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Binding Force Dynamics of *Streptococcus mutans*–glucosyltransferase B to *Candida albicans*

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Appendix

Immobilization of Microbial Cells and Functionalization of Atomic Force Microscopy with Glucosyltransferase B and C

The immobilized cells on the glass slide were stained with the BacLight Live/Dead viability kit (Molecular Probes Inc., Eugene, OR, USA) to determine viability (Xiao et al. 2012). SYTO9 (2.5 μM) and propidium iodide (15 μM) were added to the cells immobilized on the glass slide and incubated for 30 min in the dark. Images were acquired in confocal microscopy (Leica DMI4000 with Yokogawa CSU-X1 Spinning Disk Confocal Attachment with excitation at 488 nm and emission detected using a dual-band emission filter [500 to 550 nm/604 to 644 nm]).

To functionalize the atomic force microscopy (AFM) tips with glucosyltransferase B (GtfB) or C (GtfC), AFM tips were cleaned by immersing them in nitric acid for 5 min, followed by washing them in demineralized water 3 times. Then, AFM tips were exposed to saturating amounts of GtfB or GtfC solution (30 to 60 μg/mL) for 1 h at room temperature. The GtfB tips were dipped in 1% bovine serum albumin solution (dissolved in phosphate-buffered saline) to block other active sites on the tips and washed. GtfB functionalization was verified via anti-GtfB monoclonal antibody (mouse IgG; the anti-GtfB mAB was kindly provided by Dr. William H. Bowen, Center for Oral Biology, University of Rochester Medical Center). Briefly, the GtfB tips were incubated with anti-GtfB mAb for 1 h. Then, the tips were incubated another 30 min with Alexa 488–labeled (absorbance/fluorescence emission maxima, 488/519 nm) goat anti-mouse IgG secondary antibody (Life Technologies, Inc.).

Enzymatic activity of GtfB or GtfC tips was verified by incubation with sucrose substrate (final concentration of 100 mM) in adsorption buffer (pH 6.5) containing Alexa Fluor 647–labeled dextran conjugate (10,000 molecular weight; absorbance/fluorescence emission maxima, 647/668 nm; Molecular Probes Inc., Eugene, OR, USA) for 1 h at room temperature as described by Xiao et al. (2012). Fluorescence imaging of AFM tips was done by total internal reflection fluorescence AFM.

Glucosyltransferase Binding to the Candida albicans *and* Streptococcus mutans *Surfaces*

The GtfB was adsorbed to the microbial cells, as detailed elsewhere (Schilling and Bowen 1992; Vacca-Smith and Bowen 1998). Microbial cells (1×10^9 cells/mL) were mixed with saturating amounts of GtfB in adsorption buffer and incubated for 60 min at 37 °C; cells in buffer alone (without GtfB) were also incubated as controls. After incubation, the cells were pelleted by centrifugation (6,000 × g, 10 min, 4 °C), and the unbound GtfB (supernatant) was saved. The pellet was resuspended; loosely bound GtfB was removed by washing with adsorption buffer twice; and each supernatant was saved. The washed cells with and without adsorbed GtfB were resuspended in 250 μL of adsorption buffer and mixed with 250 μL of [^{14}C]glucose-sucrose substrate (0.2 μCi/mL; 100.0 mmol of sucrose/L, 20 μmol of dextran 9000/L, and 0.02% sodium azide in adsorption buffer, pH 6.5) for 2 h at 37 °C. The amount of glucans formed on the surface was determined using scintillation counting (Schilling and Bowen 1992).

