Inhibitory Effects of Green Tea Polyphenols on Glucan Synthesis and Cellular Adherence of Cariogenic Streptococci

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Inhibitory effects of green tea polyphenols on glucan synthesis by glucosyltransferases of Streptococcus mutans MT8148 and Streptococcus sobrinus 6715DP and on sucrose-dependent adherence of the bacterial cells were examined in vitro. The glucan synthesis by the bacterial glucosyltransferase was strongly inhibited by (−)-epicatechin gallate (ECg) and (−)-epigallocatechin gallate (EGCg), the main components of the tea polyphenols. It was also demonstrated that ECg and EGCg interfered with the sucrose-dependent adherence of those bacterial cells at much smaller concentrations than those which were needed to inhibit the growth of the bacteria.

Our previous paper reported that several polyphenols contained in green tea extract showed inhibitory effects on the growth of Streptococcus mutans, a cariogenic bacterium.1) This bacterium is known to synthesize glucan of two types: water-soluble and water-insoluble glucans. It is also known that the two glucans are synthesized by two different groups of glucosyltransferases (GTase; EC 2.4.1.5). The two GTases cooperatively synthesize an adherent and water-insoluble highly branched glucan that is responsible for the bacterial cell adherence to tooth surface.2)

In this paper, effects of tea polyphenols on the glucan synthesis by enzymes from several cariogenic streptococci were examined and the results showed that some of the phenolic compounds were strongly inhibitory toward the synthesis of glucan by the bacterial enzymes, and suggested that the tea polyphenols might be effective to protect our tooth surfaces from bacterial adherence. Also, the inhibitory effect of the tea polyphenols was discussed in regard to their chemical structures.

Materials and Methods

Bacterial strains and chemicals used: S. mutans MT8148 (serotype C) and S. sobrinus 6715DP (serotype G) were used, which are known to be the most common serotypes of cariogenic bacteria among Japanese people. Todd-Hewitt broth (Difco Laboratories, Detroit, U.S.A.) was used as the medium for the bacterial culture. Dextran T10 (Pharmacia Fine Chemicals, Uppsala, Sweden) was used as a primer of glucan synthesis.

The glucosyltransferase (GTase) of S. mutans MT8148 and S. sobrinus 6715DP were obtained from the supernatants of their culture broth in TTY medium at 37°C for 18 h. The TTY medium contained (per liter): trypticase (Baltimore Biological Laboratory, Cockeysville, U.S.A.), 15 g; tryptose (Difco), 4 g; yeast extract, 4 g; K2HPO4, 2 g; KH2PO4, 5 g; Na2CO3, 2 g; NaCl, 2 g; and glucose, 10 g; at pH 7.4.3) Ammonium sulfate was added to the supernatants, until they were 50% saturated. The resulting precipitate was collected by centrifugation, dissolved in 0.1 M sodium phosphate buffer, pH 6.5, and dialyzed against the same buffer. The dialyzed solution was centrifuged and the supernatant was used as GTase.

For preparation of the “cell-associated GTase” of S. mutans MT8148, cells grown in 1000 ml TTY medium overnight were collected, washed extensively with 0.1 M sodium phosphate buffer, pH 6.5, and suspended in 30 ml of 8 M urea. The cell suspension was incubated at 5°C for 1 hr with occasional shaking, and centrifuged. The cells were then extracted three times in that way, and the supernatants were combined and dialyzed against 0.1 M sodium phosphate buffer, pH 6.5, and its supernatant was used as the “cell-associated GTase.”

GTase was assayed by the method of Shimamura et al.4) An appropriate amount of enzyme solution was incubated at 37°C for 15 hr with 0.1 M sodium phosphate buffer, pH
Results

Effects of several polyphenols isolated from Japanese green tea on water-soluble and -insoluble glucan synthesis by enzymes are shown in Table I. ECg, GCg, and EGCg strongly inhibited glucan synthesis by any of GTases used. However, other polyphenols examined were not as inhibitory as the above compounds.

The inhibition of glucan synthesis by ECg and EGCG, which are the major components of tea polyphenols, was investigated as a function of sucrose concentration.

As shown in Fig. 1, the glucan synthesis was inhibited almost proportionally to ECg and EGCG concentrations and this inhibition was independent on the concentration of sucrose. This result may indicate that ECg and EGCG bind GTases to inactivate them irreversibly.

The inhibitory effects of ECg and EGCG on glucan synthesis were also examined as functions of their concentrations. As shown in Fig. 2, both ECg and EGCG of concentrations more than 25 to 30 μg/ml almost completely inhibited glucan synthesis by the enzymes from 6715DP and MT8148-I. However, for the complete inhibition of that by MT8148-S enzyme, more ECg and EGCG were necessary.

