Inhibitory Effects of Apple Polyphenols and Related Compounds on Cariogenic Factors of Mutans Streptococci

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The inhibitory effects of apple polyphenols (APP) on the synthesis of water-insoluble glucans by glucosyltransferases (GTF) of streptococci of the mutans group and on the sucrose-dependent adherence of the bacterial cells were examined in vitro. APP markedly inhibited the activity of GTF purified from the cariogenic bacterial cells. However, APP showed no significant effect on the growth of the cariogenic bacteria. The strongest GTF inhibitors in APP were apple condensed tannins (ACT), a mixture of procyanidins. The 50% inhibitory doses of ACT against the GTF of *S. sobrinus* and that of *S. mutans* were 1.5 μg/mL and 5 μg/mL, respectively. The ACT efficacy largely depended upon the degree of polymerization. Interestingly, while the other polyphenols known to inhibit GTF such as tannic acid markedly inhibited salivary α-amylase activity, APP and ACT only scarcely inhibited that enzyme activity. This means that APP and ACT might selectively inhibit the bacterial GTF activity under oral conditions.

Keywords: *Polyphenol; procyanidin; apple; mutans streptococci; glucosyltransferase*

INTRODUCTION

Among the members of the oral bacterial flora, streptcocci of the mutans group including *S. mutans* and *S. sobrinus* have been confirmed to be highly cariogenic pathogens in humans (Loesche, 1986). Considerable evidence has indicated that the streptococci of the mutans group produce extracellular glucans from sucrose through the action of glucosyltransferases (GTFs) (Rölla et al., 1985). In particular, water-insoluble glucans mediate the accumulation of streptococci of the mutans group on tooth surfaces, causing the aggregation of bacteria as dental plaque which finally leads to dental caries (Freedman and Tanzer, 1974, Hamada and Torii, 1978).

Over the past two decades many studies on the anticariogenic effect of polyphenols extracted from different types of plants have been reported (Ito et al., 1984; Kakiuchi et al., 1986; Sawamura et al., 1992; Tagashira et al., 1997; Mitsunaga and Abe, 1997). Particularly, the anticaries effects of tea polyphenols have been confirmed by many Japanese investigators (Hattori et al., 1990; Sakanaka et al., 1989, 1990, 1992; Otake et al., 1991; Nakahara et al., 1993; Ooshima et al., 1993, 1994, 1998). Tea polyphenols markedly inhibit the growth of cariogenic bacteria as well as the production of insoluble glucans by their GTFs. These reports have shown that epigallocatechin gallate (EGCg) in tea leaves and some oxidized polymers produced through fermentation of tea leaves are the main GTF inhibitors.

In the plant kingdom, edible fruits are one of the richest sources of polyphenols. We consume these compounds every day from natural foods including fresh fruits, juices, wines, and the other processed foods made from edible fruits. However, little is known about the daily intake of these fruit polyphenols or about their impact on dental health. For example, apples have been questionably related to better dental health (Slack and Martin, 1958; Bibby, 1983), and there is no report regarding the anticariogenicity of apple polyphenols (APP). Recently, apple condensed tannins (ACT) in APP have been shown to display strong antiallergic effects such as inhibition of hyaluronidase activity and inhibition of histamine release from rat peritoneal mast cells (Kanda et al., 1998). Like tea polyphenols, APP and their components may have important anticaries properties of interest to the dental profession.

The aim of this study was to determine the effects of APP extracted from immature apples on the synthesis of water-insoluble glucans by the GTFs from *S. mutans* and *S. sobrinus* and on the sucrose-dependent adherence of these bacteria in vitro. Furthermore, the structural features of common anticariogenic plant polyphenols are described with reference to the utilization of apple polyphenols for caries prevention.

MATERIALS AND METHODS

Polyphenol Compounds. Epicatechin (EC), epigallocatechin gallate (EGCg), and chlorogenic acid were purchased from Wako Pure Chemicals (Osaka, Japan). Gallic acid, ellagic

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acid, caffeic acid, *p*-coumaric acid, and phloridzin were obtained from Tokyo Kasei (Tokyo, Japan), quercetin, rutin, and tannic acid from Kanto Chemicals (Tokyo, Japan), and procyanidin B2 and phloretin from Funakoshi (Tokyo, Japan). All other chemicals and solvents were of analytical-reagent grade.

