Note

Inhibitory Effects of Polysaccharides on the Cariogenic Activities of *Streptococcus mutans*

Akira YANO,¹, ¹⁴ Naotake KONNO,¹ Susumu IMAI,² and Hirohisa KATO³

¹Iwate Biotechnology Research Center, 22-174-4 Narita, Kitakami, Iwate 024-0003, Japan
²Translational Research, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Yokohama, Kanagawa 230-8501, Japan
³Basic Medicine, Iwate Medical University, 19-1 Uchimaru, Morioka, Iwate 020-8505, Japan

Received June 15, 2012; Accepted September 19, 2012; Online Publication, December 7, 2012

Many carbohydrates are involved in the biofilm formation and activities of glucosyltransferases (Gtfs) of *Streptococcus mutans*, and the effects of various disaccharides and polysaccharides were investigated in this study, including the hot water-extracted glucan fraction of the *Lentinula edodes* fruiting body (HWG). HWG was found to inhibit the initial adhesion of *S. mutans* to saliva-coated hydroxyapatite (sHA), and also laminarin to inhibit glucan synthesis by Gtfs. However, sucrose-dependent biofilm formation by *S. mutans* was not inhibited by these materials. Interestingly, dextran was found to have an inhibitory effect on the sucrose-dependent biofilm formation. The data suggest that the presence of such an edible glucan as dextran in daily foods would act to some degree on *S. mutans* for suppressing the cariogenic activity.

Key words: polysaccharide; glucan; *Streptococcus mutans*; Gtfs; biofilm

*Streptococcus mutans* is the causative pathogen for dental caries.¹,² An early attachment to the pellicle of the tooth surface, where it becomes rigidly anchored by producing extracellular adherent glucans. These glucans, which are synthesized by glucosyltransferases (Gtfs) from sucrose, are used by other oral bacteria as the scaffold to promote the formation of a biofilm on the tooth surface.² Organic acids are produced from dietary sugar by bacterial fermentation in the biofilm, resulting in demineralization of the tooth surface and generation of dental caries.¹³–¹⁴

Non-cariogenic sweeteners have been studied for many years to reduce the risk of caries.³ Such disaccharides as trehalose, palatinose, and isomaltose are less cariogenic than sucrose.³ Sugar alcohols, typified by xylitol, have been well studied as caries-preventive sugar substitutes.⁶ Maltitol and erythritol are other sugar alcohols that are used as sweeteners for foods and oral care products.⁷ Sugar alcohols can be expected to be less cariogenic than sucrose because they are not converted to acids by oral bacteria. Several clinical studies have shown that the use of xylitol can decrease the occurrence of dental caries.⁷–⁹

The major sugar substitutes are mono- and disaccharides or their derivatives. Polysaccharides are the major carbohydrates in foods, but their properties related to dental caries are almost unknown. We investigated in this study the anticariogenic activities of several food-derived polysaccharides in vitro. We particularly studied the inhibitory effects of polysaccharides on the glucosyltransferases (Gtfs) of *S. mutans* by a previously reported method (Fig. 1).¹⁰ Maltose and cellobiose (Wako Pure Chemicals, Osaka, Japan) were respectively used as positive and negative controls. Maltose is a saccharide formed from two units of glucose with an α-1,4 junction, and Ooshima¹¹ has reported that maltose inhibited the synthesis of the water-insoluble glucan by Gtfs of *S. mutans*. Glycogen and dextran (Wako Pure Chemicals) were used as representative α-glucans with respective 1,4-1,6 and 1,6 junctions. Laminarin and gentio-oligosaccharide (Wako Pure Chemicals) are β-glucans with 1,3-1,6 and 1,6 junctions.

We also investigated the hot water-extracted glucan (HWG) of *Lentinula edodes*. Although we had previously studied the anticariogenic activities of water-soluble extracts of *L. edodes*,¹² those activities were heat-labile and difficult to use for oral care. We examined in this present study the thermo-stable fraction of the mushroom. The fruiting body of *L. edodes* was autoclaved at 121°C for 15 min with 10 volumes of water per dried weight after extracting with phenol/chloroform. The polysaccharides in the extract were precipitated by adding a two-fold volume of ethanol. The mushroom extract is designated as HWG. Glucan extracts of the mushroom are usually reported to contain β-glucans.¹³,¹⁴ Figures 1A and B respectively show the amounts of water-insoluble glucan synthesized by recombinant GtfB and of water-soluble glucan synthesized by GtfD.

