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Synthesis and characterization of ampelopsin glucosides using dextransucrase from *Leuconostoc mesenteroides* B-1299CB4: Glucosylation enhancing physicochemical properties

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ABSTRACT

Novel ampelopsin glucosides (AMPLS-Gs) were enzymatically synthesized and purified using a Sephadex LH-20 column. Each structure of the purified AMPLS-Gs was determined by nuclear magnetic resonance, and the ionic product of AMPLS-G1 was observed at *m/z* 505 $(C_{21}H_{22}O_{13}{\cdot}Na)^+$ using matrix-assisted laser desorption ionization time-of-flight mass spectrometry. AMPLS-G1 was identified as ampelopsin-4′-*O*-α-D-glucopyranoside. The optimum condition for AMPLS-G1, determined using response surface methodology, was 70 mM ampelopsin, 150 mM sucrose, and 1 U/mL dextransucrase, which resulted in an AMPLS-G1 yield of 34 g/L. The purified AMPLS-G1 displayed 89-fold increased water solubility and 14.5-fold browning resistance compared to those of AMPLS and competitive inhibition against tyrosinase with a K_i value of 40.16 μM. This value was smaller than that of AMPLS (K_i = 62.56 μM) and much smaller than that of β-arbutin (K_i = 514.84 μM), a commercial active ingredient of whitening cosmetics. These results indicate the potential of AMPLS and AMPLS-G1 as superior ingredients for functional cosmetics.

1. Introduction

The ampelopsin (3,5,7,3′,4′,5′-hexahydroxyl 2,3 dihydrogen flavanonol, AMPLS) is one of the most common flavonoids isolated from the tender stem and leaves of the plant species *Ampelopsis grossedentata* (*Hand-Mazz*) W.T. Wang, known as rattan tea, a health beverage in China. Ampelopsin possesses numerous pharmacological activities, such as antiinflammatory and antimicrobial activity, relief of cough, as well as antioxidation, antihypertension, hepatoprotective, and anticarcinogenic effects [1–3]. A recent study reported the anti-human immunodeficiency virus (HIV) effects of AMPLS and its interaction with the HIV-1 receptor CXCR4. The antiviral effect of AMPLS might be related to its antioxidative properties [3].

Systematic research on AMPLS has been conducted to characterize its pharmacological potential and the possibility for drug product development. AMPLS is poorly soluble in water (0.67 mM at 25 °C) and in aqueous and organic solutions, AMPLS decomposes easily when exposed to light, forming a colored photolysis product. The low water solubility, low intestinal permeability, and ease of degradation in solution have limited the potential of AMPLS in most pharmaceutical preparations and as an experimental compound. Various techniques have been used to improve the solubility/dissolution rate of poorly water-soluble drugs such as AMPLS. Among them, the solid dispersion technique [4,5] and complex with cyclodextrins [6] are most frequently used.

Glucansucrases or dextransucrases are enzymes that synthesize either dextrans or glucans. Glucansucrases catalyze the transfer of a sucrose-derived glucose to other carbohydrates using sucrose as

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the substrate [7], thereby allowing oligosaccharide synthesis [8]. This reaction is referred to as an acceptor reaction [9]. Glucansucrases also catalyze the transfer of mono-, di-, or higher glucose units to other carbohydrate acceptors via a variety of glycosidic linkages [10].

Transglycosylation catalyzed by enzymes from various bacteria has been used to improve physicochemical properties such as water solubility and oxidative stability of various compounds [7]. Epigallocatechin gallate glucosides [11], L-DOPA α-glycosides [12], hydroquinone-glucoside, -fructoside, -galactoside [13–15] and astragalin-glucosides [16] were synthesized using the acceptor reaction with sucrose and dextransucrases from *Leuconostoc mesenteroides* [11] and the glycosides display varied anti-oxidant effects, strong browning resistance, and increased water solubility compared to particular acceptor according to their structures. Four kinds of ascorbic acid derivatives using maltogenic amylase from *Bacillus stearothermophilus* [17,18] and two kinds of caffeic acid glucosides using α-amylase from *Bacillus subtilis* [19] were synthesized and the glycosylated compounds exhibit rather effective antioxidant properties, prevention of lipid oxidation, and/or antimutagenic properties.