Apple Polyphenols. The methods of preparation of apple polyphenols (APP) and apple condensed tannins (ACT) from immature fruits (*Malus Pumila* cv. Fuji) have been described elsewhere (Ohnishi-Kameyana et al., 1997; Kanda et al., 1998; Yanagida et al., 1999). APP is a mixture of polyphenols consisting of phenolic acids (about 25 w/w %; mainly, chlorogenic acid), phloretin glycosides (about 10 w/w %; mainly, phloridzin), monomeric flavan-3-ols (about 15 w/w %; catechin and EC), oligomeric procyanidins (about 45 w/w %), and other compounds (about 5 w/w %; mainly, quercetin glycosides) (Kanda et al., 1998). Purified ACT is a mixture of monomeric flavan-3-ols (catechin and EC) and procyanidin oligomers ranging in size from dimers (2-mer) to pentadecamers (15-mer) (Ohnishi-Kameyana et al., 1997).

Preparation of Procyanidin Oligomers from ACT. Low molecular mass procyanidins ranging in size from dimers to pentamers were selectively extracted from ACT by methyl acetate extraction, and each oligomer in this extract was isorated by normal-phase high-performance liquid chromatography (NP-HPLC) (Yanagida et al., 2000). The methods of methyl acetate extraction and NP-HPLC fractionation are briefly described below.

First, 1 g of lyophilized ACT powder was added into 100 mL of methyl acetate. The mixture was stirred for 30 min at room temperature and filtered. The extraction residue was reextracted with methyl acetate under the same conditions. For example, the extraction (100 mL, 2 times) of 1 g of ACT powder yielded 0.583 g of extract (58.3 w/w %) and 0.417 g of residue (41.7 w/w %), both as the lyophilized material. Through this treatment, monomeric flavan-3-ols and most of the low molecular mass procyanidins in ACT, ranging in size from dimers to pentamers, were selectively recovered in the extract fraction. On the other hand, the high molecular mass procyanidins in ACT remained in the residue fraction (Yanagida et al., 2000).

Next, this methyl acetate extract in the form of a lyophilized powder was redissolved in a small volume of methyl acetate and fractionated by NP-HPLC. Sokensil s-15/30 (Soken Chem. & Eng., Tokyo, Japan), consisting of porous spherical silica-beads (15−30 mm particle size), was packed in stainless steel columns (500 μm × 50 mm i.d.). As the mobile phase, solvents A and B were used, each consisting of a mixture of hexane-acetone at volume ratios of (A) 35:65 and (B) 20:80, respectively. A 12 g portion of the extract in the form of a lyophilized powder was dissolved in 30 mL of methyl acetate as the sample solution, and this solution was injected and eluted with solvent A for 80 min. Then, linear gradient elution from 0 to 100% solvent B was applied for 110 min. The components that remained in the column were then eluted with solvent B for 110 min. Elution was performed at a flow rate of 57 mL/min, and the absorbance of the eluate was monitored at 230 nm. The NP-HPLC fractions corresponding to the peaks derived from procyanidin oligomers of each size ranging from dimers to pentamers were combined separately, the organic solvent in each fraction was removed by evaporation, and the remaining material was lyophilized. The degree of polymerization of the procyanidin oligomers in each lyophilized sample was calculated based on the mass $[M + Na]^+$ of the molecular ions detected by MALDI-TOF MS analysis (Ohnishi-Kameyama et al., 1997; Yanagida et al., 2000) (data not shown). Finally, from 12 g of methyl acetate extract of ACT, 1.69 g, 2.17 g, 2.06, 1.67, and 1.14 g of lyophilized monomers, dimers, trimers, tetramers, and pentamers, respectively, were obtained.

Bacterial Strains. *S. sobrinus* 6715 (serotype g) and *S. mutans* MT8148 (serotype c) were kindly provided by Dr. I. Nasu from Nihon University, School of Dentistry at Matsudo.

