APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1993, p. 968–973
0099-2240/93/040968-06$02.00/0

Vol. 59, No. 4

Inhibitory Effect of Oolong Tea Polyphenols on Glucosyltransferases of Mutans Streptococci

KOICHI NAKAHARA,[1] SHIGETADA KAWABATA,[2] HIROYUKI ONO,[1] KYOICHI OGURA,[1] TAKAHARU TANAKA,[1] TAKASHI OOSHIMA,[3] AND SHIGEYUKI HAMADA[2]*

Institute for Biomedical Research, Suntory Ltd., Shimamotocho, Osaka 618,[1] and Departments of Oral Microbiology[2] and Pedodontics,[3] Osaka University Faculty of Dentistry, Yamadaoka, Suita-Osaka 565, Japan

Received 23 September 1992/Accepted 14 January 1993

Oolong tea extract (OTE) was found to inhibit the water-insoluble glucan-synthesizing enzyme, glucosyltransferase I (GTase-I), of *Streptococcus sobrinus* 6715. The GTase-inhibitory substance in the OTE was purified by successive adsorption chromatography on Diaion HP-21 and HP-20 columns; this was followed by further purification by Sephadex LH-20 column chromatography. A major fraction that inhibited GTase activity (fraction OTF10) was obtained, and the chemical analysis of OTF10 indicated that it was a novel polymeric polyphenol compound that had a molecular weight of approximately 2,000 and differed from other tea polyphenols. Catechins and all other low-molecular-weight polyphenols except theaflavin derived from black tea did not show significant GTase-inhibitory activities. It was found that OTE and OTF10 markedly inhibit GTase-I and yeast α-glucosidase, but not salivary α-amylase. Various GTases purified from *S. sobrinus* and *Streptococcus mutans* were examined for inhibition by OTE and OTF10. It was determined that *S. sobrinus* GTase-I and *S. mutans* cell-free GTase synthesizing water-soluble glucan were most susceptible to the inhibitory action of OTF10, while *S. sobrinus* GTase-Sa and *S. mutans* cell-associated GTase were moderately inhibited; no inhibition of *S. sobrinus* GTase-Sb was observed. Inhibition of a specific GTase or specific GTases of mutants streptococci resulted in decreased adherence of the growing cells of these organisms. The inhibitory effect of OTF10 on cellular adherence was significantly stronger than that of OTE.

Mutans streptococci have been implicated as primary causative agents of dental caries in humans and experimental animals (9). Cariogenicity is considered to be strongly associated with the ability of these organisms to synthesize extracellular water-insoluble glucans by using glucosyltransferases (GTases). The glucans are synthesized from sucrose by cooperative actions of GTases and are highly adherent to various solid surfaces, including the tooth surface. This biochemical process results in firm, irreversible adherence of mutans streptococci to the tooth surface, which eventually leads to formation of dental plaque and development of dental caries (20).

A variety of compounds capable of controlling dental caries have been extensively surveyed on the basis of the following criteria: antimicrobial activity (6, 24), inhibition of GTase by immunological neutralization (7), enzyme inhibitors (15), and replacement of sucrose with other sweeteners (25, 27, 29). However, only limited numbers of compounds from natural products are available because of effectiveness, stability, odor, taste, and economic feasibility. Tea is one of the most widely consumed beverages in the world. It is classified into four types, green tea, oolong tea, black tea, and puer tea, on the basis of the manufacturing process. All of these teas are prepared from leaves of *Camellia sinensis* and its varieties. Kubo et al. (21) reported that green tea extract (GTE) contained some cariostatic substances, and the active principles were ascribed to volatile components with flavor. Recently, it has been claimed that several polyphenolic compounds in GTE can suppress the growth of mutans streptococci and inhibit glucan synthesis from sucrose by GTases (12, 30).

* Corresponding author.

In an attempt to survey different kinds of tea extracts that inhibit GTase activity of mutans streptococci, we have found that certain polymeric polyphenols from oolong tea extract (OTE) can exhibit strong inhibitory activities against GTases of mutans streptococci. In this paper we describe the preparation, chemical characterization, and GTase inhibition of oolong tea polyphenolic compounds.

