Glucosyltransferase from *Streptococcus sobrinus* Catalyzes Glucosylation of Catechin

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We previously showed that the polymeric forms of polyphenols present in oolong tea extract exhibited strong inhibitory activities against glucosyltransferases (GTases) of mutans streptococci, while green tea extract, which is rich in catechins, did not show such GTase-inhibitory activities. In this study, (+)-catechin [2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,5,7-triol] was found to be glucosylated by the GTase of *Streptococcus sobrinus* 6715 with sucrose as the substrate, and the product was identified as 4'-O- α -D-glucopyranosyl-(+)-catechin. The α -glucosylated (+)-catechin did not exhibit significant inhibition of glucan synthesis from sucrose by the GTase, which is in contrast to the polymeric polyphenols isolated from oolong tea leaves.

Glucans synthesized from sucrose by the cooperative action of glucosyltransferases (GTases) produced by mutans streptococci, including *Streptococcus mutans* and *Streptococcus sobrinus*, are highly adherent to various solid surfaces, including the tooth surface. This property of these organisms as well as production of acid from various sugars are considered to be essential virulence attributes that contribute to the development of dental caries in humans and experimental animals (5). Inhibition of the GTase activity of mutans streptococci hindered sucrose-dependent in vitro cellular adherence and the development of dental caries in rats that had been infected with mutans streptococci and fed a high-sucrose diet (6). We recently found that polymeric polyphenols from oolong tea extract strongly inhibited glucan synthesis from sucrose by GTases of mutans streptococci (13). On the other hand, green tea extract and catechins did not exhibit such inhibitory effects (8, 13). It is of interest to study the mechanism of GTase inhibition by polymeric, but not monomeric polyphenols.

GTase was prepared from the culture supernatant of *S. sobrinus* 6715 (serotype g) essentially as described by Furuta et al. (3). In brief, *S. sobrinus* 6715 was grown in 3 liters of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) for 22 h at 37°C. After the cells were removed by centrifugation, the culture supernatant was precipitated with 50% saturated ammonium sulfate. The centrifuged precipitate was suspended in 10 mM sodium phosphate buffer (pH 6.0) and was dialyzed overnight against the buffer. The dialysate (75 ml) was used as a GTase preparation.

(+)-Catechin (60 mg) (Nacalai Tesque Ltd., Kyoto, Japan) [2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,5,7-triol] was added to 60 ml of 100 mM sodium phosphate buffer (pH 6.0) containing 2% sucrose. The GTase reaction was initiated by the addition of GTase (4 ml), and the mixture was incubated at 37°C for 24 h. The enzymatic reaction was stopped by adding 120 ml of ethanol, and the product was filtered with a MILLEX-PF 0.8- μ m-pore-size filter unit (Mil-

lipore Products Division, Bedford, Mass.) and concentrated by evaporation. The concentrate was resuspended in 120 ml of H₂O and adsorbed to a column of Diaion HP-20 packed with a stylenedivinylbenzene resin (2 by 20 cm; Mitsubishi Chemical Industry, Tokyo, Japan). The column was washed with 1,000 ml

FIG. 1. ¹H-NMR spectra and NOE, showing the difference between (+)-catechin and the reaction product [4'-O- α -D-glucopyranosyl-(+)-catechin]. (A) ¹H-NMR spectrum of (+)-catechin; (B) ¹H-NMR spectrum of the reaction product; (C) NOE for the reaction product.

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FIG. 2. Synthesis of glucosylated (+)-catechin from (+)-catechin and sucrose due to enzymatic action of *S. sobrinus* GTase.

of H₂O and then eluted with 50% ethanol. Major fractions containing (+)-catechin were further purified by high-pressure liquid chromatography with a Develosil octadecyl silane column (4.6 by 150 mm; Nomura Chemical Co., Seto, Japan) equipped with a model 305 pump, a model 805 manometric module, and a model 1001 UV detector (MS Gilson, Osaka, Japan). The column was eluted with 25% methanol at a flow rate of 1 ml/min with monitoring of A_{280} . Two peaks were eluted; the second peak, giving a retention time of 4.7 min, was (+)-catechin, while the first peak may have been a derivative of (+)-catechin, judging from its retention time of 3.7 min. The dry weight of the reaction product was 12.8 mg.