Preparation of Glucosyltransferases (GTFs). It is known that the water-insoluble glucan-synthesizing GTF of *S. sobrinus* 6715 is released into the culture medium and that GTF of *S. mutans* MT8148 is present mainly on the cell surface (Hamada and Torii, 1978). *S. sobrinus* 6715 and *S. mutans* MT8148 were grown for 20 h at 37 °C in 1 L of TTY broth (Sakanaka et al., 1990). In the case of the former, the culture medium was centrifuged at 3500x*g*, and the protein in the supernatant was precipitated with 50% saturated ammonium sulfate at 4 °C. The precipitate collected by recentrifugation was dissolved in 10 mM potassium phosphate buffer (pH 6.5) and dialyzed against the same buffer, and the resulting solution was designated as the crude cell-free GTF (CF-GTF) of *S. sobrinus*. In the case of the latter, *S. mutans* cells collected by centrifugation of the culture medium were washed with distilled water and treated with 8 M urea for 1 h at 4 °C. The cell-extract was dialyzed against phosphate buffer (pH 6.5), and the resulting solution was designated as the crude cell-associated GTF (CA-GTF) of *S. mutans*. Furthermore, both GTFs were purified by hydroxylapatite chromatography. Each crude-GTF solution was loaded onto a Bio-gel HTP (Bio-rad, California, U.S.A.) column (250 mm × 15 mm i.d.). The column was first washed with 50 mL of 0.05 M phosphate buffer (pH 6.5), and the concentration of potassium phosphate in the mobile phase was increased linearly from 0.05 to 1 M. In each instance, the GTF was eluted with 0.4 M phosphate buffer, and this preparation was used as the purified enzyme solution in the subsequent assays.

Enzymatic Assay for GTF. The polyphenols to be tested were dissolved in either 50% or 100% dimethyl sulfoxide as sample solutions. A phosphate buffer (0.1 M, pH 6.5) containing 2% sucrose, 0.1% N_3Na, and 0.04 M dextran T10 (Pharmacia, Uppsala, Sweden) was prepared as the substrate solution. To measure GTF activity and inhibition by polyphenols, the purified GTF solution (*S. sobrinus* CF-GTF 20 μL, *S. mutans* CA-GTF 80 μL) was incubated with 50 μL of the sample solution, 1 mL of the substrate solution, and distilled water (930 μL or 870 μL) at 37 °C for 18 h. The amount of insoluble glucan produced in the mixture was measured turbidimetrically by determining the increase in o.d. at 600 nm. In this assay, the relative amount (%) of insoluble glucan produced at a certain polyphenol concentration (as compared to the amount produced in the absence of any polyphenol) was calculated, and a relative amount (%) of insoluble glucan was plotted against the polyphenol concentration in the reaction mixture. The 50% inhibitory dose (ID_{50}) of the polyphenol was estimated from a plot of the average values of triplicate and/or duplicate assays.

Enzymatic Assay for α-Amylase. The effects of polyphenols on α-amylase obtained from human saliva (Sigma, St. Louis, U.S.A.) were determined by measuring the release of reducing sugars from soluble starch. The polyphenols to be tested were dissolved in 0.05 M phosphate buffer (pH 7.0) containing 20% methanol as sample assays. Salivary α-amylase (0.25 units per 0.5 mL buffer) was incubated with 1 mL of 0.5% soluble starch and 0.5 mL of sample solution at 37 °C for 1 h. The reducing sugars liberated were quantified by the dinitrosalicylate method (Hostettler et al., 1995). The % inhibition was plotted against the polyphenol concentration in the reaction mixture, and the 50% inhibitory dose (ID_{50}) of polyphenol was estimated from a plot of the average values of triplicate assays.

Measurement of Cellular Adherence. For the measurement of sucrose-dependent cellular adherence, growing cells of *S. sobrinus* were used. *S. sobrinus* cells cultured in TTY-broth were collected in the logarithmic phase of growth by centrifugation, washed with distilled water, and suspended in 0.1 M phosphate buffer (pH 6.8). This cell suspension was diluted with the same buffer ($OD_{600} = 3.0$). The polyphenols to be tested were dissolved in phosphate buffer containing 10% ethanol as sample solutions. Equal volumes (0.7 mL) of the cell suspension, sample solution, and substrate solution (3% sucrose, 0.06% N_3Na in buffer) were mixed (total 2.1 mL) in a glass tube (7 mm i.d. × 70 mm), and the mixture was incubated at 37 °C at a 30-degree inclination for 18 h. Furthermore, after removal of the supernatant containing nonadherent cells by means of a pipet, 1 mL of distilled water was added to the glass tube, and the adherent cells were suspended using a vortex mixer. This cell suspension was lyophilized, and the weight of adherent cells was then mea-