Dextran (α-1,6-glucan) is a primer of glucan synthesis by Gtfs that promotes glucan synthesis by GtfB and GtfD.¹⁵ Maltose and glycogen, the latter containing the α-1,4-junction of glucose, increased the synthesis of water-soluble glucan by GtfD. Those saccharides might work as primers for α-1,6-glucan synthesis by GtfD. Interestingly, laminarin (a β-1,3-1,6-glucan) inhibited the synthesis of the water-insoluble glucan by GtfB and increased the synthesis of the water-soluble glucan by GtfD. The β-1,3-motif of laminarin should effectively suppress the GtfB activities, because the gentio-oligosaccharide (β-1,6-oligo glucan) did not inhibit glucan synthesis by GtfB. We also investigated the effect of

¹ To whom correspondence should be addressed. Tel: +81-197-68-2911; Fax: +81-197-68-3881; E-mail: akiray@ibrc.or.jp
those saccharides on the acid produced from glucose fermentation by *S. mutans*, but did not find any inhibitory effect (data not shown).

We examined the effects of the saccharides on the initial adhesion of *S. mutans* to saliva-coated hydroxyapatite (sHA) by using our previously described method (Fig. 2). Briefly, HA beads (Bio-Rad) were coated with clarified saliva at 37°C for 1 h and then gently washed twice by an adhesion buffer (50 mM KCl, 1 mM CaCl₂, 0.1 mM MgCl₂, and 1 mM KPO₄ at pH 6.5). *S. mutans* in the mid-exponential phase was labeled with propidium iodide (Molecular Probes, Eugene, OR, USA) and suspended with sHA beads in a 96-well microtiter plate. The sHA-binding cells were measured with a fluorescence microplate reader (Molecular Devices, Sunnyvale, CA, USA) after washing out the non-binding cells. *S. mutans* binding to sHA was not influenced by most of the saccharides, but a high concentration of laminarin showed an inhibitory effect. Interestingly, HWG inhibited *S. mutans*-sHA binding in a dose-dependent manner (Fig. 2). To our knowledge, this is the first report of dietary polysaccharides having inhibitory activity against the initial binding of *S. mutans*.

After attaching to the tooth surface, *S. mutans* forms a rigid biofilm on the surface via glucan synthesis by Gtfs. We investigated the inhibitory effects of saccharides on sucrose-dependent biofilm formation on the sHA surface (Fig. 3). HA-coated 24-well plates were incubated with clarified saliva. *S. mutans* was cultured in each well with saccharides and 1% sucrose containing a THB medium as previously described.12) HWG, which had effectively inhibited cell binding on sHA beads, unexpectedly did not reduce biofilm formation in the wells. After inhibiting the initial adhesion, sucrose induced the formation of a thick biofilm during several hours. HWG might have been among the newly synthesized glucans and unable to alter the glucan-bacteria interaction which was important for
biofilm formation. Laminarin, which inhibited water-insoluble glucan synthesis by GtfB (Fig. 2), did not inhibit the biofilm formation by *S. mutans*. Higher specific inhibitory activity toward glucan synthesis would be necessary for reducing the biofilm formation.

Dextran surprisingly reduced the sucrose-dependent biofilm formation (Fig. 3). This α-1,6-glucan enhanced the glucan synthesis by GtfB and GtfD, and was expected to enhance biofilm formation.

Our results show that the biofilm formed with dextran was loosely bound to sHA and *S. mutans* and easily washed out by gentle rinsing. Imbalance of the extracellular polysaccharide composition might have changed the physical properties of the sucrose-dependent biofilm. If an excess volume of glucans had surrounded the *S. mutans* cells, the cells might then have become separated in the in vitro assay.

In conclusion, we identified the novel properties of several polysaccharides in terms of their *S. mutans* cariogenic activities. The polysaccharide HWG of *L. edodes* inhibited the initial adhesion of *S. mutans* to a saliva-coated HA surface. Laminarin reduced the

---

**Fig. 2.** Inhibition of Bacterial Adhesion to Saliva-Coated Hydroxyapatite Beads by Saccharides.

The number of cells adhering to the beads in the presence of saccharide is expressed relative to the number in the absence of saccharide (gray box). *p < 0.05; **p < 0.01, t-test. Error bars indicate a standard deviation of n = 3.

**Fig. 3.** Inhibitory Effects of Polysaccharides on Sucrose-Dependent Biofilm Formation by *S. mutans* on Saliva-Coated Hydroxyapatite.

*S. mutans* was incubated in a THB medium containing 1% sucrose (w/v) and the indicated saccharide concentrations at 37°C for 20 h. Saliva-coated hydroxyapatite was on the bottom of the wells. The amount of biofilm formed in the presence of polysaccharide is expressed relative to that in the absence of polysaccharide. *p < 0.05, t-test. Error bars indicate a standard deviation of n = 3.
water-insoluble glucan synthesis by GtfB. Dextran inhibited the sucrose-dependent biofilm formation by S. mutans. Although polysaccharides are not sweet and are not used as sucrose substitutes, they might serve as a novel type of anticariogenic natural food additive.

Acknowledgment

This study was supported, in part, by grant-aid for scientific research (nos. 23593118 and 23780169) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References