

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

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Abstract

Candida albicans cells are often detected with *Streptococcus mutans* in plaque biofilms from children affected with early childhood caries. The coadhesion between these 2 organisms appears to be largely mediated by the *S. mutans*–derived exoenzyme glucosyltransferase B (GtfB); GtfB readily binds to *C. albicans* cells in an active form, producing glucans locally that provide enhanced binding sites for *S. mutans*. However, knowledge is limited about the mechanisms by which the bacterial exoenzyme binds to and functions on the fungal surface to promote this unique cross-kingdom interaction. In this study, we use atomic force microscopy to understand the strength and binding dynamics modulating GtfB–*C. albicans* adhesive interactions in situ. Single-molecule force spectroscopy with GtfB-functionalized atomic force microscopy tips demonstrated that the enzyme binds with remarkable strength to the *C. albicans* cell surface (~2 nN) and showed a low dissociation rate, suggesting a highly stable bond. Strikingly, the binding strength of GtfB to the *C. albicans* surface was ~2.5-fold higher and the binding stability, ~20 times higher, as compared with the enzyme adhesion to *S. mutans*. Furthermore, adhesion force maps showed an intriguing pattern of GtfB binding. GtfB adhered heterogeneously on the surface of *C. albicans*, showing a higher frequency of adhesion failure but large sections of remarkably strong binding forces, suggesting the presence of GtfB binding domains unevenly distributed on the fungal surface. In contrast, GtfB bound uniformly across the *S. mutans* cell surface with less adhesion failure and a narrower range of binding forces (vs. the *C. albicans* surface). The data provide the first insights into the mechanisms underlying the adhesive and mechanical properties governing GtfB interactions with *C. albicans*. The strong and highly stable GtfB binding to *C. albicans* could explain, at least in part, why this bacterially derived exoenzyme effectively modulates this virulent cross-kingdom interaction.

Keywords: nanotechnology, bioengineering, caries, single-molecule force spectroscopy, dissociation rate, glucans

Introduction

Streptococcus mutans is often regarded as one of the main bacterial pathogens in dental caries, particularly in early childhood caries (ECC). ECC is characterized by heavy *S. mutans* infection (often >30% of the cultivable plaque flora), accompanied by protracted feeding of dietary sugars, such as sucrose (Gross et al. 2010; Parisotto et al. 2010; Hughes et al. 2012). This bacterial pathogen can rapidly orchestrate the assembly of cariogenic biofilms when frequently exposed to sucrose. Sucrose is utilized by *S. mutans*–derived exoenzymes (e.g., glucosyltransferases) to produce extracellular glucans, which enhance local accumulation of microbes and facilitate the buildup of cariogenic biofilms on the tooth surface (Paes Leme et al. 2006; Bowen and Koo 2011). However, *S. mutans* may not act alone in ECC, as other organisms contribute to caries pathogenesis (Takahashi and Nyvad 2011).

Results from previous clinical studies reveal that high numbers of the fungus *Candida albicans* are frequently detected with heavy infection by *S. mutans* in plaque biofilms from children with ECC (de Carvalho et al. 2006; Raja et al. 2010; Yang et al. 2012). These findings are intriguing because *C. albicans* usually does not bind well with *S. mutans* (Jenkinson et al.

1990; Gregoire et al. 2011). Rather, *C. albicans* adheres to oral mucosal and prosthetic surfaces and interacts with commensal streptococci to cause mucosal infections (Thein et al. 2009; Xu, Jenkinson, et al. 2014). *C. albicans* is well known to coadhere with *Streptococcus gordonii* and *Streptococcus oralis* (Jenkinson et al. 1990; Jenkinson and Douglas 2002; Diaz et al. 2012), enhancing fungal carriage and infectivity in vivo (Xu, Sobue, et al. 2014). Yet *Candida* was initially regarded as having little to no physical coadhesion with the pathogen *S. mutans* in the absence of sucrose. However, evidence from prior in

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vitro studies revealed that the adhesive interactions between *C. albicans* and *S. mutans* are greatly enhanced in the presence of sucrose (Branting et al. 1989; Gregoire et al. 2011) and that these conditions also promote biofilm formation (Pereira-Cenci et al. 2008; Metwalli et al. 2013; Falsetta et al. 2014).