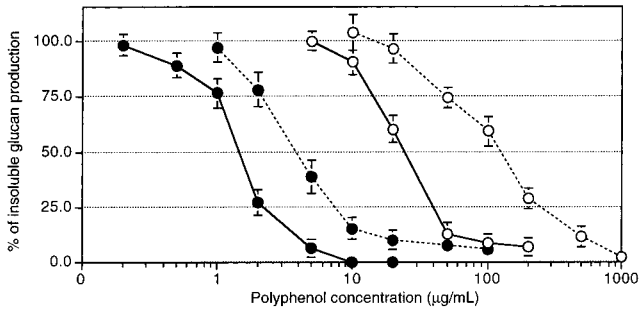

Figure 1. Inhibitory effects of apple polyphenols (○ = APP) and apple condensed tannins (● = ACT) on insoluble glucan synthesis by cell-free GTF of *S. sobrinus* (straight lines) and the cell-associated GTF of *S. mutans* (dotted lines). The % glucan synthesis means the relative amount (%) of insoluble glucan produced at a certain polyphenol concentration as compared to the amount produced in the absence of any polyphenol. Each value is the average of triplicate assays and each bar indicates mean ± SE ($n = 3$).

sured. The relative amount (%) of adherent cells at a certain polyphenol concentration (as compared to the amount in the absence of any polyphenol) was calculated. The relative amount (%) of adherent cells was plotted against the polyphenol concentration in the reaction mixture, and the 50% inhibitory dose (ID_{50}) of the polyphenolic compound was estimated from a plot of the average values of triplicate assays.

RESULTS

Effects of APP and Related Compounds on GTF Activity of Mutans Streptococci. In our preliminary study, APP at concentrations up to 2000 μg/mL showed no substantial effect on the growth of *S. sobrinus* 6715 or *S. mutans* MT8148 on TTY-agar plates under aerobic conditions and no substantial effects on the growth of these bacteria on mitis salivarius agar (DIFCO Laboratories, Detroit, U.S.A.) plates containing 5 mg/L bacitracin under anaerobic conditions (data not shown). On the other hand, APP and ACT (purified from APP) markedly inhibited the synthesis of insoluble glucan by *S. sobrinus* CF-GTF and *S. mutans* CA-GTF.

Figure 1 shows the inhibition of insoluble glucan synthesis (%) with increasing polyphenol concentrations (μg/mL). Table 1 shows a comparison of the 50% inhibitory dose (ID_{50}) values for various polyphenols present in APP and ACT for inhibition of GTF activity. As shown in Figure 1, in the case of both APP and ACT, the effectiveness in inhibition of insoluble glucan synthesis was dependent on the polyphenol concentration in the reaction mixture. The inhibitory effect of both APP and ACT on the GTF of *S. sobrinus* (straight lines) was stronger than that on the GTF of *S. mutans* (dotted lines). Moreover, the ID_{50} values of APP and ACT against the GTF of *S. sobrinus* were 25 μg/mL, and 1.5 μg/mL, respectively. The ID_{50} values against the GTF of *S. mutans* were 120 μg/mL and 5 μg/mL, respectively (Figure 1 and Table 1).

