Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Molecular Catalysis B: Enzymatic 40 (2006) 1–7
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Enzymatic synthesis and characterization of novel epigallocatechin gallate glucosides

Young-Hwan Moon [a], Ghahyun Kim [b], Jin-Ha Lee [c], Xing-Ji Jin [d,h], Do-Won Kim [e], Doman Kim [f,g,h,*]

[a] *Laboratory of Functional Carbohydrate Enzymes and Microbial Genomics, Department of Material and Chemical and Biochemical Engineering, Chonnam National University, Gwangju 500-757, South Korea*
[b] *Korean Minjok Leadership Academy, Gangwondo 225-823, South Korea*
[c] *Engineering Research Institute, Chonnam National University, Gwangju 500-757, South Korea*
[d] *Department of Fine Chemical Engineering, Chonnam National University, Gwangju 500-757, South Korea*
[e] *Department of Physics, Kangnung National University, Kangnung 210-702, South Korea*
[f] *Biology Research Center for Industrial Accelerator, Dongshin University, Naju, Jeollanamdo 520-714, South Korea*
[g] *School of Biological Sciences and Technology, and The Research Institute for Catalysis, Chonnam National University, Gwangju 500-757, South Korea*
[h] *Institute of Bioindustrial Technology, School of Biological Sciences, Chonnam National University, Gwangju 500-757, South Korea*

Received 8 November 2005; received in revised form 19 January 2006; accepted 21 January 2006
Available online 28 February 2006

Abstract

Three epigallocatechin gallate (EGCG) glucosides were synthesized by the acceptor reaction of a glucansucrase from *Leuconostoc mesenteroides* B-1299CB with EGCG and sucrose. After ^{1}H, ^{13}C, heteronuclear single quantum coherence, ^{1}H–^{1}H correlation spectroscopy, and heteronuclear multiple bond correlation nuclear magnetic resonance analyses, the glucosides were identified as (−)-epigallocatechin gallate 4″-*O*-α-D-glucopyranoside (EGCG-G1), (−)-epigallocatechin gallate 7,4″-di-*O*-α-D-glucopyranoside (EGCG-G2A), and (−)-epigallocatechin gallate 4′,4″-di-*O*-α-D-glucopyranoside (EGCG-G2B). Two of the compounds (EGCG-G1 and EGCG-G2A) are reported for the first time. The EGCG glucosides exhibited antioxidant effects, depending on their structures (EGCG > EGCG-G1 > EGCG-G2A > EGCG-G2B). They also uniformly exhibited greater browning resistance than was observed in EGCG. Also, the water solubility of EGCG-G1, EGCG-G2A, and EGCG-G2B were 69, 126, and 122 times higher, respectively, than that of EGCG.

Keywords: *Leuconostoc mesenteroides* glucansucrase; EGCG; Glucoside; Acceptor reaction

1. Introduction

Glucosyltransferases (GTFs) are enzymes that synthesize either dextrans or glucans, using sucrose as a substrate [1]. Robyt et al. first reported that GTFs can catalyze the transfer of a sucrose-derived glucose to another carbohydrates, thereby, allowing oligosaccharide synthesis [2]. This reaction is an *acceptor reaction*, where the added carbohydrates are *acceptors* [3]. GTFs are also able to transfer mono-, di-, or higher glucose units to other acceptors, forming a variety of glycosidic linkages [4].

Enzymatic transglycosylation, using *L. mesenteroides* glucansucrases, has been applied to modify a variety of bioactive substances, in efforts to improve functionality. Acarbose analogues have been synthesized by the reaction of acarbose with

Abbreviations: COSY, homonuclear correlation spectroscopy; DHB, 2,5-dihydroxybenzoic acid; DMSO-d_6, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EGCG, epigallocatechin gallate; EGCG-G1, (−)-epigallocatechin gallate 4″-*O*-α-D-glucopyranoside; EGCG-G2A, (−)-epigallocatechin gallate 7,4″-di-*O*-α-D-glucopyranoside; EGCG-G2B, (−)-epigallocatechin gallate 4′,4″-di-*O*-α-D-glucopyranoside; EMS, ethyl methane sulphonate; GTFs, glucosyltransferases; HPLC, high performance liquid chromatography; HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single quantum coherence; *L. mesenteroides*, *Leuconostoc mesenteroides*; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; NMR, nuclear magnetic resonance

* Corresponding author. Fax: +82 62 530 0874.
E-mail address: dmkim@chonnam.ac.kr (D. Kim).

1381-1177/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2006.01.030

sucrose and glucansucrases from *L. mesenteroides* B-512FMC and B-742CB [5]. These acarbose analogues inhibited a variety of enzymes related to diabetes, including α-glucosidase[6]. Salicin analogues have also been synthesized using glucansucrases, with sucrose as a substrate, by both Seo et al. and Yoon et al. [4,7]. They reported that the salicin analogues inhibited blood coagulation.