The UV absorption spectrum of the product (i.e., of the first peak) at neutral pH had a maximum at 276 nm. Fast atom bombardment mass spectrometry of the product with a JEOL JMS-HX110/110A tandem mass spectrometry system revealed an $(M + H)^+$ peak at m/z 453, and the molecular weight of the product was calculated to be 452. Therefore, the yield of the reaction product of the GTase reaction was 13.7%. The ¹H nuclear magnetic resonance (¹H-NMR) spectrum of the product in methanol-*d*₄, obtained with a JEOL JNM-EX400 spectrometer, was analyzed by comparison with that of (+)-catechin (Fig. 1). The ¹H-NMR spectrum of the reaction product indicated the presence of one catechin residue and one glucose residue. Briefly, this spectrum indicated that nine signals were generated as a result of nine protons of a (+)-catechin moiety, at 7.24, 6.90, 6.82, 5.93, 5.86, 4.60, 3.97, 2.83, and 2.50 ppm, and that signals due to the seven protons of a glucopyranose moiety were present at 5.37 ppm (an anomeric proton; $J = 3.6$ Hz) and

FIG. 3. Inhibitory effects of (+)-catechin and glucosylated catechin on glucan synthesis by *S. sobrinus* GTase from sucrose as the substrate. Inhibitory activities of samples were measured at various concentrations. Insoluble glucan synthesis by *S. sobrinus* GTase was measured turbidimetrically by determining the increase in A_{550} . ○, (+)-catechin; ●, glucosylated catechin; □, oolong tea extract.

between 3.92 and 3.37 ppm (six protons). These results indicate that the coupling constant of the anomeric proton is characteristic of an α -linked glucosidic residue. The linkage of the (+)-catechin residue and the glucose residue was studied by use of the nuclear Overhauser effect (NOE). As shown in Fig. 1 and 2, an NOE was observed between the anomeric proton of the glucose and the 5' proton of the catechin moiety. From these results, it was concluded that the hydroxyl group at the 4' position of the catechin was α -glucosylated, and the chemical structure of the reaction product was determined to be 4'-O- α -D-glucopyranosyl-(+)-catechin (4'-GC) (Fig. 2). Other investigators have reported catechin-glucosides linked at the 3'-O-position (3'-GC), which were synthesized by using cyclodextrin glucanotransferase (2) or sucrose phosphorylase from *Leuconostoc mesenteroides* (10). Those compounds are clearly different from our 4'-GC as determined in this study.

The catechins have been reported to exhibit a variety of physiological functions, such as antioxidative activities (9, 15), antitumor activities (1, 4), and inhibitory activities against enzymes such as reverse transcriptases (9, 10) and amylase (7). The 3'-GC was shown to inhibit noncompetitively the activity of mushroom tyrosinase (EC 1.14.18.1) but not that of mouse melanoma tyrosinase (2, 10). Arbutin (4-hydroxyphenyl- β -D-glucopyranoside) inhibited both types of tyrosinase (2, 11). Further, the antioxidative effect of 3'-GC was almost equal to that of (+)-catechin (10). We examined the effects of (+)-catechin and 4'-GC against *S. sobrinus* GTase. Oolong tea extract prepared as described previously (13) was also included in this experiment for comparison. The results indicated that (+)-catechin only weakly inhibited GTase in a dose-dependent manner and that the dose of (+)-catechin required for 50% inhibition was 350 μ g/ml, while oolong tea extract gave a marked inhibition under the same experimental conditions (Fig. 3). However, we have found that 4'-GC scarcely inhibited GTase; only 10% inhibition was obtained even when 1,000 μ g of GC per ml was used. Therefore, it appears logical to assume that the inhibitory effect of (+)-catechin on GTase was mark-

edly hampered as the transglucosylation reaction proceeded from sucrose to catechin, which suggests that the formation of the 4'-hydroxyl group of (+)-catechin resulted in blockage of the GTase-inhibitory activity of catechin.

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