The first and second columns of Table 1 show the groups of polyphenols and phenolic compounds present in plants. The underlined compounds in the second column are the main polyphenols found in APP, which are chlorogenic acid, epicatechin (EC), procyanidin B2, ACT, rutin, and phloridzin. All of these compounds are low-molecular-weight (LMW) polyphenols, with the exception of ACT which is a mixture of catechin oligomers. Procyanidin B2 is an EC dimer. The ID_{50} values of the LMW compounds for inhibition of GTF activity were higher than 1000 μg/mL, and thus these compounds were ineffective. This means that ACT is the only GTF inhibitor in APP. Of the groups of polyphenols in the first column, the compounds which are phenolic acids did not inhibit GTF activity. Gallic acid did not inhibit the enzyme either, but its ester-form derivatives (i.e., hydrolyzable tannins) such as ellagic acid showed some GTF inhibitory activities. Especially tannic acid, which is a mixture of polygalloylglucose, was found to be a very strong GTF inhibitor. The inhibitory effectiveness of tannic acid was nearly equal to that of ACT, and its ID_{50} values against the GTF of *S. sobrinus* and the GTF of *S. mutans* were 0.4 μg/mL and 20 μg/mL, respectively. In the case of the flavonols and dihydrocalcones, the aglycone-form compounds (quercetin and phloretin) weakly inhibited the GTF activity, but the glycoside-form compounds (rutin and phloridzin) did not. Furthermore, in the case of the flavan-3-ols and their oligomers (i.e., condensed tannins), monomeric catechins (EC) and dimeric procyanidin (procyanidin B2) showed no effect on the insoluble glucan synthesis by these GTFs. However, the mixture of catechin oligomers (i.e., ACT) showed very strong inhibitory action. Epigallocatechin gallate (EGCg), which is known to be the main GTF-inhibitor in Japanese green tea (Hattori et al., 1990; Sakanaka et al., 1990), showed weak inhibitory action against only *S. sobrinus* CF-GTF. The inhibition intensity of EGCg (ID_{50}: 480 μg/mL) was very much weaker than that of ACT.

Table 2 shows a comparison of the ID_{50} values of catechin oligomers in ACT with different degrees of polymerization against *S. sobrinus* CF-GTF. The catechin monomers, dimers and trimers had no effect on the activity of the GTF of *S. sobrinus*, but the tetramers showed some inhibition (ID_{50}: 270 μg/mL). The effectiveness in inhibition of GTF increased in proportion to the degree of polymerization of the inhibitors and the fraction of highly polymerized oligomers of sizes larger than pentamers showed the strongest inhibitory action among the compounds in ACT (ID_{50}: 1.0 μg/mL).

Effects of APP on Salivary α-Amylase. Table 3 shows a comparison of the ID_{50} values of various polyphenols for inhibition of the GTF and salivary α-amylase activities. EC had no effect on either enzyme activity. However, the GTF inhibitors with galloyl-ester bonds in their chemical structure, such as EGCg and tannic acid, showed a strong inhibition of α-amylase activity (ID_{50}: 250 μg/mL and 2 μg/mL, respectively). On the other hand, APP and ACT which do not contain gallic acid or its esters scarcely inhibited the α-amylase activity (ID_{50}: 1000 μg/mL and 480 μg/mL, respectively) as compared to their inhibition of the GTF activity.

Effects of APP on Cellular Adherence. The inhibitory effect of APP on sucrose-dependent adherence of cells to the glass surface in a test tube was examined using growing cells of *S. sobrinus* 6715. As shown in Figure 2, APP and ACT markedly inhibited the adherence of the growing cells, and the inhibitory effectiveness was dependent on the concentration of APP or ACT in the reaction mixture. Furthermore, the inhibitory effect of ACT on cellular adherence was significantly stronger than that of APP, and the ID_{50} values of APP and ACT were about 200 μg/mL and under 80 μg/mL, respectively. On the other hand, the LMW compounds present in apple, such as chlorogenic acid and EC, showed no significant effect on adherence of the growing cells.

Table 1. Comparison of the Inhibitory Effects of Various Polyphenols on GTF Activity[d]

polyphenols		ID_{50} (μg/mL) for inhibition of GTF activity[a]	
groups	compounds[b]	*S. sobrinus* CF-GTF	*S. mutans* CA-GTF
	APP	25	120
phenolic acids	*p*-coumaric acid	>1000	>1000
	caffeic acid	>1000	>1000
	chlorogenic acid	>1000	>1000
flavan-3-ol and oligomers	EC	>1000	>1000
(condensed tannins)	procyanidin B2	>1000	>1000
	ACT	1.5	5
gallic acid and derivatives	gallic acid	>1000	>1000
(hydrolyzable tannins)	ellagic acid	20	ND[c]
	EGCg	480	>1000
	tannic acid	0.4	20
flavonols	quercetin	120	ND
	rutin	>1000	>1000
dihydrochalcones	phloretin	200	250
	phloridzin	>1000	>1000

[a] The ID_{50} of each polyphenol was estimated from a plot of the average values of triplicate assays. [b] Polyphenolic compounds underlined are the constituents in APP. [c] ND means no data. [d] APP: apple polyphenols, ACT: apple condensed tannins, EC: epicatechin, EGCg: epigallocatechin gallate, ID_{50}: 50% inhibitory dose.