MATERIALS AND METHODS

Extraction and preparation of GTase-inhibitory compounds. Pulverized oolong tea leaves (435 g) produced in the Fukien province of the People's Republic of China were suspended in 1 liter of 45% (vol/vol) ethanol and kept at room temperature for 1 day. After filtration and evaporation of the ethanol, the remaining extract was lyophilized to give a powder (OTE) (yield, 100 g). The OTE preparation (100 g) was further fractionated by adsorption chromatography by using a Diaion HP-21 column (8 by 20 cm; Mitsubishi Chemical Industries, Tokyo, Japan). The column was eluted with deionized water (10 liters), then with 30% ethanol (5 liters), and finally with 100% ethanol (5 liters), and three fractions, OTF1, OTF2, and OTF3, were obtained. GTase-inhibitory fraction OTF1 was further purified by successive column chromatography with a Diaion HP-20 column (8 by 20 cm) and a Sephadex LH-20 column (3 by 120 cm; Pharmacia-LKB Biotechnology, Uppsala, Sweden). For comparison, GTE was prepared similarly, and the level of recovery was 28% of the starting green tea leaves on a weight basis. The GTE preparation was treated with ethyl acetate-water (1:1, vol/vol), and the upper ethyl acetate layer was separated and evaporated in vacuo. The resultant extract was found to be mainly composed of catechins, as well

as trace amounts of unknown polyphenols, and was designated the crude catechin preparation (CCP).

Other polyphenolic compounds, such as (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate were extracted from green tea and purified by the procedures of Matsuzaki and Hara (22). Theaflavin was extracted from black tea and purified by reverse-phase high-performance liquid chromatography by using a Develosil octadecyl silane column (4.5 by 150 mm; Nomura Chemical Co., Seto, Japan) after caffeine was removed with chloroform. Other reagent grade chemicals were obtained from Nacalai Tesque, Inc., Kyoto, Japan, and Wako Pure Chemical Co., Osaka, Japan.

Chemical differentiation of various tea polyphenols. Differences in chemical structure among the polyphenol compounds prepared as described above were determined by using the following specific coloration reactions (31): the Folin-Denis method for the determination of total phenol content (36), the vanillin-HCl method to determine total flavanol content (3), the *n*-butanol–HCl method to determine procyanidine content (2), the $NaNO_2$ method to determine ellagitannin content (1), and the $FeCl_3$ method to determine total tannin content (4).

Preparation of GTases. *Streptococcus mutans* MT8148 and *Streptococcus sobrinus* 6715 were grown for 18 h at 37°C in 8 liters of diffusate of TTY broth containing Trypticase, tryptose, yeast extract, salts, and 1% glucose (10). The centrifuged supernatant from each culture was concentrated by filtration through a module (model ACL-1010; Asahi Chemical Industry, Tokyo, Japan) and was precipitated with 50% saturated ammonium sulfate. The precipitate was extensively dialyzed against 10 mM potassium phosphate buffer (pH 6.0), and this preparation was designated the crude cell-free GTase (CF-GTase). The crude CF-GTase of *S. mutans* was further purified by the chromatofocusing method by using a Polybuffer exchanger PBE94 (Pharmacia-LKB Biotechnology) as described by Sato et al. (33). Purified CF-GTase synthesized water-soluble glucan from sucrose. *S. mutans* cells obtained after centrifugation of a TTY culture were extensively washed with saline and water and then treated with 8 M urea at 25°C for 1 h, and the extract was dialyzed, concentrated, and purified by DEAE-Sephacel (Pharmacia-LKB Biotechnology) column chromatography as reported previously (8). This cell-associated GTase (CA-GTase) was found to synthesize almost exclusively water-insoluble glucan from sucrose. On the other hand, GTase-I and water-soluble-glucan-synthesizing GTases (GTase-Sa and GTase-Sb) were purified from a cell-free supernatant of the *S. sobrinus* 6715 culture by chromatofocusing (5). It was noted that GTase-Sa was primer independent, while GTase-I and GTase-Sb were primer dependent.