Mutanase and Dextranase Susceptibility

The glucans formed on *C. albicans* and *S. mutans* cells by surface-adsorbed GtfB were examined for susceptibility to glucanohydrolases as described by Kopec et al. (1997). In this assay, mutanase, α(1,3)-glucanase (EC 3.2.1.59), produced by *Trichoderma harzianum* and dextranase, α(1,6)-glucanase (EC 3.2.1.11), produced by *Penicillium aculeatum* were used to determine the proportion of α-1,3- and α-1,6-linked glucose in the glucan structure formed on the bacterial and fungal surface. These enzymes (Novo Nordisk, Denmark) were kindly

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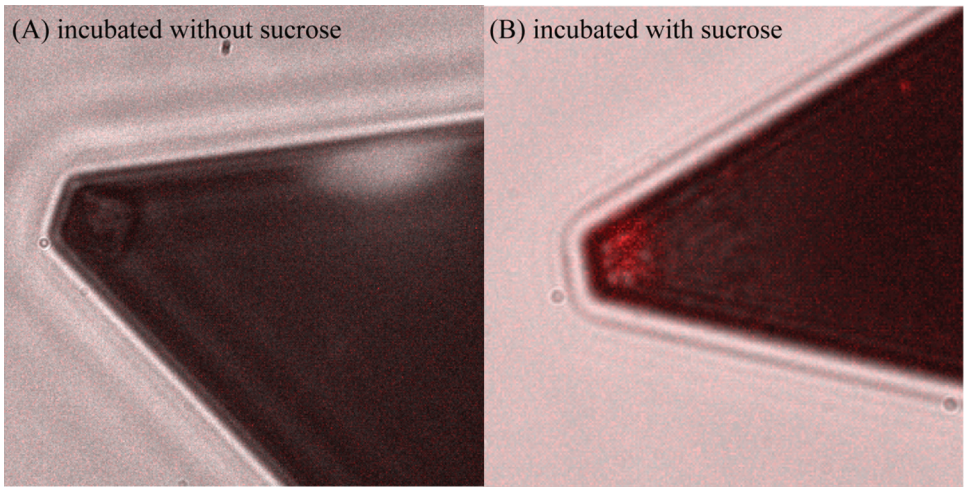
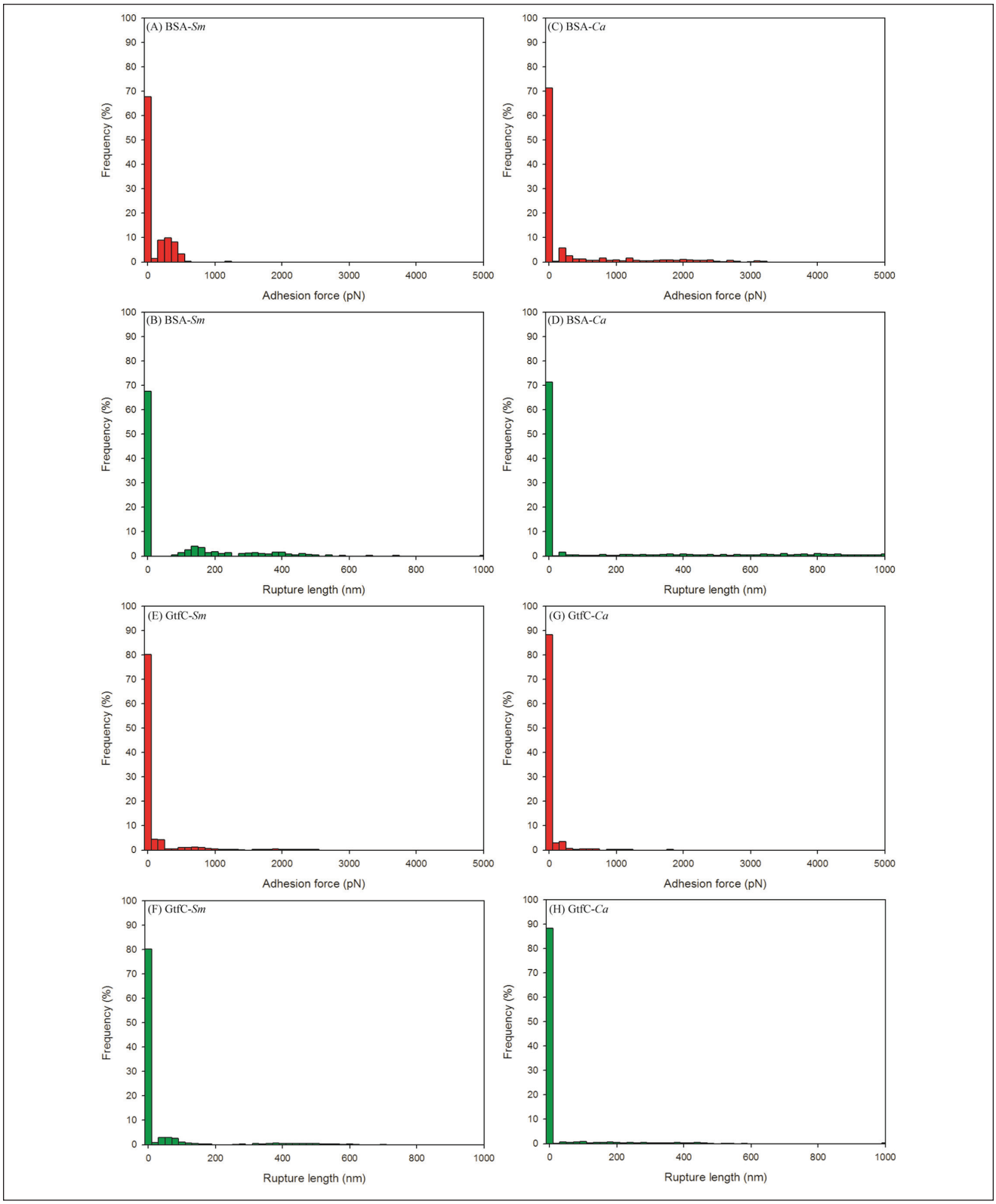
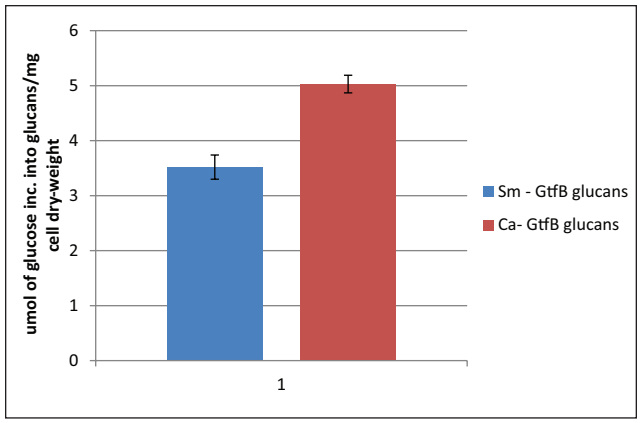


