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Enzymatic synthesis of catechol-functionalized polyphenols with excellent selectivity and productivity

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ABSTRACT

Polyphenol products have become more and more attractive due to their strong anti-oxidant properties and a great variety of promising pharmacological activities and beneficial effects on human health. In this study, mushroom tyrosinase immobilized as cross-linked enzyme aggregates (CLEAs) was used as the catalyst for *ortho*-hydroxylation reactions to produce 3,4-dihydroxyphenylacetic acid, piceatannol and 3′-hydroxypterostilbene from 4-hydroxyphenylacetic acid, resveratrol and pterostilbene, respectively, with excellent selectivity and productivity. This is the first report of synthesizing these three polyphenolic compounds with tyrosinase CLEAs as catalyst, and the first study of biocatalytic production of 3′-hydroxypterostilbene. Introducing a deep eutectic solvent (DES) into the tyrosinase CLEA preparation exhibited a positive effect in terms of enhancing the catalytic activity of the immobilized enzyme and also promoting the synthesis of the polyphenol products.

1. Introduction

Polyphenols are an ample family of phenolic compounds such as phenolic acids, stilbenes, flavonoids and lignans, which are characterized by the presence of several hydroxyl groups attached on aromatic rings [1,2]. Nowadays, polyphenols have attracted more and more attentions mainly due to the recognition of their antioxidant properties and, as a result, their probable role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases [3]. The structure-activity relationship of stilbene analogues has revealed that an increased number of hydroxyl groups on the aromatic ring structure signifies better antitumor and free radical scavenging capacities; and in terms of the position of hydroxylation, *ortho*-hydroxystilbenes are more effective than other hydroxyl-substituted stilbenoid derivatives [4]. Piceatannol (Pic), 3′-hydroxypterostilbene (HPS) and 3,4-dihydroxyphenylacetic acid (DHPAA) are three of the typical examples (Scheme 1).

Piceatannol is an *ortho*-hydroxylated derivative of resveratrol (Res), a well-known naturally occurring stilbene endowed with a plethora of health-promoting effects such as anti-inflammation, −cancer, −diabetes, −obesity and −aging activities [5]. Although having been found to display a similarly broad spectrum of biological functions as resveratrol, piceatannol has shown to exhibit much higher antitumor and antioxidant properties [4]. In addition, this compound also possesses some other pharmacological activities as reviewed in [6]. All these beneficial properties have encouraged the use of piceatannol, more potent as complementary to its congener resveratrol, in health and functional foods as well as in pharmaceutical and cosmetic products.

3′-Hydroxypterostilbene is another naturally occurring *o*-dihydroxyl stilbenoid compound. The original molecule it derives from is pterostilbene (PS), which has received tremendous attention recently due to its much greater bioavailability and better biological activity, relative to its original analogue, resveratrol [5]. Although there is a paucity of published data regarding the biological activities of 3′-hydroxypterostilbene, a few studies have revealed that this compound possesses similar pharmacological activities such as being anti-oxidant, anti-inflammatory, and anti-adipogenic [7], and that it is more potent than pterostilbene against the growth of human cancer cells [8] and remarkably more effective in inducing apoptosis of leukemia cells than not only pterostilbene but also piceatannol and resveratrol [9], all suggesting that it may be a promising antitumor agent.

Although less studied, 3,4-dihydroxyphenylacetic acid is also a strong anti-oxidant agent [10]. Limited research has shown that it has anti-proliferative activity in prostate and colon cancer cells [11], and can protect against cholesterol-induced dysfunction of pancreatic β-cells [12]. In addition, this compound can also be used as a precursor for the synthesis of hydroxytyrosol and hydroxystilbenes, both of which are also natural polyphenols with a broad spectrum of beneficial effects

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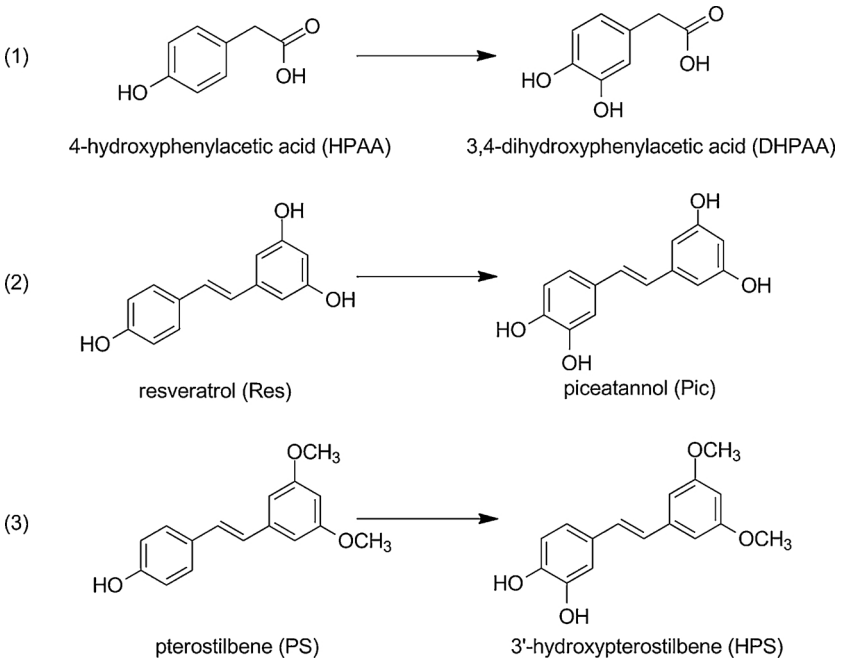