Enzymatic transglycosylation, using other enzymes, have been also employed in the modification of natural bioactive compounds, improving their physicochemical properties. Lee et al. reported that glycosylated ascorbic acids, synthesized using amylase, prevented lipid oxidation, and manifested synergistic effects superior to those associated with ascorbic acid [8]. With regard to solubility, glycosylated naringin, synthesized using amylase, was found to be 250 times more soluble in water than naringin, and 10 times less bitter [9]. Li et al. also reported that the solubility of transglycosylated puerarin, synthesized using amylase, was 14–168 times higher than that of puerarin [10]. Glycosylated catechin, synthesized using sucrose phosphorylase, has proven to be quite stable against UV radiation, although catechin itself is readily degraded [11].

Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea. It exhibits antioxidant [12], anticancer [13], antitumour [14], and antibacterial effects [15], appears to prevent dental caries [16], and regulate plasma lipid levels [17]. However, EGCG is not very soluble in water, and degrades easily in aqueous solutions [18]. Due to these disadvantages, the uses of EGCG in the food, drug, and cosmetic industries remains somewhat limited. In order to circumvent these disadvantages, researchers have focused on the glycosylations of a host of polyphenols [11,18,19]. Transglycosylated compounds showed enhanced functionalities in water solubility, stability on light or oxidation, lower bitter taste, and potent tyrosinase inhibition [11,18–20].

The dextransucrase from *L. mesenteroides* NRRL B-1299 synthesizes two types of dextrans: fraction L, which is precipitated by 38% ethanol and contains 27% α-1,2 and 1% α-1,3 branched glucan; and fraction S, which is precipitated by 40% ethanol, and contains 35% α-1,2 branch glucan [21–23]. Dols et al. using dextransucrase from B-1299 in the presence of maltose and sucrose generated a primarily α-1,6 linked glucooligosaccharides, with some α-1,2 branch linkages [24]. *L. mesenteroides* NRRL B-1299CB is a constitutive mutant derived from *L. mesenteroides* NRRL B-1299, using the ethyl methane sulphonate (EMS) method developed by Kim and Robyt [25]. This mutant is constitutive for glucansucrases and therefore is devoid of contaminating dextran.

In this paper, we report the synthesis of a set of novel EGCG glucosides where a D-glucopyranosyl residue is attached to the 4″-hydroxyl group of the galloyl residue (EGCG-G1), the 7- and 4″-hydroxyl groups of EGCG (EGCG-G2A) or the 4′- and 4″-hydroxyl groups of EGCG (EGCG-G2B). This is the first report of EGCG-G1 and EGCG-G2A. In comparison to EGCG, EGCG glucosides were more stable to UV irradiation with increased browning resistance and water solubility.

2. Experimental

2.1. Materials

The EGCG, dimethyl sulfoxide (DMSO-d_6), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,5-dihydroxybenzoic acid (DHB) were obtained from the Sigma Chemical Co. (St. Louis, USA). The Sephadex LH-20 gel was acquired from Amersham Biosciences (Uppsala, Sweden).

2.2. Enzyme preparation

L. mesenteroides B-1299CB mutant was grown at 28 °C on LM medium containing 2% (w/v) glucose as a carbon source. The LM medium consists of 4 g yeast extract, 2 g peptone, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.01 g NaCl, 0.01 g $MnSO_4{\cdot}H_2O$, 0.015 g $CaCl_2{\cdot}2H_2O$, and 2 g K_2HPO_4 per liter of deionized water. After fermentation, the cultures were harvested, centrifuged, and concentrated using a 30 K cut-off hollow fiber membrane (Millipore, Bedford, MA).

2.3. Assay of glucansucrase activity

Enzyme activity was evaluated by the incubation of the enzyme for different reaction periods at 28 °C with 100 mM sucrose, in 20 mM sodium acetate (pH 5.2) as a substrate. Standard assay mixtures contained 200 μL of 200 mM sucrose and 200 μL of an enzyme solution. Each of the reaction samples was spotted on Whatman K5 TLC plates (Whatman Inc., Clifton, NJ). The TLC plates were developed using a double ascension of an acetonitrile–water (85:15, v/v) solvent system. The carbohydrates were visualized using 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 heating for 10 min at 121 °C [26]. The fructose concentration was determined using an NIH Image Program, against standards [27]. One unit of glucansucrase activity was defined as the amount of enzyme required to generate 1 μmol of fructose per minute at 28 °C and pH 5.2, in 20 mM sodium acetate buffer.

2.4. Glucosylation of EGCG

Glucosylation was conducted in reaction mixture (250 mL) containing 0.2% EGCG, 80 mM sucrose, and B-1299CB glucansucrase (2.4 U mL^{-1}). It was incubated at 28 °C for 6.5 h, and the reaction mixture was then boiled for 8 min to halt the enzyme reaction.

2.5. Analysis of acceptor reaction products using thin layer chromatography (TLC)

TLC was conducted at room temperature using silica gel 60 F_{254} TLC plates (Merck Co.). One microlitre of a reaction mixture was spotted onto a lane in a silica gel plate, and the plate was then developed using a solvent mixture of ethyl acetate–acetic acid–water (3:1:1, v/v/v). The developed plate was then dried and visualized as previously described [26].