Measurement of glucan synthesis and cellular adherence. To measure soluble glucan synthesis, a reaction mixture containing *S. mutans* CF-GTase or *S. sobrinus* GTase-Sa or GTase-Sb, 10 mM [^{14}C-glucose]sucrose (1.85 GBq/mol), and polyphenols was incubated with or without primer dextran T10 at 37°C for 1 h. The reaction product was spotted on a small square of filter paper, washed with 100% methanol to remove nonpolymerized sugars, and dried in air. The radioactivity remaining on the filter paper square was quantified with a model 1214 scintillation counter (Pharmacia-LKB Biotechnology) (17). Insoluble glucan synthesis by *S. mutans* CA-GTase and *S. sobrinus* GTase-I was measured turbidimetrically by determining the increase in A_{550}. CA-GTases or GTase-I (10 to 20 mU) was incubated in 2 ml of 0.05 M phosphate buffer (pH 6.0) containing 1% sucrose, 0.05% sodium azide, and polyphenols in the presence or absence of primer dextran T10 (20 μM; Pharmacia-LKB Biotechnology) at 37°C for 18 h.

Sucrose-dependent cellular adherence was determined as described previously (20). Mutans streptococci were grown at 37°C at a 30° angle to the horizon for 18 h in 3 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) containing 1% sucrose. The quantity of adherent cells after gentle washing of each test tube was determined turbidimetrically and expressed as a percentage of the total cell mass (percentage of total cell adherence). The culture tube was then vigorously vibrated with a Vortex mixer for 2 s, and the percentage of adherence was determined (percentage of firm cell adherence).

Assays for α-glucosidase and α-amylase. The effects of polyphenols on yeast α-glucosidase (Toyobo Co., Osaka, Japan) and salivary α-amylase (Sigma Chemical Co., St. Louis, Mo.) were measured by determining the release of reducing sugars from sucrose and soluble starch, respectively. Yeast α-glucosidase (0.1 U in 500 μl) was incubated with 1 ml of 50 mM sucrose and 400 μl of 0.05 M phosphate buffer (pH 7.0) in the presence of a polyphenol solution (100 μl) or distilled water (100 μl) at 37°C for 30 min. The reducing sugars released were quantified by the dinitrosalicylate method (13). Salivary α-amylase (0.26 U in 500 μl) was incubated with 1 ml of 0.5% soluble starch and 400 μl of 0.05 M phosphate buffer (pH 7.0) in the presence of a polyphenol solution (100 μl) or distilled water (100 μl) at 37°C for 60 min. The reducing sugars liberated were measured as described above.

Measurement of molecular weights of oolong tea polyphenols. To estimate the molecular weights of oolong tea polyphenols, these compounds at a concentration of 1 mg/ml of methanol were incubated in an excess of diazomethane ether at 4°C for 2 days (32). The ether was then removed under reduced pressure, and the methylated product was extracted with chloroform. The apparent molecular weights of the methylated GTase inhibitors from oolong tea were determined by comparing the elution profiles on a TSK G3000H-XL gel (Toso Co., Tokyo, Japan) column (7.5 by 300 mm) with the elution profiles of polystyrenes having known molecular weights and methylated catechin.

Statistics. The results for [^{14}C]glucan synthesized from [^{14}C]sucrose and cellular adherence were expressed as the means of triplicate assays ± standard deviations. The standard deviation values obtained were found to be within 5% of the mean values. All values are expressed as percentages of the mean for the control experiment without polyphenol.

RESULTS

Isolation of GTase-inhibitory polyphenols. GTase-inhibitory polyphenols were chromatographically isolated and purified. Chromatography of OTE with a Diaion HP-21 column yielded three fractions, OTF1, OTF2, and OTF3. OTF1 released from the column without adsorption exhibited clear inhibitory activities against *S. sobrinus* GTase-I. This fraction was further chromatographed on a Diaion HP-20 column and was released by stepwise elution with H_2O and 20, 40, and 100% ethanol solutions. This chromatography yielded four fractions, OTF4 to OTF7. Since the 40% ethanol eluate (i.e., OTF6) was found to inhibit GTase-I, it was then applied to a Sephadex LH-20 column for further purification, which produced three peak fractions, OTF8 to OTF10. These fractions exhibited strong GTase-inhibitory activities and similar chemical reactivities

TABLE 1. Purification of oolong tea polyphenols inhibiting *S. sobrinus* GTase-I

Purification step	Dry wt (g)	ID_{50} (μg/ml)[a]	Yield (%)	Purification (fold)
OTE	100.0	40.0	100.0	1
Diaion HP-21 effluent	40.0	20.0	80.0	2
Diaion HP-20 40% ethanol fraction	5.0	3.0	66.8	13
Sephadex LH-20 fractions				
OTF8	3.2	5.0	25.6	8
OTF9	0.7	2.5	11.2	16
OTF10	0.8	2.0	16.0	20

[a] ID_{50}, 50% inhibitory dose (the concentration giving 50% inhibition of GTase-I activity).

with specific coloration reagents. It was found that OTF10 had the smallest molecular weight, and this fraction was used for most of the chemical characterization and GTase inhibition studies described below. The purification and yields of GTase-inhibitory OTE fractions are summarized in Table 1.