Table 2. Relationship between the Degree of Polymerization of Catechins and the Inhibitory Activity against *S. sobrinus* CF-GTF

catechin oligomers purified from ACT[a]	degree of polymerization[b]	ID_{50} (μg/mL) against *S. sobrinus* CF-GTF[c]
ACT	mixture from 1 to 15	1.5
monomers	1	>1000
dimers	2	>1000
trimers	3	1000
tetramers	4	270
pentamers	5	150
polymer fraction[d]	mainly more than 5[e]	1.0

[a] Catechin oligomers differing in terms of the degree of polymerization were purified from ACT by normal-phase HPLC. [b] The degree of polymerization was determined in the case of each oligomer by MALDI-TOF MS and/or size-exclusion chromatography (see (Yanagida et al. 2000)). [c] The ID_{50} of each oligomer was estimated from a plot of the average values of duplicate assays. [d] The residue obtained upon methyl acetate extraction of ACT was used as the polymer fraction in this assay. [e] Small amounts of 3-mers, 4-mers, and 5-mers and trace amount of 2-mers were found as contaminants in this fraction.

Table 3. 50% Inhibitory Doses (ID_{50}) of Various Polyphenols for Inhibition of GTFs and α-Amylase Activities

	ID_{50} for inhibition of enzymatic activity (μg/mL)[b]		
polyphenols[a]	*S. sobrinus* CF-GTF	*S. mutans* CA-GTF	salivary α-amylase
APP	25	120	1000
EC	>1000	>1000	>1000
ACT	1.5	5	480
EGCg	480	>1000	250
tannic acid	0.4	20	2

[a] Polyphenolic compounds underlined are the constituents in APP. [b] The ID_{50} of each polyphenol was estimated from a plot of the average values of triplicate assays.

DISCUSSION

It is well-known that many kinds of plant polyphenols display various types of physiological activities, but information on the potential of these substances for prevention of dental caries is fragmentary. Over the past years, many studies showing the anticariogenicity of tea polyphenols have been published in the dental literature. However, there have been no studies comparing the anticariogenicity of polyphenols present in tea and that of polyphenols found in other plants. Thus, many questions still remain unanswered. What kinds of plant polyphenols show anticariogenic action? Can the polyphenols present in other plants (such as edible fruits) show the same anticariogenicity as tea polyphenols do? Is there a common structural feature among all plant polyphenols related to their anticariogenicity, especially regarding the inhibition of bacterial GTF activity? In this study, an attempt was made to answer some of the above questions.

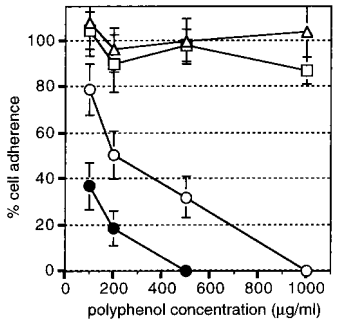

Figure 2. Inhibitory effects of various apple polyphenols on the extent of sucrose-dependent cell adherence of *S. sobrinus* 6715: ○ = APP; ● = ACT; △ = chlorogenic acid; □ = epicatechin. The % cell adherence means the relative amount (%) of adherent cells at a certain polyphenol concentration as compared to the amount detected in the absence of any polyphenol. Each value is the average of triplicate assays and each bar indicates mean ± SE (n = 3).