2.6. Purification of EGCG acceptor reaction products

The reaction products (250 mL) were separated using Sephadex LH-20 column (47 mm × 200 mm). After loading sample, unbound sugars (sugar-polymer, fructose, and glucose) were removed with distilled water (3 L, 1 mL/min), and then successively eluted with 70% (v/v) ethanol (1 L). The eluent (which contained EGCG glucosides) was then concentrated at 47 °C with a rotary evaporator. The eluant was subjected to HPLC on a LC-10AD instrument (Shimadzu, Koyto, Japan) under the following conditions: reverse column, μ-Bondapak C_{18} (7.8 mm × 300 mm; Waters, Milford, MA); mobile phase, 23% methanol; flow rate, 3 mL/min; room temperature; detection, Waters 2487 UV detector at 280 nm (Waters, Milford, MA).

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

The purified EGCG glucosides (3 mg/mL) were diluted with deionized water, then mixed with 2,5-dihydroxybenzoic acid (1 mg/mL) dissolved in acetonitrile, at a 1:1 ratio. The resultant solution (1 μL) was spotted onto a stainless steel plate, and slowly dried at room temperature. The mass spectrum was obtained using a Voyager DE-STR MALDI-TOF mass spectrometer (Applied Biosystems, Foster, CA). The mass spectra were obtained in the positive linear mode with delayed extraction (average of 75 laser shots) with a 65 kV acceleration voltage.

2.8. Nuclear magnetic resonance (NMR) analysis

About 2–3 mg of the purified EGCG glucosides were dissolved in DMSO-d_6 (250 μL) and placed into 3 mm NMR tubes. NMR spectra were obtained using a Unity Inova 500 spectrometer (Varian Inc., Charlotte, NC), operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR at 25 °C. Linkages between EGCG and glucose were characterized using the spectra obtained from homonuclear correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC). Specific rotations were measured with a JASCO DIP-1000 digital polarimeter (JASCO Inc., Easton, MD).

2.9. Browning resistance effect of EGCG and its glucosides

Browning resistance to UV irradiation was examined in water solution (1.0 mL) containing 0.25% (w/v) of either epigallocatechin gallate or the EGCG glucosides. The sample solutions were then exposed to UV irradiation at a distance of 10 cm from the 254 nm, 10 W, G10T8-AN UV source (Sankyo Denki, Tokyo, Japan) for 24 h at room temperature. The increases in absorbance were determined at 460 nm, using a BIO-RAD SmartSpec™ 3000 spectrophotometer.

2.10. Antioxidant activity

The antioxidant activity of EGCG and of each of the EGCG glucosides was evaluated using DPPH radical scavenging [28]. Each of the samples (10, 12.5, 25, 50, 100, 200 μM) was dissolved in ethanol (30 μL) and mixed thoroughly with a 100 μM DPPH ethanol solution (270 μL). After 10 min in the absence of light, at room temperature, the absorbance of the mixture was measured at a wavelength of 517 nm using a BIO-RAD SmartSpec™ 3000 spectrophotometer. DPPH radical-scavenging activity was then evaluated by the decrease in the absorbance in the samples compared to a blank (ethanol). The SC_{50} value designates the sample concentration at which the absorbance was reduced by 50%.

2.11. Water solubility analysis

The excess EGCG and EGCG glucosides were mixed with 200 μL of water in an Eppendorf tube at room temperature. An ultrasonic cleaner (3510R-DTH, Branson, Danbury, CT) was used to maximize the solubility. After vortexing for 20 min at room temperature (23 °C—after 5 min vortexing, each sample was kept at 20 °C water bath for 2 min), each sample was diluted and filtered through a 0.45 μm MFS membrane (Adventec, Peasanton, CA) for HPLC analysis, in order to determine the concentrations. A Waters 1525 HPLC system connected to a μ-Bondapak C_{18} (3.9 mm × 400 mm, Waters, Milford, MA) and a UV detector (Waters 2487, Waters, Milford, MA) at 280 nm was used to determine the quantities of EGCG or EGCG glucosides. The mobile phase consisted of 23% methanol by the isocratic method, at a flow rate of 0.5 mL/min. The concentrations of EGCG and the EGCG glucosides were calculated according to the method previously described by Li et al. [10].

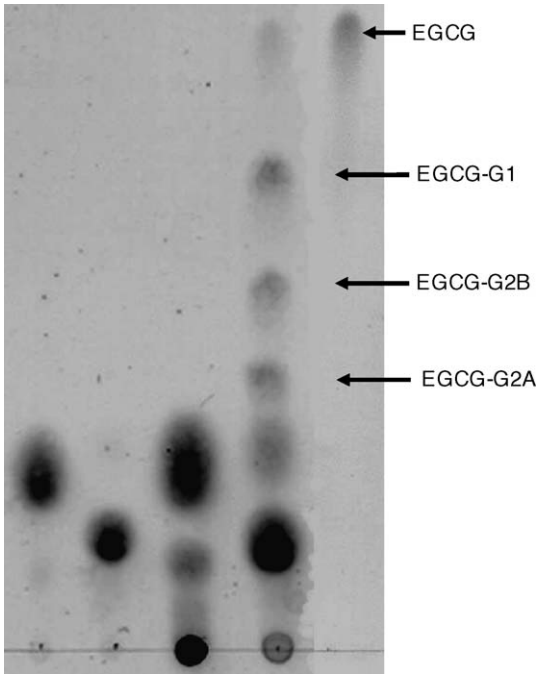

Fig. 1. Thin-layer chromatogram of the glucansucrase acceptor reaction mixture. Lane 1, fructose; lane 2, sucrose; lane 3, enzyme reaction mixture (without EGCG); lane 4, enzyme reaction mixture with EGCG. Arrows indicate EGCG and its acceptor products.