Properties of OTF10. The UV spectrum of OTF10 dissolved in a neutral solution exhibited maximum adsorption at 274 nm and a trough at 204 nm. The Fourier transform infrared spectrum determined by the diffuse reflection method, using KBr powder, exhibited absorption bands at 3,312, 1,696, 1,611, 1,514, 1,450, 1,373, and 1,230 cm^{-1} (data not shown). A positive color reaction of OTF10 with the Folin-Denis reagent indicated that OTF10 contained phenolic substances. Aberrant reactions with vanillin-HCl and $FeCl_3$ reagents showed the presence of polyphenols possessing structures that mimic flavanol and ellagitannin molecules. The overall reactions of OTF10 with reagents reactive with representative tannic compounds are summarized in Table 2. For reference, typical tannic compounds were also included. The results shown in Table 2 clearly indicate that OTF10 was different from any other tannic substance examined. The molecular weight of OTF10 was estimated to be 2,000 on the basis of the elution profile from gel permeation chromatography (data not shown). OTF10 and OTF6 were very stable against heat treatment (up to 100°C for 1 h) and photoradiation (12,000 lux at 20°C for 1 week).

Inhibition of GTases by tea polyphenols. Various polyphenols were examined for their inhibitory activities against *S. sobrinus* GTase-I. OTF10 and OTE markedly inhibited GTase-I, while GTE or CCP only slightly inhibited the enzyme. In addition, complete inhibition of GTase-I by OTF10 was obtained at concentrations of ≥20 μg/ml of reaction mixture (Fig. 1). It was also found that OTF10 and theaflavin derived from black tea extract produced 50% inhibition of GTase-I at concentrations of 1.0 and 10 μM, respectively. On the other hand, other polyphenols, such as (+)-catechin and other related compounds, resulted in very weak GTase inhibition (≤28%) at concentrations of more than 500 μM (Table 3).

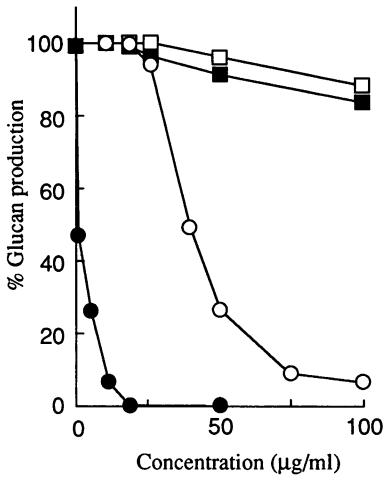

FIG. 1. Inhibitory effects OTE, OTF10, and GTE on glucan synthesis by *S. sobrinus* GTase-I from sucrose. Inhibitory activities of samples were measured at various concentrations. ●, OTF10; ○, OTE; ■, CCP; □, GTE.

The specificity of enzyme inhibition by tea extracts and purified polyphenols was examined by using enzymes that split the glucose residue from the substrate. Table 4 shows that OTE and OTF10 specifically inhibited GTase-I and α-glucosidase, but not α-amylase. On the other hand, GTE and CCP inhibited α-glucosidase, but not GTase-I and α-amylase.

Effects of OTE and OTF10 on various GTases and cell adherence. *S. sobrinus* produces two GTases which synthesize water-soluble glucans in addition to GTase-I (GTase-Sa and GTase-Sb). It was evident that neither OTE nor OTF10 affected GTase-Sb, while GTase-Sa was moderately inhibited (Fig. 2). In *S. mutans* GTases, however, the CF-GTase

TABLE 2. Comparison of properties of OTF10 and other authentic polyphenols on the basis of specific coloration reactions

Polyphenol	Colorimetric detection				
	Total phenols (Folin-Denis method)	Total flavanols (vanillin-HCl method)	Procyanidine (butanol-HCl method)	Ellagitannins ($NaNO_2$ method)	Total tannins ($FeCl_3$ method)
OTF10	+ (blue)[a]	+ (yellow)	−	−	+ (brown)
Condensed tannins					
Proanthocyanidin	+ (blue)	+ (red)	+ (red)	−	+ (green)
Flavanol (catechins)	+ (blue)	+ (red)	−	−	+ (blue)
Hydrolyzable tannins					
Gallotannin	+ (blue)	−	−	−	+ (blue)
Ellagitannin	+ (blue)	−	−	+ (blue)	+ (blue)
Complex tannins	+ (blue)	+ (red)	−	+ (blue)	+ (blue)

[a] +, positive coloration reaction (color developed); −, negative color reaction.