Additional in vitro studies demonstrated that *S. mutans*–derived glucosyltransferase B (GtfB) binds avidly to *C. albicans* cell surfaces in an enzymatically active form (Gregoire et al. 2011). When sucrose is available, the surface-bound GtfB produces large amounts of glucans on the fungal surface. The glucans produced in situ provide enhanced binding sites for *S. mutans*, greatly promoting their adhesive interactions (Branting et al., 1989; Gregoire et al. 2011) and the development of highly virulent cospecies biofilms in vivo (Falsetta et al. 2014). Indeed, lack of *gtfB* expression by *S. mutans* impaired the ability of the bacterium to interact with *C. albicans* and form cospecies biofilms in the presence of sucrose (Falsetta et al. 2014). Clearly, GtfB plays a critical role mediating *S. mutans*–*C. albicans* coadherence. However, how the bacterial exoenzyme (GtfB) binds to and functions on the fungal surface to orchestrate this unique adhesive interaction between a bacterial oral pathogen and an opportunistic fungus remains to be elucidated.

Here, we explore the mechanical properties mediating GtfB binding to *C. albicans* and *S. mutans* surfaces by measuring the magnitude of adhesion force and stability using a single-molecule force spectroscopy (SMFS). The adhesion force (which indicates binding strength), dissociation rate (i.e., binding stability), and force map distribution (i.e., force localization) were measured between GtfB and microbial cell surfaces with atomic force microscopy (AFM) tips functionalized with GtfB. Our data reveal remarkable binding strength and binding stability of GtfB on *C. albicans* surfaces, both of which were several-fold higher than those observed for the same enzyme on *S. mutans* surfaces. Furthermore, we observed that GtfB, when bound to *C. albicans*, is highly active and produces more glucans (with distinct structure) than does GtfB bound to *S. mutans* surfaces. The results presented in this study provide the first insight into the biophysical underpinnings governing the adhesive interactions between a bacterially derived exoenzyme (GtfB) and the *C. albicans* cell surface, which could explain, at least in part, how GtfB effectively modulates this intriguing and virulent cross-kingdom association.

Materials and Methods

Microbial Strains and Culture Conditions

Candida albicans SC5314, a well-characterized strain whose genome has been sequenced, and *Streptococcus mutans* UA159, a proven virulent cariogenic pathogen, were used for single-molecule force measurements. *C. albicans* and *S. mutans* cultures were stored at -80°C in tryptic soy broth and Sabouraud dextrose broth containing 20% glycerol. *C. albicans* and *S. mutans* cells were grown to midexponential phase (optical densities at 600 nm of 0.65 and 0.5, respectively) in ultrafiltered (10-kDa molecular-mass cutoff; Millipore, Billerica, MA, USA) yeast–tryptone extract broth

(UFTYE; pH 5.5 and 7.0 for *C. albicans* and *S. mutans*, respectively) containing 1% (wt/vol) glucose and harvested by centrifugation ($6,000 \times g$, 10 min, 4°C) as described previously (Gregoire et al. 2011). The cells were then washed 3 times in phosphate-buffered saline (pH 7.0 to 7.2; HyClone Laboratories Inc., Logan, UT, USA) before cell immobilization.

Glucosyltransferase Enzymes

The GtfB (or GtfC) enzyme was prepared and purified to near homogeneity via hydroxylapatite column chromatography as detailed elsewhere (Koo et al. 2002). Glucosyltransferase activity was measured by the incorporation of [^{14}C]-glucose from labeled sucrose (NEN Research Products, Boston, MA, USA) into glucans (Schilling and Bowen 1992; Koo et al. 2002). One unit of enzyme activity was defined as the amount of glucosyltransferase enzyme that incorporates 1 μmol of glucose into glucans over the 2-h reaction.