The experimental result of effects of ECg and EGCG on adherence of resting cells of S. mutans MT8148 and S. sobrinus 6715DP is shown in Fig. 3. The bacterial adherence to glass surfaces is known to be due to physical properties of glucans produced by cell-bound GTases. The result of Fig. 3 indicates that both EGCG conc.

### Table I. Inhibitory Effects of Tea Polyphenols on Glucosyltransferase Activity

<table>
<thead>
<tr>
<th>Test compounds conc., μg/ml</th>
<th>6715DP</th>
<th>MT8148 GTase-I*</th>
<th>MT8148 GTase-S*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>C</td>
<td>96.7</td>
<td>73.1</td>
<td>83.7</td>
</tr>
<tr>
<td>EC</td>
<td>85.4</td>
<td>71.2</td>
<td>81.4</td>
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<tr>
<td>GC</td>
<td>31.7</td>
<td>9.7</td>
<td>97.2</td>
</tr>
<tr>
<td>EGC</td>
<td>84.7</td>
<td>61.0</td>
<td>94.0</td>
</tr>
<tr>
<td>ECg</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GCg</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EGCG</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* GTase-I and GTase-S synthesized insoluble- and soluble glucan, respectively.

* Test compounds: C, (+)-catechin; EC, (-)-epicatechin; GC, (+)-gallocatechin; EGC, (-)-epigallocatechin; ECg, (-)-epicatechin gallate; GCg, (-)-gallocatechin gallate; EGCG, (-)-epigallocatechin gallate.
of 50 μg/ml almost completely inhibited adherence of the bacterial cells to the glass surface. However, IC₅₀ (concentration needed for 50% inhibition) for bacterial strain MT8148 was slightly different between EGCg and ECg, that is, IC₅₀ of EGCg and ECg were 5.0 μg/ml and 11.7 μg/ml, respectively.

**Discussion**

The cariogenic bacteria, *Streptococcus mutans* and *Streptococcus sobrinus*, produce insoluble glucan from sucrose and adhere firmly to tooth surfaces.

This study showed that EGCg and ECg isolated from Japanese green tea strongly interfered not only with the synthesis of soluble and insoluble glucans by the bacterial enzymes, but also with the bacterial cell adherence to glass surface that was caused by insoluble glucan produced on the bacterial cell surface.

Of interest is the fact that the concentrations of the above polyphenols effective to inhibit enzymatic glucan synthesis or cellular adherence of the bacteria are much less than those which are necessary to inhibit the bacterial growth. It is noteworthy that a cup of green tea (100 ml) usually contains 50 to 100 mg polyphenols, of which about 60% are ECg and EGCg. The combined concentrations of these two major compounds (which could range from 300 to 500 μg/ml) is higher than those used in this study, and therefore a cup of green tea is sufficient to inhibit enzymatic glucan synthesis or cellular adherence of the bacteria.

The polyphenols, ECg and EGCg, all with
Fig. 2. Effects of EGCg and ECg on Enzymatic Glucan Synthesis.
Sucrose conc., 41.7 mM; (A), GTase from S. sobrinus 6715DP; (B), GTase-I from S. mutans MT8148; (C), GTase-S from S. mutans MT8148. Other conditions were the same as in Fig. 1. ●, EGCg added; ▲, ECg added.

Fig. 3. Effects of EGCg and ECg on Adherence of Resting Cells of S. mutans MT8148 and S. sobrinus 6715DP.
PH, 6.8; temp., 37°C; angle of inclination, 30°; incubation period, 18 hr. Sucrose (20 mg) and S. sobrinus 6715DP (A) or S. mutans MT8148 (B) cells (1 mg as dry weight) were added to 2.4 ml reaction mixture. ●, EGCg added; ▲, ECg added.
a galloyl moiety in their molecules, showed inhibitory effects on glucan synthesis by cariogenic bacterial GTase. The degrees of inhibition by these compounds were pronounced as compared with those by other tea polyphenols examined. Nevertheless, it was found that gallic acid itself showed no inhibitory effect. Therefore, the inhibitory effect shown by above galloyl compounds may be concluded to be due to the chemical groups and their configuration other than galloyl moiety. It is highly likely that the structure in question affects the catalytic sites of GTase irreversibly. But further study will be to clearly elucidate the relationships between the chemical structures of the effective polyphenols and inhibition of GTase.

Smith and Taubman published a paper on the antigenic relatedness of cariogenic bacterial GTases, suggesting that serotypes a, d, g; b; and c, e might be distinct biotypes of S. mutans. Concerning this report, it is of interest that tea polyphenols independently investigated showed no particular specificity in their inhibitory effects on enzymatic glucan synthesis as well as on cell adherence of S. mutans MT8148 (serotype c) and S. sobrinus 6715DP (serotype g).

This paper together with our previous work may also indicate the possibility that Japanese green tea polyphenols, if applied, are effective for protection from dental plaque formation that subsequently develops dental caries.

References