TABLE 3. Effects of various polyphenols on *S. sobrinus* GTase-I

Polyphenol	Polyphenol concn		% Inhibition[a]
	mg/ml	mM	
(+)-Catechin	0.250	0.862	13
(−)-Epicatechin	0.250	0.862	9
(−)-Epigallocatechin	0.250	0.817	8
(−)-Epicatechin gallate	0.250	0.566	28
(−)-Epigallocatechin gallate	0.250	0.546	23
Theaflavin	0.008	0.010	50
OTF10	0.002	0.001	50

[a] Insoluble glucan synthesis was determined turbidimetrically.

synthesizing water-soluble glucan from sucrose was almost completely suppressed upon addition of more than 0.025% OTE or OTF10. On the other hand, CA-GTase producing insoluble glucan from sucrose was gradually inhibited by increasing concentrations of OTF10 and, to a lesser extent, by OTE (Fig. 3). Inhibition of cellular adherence of growing cells of mutans streptococci to a glass surface was examined; addition of OTF10 and OTE inhibited cell adherence in a dose-dependent manner (Fig. 4).

DISCUSSION

Our preliminary chemical analyses of polyphenolic compounds in various tea leaves have revealed that OTE and black tea extract contain significant quantities of unknown polyphenols that are not detected in GTE. Green tea leaves contain 14.7% polyphenols (mainly condensed and hydrolyzable monomeric polyphenols), while oolong tea leaves contain 16.0% polyphenols, including 9.7% monomeric polyphenols and 6.4% unknown polymeric polyphenols (unpublished data). Another interesting finding is that OTE exhibits the most prominent inhibitory action among various tea extracts. In this study, we have shown that GTase inhibition was caused by polymeric polyphenols free of low-molecular-weight catechins. OTF10, a polymeric polyphenol fraction, was found to exhibit strong GTase inhibition, while catechins from different sources did not inhibit GTase significantly (Table 3). The chemical structure of OTF10 is suggested to be unique on the basis of specific coloration reactions (Table 2).

It is of interest to know how GTase-inhibitory polyphenols are produced in oolong tea or black tea despite the fact that all teas used in this study are manufactured from leaves of the same plant species, *C. sinensis*. Unlike green teas, oolong and black teas are semifermented and fermented, respectively, during the manufacturing process. The fermentation and heating of tea leaves result in polymerization of

TABLE 4. 50% Inhibitory doses of tea extracts and catechins against enzymatic activities of *S. sobrinus* GTase-I, yeast α-glucosidase, and salivary α-amylase

Prepn	ID_{50} (mg/ml)[a]		
	GTase-I	α-Glucosidase	α-Amylase
OTE	0.040	0.020	5.0
OTF10	0.002	0.003	>10.0
GTE	0.250	0.020	1.5
CCP	0.250	0.010	0.5

[a] ID_{50}, 50% inhibitory dose (the concentration giving 50% inhibition of enzyme activity).

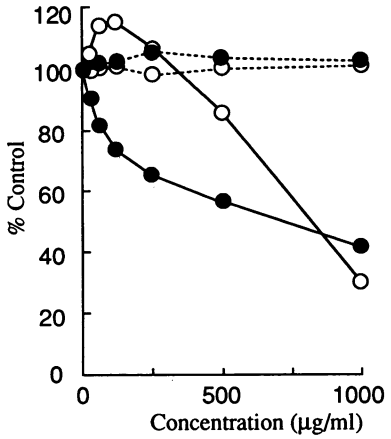

FIG. 2. Effects of OTE and OTF10 on enzyme activity of *S. sobrinus* GTase-Sa (——) and GTase-Sb (– – –) synthesizing soluble glucans. ○, OTE; ●, OTF10.