In this study, we report the synthesis and characterization of pure AMPLS glucosides, in which a D-glucopyranosyl residue is attached as an α-1,6 linkage to the B ring of AMPLS and, alternatively, the glucose unit of AMPLS with different degrees of polymerization (AMPLS-Gs) using dextransucrase prepared from *L. mesenteroides* B-1299CB4. The purified AMPLS-G1 (one glucose linked AMPLS) was studied regarding various activities as a potential functional food ingredient or as a raw material for medicine and cosmetic regents.

2. Materials and methods

2.1. Materials

AMPLS was purchased from ZR Chemicals (Shanghai, China). Sucrose, 1,1-diphenyl-2-pricrylhydrazyl (DPPH), deuterium oxide (D_2O), and mushroom tyrosinase were purchased form Sigma–Aldrich (St. Louis, MO, USA). Sephadex LH-20 was purchased from GE Healthcare (Uppsala, Sweden). Other chemical reagents were commercially available and a chemically pure grade.

2.2. Enzyme preparation

Dextransucrase was prepared by culturing *L. mesenteroides* B-1299CB4 which produced 4.5 times higher activity and showed complete constitutivity for dextransucrase production in glucose medium [11,20]. The partially purified dextransucrase was obtained as described previously [11]. Enzyme activity was evaluated via the incubation of the enzyme for different reaction periods at 28 °C with 100 mM sucrose in 20 mM sodium acetate buffer (pH 5.2) as substrate. Each of enzyme reaction samples was spotted on a silica gel $60F_{254}$ thin layer chromatography (TLC) plate (Merck, Darmstadt, Germany). The TLC plate was ascended twice on acetonitrile:water (85:15, v/v) solvent system. Each of the carbohydrates was visualized via the dipping of the plates into 0.3% (w/v) N-(1-naphthyl)-ethylenediamine and 5% (v/v) H_2SO_4 in methanol, followed by 10 min of heating at 121 °C [11,16]. The amount of fructose released from the sucrose was then analyzed using the AlphaEase® FC 4.0 Image Program (Alpha Inotech, San Leandro, CA, USA), with standard materials. One unit was defined as the amount of enzyme that catalyzed the formation of 1 μmol of fructose per min at 28 °C.

2.3. Glucosylation of AMPLS

The reaction mixture (100 mL) in 20 mM sodium acetate buffer (pH 5.2) and 20% dimethyl sulfoxide consisting 70 mM AMPLS (224 mg), 150 mM sucrose, and 1299CB4 dextransucrase (1 U/mL) was incubated at 28 °C for 1 h. The reaction mixture was then boiled for 10 min to halt the enzyme reaction. The synthesis of AMPLS accepter reaction products was confirmed using TLC as described previously.

2.4. Analysis of acceptor reaction products by TLC

TLC was conducted at room temperature using a silica gel $60F_{254}$ TLC plate (Merck, Darmstadt, Germany). The reaction mixtures were spotted onto the silica gel plate, and the plate was doubly developed first using a solvent mixture of ethyl acetate:acetic acid:water (3:1:1, v/v/v) and then using acetonitrile:water (85:15, v/v). The compounds were developed by rapidly dipping each plate into a solution as described above; each plate was dried and then placed in an oven for 10 min at 120 °C [11,16].

2.5. Experimental design for optimization of acceptor reaction

The experimental response surface method (RSM) data were fitted via the response surface regression procedure using the following second-order polynomial equation:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3.$$

Y is the predicted response and x_1, x_2, x_3 are the independent variables. Design-Expert 7.1.5 CCD RSM software (State-Ease, Minneapolis, MN, USA) was used for regression analysis and graphical analysis of the data obtained during all experiments. Analysis of variance (ANOVA) was used to estimate the statistical parameters. The significance of the model equation and model terms was evaluated by Fisher's test [21]. The quality of fit for the polynomial model equation was expressed by the coefficient of determination (R^2) and adjusted R^2. The combination of different parameters producing the maximum response was determined to verify the model. A preliminary experiment indicated that three factors (dextransucrase unit, sucrose and ampelopsin concentration) were needed to optimize the AMPLS-G1synthesis of: dextransucrase from *L. mesenteroides* B-1299CB4, 0.1–3 U/mL; 50–300 mM sucrose concentration, and 10–150 mM AMPLS.

