activated hydrocarbons under mild reaction conditions is of special interest for a range of applications in fine chemical production and lead diversification [31]. Thus, P450-catalyzed reactions can accomplish chemical transformations that are significantly challenging tasks in chemical synthesis. CYP102A1 has been extensively engineered to catalyze the oxidation of non-natural substrates, such as alkanes, terpenes, heteroaromatics, alkaloids, steroids, and pharmaceuticals [31–34]. A large set of CYP102A1 mutants generated through rational design or directed evolution can oxidize several human P450 substrates to produce their authentic human metabolites with higher activity [33,35–39]. These results suggest that CYP102A1 can be developed as a biocatalyst for drug discovery and synthesis [40,41].

The aim of this study was to develop a simple strategy for the highly efficient one-step synthesis of a highly expensive (*E*)-astringin from polydatin, an inexpensive substrate, using P450. Fig. 1 illustrates the concept of the highly regioselective C—H bond hydroxylation of polydatin by CYP102A1. Resveratrol, an aglycone of polydatin, is known to be regioselectively hydroxylated into piceatannol by P450 BM3 [11]. As polydatin is a resveratrol glucoside, we compared the kinetic parameters of polydatin hydroxylation to those of resveratrol hydroxylation catalyzed by selected CYP102A1 mutants.