

Characterization of novel EGCG (Epigallocatechin gallate) analogues synthesized by glucansucrase from *Leuconostoc mesenteroides* B-1299CB

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Abstract

Three EGCG analogues were synthesized by the acceptor reaction of a glucansucrase from *Leuconostoc mesenteroides* B-1299CB with EGCG and sucrose. The transfer products was purified using Sephadex LH-20 column chromatography and high performance liquid chromatography (HPLC). EGCG-G1 and EGCG-G2 were novel compounds for the first time reported in this paper. EGCG glycosides showed similar or slower antioxidative effects according to their structures (EGCG ≥ EGCG-G1 > EGCG-G1' > EGCG-G2). However, the water solubilities of the EGCG-G1, EGCG-G1' and EGCG-G2 were 52, 76 and 140 times higher than that of EGCG. Furthermore, they showed more browning resistance against UV irradiation than EGCG.

Introduction

Green tea, a popular beverage commonly consumed in Asia, is an important

source of flavonoids called catechin. The green tea catechins, namely EGCG (epigallocatechin gallate), EGC (epigallocatechin), ECG (epicatechin gallate), and EC (epicatechin), have been studied as bioactive substances for antioxidant and cancer [1,2]. Among these catechins, EGCG is a most abundant compound in green tea catechins [3]. However, EGCG are poorly soluble in water, and degraded easily in aqueous solution [4]. Because of these defects, EGCG is limited in its use in food, drug, and cosmetic industries. To overcome these weak points, glycosylations of various polyphenols have been studied in various aspects [4-6]. The transglycosylated compounds sometimes acquired increased solubility in water, increased stability against light or oxidation, improved quality of taste and stronger inhibition effect of tyrosinase [4-7]. Because of these preferable properties, glycosylated bioactive substances are expected to be used as additives of medications and cosmetics.

In this paper, we report the enzymatic synthesis of novel EGCG glycosides (EGCG-G1, EGCG-G2) using glucansucrase from *Leuconostoc mesenteroides* B-1299CB. Both EGCG-G1 and EGCG-G2 were the first time reported enzymatically synthesized compounds and these EGCG glycosides had similar but slower antioxidant activities compared to that of EGCG, but showed stronger stabilities in a browning resistance and much higher solubilities in water.

MATERIALS AND METHODS

Preparation of enzyme. A mutant of *L. mesenteroides* B-1299CB was grown at 28°C on the LM medium that contained 2% (w/v) glucose as carbon sources. LM medium consists of 4 g yeast extract, 2 g peptone, 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g NaCl, 0.01 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.015 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 2 g K_2HPO_4 per liter of deionized water. After fermentation, the culture was harvested, centrifuged and concentrated using hollow fiber (30 K cut-off, Millipore, Japan).

Glucosylation of EGCG as an acceptor. A reaction mixture (250 mL) was consisted of the final 0.2% EGCG, final 80 mM sucrose and B-1299CB glucansucrase (final activity: 2.4 U mL^{-1}). It was incubated at 28°C for 6.5 h and sucrose was completely decomposed into monosaccharide. The reaction mixture was boiled for 5 minutes to stop the enzyme reaction.

Purification of EGCG acceptor reaction products. The reaction digest (250 mL) was applied to a Sephadex LH-20 column (47×200 mm) chromatography. The transfer products were washed with distilled water (3 L, 1 mL/min) to remove sugars (sugar-polymer, fructose and glucose) from the reaction digest and successively eluted with 70% (v/v) ethanol (1 L). The eluant (containing EGCG glycosides) was concentrated at 47°C using a rotary evaporator (N-N series, EYELA, Tokyo, Japan). The eluant was subjected to HPLC (LC-10AD, Shimadzu, Koyto, Japan) under the following conditions: reverse column, μ -Bondapak C₁₈ (3.9×400 mm, waters, U.S.A.); mobile phase, 23% methanol; flow rate, 0.5 mL/min; room temperature; detection, RI detector (RID-10A, Shimadzu, Japan).

Structural determination of EGCG glycosides. The number of glucose units attached on the purified EGCG-G1, EGCG-G1' or EGCG-G2 was confirmed by MALDI-TOF MS analysis. The glucosidic linkages were determined by ¹H, ¹³C, ¹H-COSY, HSQC and HMBC analyses.

RESULTS AND DISCUSSION

After acceptor reaction using *L. mesenteroides* B-1299CB glucansucrase (600 U per reaction digest) with EGCG (500 mg) and sucrose (final 80 mM), three reaction products were identified by TLC analysis (Fig. 1, lane 4)

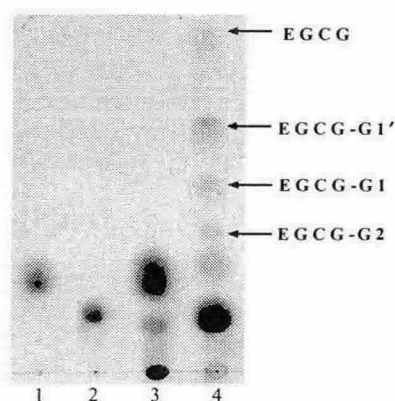


Fig. 1. Thin-layer chromatogram of the glucansucrase acceptor reaction digests. Lane 1, glucose; lane 2, sucrose; lane 3, enzyme reaction digest (without EGCG); lane 4, enzyme reaction digest with EGCG. Arrows indicates EGCG acceptor reaction products.

The number of glucose units attached on the purified EGCG-G1, EGCG-G1' or EGCG-G2 was confirmed by MALDI-TOF MS analysis. The molecular weights of glycosides have been increased over that of EGCG by exactly a single glucose residue addition; EGCG-G1 and EGCG-G1' have one glucose was attached and EGCG-G2 has two glucose was attached. The glucosidic linkages were determined by ^1H , ^{13}C , ^1H -COSY, HSQC and HMBC analyses. EGCG and its glycosides showed different antioxidant activities according to their structural configuration. EGCG-G1 showed similar activity comparing with that of purified EGCG. On the other hand, EGCG-G1' and EGCG-G2 were lower than that of purified EGCG. However, the water solubilities of the EGCG-G1, EGCG-G1' and EGCG-G2 were 52, 76 and 140 times higher than that of EGCG. Furthermore, they showed more browning resistance against UV irradiation than EGCG.

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