monomeric polyphenolic compounds, such as catechins (11). These findings clearly indicate that conformational changes due to polymerization of catechins should be critically important for exerting an inhibitory effect on GTases of mutans streptococci. However, the mode of GTase-inhibitory action of OTE and OTF10 is unknown at this time. In this respect, it is necessary to point out that the oolong tea polyphenols inhibit GTase and α-glucosidase rather specifically (Table 3) and that not all GTases prepared from *S. mutans* and *S. sobrinus* are inhibited by these substances. For example, *S. mutans* CF-GTase synthesizing water-soluble glucan was markedly inhibited upon addition of OTE or OTF10. On the other hand, *S. sobrinus* GTase-I synthesizing water-insoluble glucan was very sensitive to inhibition resulting from the action of these polyphenols (Fig. 1 through 3).

Diminished glucan synthesis due to specific inhibition of a GTase(s) in growing cells of *S. mutans* or *S. sobrinus* resulted in decreased cellular adherence to a glass surface. This phenomenon may be induced because of unbalanced synthesis of soluble and insoluble glucans (14). Some polyphenolic and related compounds from several tea leaves and betel nuts were shown to inhibit GTase from *S. mutans* (15,

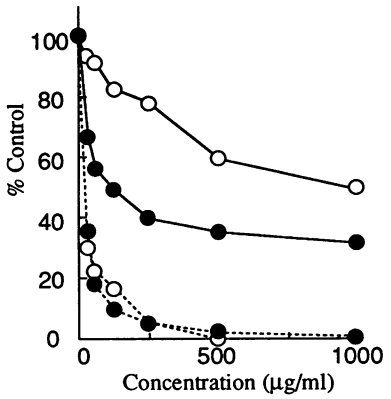

FIG. 3. Effects of OTE and OTF10 on enzyme activity of *S. mutans* CA-GTase (——) and CF-GTase (– – –). ○, OTE; ●, OTF10.

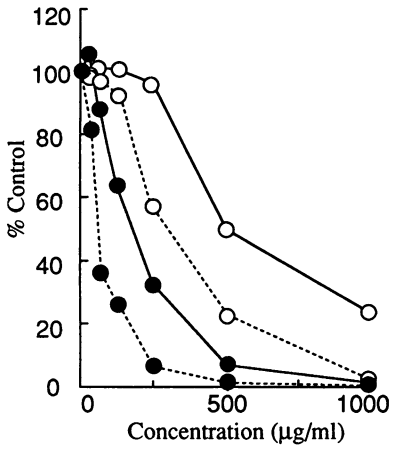

FIG. 4. Adherence inhibition of growing cells of *S. mutans* (——) and *S. sobrinus* (– – –) in the presence of OTE and OTF10. Mutans streptococci were inoculated into brain heart infusion broth containing 1% sucrose and various concentrations of polyphenol preparations, and the relative adherence to the surface of the culture tube was estimated. ○, OTE; ●, OTF10.

16, 34). In these studies, however, no attempts were made to use purified GTase enzymes or to characterize the chemical properties of polyphenols. Other GTase inhibitors of different entities and origins have been reported. A proteinaceous inhibitor, mutastein, was isolated from culture liquor of *Aspergillus terreus* (18), while a ribose oligosaccharide, ribocitrin, was obtained from *Streptomyces neyagawaensis* culture supernatant (37). Glycyrrhizin found in licorice was also found to inhibit *S. sobrinus* GTase (35). In addition to these compounds, oligosaccharides such as maltose, isomaltose, panose (19), and structural isomers of sucrose like palatinose and trehalulose clearly inhibit GTase activities (23, 26, 28).

In conclusion, oolong tea polyphenols strongly inhibited the enzyme activities of some types of GTases of mutans streptococci. To our knowledge, the chemical structure of the polyphenols should differ from the chemical structures of known types of polyphenolic or tannic substances. Since the compounds in oolong tea have been ingested by many people for many years, no side effects would be expected, and oolong tea should be a useful cariostatic material when it is given orally.