Structural Features of GTF-Inhibiting Polyphenols. Tea polyphenols are effective in reducing caries both in vitro and in vivo. Earlier studies with tea polyphenols indicated that epigallocatechin gallate (EGCg) was the strong GTF inhibitor in green tea leaves (Sakanaka et al., 1990; Otake et al., 1991). Subsequent studies also showed that GTF-inhibitory activity of polymerized polyphenols from fermented teas such as oolong tea (Nakahara et al., 1993; Ooshima et al., 1993) and black tea (Hattori et al., 1990) is stronger than that of EGCg. Furthermore, other reports proved that galloyl-ester derivatives (hydrolyzable tannins) show strong inhibition of GTF activity (Kakiuchi et al., 1986; Ito et al., 1991; Sawamura et al., 1992). However, these compounds are just a few of the various polyphenols in the plant kingdom, and no report has clearly shown the structure−activity relationship between GTF inhibition and the major groups of plant polyphenols. Regarding that matter, we compared the GTF-inhibitory activity

of the compounds present in APP with that of other types of plant polyphenols, as shown in Tables 1 and 2. On one hand, our data revealed that most LMW polyphenols do not inhibit the GTF activity, except for some aglicone-form compounds (such as quercetin and phloretin) and gallate-ester form compounds (such as EGCg and ellagic acid). On the other hand, high-molecular-weight (HMW) polyphenols such as the oligomers larger than trimers in ACT and tannic acid showed strong inhibitory action. This indicates that the strong GTF inhibitors in plant polyphenols have a common structural feature shared with either catechin-based oligomeric forms (condensed tannin: C-Tan) such as ACT and/or gallate-ester form compounds (hydrolyzable tannin: H-Tan) like EGCg and tannic acid. Other established GTF-inhibiting polyphenols such as those in oolong tea and black tea are classified as complex-tannin form compounds.

Mechanism of GTF Inhibition by HMW Polyphenols. It is well-known that many kinds of polyphenols, in particular those having an *ortho*-diphenolic moiety in their structure, are strong natural antioxidants. However, the mechanism of inhibition of GTF by some HMW polyphenols shown in our assay is probably based on their protein binding activity rather than their nature as antioxidant. Among the GTF inhibitors, H-Tan such as tannic acid and EGCg strongly inhibited salivary α-amylase activity in our assay as shown in Table 3. However, C-Tan such as ACT hardly inhibited the α-amylase activity. Additionally, in another assay (Okuda et al., 1985), H-Tan showed an activity in binding with proteins such as hemoglobin, and the activity was directly proportional to the increase in molecular mass of the H-Tan, most likely due to galloylation. Notably, C-Tan did not show the same activity in this hemoglobin binding assay. These observations indicate that the mechanism of inhibition of GTF and the protein binding ability in case of H-Tan differ from those in the case of C-Tan and that nonspecific binding with enzymes is similarly applicable to the mechanism of GTF inhibition by H-Tan. Our data shown in Table 3 indicate that a C-Tan such as ACT might selectively inhibit the bacterial GTF activity under oral conditions. However, it has been reported that C-Tan from quebracho binds strongly to the proline-rich proteins in saliva and coprecipitates with them (Naurato et al., 1999). To promote a better understanding of the relationship between the protein binding ability of C-Tan and the selectivity under oral conditions, a new assay system for direct detection of protein binding is required, and further studies on the purified procyanidin oligomers according to their degree of polymerization seems to be necessary.

Utilization of APP in Prevention of Dental Caries. In this study, polyphenols extracted from immature apples markedly inhibited bacterial GTF activity as shown in Figure 1 and the adherence of growing cariogenic bacteria as shown in Figure 2. These findings indicate that the inhibition of GTF activity is one of the important factors in inhibition of bacterial cellular adherence in caries prevention. As these happened together, they are likely related, and possibly there is a cause and effect relation in our assay system.

Recently, it has been demonstrated that APP is effective in inhibiting plaque formation in vivo in human subjects (Matsudaira et al., 1998), but how APP influences on the severity of dental caries is still unknown. Now, we are conducting animal studies in an effort to understand the relationship between APP and dental caries. Many plant extracts or derivatives of natural plant products have been successfully incorporated into dentifrices in some countries around the world (Wu-Yuan et al., 1990). The incorporation of plant polyphenols into dentifrices may be an important means of controlling dental and oral diseases. The polyphenols extracted from apple might be used in the same way if incorporated into foodstuffs and dentifrices.

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