Immobilization of Microbial Cells and Functionalization of AFM Tips with GtfB

Prior to AFM analysis, microbial cells were immobilized on a poly-L-lysine-coated glass slide (Schaer-Zammaretti and Ubbink 2003). Briefly, a glass slide was cleaned by piranha solution (a mixture of sulfuric acid and hydrogen peroxide), followed by deposition of poly-L-lysine solution (0.1% w/v in H_2O ; Sigma-Aldrich, St. Louis, MO, USA) by overnight incubation. Washed cells were immobilized on a positively charged poly-L-lysine-coated glass slide for 1 h at room temperature. Then, the glass slide was gently washed with demineralized water to remove loosely adhered cells, and the slide was kept hydrated prior to AFM analysis. The immobilized cells were verified for viability using BacLight Live/Dead, as detailed in the Appendix.

To functionalize the AFM tips with GtfB, the cantilever 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 for 1 h at room temperature. The glucosyltransferase tips were dipped in 1% bovine serum albumin solution to block other active sites on the tips. GtfB functionalization was verified using our monoclonal antibody to GtfB and fluorescence imaging via Alexa Fluor 488–labeled goat anti-mouse IgG secondary antibody (488/519 nm; Life Technologies, Inc.). The enzymatic activity of the GtfB immobilized onto AFM tips was also verified by incubating GtfB tips with sucrose substrate in adsorption buffer containing Alexa Fluor 647–labeled dextran conjugate (647/668 nm; Molecular Probes Inc.) for 1 h at room temperature as described previously (Gregoire et al. 2011). Details of GtfB (and GtfC) functionalization of AFM tips and verification can be found in the Appendix.

AFM Methodology

All force measurements were conducted in fluid-phase at room temperature under phosphate-buffered saline (HyClone

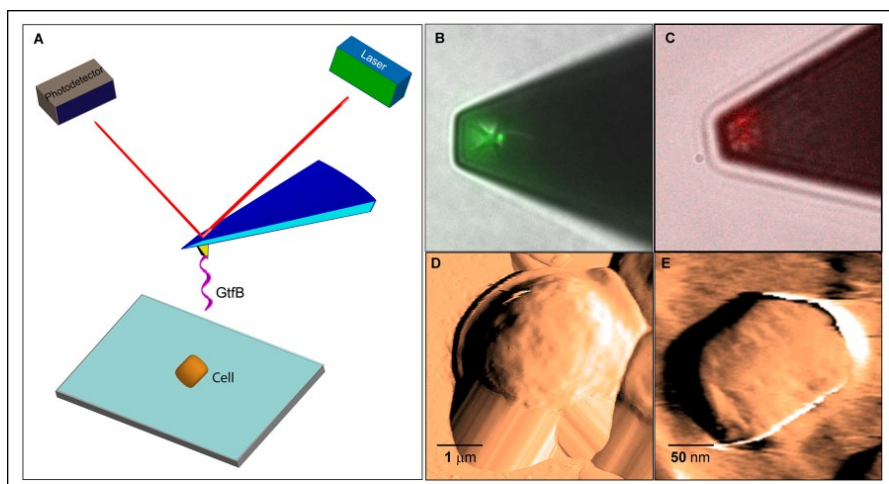


Figure 1. Single-molecule atomic force microscopy (AFM) analysis of the adhesive interactions between glucosyltransferase B (GtfB) and microbial cell surfaces (*Candida albicans* or *Streptococcus mutans*). **(A)** AFM tips were functionalized with GtfB, and cells were immobilized on the glass surface. **(B)** Fluorescent image of GtfB-functionalized AFM tip. Alexa 488-labeled monoclonal antibody (goat anti-mouse IgG [H+L]-HRP) was bound to GtfB on the AFM tips to verify the local functionalization. **(C)** Fluorescent image of glucans formed on the GtfB-functionalized AFM tip. In situ glucan production was determined by incubating GtfB-functionalized AFM tips with sucrose substrate containing Alexa Fluor 647-labeled dextran conjugate to verify the enzymatic activity of GtfB on the AFM tips. **(D)** AFM scanning image of immobilized *C. albicans* on poly-L-lysine-coated glass slide. **(E)** AFM scanning image of immobilized *S. mutans* on poly-L-lysine-coated glass slide.