Table 1
^{13}C and ^{1}H NMR data of EGCG and EGCG glucosides (units: ppm)

Carbon position	EGCG (δ)		EGCG-G1 (δ_1)			EGCG-G2A (δ_2)			EGCG-G2B (δ_3)		
	δ_C	δ_H	δ_{1C}	($\delta_1 - \delta$)	δ_H	δ_{2C}	($\delta_2 - \delta$)	δ_H	δ_{3C}	($\delta_3 - \delta$	δ_H
EGCG											
2	76.6	4.95 (br s)	76.6	(−0.3)	4.97 (br s)	76.9	(0.3)	5.05 (br s)	76.6	(0.0)	5.04 (br s)
3	68.2	5.36 (br s)	68.7	(0.5)	5.38 (br s)	68.9	(0.5)	5.43 (br s)	69.1	(0.9)	5.40 (br s)
4	25.9	2.92 (dd) (J = 4.5, 17 Hz) 2.65 (br d) (J = 17 Hz)	25.6	(−0.3)	2.94 (dd) (J = 4, 17 Hz) 2.68 (br d) (J = 17 Hz)	26.1	(0.2)	3.03 (dd) (J = 4.5, 17 Hz) 2.75 (br d) (J = 17 Hz)	26.1	(0.2)	2.95 (dd) (J = 4, 17 Hz) 2.69 (br d) (J = 17 Hz)
4a	97.6		97.2	(−0.4)		100.8	**(3.2)**		97.6	(0.0)	
5	155.8		155.6	(−0.2)		155.9	(0.1)		155.8	(0.0)	
6	94.5	5.82 (d) (J = 2.5 Hz)	94.3	(−0.2)	5.83 (d) (J = 2 Hz)	96.3	**(1.8)**	6.18 (d) (J = 2.5 Hz)	94.8	(0.3)	5.86 (d) (J = 2 Hz)
7	156.7		156.6	(−0.1)		157.2	(0.5)		157.1	(0.4)	
8	95.7	5.93 (d) (J = 2.5 Hz)	95.6	(−0.1)	5.93 (d) (J = 2 Hz)	97.6	**(1.9)**	6.23 (d) (J = 2 Hz)	96.1	(0.4)	5.96 (d) (J = 2 Hz)
8a	156.7		156.5	(−0.2)		156.9	(0.2)		156.9	(0.2)	
1′	128.8		128.6	(−0.2)		128.8	(0.0)		135.4	**(6.6)**	
2′/6′	105.7	6.40 (s)	105.3	(−0.4)	6.40 (s)	105.8	(0.1)	6.45 (s)	105.8	(0.1)	6.49 (s)
3′/5′	145.6		145.7	(0.1)		146.2	(0.6)		150.7	**(5.1)**	
4′	132.5		132.4	(−0.1)		132.8	(0.3)		133.7	(1.2)	
1″	119.5		125.5	**(6.0)**	5	125.9	**(6.4)**		125.8	**(6.3)**	
2″/6″	108.8	6.81 (s)	108.4	(−0.4)	6.81 (s)	108.9	(0.1)	6.84 (s)	108.9	(0.1) 6.83 (s)	6.83 (s)
3″/5″	145.8		150.8	**(5.0)**		151.2	**(5.4)**		151.3	**(5.5)**	
4″	138.7		137.8	(−0.9)		138.3	(−0.4)		138.3	(−0.4)	
COO-	165.4		164.8	(−0.6)		165.3	(−0.1)		165.3	(−0.1)	
Glucose (−G1/−G1′)											
1‴			103.0		5.05 (d) (J = 4 Hz)	98.6		5.29 (d) (J = 3.5 Hz)	103.4		5.07 (d) (J = 3.5 Hz)
2‴			71.8		3.37 (dd) (J = 4, 9.5 Hz)	72.1		3.35 (dd) (J = 3.5, 9 Hz)	72.3		3.38 (m)
3‴			73.1		3.63 (m)	73.5		3.61 (m)	73.5		3.61 (m)
4‴			69.2		3.25 (dd) (J = 9.5, 9.5 Hz)	70.3		3.23 (dd) (J = 9, 9 Hz)	69.7		3.25 (m)
5‴			73.8		3.97 (m)	74.1		3.47 (m)	74.2		3.96 (m)
6‴			60.1		3.42/3.43 (m)	60.1		3.58/3.60 (m)	60.5		3.56/3.60 (m)
Glucose (−G2)											
1⁗						103.4		5.09 (d) (J = 3 Hz)	104.3		4.86 (d) (J = 3.5 Hz)
2⁗						72.3		3.39 (dd) (J = 3, 9.5 Hz)	72.2		3.38 (m)
3⁗						73.5		3.64 (m)	73.6		3.61 (m)
4⁗						69.7		3.28 (dd) (J = 9.5, 9.5 Hz)	69.6		3.25 (m)
5⁗						74.3		3.49 (m)	74.2		3.96 (m)
6⁗						60.5		3.54/3.61 (m)	60.5		3.59/3.63 (m)