2.6. Purification of AMPLS-Gs

The reaction mixture in 20 mM Na-acetate (pH 5.2) consisted of 70 mM AMPLS, 150 mM sucrose, 1.84 U/mL dextransucrase, and 20% dimethyl sulfoxide. Twelve milliliters of the concentrate was applied to a 3 cm × 110 cm Sephadex LH-20 column. After the column was washed with distilled water (total, 1 L; flow rate, 1 mL/min) to remove the sugars (polymers, leucrose, fructose and glucose), each AMPLS acceptor reaction product was purified with a gradient of 0–100% ethanol (total, 3.5 L; flow rate, 1 mL/min). Each AMPLS glucoside fraction was concentrated with a rotary evaporator (EYELA, Tokyo, Japan) at 50 °C [17]. Each fraction was subjected to high performance liquid chromatography (HPLC) (LC-10AD; Shimadzu, Kyoto, Japan) under the following conditions: reverse column, μ-Bondapak C_{18} (19 cm × 300 cm); mobile phase, methanol:water = 4:6 (v/v); flow rate, 10 mL/min; room temperature; detection, RID-10A model RI detector (Shimadzu). The eluents were concentrated at 65 °C with a rotary evaporator (EYELA).

2.7. Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry

Each purified AMPLS-glucoside was diluted with deionized water then mixed with a 1:1 ratio (v/v) of a 2,5-dihydroxybenzoic acid (1 mg/mL) matrix dissolved in water. The mixed solution (1 μL) was then spotted onto a stainless steel plate and slowly dried at room temperature. The water was vaporized, leaving only the re-crystallized DHB with the AMPLS-glucoside spread throughout the DHB crystals. The mass spectrum was acquired using a Voyager DE-STR MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA). The mass spectra were obtained in the positive linear mode with delayed extraction (average of 75 laser shots) of a 65 kV accelerating voltage [11].

2.8. Nuclear magnetic resonance (NMR) analysis

Approximately 5 mg of the purified AMPLS-G1 was dissolved in 250 μL of D_2O and dispensed into 3 mm NMR tubes. NMR spectra were obtained on a Unity Inova 500 spectrometer (Varian, Palo Alto, CA, USA) operating at 500 MHz for ^1H and 125 MHz for ^{13}C at 25 °C. Linkage between AMPLS and glucose was evaluated using the spectra of homonuclear correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) [15].

2.9. Water solubility analysis

Excess AMPLS, AMPLS-G1 or AMPLS-G2 was mixed with 200 μL of water in an Eppendorf tube at room temperature. A 3510R-DTH ultrasonic cleaner (Branson, Danbury, CT, USA) was used to maximize solubility. After 1 h of sonication at room temperature, each sample was diluted and then filtered through a 0.45 μm MFS membrane (Adventec, Pleasanton, CA, USA) for HPLC analyses to determine the maximum concentrations to be dissolved in water [11]. A model 1525 HPLC system, connected to a 400 mm × 3.9 mm i.d. μ-Bondapak C_{18} column (Waters, Milford, MA, USA) and a model 2487 UV detector (Waters) at 280 nm were utilized to quantify the amounts of AMPLS and AMPLS-G1 [22]. The mobile phase consisted of 23% methanol, and HPLC was conducted via the isocratic method with a flow rate of 0.5 mL/min.

2.10. Browning resistance effect of AMPLS and its glucosides (AMPLS-Gs)

Browning resistance of each AMPLS or AMPLS-Gs after UV irradiation in an aqueous system was evaluated in water (1 mL) containing 0.25% (w/v) of AMPLS,