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Short communication

Highly efficient enzymatic synthesis of novel polydatin prodrugs with potential anticancer activity

Zhao-Yu Wang[a,*], Yan-Hong Bi[a], Rong-Ling Yang[a], Xiang-Jie Zhao[a], Ling Jiang[b,*], Cheng-Xin Ding[a], Shang-Yong Zheng[c]

[a] School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huai'an 223003, China
[b] College of Food Science and Light Industry, Nanjing Tech University, Nanjing 211816, China
[c] School of Medicine, Yunnan University, Kunming 650091, China

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ABSTRACT

Efficient lipase-mediated research work was successfully exploited for synthesizing potential 6″-*O*-acyl-polydatin prodrugs in biomass-derived 2-methyltetrahydrofuran (2-MeTHF). The results of the enzyme recognitions of nine acyl donors evidently demonstrated that the position and number of the C—C double bond in acyl chains profoundly influenced the behavior of the enzyme, which could be attributable to the resonance effect between the double bond and carbonyl group. Further investigations showed that introducing various acyl groups into the polydatin apparently enhanced its pH stability and 1-octanol-water partition coefficient (log *P*). With regard to the human cervical cancer siHa cell apoptosis by a flow cytometry assay, the lipophilic 6″-*O*-sorboyl-polydatin exhibited improved apoptosis-inducing capability than the parent drug. The presence of the more lipophilic sorboyl chain in the acylated derivative could account for this.

1. Introduction

Nature offers multifarious natural products with unique chemical structures and biological, pharmacological, and medicinal properties, which represents the feasibility of drug design and discovery based on their privileged molecular frameworks [1]. Recently, Newman et al. reviewed the source of new approved clinical drugs for treating a wide variety of human diseases in the last three decades [2]. From their data, only 6% of the new 1073 therapeutic agents are directly from the original natural products, whereas approximately 44% are actually the derivatives or analogues possessing natural product pharmacophores. Therefore, the natural product modification has always been the research hotspot for exploiting structural and functional diversity of the novel drug derivatives with desirable pharmacological properties [3].

Flavonoid glucosides are a large group of secondary plant polyphenolic metabolites with high biological activities in vitro [4]. For example, polydatin (3,4′,5-trihydroxystilbene-3-β-D-glucopyranoside) is one of the most common pharmacological constituents isolated from the root and rhizome of a traditional Chinese medicinal plant of *Polygonum cuspidatum* Sieb. et Zucc. and has been widely applied in anti-inflammatory, anti-oxidant, and anti-angiogenesis chemotherapy [5,6]. Owing to the polyhydroxy structure, however, polydatin usually shows poor solubility and weak stability in lipid formulations, thus resulting in its low bioavailability and unsatisfactory processability and limiting its application in the field of functional food, pharmaceutical, and cosmetic production [6]. In view of improving these properties, several strategies have been explored to modify polyhydroxy compound and one of the most successful approaches is to regioselectively synthesize its fatty ester derivatives [7,8]. Additionally, according to the biopharmaceutical classification system (BCS), the highly lipophilic and lipid-soluble drugs possessing appropriate membrane permeability have been proved to be of great significance [9]. The acylated derivatives of rutin, pruning, and naringin, for instance, exert more potent biological activity than the corresponding parental agents [10,11].

Enzymatic selective acylation of polyhydroxy compounds has turned out to be more and more popular than the multistep chemical methods, because of its most important advantages of high specificity and efficiency of the biocatalysts [12,13]. Particularly, the used eco-friendly biomass-derived solvents (such as 2-methyltetrahydrofuran, 2-MeTHF) in biocatalytic systems display potential abilities to overcome the conflict between the

* Corresponding authors.
E-mail addresses: biowzy@126.com (Z.-Y. Wang), jiangling@njtech.edu.cn (L. Jiang).

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solubility of the polyhydroxy substrate in hydrophilic media and maintenance of the enzyme activity in hydrophobic reaction system [14,15], which seems to be irreconcilable in pure organic solvents [16].

Based on the first attempt to acylate polydatin in eco-friendly 2-MeTHF in our group [17], in this study, a further extension of synthesizing various structural polydatin derivatives with promising enhanced biological and pharmaceutical properties was explored in 2-MeTHF (Scheme 1). First detailed investigations on the pH stability, log *P* of acylated derivatives, and acyl donor recognition of the *Thermomyces lanuginosus* lipase (lipozyme TL IM) were carried out subsequently. Also, the apoptosis effect on human cervical cancer siHa cell line induced by polydatin and its sorbic acid ester was examined to evaluate their potential anticancer activity.

2. Material and methods

2.1. Materials

Lipozyme TL IM (immobilized on granulated silica, *Thermomyces lanuginosus*, 55.0 U/g) was purchased from Novozymes Co., Ltd., China. Polydatin was obtained from Sigma-Aldrich. Vinyl sorbate, vinyl hexanoate, vinyl undecenoate, vinyl laurate, vinyl butyrate, vinyl crotonate, vinyl decanoate, vinyl palmitate, and vinyl myristate were provided by TCI. 2-MeTHF was from Aladdin and dried by 4 Å molecular sieves. Human siHa cell line and AnnexinV-FITC cell apoptosis assay kit (KGA108) were purchased from Nanjing Keygen Technology (Nanjing, China). Dulbecco's modified Eagle's medium (DMEM, high glucose) and phxosphate buffered saline (PBS) were obtained from Thermo Scientific Hyclone (South Logan, USA). Fetal bovine serum (FBS) was from Gibco Life Technologies (Basel, Switzerland). All other solvents were from commercial sources and of the highest purity available.