3. Results and discussion

3.1. Formation EGCG glucosides by acceptor reaction

After reaction involving *L. mesenteroides* NRRL B-1299CB glucansucrase (600 U per reaction mixture) with EGCG (500 mg) and sucrose (80 mM), we were able to identify three products by TLC analysis (Fig. 1, lane 4). Following Sephadex LH-20 chromatography and HPLC purification, the yields of EGCG-G1, EGCG-G2A, and EGCG-G2B were as follows, respectively (%, a molar ratio): 97.6 mg (15.7% of reacted EGCG), 177.6 mg (22.7% of reacted EGCG), and 186.4 mg (23.8% of reacted EGCG).

3.2. Structural determination of EGCG glucosides

The numbers of glucose units attached to the EGCG-G1, EGCG-G2A, and EGCG-G2B were confirmed by MALDI-TOF MS analysis. EGCG-G1 featured one attached glucose, and EGCG-G2A and EGCG-G2B each featured two attached glucoses. The glucosidic linkages were characterized by ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, HSQC, and HMBC NMR analyses, and these results are summarized in Table 1.

3.2.1. EGCG-G1

The molecular ions of EGCG-G1 were observed at *m/z* 643 $(M + Na)^+$. In Table 1, a doublet signal at 5.05 ppm ($J = 4$ Hz) was assigned to the anomeric proton, indicating that only one glucosyl residue was connected to EGCG by an α-linkage. All of the carbon signals assigned to the EGCG moiety were almost identical to those of EGCG, with the exception of the assignment of signals at 125.5 ppm to C-1″ and 150.8 ppm to C-3″/5″. These signals evidenced downfield shifts of 6.0 and 5.0 ppm, indicating that the transferred glucosyl residue had attached to C-4″ in the EGCG residue. According to HMBC data, the C-4″ of the EGCG was observed at 137.8 ppm, and the coupling occurred between proton H-1‴ of the glucosyl residue and C-4″ of the EGCG. The specific rotation was $[\alpha]_D$ −35.8° ($c = 0.1$, methanol, 22 °C). The structure of EGCG-G1 was designated as (−)-epigallocatechin gallate-4″-*O*-α-D-glucopyranoside (Fig. 2B).

3.2.2. EGCG-G2A

The molecular ion signals of EGCG-G2A were observed at *m/z* 805 $(M + Na)^+$. The two doublet signals at 5.29 ppm ($J = 3.5$ Hz) and 5.09 ppm ($J = 3$ Hz) were assigned to anomeric protons, indicating that the two glucosyl residues were connected to EGCG by α-linkages. All of the carbon signals assigned to the EGCG moiety were almost identical to those of EGCG, with the exception of the assignment of signals at 100.8 ppm to C-4a, 96.3 ppm to C-6, 97.6 ppm to C-8, 125.9 ppm to C-1″, and 151.2 ppm to C-3″/5″. These signals evidenced downfield shifts of 3.2, 1.8, 1.9, 6.4, and 5.4 ppm, respectively, and revealed two glucoses had been attached to the C-7 and C-4″ of EGCG, respectively. According to the HMBC data, these couplings occurred between proton H-1‴ of the glucosyl residue and C-7 of the EGCG, and also between proton H-1⁗ of the remaining glucosyl residue and C-4″ of the EGCG residue. This indicates that two α-glucosidic link-