Laboratories Inc.) using an MFP-3D AFM (Asylum Research, Santa Barbara, CA, USA). Silicon nitride probes (TR400PSA, Olympus, Tokyo, Japan) with a resonance frequency of ~11 kHz and spring constant of ~0.02 N/m and a nominal tip radius of 20 nm were calibrated via the thermal tune method (Hutter and Bechhoefer 1993) and examined optically before every experiment was conducted. To locate microbial cells, cells immobilized on glass surfaces were imaged in contact mode at randomly selected locations at 1 Hz. When isolated *C. albicans* or *S. mutans* was located in the image, a region of interest was defined over the cell surface, and an adhesion map was obtained by recording 50×50 force-distance curves within the region ($5 \times 5 \mu\text{m}$ and $0.5 \times 0.5 \mu\text{m}$ for *C. albicans* and *S. mutans*, respectively). Cantilever deflection data upon retraction from cell surfaces were acquired and converted to force data. Force measurements obtained from loosely immobilized microbes (detached/moved during AFM probing) were discarded. The force-distance curves were obtained from at least 20 individual microbial cells from at least 3 distinct culture preparations per strain.

Statistical Analyses

The data were analyzed by pairwise comparisons of multiple groups with regression models based on the ranked values. Kruskal-Wallis tests, which are nonparametric and based on ranks, were used for 2-group comparisons. The significance level was set at 5%.

Results

Functionalization of AFM Tips and Immobilization of Microbial Cells

We used AFM tips functionalized with GtfB to investigate the physical interactions between the enzyme and single cells of *C. albicans* or *S. mutans*. Stable functionalization of AFM tips and preparation of immobilized microbial cells are key requirements for reliable detection of intermolecular forces (Hinterdorfer and Dufrene 2006). To determine whether AFM tips were successfully functionalized with GtfB, we used 2 complementary methods. We initially verified whether GtfB molecules were immobilized onto AFM tips using fluorescently labeled anti-GtfB monoclonal antibody (Alexa 488). Fluorescent imaging showed that GtfB was attached and located on the tip of the AFM cantilever probe (in green; Fig. 1B). Then, we incubated GtfB-functionalized AFM tips with sucrose containing Alexa 647-labeled dextran conjugate to assess enzymatic activity of the immobilized GtfB. As shown in Figure 1C, glucans (in red) were produced on the enzyme-functionalized AFM tip, indicating GtfB bound to the probe without affecting its biological activity. Fluorescence was not detected in “no sucrose” control (Appendix Fig. 1), indicating that the fluorescence from the probe (incubated with sucrose) is due to synthesis of glucans in situ. Immobilization of microbial cells on the glass slide was also verified before and after force measurements to confirm that the cells remained anchored to the glass surfaces during force measurement (Fig. 1D, E). Furthermore, microbial cells were viable after immobilization, as determined via BacLight Live/Dead.

Single-molecule Force Measurements

GtfB–*C. albicans* interactions. Although GtfB has been shown to bind avidly to *C. albicans*, the biophysical properties mediating GtfB–*C. albicans* adhesive interactions are unknown. Thus, we used SMFS with GtfB-attached AFM tips to measure the magnitude of force, force distribution/localization, and dynamics of GtfB binding to the surface of single microbial cells. First, we determined the adhesion force and rupture length (binding strength measurements), and the data are shown in Figure 2A and B. We observed a large portion of highly adhesive interactions ranging from 1 to 2 nN and rupture lengths between 600 and 700 nm. Interestingly, the frequency of nonadhesive GtfB–

C. albicans interaction was rather high (>25%), suggesting heterogeneous binding of GtfB on the *C. albicans* surface. To further detail the location of GtfB–*Candida* interactions, we used the GtfB tips to map the distribution of GtfB binding strength on microbial cell surface. Adhesion force maps revealed that large portions of the *C. albicans* surface have remarkably high binding affinities to GtfB, while some areas were devoid of detectable binding events (Fig. 3A).