Appendix Figure 1. Fluorescent images of glucosyltransferase B (GtfB)–functionalized atomic force microscopy (AFM) tip. (**A**) No glucans were formed on the GtfB-functionalized AFM tip incubated without sucrose (no sucrose, control). (**B**) Fluorescent image of glucans formed on the GtfB-functionalized AFM tip incubated with sucrose. In situ glucan production was determined by incubating GtfB-functionalized AFM tips with (or without) sucrose substrate containing Alexa Fluor 647–labeled dextran conjugate to verify the enzymatic activity of GtfB on the AFM tips.

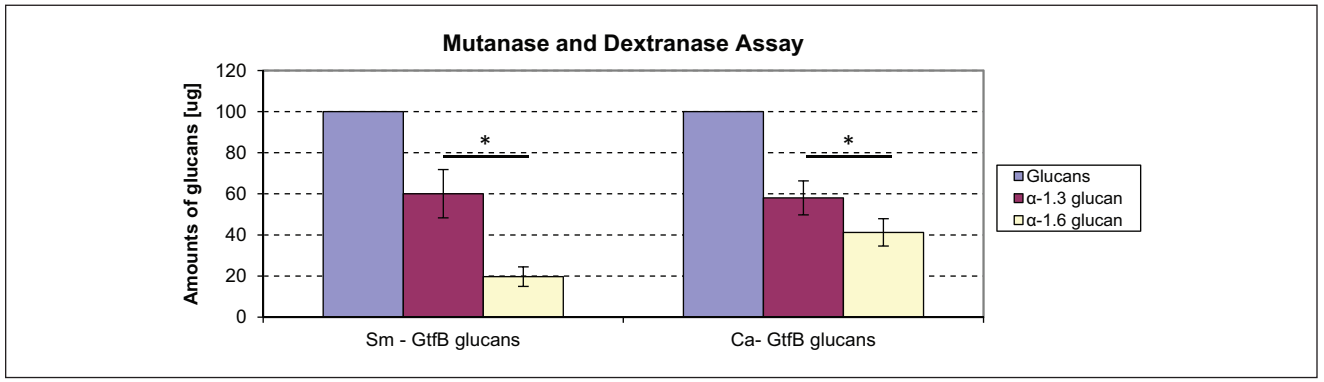
provided by William H. Bowen (Center for Oral Biology, University of Rochester Medical Center). The surface-formed glucans were precipitated in ice-cold 75% (vol/vol) ethanol, collected via centrifugation, followed by 4 times washing with 75% (vol/vol) ethanol; then, the amounts were measured by scintillation counting (Schilling and Bowen 1992). One hundred micrograms of glucans was then resuspended in 0.1M sodium acetate buffer (pH 5.5) and homogenized via probe sonication (three 10-s pulses at an output of 7 W). The suspension was mixed with 10 U of mutanase or dextranase in 0.1M sodium acetate buffer (pH 5.5; predetermined as optimum pH for enzymatic activity) in a total reaction volume of 300 μL and incubated for 4 h at 30 °C as described elsewhere (Kopec et al. 1997; Hayacibara et al. 2004). The amount of reducing sugar released during digestion of each surface-formed glucan by mutanase or dextranase was determined colorimetrically (Somogyi 1945; Kopec et al. 1997; Hayacibara et al. 2004).



Appendix Figure 2. Binding force and rupture length of bovine serum albumin (BSA)– or glucosyltransferase C (GtfC)–coated atomic force microscopy tip on microbial cell surfaces. (**A**) Adhesion force and (**B**) rupture length histograms of BSA on *Streptococcus mutans* surfaces. (**C**) Adhesion force and (**D**) rupture length histograms of BSA on *Candida albicans* surfaces. (**E**) Adhesion force and (**F**) rupture length histograms of GtfC on *S. mutans* surfaces. (**G**) Adhesion force and (**H**) rupture length histograms of GtfC on *C. albicans* surfaces. The maximum adhesion force and the rupture length from last peak were used to generate the histograms.



Appendix Figure 3. Glucosyltransferase B (GtfB) glucan production by GtfB bound to *Streptococcus mutans* and *Candida albicans*. Amount of glucose incorporated into glucans (μmol) was normalized by cell dry weight. The values of *S. mutans*–GtfB glucans and *C. albicans*–GtfB glucans are significantly different at a level of $P < 0.05$.



Appendix Figure 4. Hydrolysis of glucosyltransferase B (GtfB) glucans by glucanohydrolases, mutanase (α-1,3 glucanase), and dextranase (α-1,6 glucanase). The data indicate the proportion of α-1,3- and α-1,6-linked glucans formed on the *Streptococcus mutans* or *Candida albicans* surface. The asterisks indicate that the values are significantly different at a level of $P < 0.05$. Furthermore, the amount of α-1,6-linked glucans formed by *S. mutans*–GtfB is significantly different from that of *C. albicans*–GtfB at $P < 0.05$. Approximately 20% of the glucans are composed of other glycosyl linkages that could not be determined using the biochemical methods employed in this study.

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