REFERENCES

1. **Bate-Smith, E. C.** 1972. Detection and determination of ellagitannins. Phytochemistry **11**:1153–1156.
2. **Bate-Smith, E. C.** 1975. Phytochemistry of proanthocyanidins. Phytochemistry **14**:1107–1113.
3. **Broadhurst, R. B., and W. T. Jones.** 1978. Analysis of condensed tannins using acidified vanillin. J. Sci. Food Agric. **29**:788–794.
4. **Czerniawski, M.** 1958. The quantitative determination of vegetable tannins by the colorimetric method. Chem. Anal. (Warsaw) **3**:37–45.
5. **Furuta, T., T. Koga, T. Nisizawa, N. Okahashi, and S. Hamada.** 1985. Purification and characterization of glucosyltransferase from *Streptococcus mutans* 6715. J. Gen. Microbiol. **131**:285–293.
6. **Hada, S., N. Kakiuchi, M. Hattori, and T. Namba.** 1989. Identification of antibacterial principles against *Streptococcus mutans* and inhibitory principles against glucosyltransferase from the seed of *Areca catechu* L. Phytother. Res. **3**:140–144.
7. **Hamada, S., T. Horikoshi, T. Minami, S. Kawabata, J. Hiraoka, T. Fujiwara, and T. Ooshima.** 1991. Oral passive immunization against dental caries in rats by use of hen egg yolk antibodies specific for cell-associated glucosyltransferase of *Streptococcus mutans*. Infect. Immun. **59**:4161–4167.
8. **Hamada, S., T. Horikoshi, T. Minami, N. Okahashi, and T. Koga.** 1989. Purification and characterization of cell-associated glucosyltransferase synthesizing water-insoluble glucan from serotype *c Streptococcus mutans*. J. Gen. Microbiol. **135**:335–344.
9. **Hamada, S., T. Koga, and T. Ooshima.** 1984. Virulence factors of *Streptococcus mutans* and dental caries prevention. J. Dent. Res. **63**:407–411.
10. **Hamada, S., and M. Torii.** 1978. Effect of sucrose in culture media on the location of glucosyltransferase of *Streptococcus mutans* and cell adherence to glass surfaces. Infect. Immun. **20**:592–599.
11. **Hashimoto, F., G. Nonaka, and I. Nishioka.** 1989. Tannins and related compounds. XC. 8-*C*-Ascorbyl (−)-epigallocatechin 3-*O*-gallate and novel dimeric flavan-3-ols, oolonghomobisflavans A and B, from oolong tea. Chem. Pharm. Bull. (Tokyo) **37**:3255–3263.
12. **Hattori, M., I. Kusumoto, T. Namba, T. Ishigami, and Y. Hara.** 1990. Effect of tea polyphenols of glucan synthesis by glucosyltransferase from *Streptococcus mutans*. Chem. Pharm. Bull. (Tokyo) **38**:717–720.
13. **Hostettler, F., E. Borel, and H. Denel.** 1951. Über die Reduktion der 3,5-Dinitrosalicylsäure durch Zucker. Helv. Chim. Acta **34**:2132–2139.
14. **Inoue, M., T. Koga, S. Sato, and S. Hamada.** 1982. Synthesis of adherent insoluble glucan by the concerted action of the two glucosyltransferase components of *Streptococcus mutans*. FEBS Lett. **143**:101–104.
15. **Kakiuchi, N., M. Hattori, M. Nishizawa, T. Yamagishi, T. Okuda, and T. Namba.** 1986. Studies on dental caries prevention by traditional medicines. VIII. Inhibitory effect of various tannins on glucan synthesis by glucosyltransferase from *Streptococcus mutans*. Chem. Pharm. Bull. (Tokyo) **34**:720–725.
16. **Kashket, S., V. J. Paolino, D. A. Lewis, and J. Van Houte.** 1985. *In-vitro* inhibition of glucosyltransferase from the dental plaque bacterium *Streptococcus mutans* by common beverages and food extracts. Arch. Oral Biol. **30**:822–826.
17. **Koga, T., H. Asakawa, N. Okahashi, and S. Hamada.** 1986. Sucrose-dependent cell adherence and cariogenicity of serotype *c Streptococcus mutans*. J. Gen. Microbiol. **132**:2873–2883.
18. **Koga, T., S. Hamada, S. Murakawa, and A. Endo.** 1982. Effect of a glucosyltransferase inhibitor on glucan synthesis and cellular adherence of *Streptococcus mutans*. Infect. Immun. **38**:882–886.
19. **Koga, T., T. Horikoshi, T. Fujiwara, and S. Hamada.** 1988. Effects of panose on glucan synthesis and cellular adherence by *Streptococcus mutans*. Microbiol. Immunol. **32**:25–31.
20. **Koga, T., N. Okahashi, H. Asakawa, and S. Hamada.** 1986. Adherence of *Streptococcus mutans* to tooth surfaces, p. 111–120. *In* S. Hamada, S. M. Michalek, H. Kiyono, L. Menaker, and J. R. McGhee (ed.), Molecular microbiology and immunobiology of *Streptococcus mutans*. Elsevier Science Publishers, Amsterdam.
21. **Kubo, I., H. Muroi, and M. Himejima.** 1992. Antimicrobial activity of green tea flavor components and their combination effects. J. Agric. Food Chem. **40**:245–248.
22. **Matsuzaki, T., and Y. Hara.** 1985. Antioxidative activity of tea leaf catechins. Nippon Nogeikagaku Kaishi **59**:129–134.
23. **Minami, T., T. Fujiwara, T. Ooshima, Y. Nakajima, and S. Hamada.** 1990. Interaction of structural isomers of sucrose in the reaction between sucrose and glucosyltransferases from mutans streptococci. Oral Microbiol. Immunol. **5**:189–194.
24. **Onishi, M., F. Ozaki, F. Yoshino, and Y. Murakami.** 1981. An experimental evidence of caries preventive activity of non-fluoride component in tea. J. Dent. Health (Tokyo) **31**:158–161.
25. **Ooshima, T., T. Fujiwara, T. Takei, A. Izumitani, S. Sobue, and S. Hamada.** 1988. The caries inhibitory effects of GOS-sugar *in vitro* and in rat experiments. Microbiol. Immunol. **32**:1093–1105.