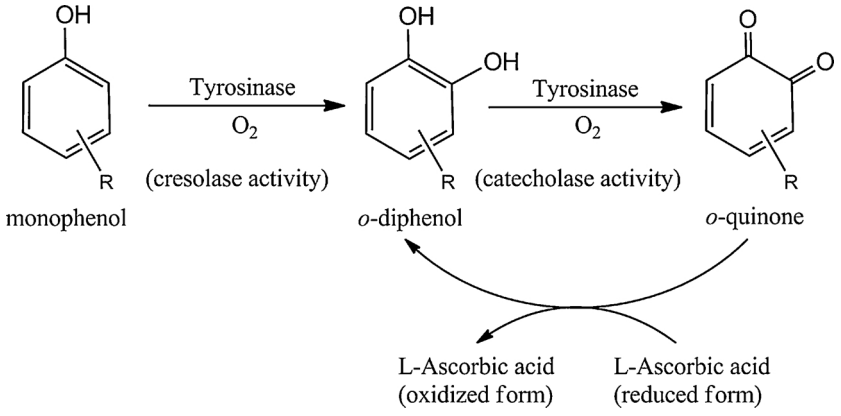
Scheme 1. The three synthetic reactions involved in this study.

on human health [13,14].

In spite of the more significant health benefits they can offer relative to their congeners, these *ortho*-hydroxylated polyphenol products are usually present in nature at much lower abundance [6]. Therefore, it is highly imperative to work out some synthetic strategies in order to supply sufficient amounts of these polyphenolic compounds for exploration and application of their pharmacological functions. Regioselective *ortho*-hydroxylation on benzene rings has always been challenging for chemical processes but can be easily achieved by means of biocatalysts. Several microbial P450 monooxygenases (CYP) and non-P450 ones (HpaBC) have been identified to be catalysts for *ortho*-hydroxylation of phenolic acids [15], stilbenoids [16–18] and flavonoids [19], however with low catalytic activity and synthetic efficiency. Tyrosinase (EC 1.14.18.1), on the other hand, is a promising candidate. The principle behind this is the strict regioselectivity of this enzyme: It is a copper-containing oxidoreductase responsible for the catalysis of the *ortho*-hydroxylation of monophenols to *o*-diphenols and their subsequent oxidation to *o*-quinones; in the presence of a reducing agent such as L-ascorbic acid the quinone product can be recycled back to the *o*-diphenol, leaving it as the sole product [20] (Scheme 2). The research conducted in our laboratory has verified that this enzyme, when immobilized in the form of cross-linked enzyme aggregates (CLEAs), is an efficient catalyst for *ortho*-hydroxylation of tyrosine with excellent productivity to synthesize L-DOPA, a drug for treatment of Parkinson's disease [21].

As a novel immobilization method more advantageous than conventional carrier-bound strategies, CLEA preparation consists of protein precipitation followed by cross-linking with each other, combining purification and immobilization into a single operation to provide easily and inexpensively prepared, yet highly stable and recyclable catalysts with remarkable catalytic efficiency. Due to these attractive features, this new immobilization method has been successfully applied to a variety of enzymes with widespread applications [22]. Our previous studies have indicated that immobilization of tyrosinase via CLEA formation can effectively improve the stability of the enzyme in aqueous solution against various deactivating conditions such as pH, temperature, denaturants, inhibitors, and organic solvents [23], and that the tyrosinase CLEAs exhibit a high catalytic power for efficient removal of phenolic compounds from wastewater [24].

The major goal of this study was to assess the feasibility of utilizing tyrosinase CLEAs as catalyst for *ortho*-hydroxylation to produce polyphenolic compounds, and DHPAA, Pic and HPS were taken as the 3 model products for this demonstration (Scheme 1). Although it is well known that biocatalytic synthesis is much more advantageous than



Scheme 2. Reaction scheme for tyrosinase-catalyzed *ortho*-hydroxylation to produce *o*-diphenols.