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Efficient enzymatic synthesis and antibacterial activity of andrographolide glycoside

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ABSTRACT

19-*O*-β-Galactosyl andrographolide, a potential novel antibacterial agent, was synthesized through enzymatic transgalactosylation of andrographolide in co-solvent systems. Organic solvents and their contents have important influences on the regioselective galactosylation of andrographolide catalyzed by β-galactosidase from bovine liver in co-solvent systems. β-Galactosidase showed high activity and stability in 5–15% (v/v) DMSO with 22–52% total molar yields of andrographolide glycosides. The addition of hydrophilic DMSO not only greatly promoted the solubility of the substrate, but also improved the reaction efficiency of the process. β-Galactosidase displayed absolute regioselectivity toward the 19-position of andrographolide. The solubility of andrographolide glycoside in water was 42.1 mg ml^{-1}, which is about 702 times that of andrographolide. The glycosylated andrographolide showed antibacterial activity against five representative species of food-borne pathogenic bacteria [with minimal inhibitory concentrations (MICs) as low as 8 μg ml^{-1}], whereas andrographolide exhibited no such activity. These results indicate an enzymatic modification was not only facile and green, but an effective method for the preparation of an andrographolide monoglycoside.

1. Introduction

Andrographolide ($C_{20}H_{30}O_5$, Scheme 1), a diterpene lactone [1], is the main and most medicinally active component isolated from *Andrographis paniculata* (Burm.f.) Nees, an important herbal medicine traditionally used to treat different range of diseases in Asian countries [2–4]. Andrographolide is found in the whole plant but is most concentrated in the leaves (about 0.5–1.0%, dry weight) and has multiple pharmacological activities such as anti-bacterial, anti-inflammatory, antiviral, and anti-allergic activities [3–5]. However, the solubility of andrographolide in water was maximal at a concentration of 60 μg ml^{-1}. Moreover, andrographolide has a poor solubility in most organic solvents. The low solubility of andrographolide is disadvantageous not only by limiting its pharmacological use in potential therapeutics but also for its modification. Andrographolide glycosides are also active components from *A. paniculata*, and have good solubility in water, but their contents are very low (about 0.01–0.03%, dry weight) [5]. In fact, andrographolide glycosides derivatives can be achieved by glycoside modification of andrographolide.

Glycosylation has been used to modify the hydrophilicity, bioactivity and chemical properties of lipophilic natural products [6]. Glycosylation strategy has been established well for the improvement of therapeutic efficacy by enhancing oral absorption, selectivity and water-solubility of the parent agents, etc. [7]. For instance, the water-solubility of the antitumor drug—geldanamycin was markedly improved upon glycosylation modification [8]. Despite the many excellent chemical methods for glycosylation, methods for the direct regioselective glycosylation of andrographolide are limited owing to the presence of multi active hydroxyl groups in the reactant (Scheme 1). In general, arduous and tedious protection–deprotection steps and environmentally unfriendly catalysts were involved in traditional chemical methods. Use of enzymes as the alternative to chemical catalysts offered many new opportunities for the regioselective glycosylation, because of excellent selectivity, simplicity, mild reaction conditions and being environmentally benign [9]. Process simplification resulted in an overall reduction in energy use and waste production because of avoiding protection–deprotection steps (Fig. 1).

However, our pre-experiments showed that enzymatic glycosylation reaction in buffer was not efficient due to the low solubility of the andrographolide. Enzymatic glycosylation in hydrophilic organic solvents provides numerous industrially attractive advan-

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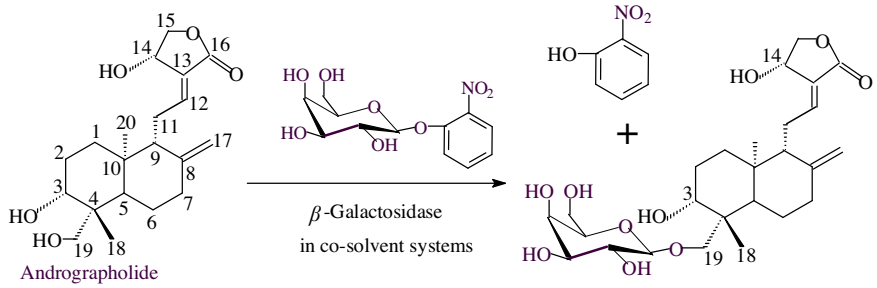

Scheme 1. Enzymatic regioselective galactosylation of andrographolide catalyzed by β-galactosidase from bovine liver.