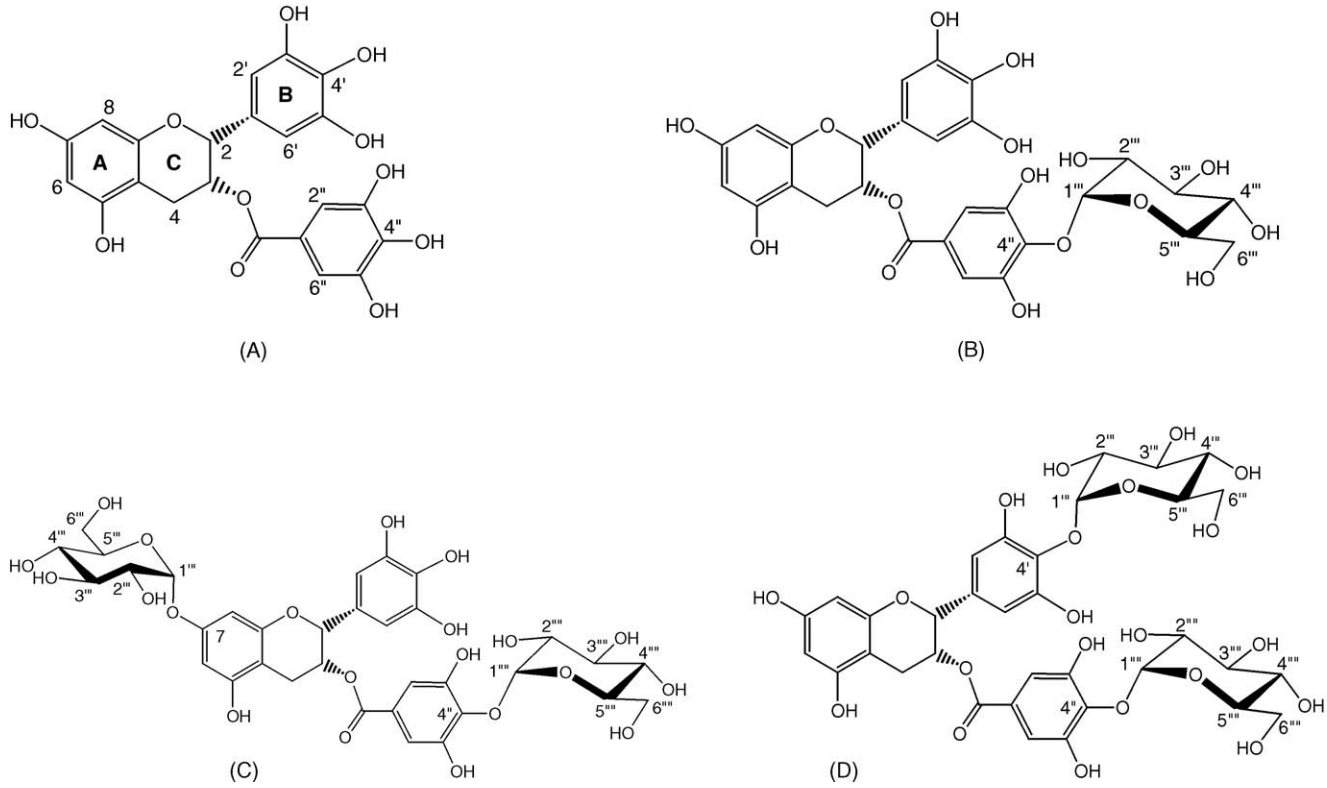

Fig. 2. The structures of EGCG (A), EGCG-G1 (B), EGCG-G2A (C), and EGCG-G2B (D).

ages (α-1 → 7 and α-1 → 4″) were formed by acceptor reaction. The specific rotation was $[\alpha]_D$ −37.2° (c = 0.1, methanol, 22 °C). Thus, EGCG-G2A could appropriately be referred to as (−)-epigallocatechin gallate-7,4″-di-*O*-α-D-glucopyranoside (Fig. 2C).

3.2.3. EGCG-G2B

The molecular ion signals of EGCG-G2B were observed at m/z 805 $(M+Na)^+$. The two doublet signals at 5.07 ppm (J = 3.5 Hz) and 4.86 ppm (J = 3.5 Hz) were assigned to anomeric protons, thereby indicating that the two glucosyl residues were connected to EGCG by α-linkages. All of the carbon signals assigned to the EGCG moiety were almost identical to those of EGCG, with the exception of the assignment of signals at 135.4 ppm to C-1′, 150.7 ppm to C-3′/5′, 125.8 ppm to C-1″, and 151.3 ppm to C-3″/5″. These signals evidenced downfield shifts of 6.6, 5.1, 6.3, and 5.5 ppm, respectively, and indicated that the two glucoses had been attached to the C-4′ and C-4″ of EGCG, respectively. According to the HMBC data, the couplings occurred between proton H-1‴ of the glucosyl residue and C-4′ of the EGCG, and also between proton H-1⁗ of the remaining glucosyl residue and the C-4″ of the EGCG residue. These results indicate that two α-glucosidic linkages (α-1 → 4′ and α-1 → 4″) were formed by the acceptor reaction. The specific rotation was $[\alpha]_D$ −44.1° (c = 0.1, methanol, 22 °C). Thus, EGCG-G2B could appropriately be referred to as (−)-epigallocatechin gallate-4′,4″-di-*O*-α-D-glucopyranoside (Fig. 2D).

Although EGCG-G2B has been previously reported by Kitao et al. [18], EGCG-G1 and EGCG-G2A were identified first in this study. Kitao et al. synthesized two EGCG glucosides, (−)-epigallocatechin gallate-4′-*O*-α-D-glucopyranoside and (−)-epigallocatechin gallate-4′,4″-di-*O*-α-D-glucopyranoside. Both of these glucosides were formed by the attachment of glucose to the B ring or to the galloyl residue. Catechin glucoside has also been previously reported to be synthesized by the attachment of glucose to the B ring, through an α-linkage [11,19]. However, this study is the first to report the attachment of a glucosyl residue to the A ring or the galloyl residue of EGCG by an α-linkage.

3.3. Properties of EGCG glucosides

3.3.1. Browning resistance effect

Catechin in water is susceptible to degradation and browning by UV irradiation [20]. The browning resistance of EGCG glucosides after UV irradiation is illustrated in Fig. 3. The UV-irradiation-associated browning of the EGCG glucosides was quite slow, even after 24 h exposure [EGCG-G1 (19.6% of that of EGCG), EGCG-G2A (17.5% of that of EGCG), EGCG-G2B (19.5% of that of EGCG)]. Catechin glucoside (3′-O-α-D-glucopyranoside), which was synthesized by transglycosylation using sucrose phosphorylase, also manifested browning resistance [11], indicating that glycosylation of EGCG compounds (catechins) confers stability against UV irradiation, regardless of the position or linkage of the glycosylation.