Force-distance curves can display distinct adhesion profiles that indicate the types of interactions taking place and provide clues to the underlying physics (Cappella and Dietler 1999). Representative retraction force-distance curves of GtfB–*C. albicans* revealed striking sawtooth-like patterns (red arrows) and force plateaus (blue arrows; Fig. 2C). Sawtooth patterns reflect the sequential unfolding of multiple tandem-repeat domains, while force plateaus correspond with the mechanical unzipping of β -sheet interactions (Dufrêne 2014). It implies that the bindings of GtfB–*C. albicans* are dissociated through initial unfolding of the bonds, followed by sequential unzipping, thereby suggesting a stable complex between GtfB and *C. albicans*. Furthermore, the observed multiple force plateaus suggest the sequential or simultaneous breakages of multiple bonds during the retraction of the GtfB tip.

GtfB–*S. mutans* interactions. Following binding measurements with *C. albicans*, we determined the adhesion strength and dynamics between GtfB and *S. mutans*. Interestingly, the adhesion strengths of GtfB–*S. mutans* interactions were substantially lower than those of GtfB–*C. albicans*. Binding forces ranged from 0.3 to 1.0 nN and rupture lengths within 200 nm (Fig. 4A, B). However, GtfB adhesive interactions on *S. mutans* cell surfaces were distributed more evenly over the cell surface (Fig. 3C), despite the lower magnitude of the binding force (vs. GtfB–*C. albicans* interactions). Indeed, the frequency of nonadhesive interactions between GtfB and *S. mutans* was <10%. Force map images show that the distribution of adhesion forces of GtfB–*S. mutans* is more homogeneous than GtfB–*C. albicans* interactions (Fig. 3A, C); furthermore, when adjusted to the same force range, the higher strength of the GtfB–*C. albicans* interactions is also evident (Fig. 3B, D). In addition, the shape of the force-distance curves for GtfB–*S. mutans* adhesions were mostly single peak with force plateaus (blue arrows), indicating that the bond is broken mainly via unzipping without unfolding.

To confirm the specificity of the observed force distribution peaks and rupture events between GtfB and microbial surfaces, 2 control force experiments using albumin- or GtfC-coated AFM tips were carried out. Both albumin and GtfC show very low binding strength with minimal adhesion events to either *S. mutans* or *C. albicans* (vs. GtfB-functionalized tips; Appendix Fig. 2), which agrees well with limited GtfC binding to microbial cell surfaces observed previously (as reviewed; Bowen and Koo 2011).

Dissociation Rates of GtfB Bindings to *C. albicans* and *S. mutans* Surfaces

In addition to adhesion strength, binding stability is an equally important factor contributing to protein interaction and function