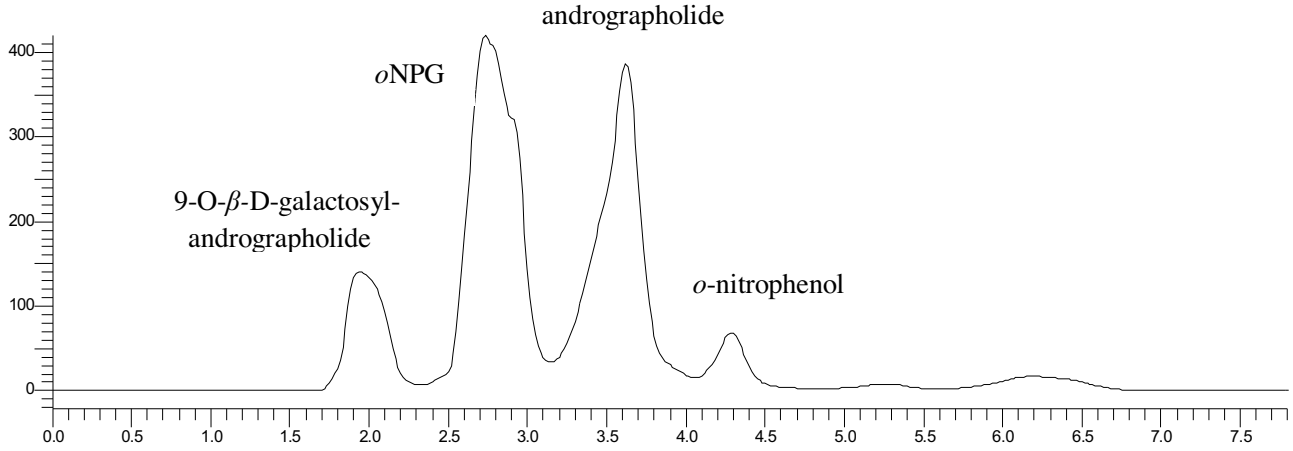

Fig. 1. HPLC of 19-*O*-β-D-galactosyl-andrographolide (1.9 min), *o*NPG (2.7 min), andrographolide (3.6 min) and *o*-nitrophenol (4.3 min).

tages, such as the increased solubility of substrate, reversal of the thermodynamic equilibrium of the hydrolysis reactions, and elimination of microbial contamination [10,11]. Obviously, the denaturation of enzymes in hydrophilic media is a major disadvantage. The synthetic utility of glycosidase would be considerably improved if its catalytic activity could be maintained in the presence of hydrophilic media.

Thus, an efficient solvent-resistant glycosidase in the presence of a certain percentage of hydrophilic organic solvent may play a pivotal role in a drastic reaction process. To the best of our knowledge, there have been no reports on the application of β-glycosidase to andrographolide glycoside syntheses. Herein we demonstrate that the β-galactosidase from bovine liver can be efficiently used for the glycosylation of andrographolide using *o*-nitrophenyl-β-D-galactoside (*o*NPG) as donor in hydrophilic organic solvents containing systems (Scheme 1). The activities of andrographolide glycoside derivatives as new antibacterial agents are also explored.

2. Materials and methods

2.1. Chemical and biological materials

The β-galactosidase from bovine liver (≥0.15 U mg^{-1} protein) was obtained from Sigm–Aldrich. Andrographolide (purity >99%) was purchased from Nanjing TCM Institute of Materia Medica, Nanjing, China. *o*-Nitrophenyl-β-D-galactoside (*o*NPG) was from Guangzhou Genebase Bioscience Co., Ltd., China. Dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), pyridine and acetone (all HPLC grade) were purchased from Sinopharm (Shanghai, China). All other chemicals were obtained from commercial sources and of the highest purity available.

Antimicrobial activities were assessed using five bacteria [*Vibrio parahemolyticus* ATCC 17802, *Listeria monocytogenes* ATCC 19115, *Salmonella enteritidis* CMCC 50041, *Staphyloccocus aureus* CICC 10307, *Escherichia coli* CICC 10372]. These test microbes were obtained from the American Type Culture Collection (ATCC), China Medical Culture Collection Center (CMCC, Beijing) and China General Microbiology Culture Collection Center (CGMCC, Beijing).

2.2. Enzyme activity assay

The enzyme powder was dissolved in phosphate buffer (100 mM, pH 7.0) at 4 °C to form the enzyme solution (10 mg ml^{-1}). Enzyme solution (20 μl) was added to 0.6 ml phosphate buffer (100 mM, pH 7.0) containing *o*NPG (50 mM). The reaction was conducted for 30 min at 45 °C, and then stopped by adding 5.38 ml 1 M Na_2CO_3. The released *o*-nitrophenol was assayed at 420 nm. One unit of glycosidase activity was defined as the amount of enzyme required to catalyze the release of 1 μmol *o*-nitrophenol per minute under the conditions given. The specific activity of the enzyme was 5 U ml^{-1}.

2.3. Solubility determination

Andrographolide or andrographolide glycoside was mixed with 0.5 ml of solvent in an Eppendorf tube at 45 °C. An ultrasonic cleaner (Type NP-B-400-15; Newpower Co., Ltd., Kunshan, China) was used to maximize the solubility of each compound. After 1 h sonication and centrifugation at 10,000 × *g* for 20 min to remove insoluble material, the sample was diluted and filtered through a 0.45 μm membrane, and used for HPLC analysis to determine the sample solution concentration.