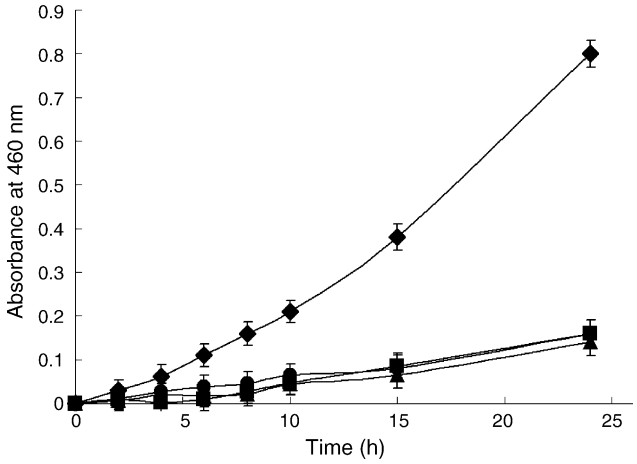

Fig. 3. Browning resistance to UV irradiation of EGCG and the EGCG glucosides. A solution (1.0 mL) containing 0.25% (w/v) EGCG (♦), EGCG-G1 (■), EGCG-G2A (▲), EGCG-G2B (●) was exposed to UV irradiation and then the absorbance at 460 nm was monitored for 24 h. Each value is the mean ± standard deviation (n = 3).

3.3.2. Antioxidant activity

Both EGCG and its glucosides evidenced distinct antioxidant activities, depending on their structural configuration (Fig. 4). The SC_{50} values of EGCG-G1 and EGCG-G2A, on the basis of the DPPH radical scavenging method, were found to be 2.1 and 3.2 μM, representing a lower degree of activity than evidenced by purified EGCG (SC_{50} = 0.9 μM). However, EGCG-G2B exhibited markedly reduced antioxidant activity. These results confirmed that both the 4′-hydroxyl moiety at the B ring and the 4″-hydroxyl moiety at the galloyl residue are essential to DPPH radical scavenging, as had been previously concluded by Valcic et al. [29].

3.3.3. Effect of glycosylation on water solubility

The water solubility of each of the EGCG glucosides was compared to that of EGCG. The solubility of EGCG was 5 mM,

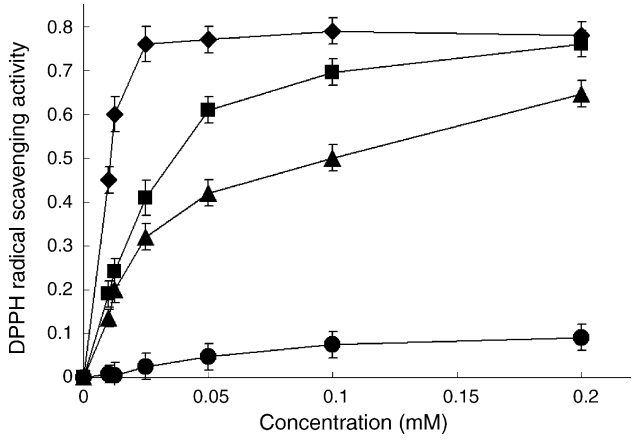

Fig. 4. DPPH radical-scavenging activities of EGCG and the EGCG glucosides. Each sample (30 μL), EGCG (♦), EGCG-G1 (■), EGCG-G2A (▲), EGCG-G2B (●) (10, 12.5, 25, 50, 100, 200 μM), was mixed with a 100 μM 1,1-diphenyl-2-picrylhydrazyl (270 μL) in darkness at room temperature for 10 min, and then the absorbance was monitored at 517 nm. Each value is the mean ± standard deviation (n = 3).

whereas the solubility of EGCG-G1, EGCG-G2A, and EGCG-G2B were 351.7, 644.4, and 623.1 mM, corresponding to 69, 126, and 122 times as high as that of EGCG. Kitao et al. also reported a 50-fold increase in the solubility of a glycosylated catechin (3′-*O*-α-D-glucopyranoside), compared to purified catechin [12]. The attachment of a glucosyl residue to EGCG results in an increase in the water solubility of the EGCG glucosides over that of EGCG, and that the number of attached glucosyl residues constitutes an important factor with regard to water solubility.

4. Conclusion

In this report, we have demonstrated the synthesis of two novel EGCG glucosides (EGCG-G1, EGCG-G2A), which contains a glucosyl residue either at the A ring or galloyl residue of EGCG. We have also elucidated, to some degree, the biochemical properties of these novel compounds. EGCG has become a focus of increasing interest for its various medicinal possibilities and EGCG glucosides are expected to prove useful as additives in the drug, cosmetic, and food industries. The observed increases in water solubility and browning resistance activities of these EGCG compounds should facilitate broader industrial application. Currently, research is underway to optimize the yields of specific EGCG glucosides, using glucansucrase engineering and bioreactor design experiments.

Acknowledgments

This work was supported by International Cooperative Research Program, Korea Science and Engineering Foundation (Grant M60402010002-05A0201-00210). We are thankful to Korea Basic Science Institute Gwangju Branch for the NMR analysis.