2.2. Enzymatic acylation procedure

The acylation was carried out in a 10 mL Erlenmeyer shaking flask with an anhydrous 2-MeTHF (3 mL) containing 0.03 mmol polydatin and 0.27 mmol vinyl donors. The reactants were well blended in an air-bath shaker with orbital stirring (200 rpm), and enzyme (5.5 U) was followed when the reaction temperature reached the desired level. Aliquot fractions of reaction mixture were periodically withdrawn during the reaction process and analyzed by HPLC. Each acylation experiment was performed at least in triplicate and the results were based on the average values.

2.3. HPLC analysis

The reaction mixture was analyzed by RP-HPLC on a 4.6 mm × 250 mm (5 μm) Eclips Plus-C18 column (Agilent Technologies Industries Co., Ltd., USA) using an Agilent G1311A pump and a UV detector at 275 nm. The mobile phase is a mixture of water and methanol at a flow rate of 1.0 mL/min. The volumetric ratio of methanol to water and the retention times for polydatin and its 6″-*O*-acyl-polydatin were 60/40, 2.54 and 4.01 min (butyrylation), 60/40, 2.63 and 3.87 min (crotonylation), 60/40, 2.55 and 7.34 min (hexanoylation), 60/40, 2.59 and 5.27 min (sorboylation), 80/20, 2.31 and 4.93 min (decanoylation), 80/20, 2.29 and 4.65 min (undecenoylation), 90/10, 2.31 and 8.19 min (lauroylation), 90/10, 2.24 and 4.41 min (myristoylation), 90/10, 2.26 and 6.57 min (palmitoylation). The initial rate, maximum substrate conversion and regioselectivity were calculated from the HPLC data.

2.4. Purification and NMR spectral analysis of the esters

Flash column chromatography using ethyl acetate (EA)/petroleum ether (PE) gave the pure acylated derivative. Structural assignments are made on the basis of the changes in the ^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) spectra caused by the acylation (Bruker DRX-400 NMR Spectrometer, Bruker Co., Germany). Results from the NMR spectroscopy are given in the Supplementary material.

2.5. pH stability assay of the acylated derivatives

In a typical experiment, a certain amount of the compound was dissolved completely in phosphate buffer solution at a given pH value in a sealed vial. After 24 h incubation at 40 °C and 200 rpm, aliquots were withdrawn and immediately analyzed by HPLC.

2.6. Determination of the log P values of the acylated derivatives

The 1-octanol-water partition coefficients (log *P*) of three unsaturated (C4, C6, and C11) and four saturated (C10, C12, C14, and C16) ester derivatives of polydatin were measured according to the previously reported method [18]. A certain amount of the compound was fully mixed with water and incubated in a shaking flask in an air-bath shaker at 37 °C and 200 rpm for 24 h. Aliquots of the mixture were withdrawn after centrifugation at 10000 rpm for 10 min and the concentration of the compound in each layer was calculated from the HPLC data.

2.7. Cell line culture

Human cervical cancer siHa cell line was maintained in Dulbecco's modified eagle's medium (DMEM, high glucose) containing 10% fetal bovine serum (FBS) at 37 °C in a humidified 5% CO_2 atmosphere. Cells in the logarithmic phase were used in the following experiments.

2.8. Cell apoptosis assay

For the flow cytometry assay, siHa cells were seeded at a density of 3×10^5 cells per well in 6-well plates for 24 h. Then, cells were treated with DMSO (0.1%, control) or an increasing dosage of the compound (0, 2.0, 4.0, 6.0, and 8.0 μM) for 48 h. After an incubation period, adherent and floating cells were collected, centrifuged and washed twice with PBS and stained with Annexin V-FITC and PI. Apoptotic cells were analyzed on a BD Accuri C6 flow cytometer (BD, USA), and data analysis was performed with CFlow Plus software.

3. Results and discussions

3.1. Effect of substrate structures on enzymatic acylation

Based on the optimization of the enzymatic acylation (data not shown), various acyl donors bearing different aliphatic chains and unsaturated bonds were examined to evaluate the universal applicability of the enzymatic synthesis of polydatin ester derivatives and understand the substrate recognition of lipozyme TL IM in 2-MeTHF. As shown in Table 1, in all nine cases in 2-MeTHF, polydatin could be successful converted to the desired product in excellent conversion (100%), which indicated that lipozyme TL IM had a broad substrate spectrum for different acyl donors. For the initial reaction rates, slight increases (from 14.3 to 16.2 mM/h) were found with elongating the chain length of saturated vinyl esters from C4 to C16. X-ray crystal structure study has demonstrated that the large hydrophobic substrate-binding site in active center of

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