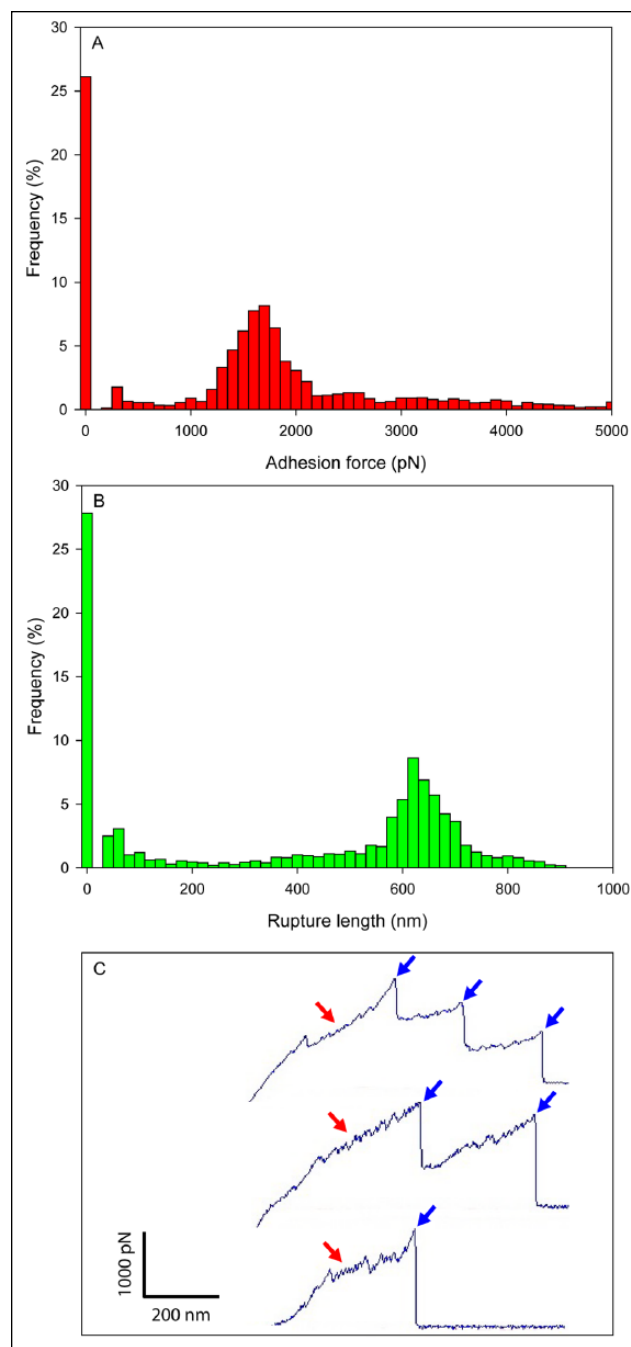


Figure 2. Binding force and rupture length of glucosyltransferase B on *Candida albicans* surfaces. (A) Adhesion force histogram, (B) rupture length histogram, and (C) representative retraction force curves. Blue arrows indicate force plateau, while red arrows indicate sawtooth pattern of force curves. The maximum adhesion force and the rupture length from last peak were used to generate the histograms. This figure is available in color online at <http://jdr.sagepub.com>.

on microbial surfaces (Dufrêne 2014). Binding stability can be determined from the kinetic off-rate by exploring the dependence of the binding strength with the force loading rate (Evans and Ritchie 1997; Lee et al. 2007). Thus, we determined the mean adhesion forces of GtfB–*C. albicans* (and GtfB–*S. mutans*) at various loading rates. Then, the characteristic