References

[1] J.F. Robyt, P.J. Martin, Carbohydr. Res. 113 (1983) 301.
[2] J.F. Robyt, Adv. Carbohydr. Chem. Biochem. 51 (1995) 133.
[3] H.J. Koepsell, H.M. Tsuchiya, N.N. Hellman, A. Kasenko, C.A. Hoffman, E.S. Shape, R.W. Jackson, J. Biol. Chem. 200 (1953) 793.
[4] E.S. Seo, J.H. Lee, J.Y. Park, D. Kim, H.J. Han, J.F. Robyt, J. Biotechnol. 117 (2005) 31.
[5] S.H. Yoon, J.F. Robyt, Carbohydr. Res. 337 (2002) 2427.
[6] M.J. Kim, S.B. Lee, H.S. Lee, S.Y. Lee, J.S. Baek, D. Kim, T.W. Moon, J.F. Robyt, K.H. Park, Arch. Biochem. Biophys. 371 (1999) 277.
[7] S.H. Yoon, D.B. Fulton, J.F. Robyt, Carbohydr. Res. 339 (2004) 1517.
[8] S.B. Lee, K.C. Nam, S.J. Lee, J.H. Lee, K. Inouye, K.H. Park, Biosci. Biotechnol. Biochem. 68 (2004) 36.
[9] S.J. Lee, J.C. Kim, M.J. Kim, M. Kitaoka, C.S. Park, S.Y. Lee, M.J. Ra, T.W. Moon, J.F. Robyt, K.H. Park, J. Agric. Food Chem. 47 (1999) 3669.
[10] D. Li, S.H. Park, J.H. Shim, H.S. Lee, S.Y. Tang, C.S. Park, K.H. Park, Carbohydr. Res. 339 (2004) 2789.
[11] S. Kitao, T. Ariga, T. Matsudo, H. Sekine, Biosci. Biotech. Biochem. 57 (1993) 2010.
[12] N. Salah, N.J. Miller, G. Paganga, L. Tijburg, G.P. Bolwell, C. Rice-Evans, Arch. Biochem. Biophys. 322 (1995) 339.
[13] S.W. Huh, S.M. Bae, Y.W. Kim, J.M. Lee, S.E. Namkoong, I.P. Lee, S.H. Kim, C.K. Kim, W.S. Ahn, Gynecol. Oncol. 94 (2004) 760.
[14] Y.D. Jung, M.S. Kim, B.A. Shin, K.O. Chay, B.W. Ahn, W. Liu, C.D. Bucana, G.E. Gallick, L.M. Ellis, Br. J. Cancer 84 (2001) 844.
[15] K.M. Lee, M. Yeo, J.S. Choue, J.H. Jin, S.J. Park, J.Y. Cheong, K.J. Lee, J.H. Kim, K.B. Hahm, Helicobacter 9 (2004) 632.
[16] S. Otake, M. Makimura, T. Kuroki, Y. Nishihara, M. Hirasawa, Caries Res. 25 (1991) 438.
[17] D.G. Raederstorff, M.F. Schlachter, V. Elste, P. Weber, J. Nutr. Biochem. 14 (2003) 326.
[18] S. Kitao, T. Matsudo, M. Saitoh, T. Horiuchi, H. Sekine, Biosci. Biotech. Biochem. 59 (1995) 2167.
[19] T. Sato, H. Nakagawa, J. Kurosu, K. Yoshida, T. Tsugane, S. Shimura, K. Kirimura, K. Kino, S. Usami, J. Biosci. Bioeng. 90 (2000) 625.
[20] H. Nakano, K. Hamayasu, K. Nakagawa, A. Tabata, K. Fujita, K. Hara, T. Kiso, H. Murakami, S. Kitahata, J. Appl. Glycosci. 49 (2002) 115.
[21] F.R. Seymour, M.E. Slodki, R.D. Plattner, A. Jeanes, Carbohydr. Res. 53 (1977) 153.
[22] F.R. Seymour, E.C.M. Chen, S.H. Bishop, Carbohydr. Res. 68 (1979) 113.
[23] F.R. Seymour, R.D. Knapp, E.C.M. Chen, A. Jeanes, S.H. Bishop, Carbohydr. Res. 71 (1978) 231.
[24] M. Dols, M.R. Simeon, R.M. Willemot, M.R. Vignon, P.F. Monsan, Carbohydr. Res. 305 (1998) 549.
[25] D. Kim, J.F. Robyt, Enzyme Microb. Technol. 17 (1995) 1050.
[26] R. Mukerjea, D. Kim, J.F. Robyt, Carbohydr. Res. 292 (1996) 11.
[27] D. Kim, J.F. Robyt, S.Y. Lee, J.H. Lee, Y.M. Kim, Carbohydr. Res. 338 (2003) 1183.
[28] N. Abe, A. Nemoto, Y. Tsuchiya, H. Hojo, A. Hirota, Biosci. Biotech. Biochem. 64 (2000) 306.
[29] S. Valcic, A. Muders, N.E. Jacobsen, D.C. Liebler, B.N. Timmermann, Chem. Res. Toxicol. 12 (1999) 382.