26. **Ooshima, T., A. Izumitani, T. Minami, T. Fujiwara, Y. Nakajima, and S. Hamada.** 1991. Trehalulose does not induce dental caries in rats infected with mutans streptococci. Caries Res. **25:**277–282.
27. **Ooshima, T., A. Izumitani, T. Minami, T. Yoshida, S. Sobue, and S. Hamada.** 1992. Noncariogenicity of maltitol in specific pathogen-free rats infected with mutans streptococci. Caries Res. **26:**33–37.
28. **Ooshima, T., A. Izumitani, S. Sobue, and S. Hamada.** 1983. Cariostatic effect of palatinose on experimental dental caries in rats. Jpn. J. Med. Sci. Biol. **36:**219–223.
29. **Ooshima, T., A. Izumitani, S. Sobue, N. Okahashi, and S. Hamada.** 1983. Noncariogenicity of the disaccharide palatinose in experimental dental caries of rats. Infect. Immun. **39:**43–49.
30. **Sakanaka, S., M. Kim, M. Taniguchi, and T. Yamamoto.** 1989. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. Agric. Biol. Chem. **53:**2307–2311.
31. **Salunkhe, D. K., J. K. Chavan, and S. S. Kadam.** 1989. Dietary tannins: consequences and remedies, p. 77–111. CRC Press, Inc., Boca Raton, Fla.
32. **Samejima, M., and T. Yoshimoto.** 1981. General aspects of phenolic extractives from coniferous barks. Mokuzai Gakkaishi **27:**491–497.
33. **Sato, S., T. Koga, and M. Inoue.** 1984. Isolation and some properties of extracellular D-glucosyltransferases and D-fructosyltransferases from *Streptococcus mutans* serotypes *c*, *e*, and *f*. Carbohydr. Res. **134:**293–304.
34. **Sawamura, S., Y. Tonosaki, and S. Hamada.** 1992. Inhibitory effects of ellagic acid on glucosyltransferases from mutans streptococci. Biosci. Biotechnol. Biochem. **56:**766–768.
35. **Sela, M. N., D. Steinberg, and R. Segal.** 1987. Inhibition of activity of glucosyltransferase from *Streptococcus mutans* by glycyrrhizin. Oral Microbiol. Immunol. **2:**125–128.
36. **Swain, T., and W. E. Hills.** 1959. Phenolic constituents of *Prunus domestica*. I. Quantitative analysis of phenolic constituents. J. Sci. Food Agric. **10:**63–68.
37. **Takashio, M., T. Ohnuki, and Y. Okami.** 1982. The structure of ribocitrin and its structure-activity relationship in the inhibition of dextransucrase of *Streptococcus mutans* E49. Agric. Biol. Chem. **46:**2449–2456.