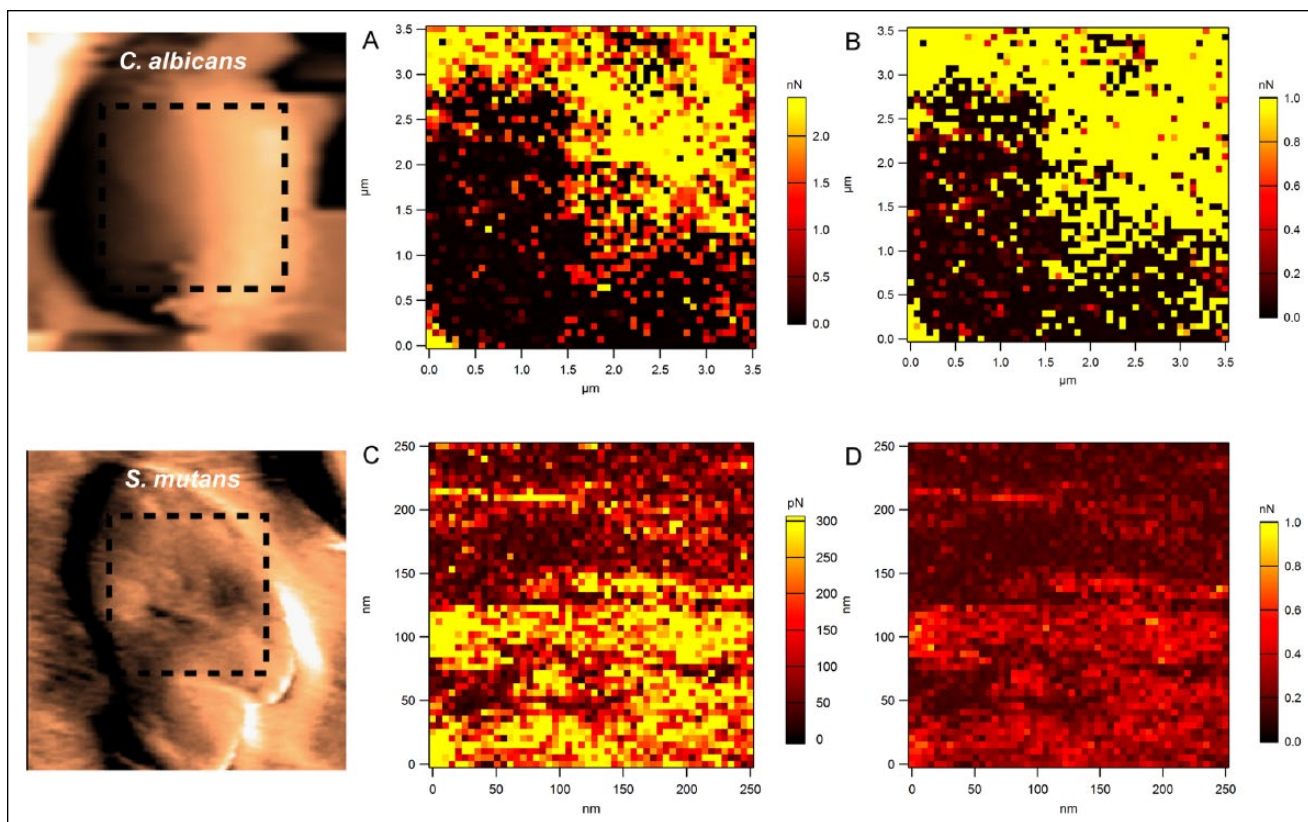


Figure 3. Adhesion force maps of *Candida albicans* and *Streptococcus mutans* surfaces. (A) Adhesion force map of *C. albicans*, (C) adhesion force map of *S. mutans*, and (B, D) adhesion force maps of *C. albicans* and *S. mutans* under same range.

dissociation rate—kinetic off rate at zero force, $k_{off}(0)$ —was estimated by equation 1:

$$F = \frac{k_B T}{x_\beta} \cdot \ln \left(\frac{r x_\beta}{k_{off} k_B T} \right), \quad (1)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and r is the loading rate. From the plot of F vs. $\ln r$ (Fig. 5), we can obtain the slope $k_B T/x_\beta$ from equation 1. Extrapolated to $F = 0$, equation 1 gives a value for $k_{off} = r x_\beta / k_B T$.

As shown in Figure 5, the most probable forces for bond breakage (F) of GtfB to both cell surfaces increased approximately linearly with the logarithm of the loading rate (r), in line with previous observations from receptor-ligand systems (Evans and Ritchie 1997; Evans and Ludwig 2000; Lee et al. 2007). However, loading rate at zero force estimated from the plot of GtfB–*C. albicans* (0.1 pNs⁻¹) was ~11 times lower than that of GtfB–*S. mutans* (1.08 pNs⁻¹), resulting in substantially (~20 times) lower dissociation rate of GtfB–*C. albicans* (4·10⁻⁴s⁻¹; vs. GtfB–*S. mutans*, 7·10⁻³s⁻¹). These findings suggest that the GtfB–*C. albicans* complex dissociates much slower than GtfB–*S. mutans*, implying that the binding stability of GtfB–*C. albicans* is substantially higher than that of GtfB–*S. mutans*.

The distinct pattern of GtfB binding to *C. albicans* and *S. mutans* surfaces also affected the enzymatic function on the microbial surfaces. GtfB adsorbed on *C. albicans* surfaces produced more glucans containing a higher proportion of α -1,6 glucosyl linkages than those formed on *S. mutans* surfaces (Appendix Figs. 3, 4), confirming previous findings (Gregoire et al. 2011). It is possible that the differences in the binding properties of GtfB observed on each microbial cell surface cause distinctive conformational changes of GtfB structure, which could affect the catalytic functions of the surface-adsorbed enzyme. Collectively, our data clearly show that GtfB has a remarkably strong and stable binding to *C. albicans* surfaces, while showing very distinctive biophysical properties compared with GtfB–*S. mutans* adhesive interactions.

Discussion

AFM is a powerful tool for measuring the binding properties between 2 interacting surfaces (Busscher et al. 2008; van der Mei et al. 2008; Wessel et al. 2014). Importantly, the AFM cantilever can also be functionalized with biomolecules (e.g., proteins) to determine adhesive interactions between specific proteins and host or bacterial surfaces at the single molecule level (Dupres et al. 2007; Hinterdorfer and Dufrêne 2006;

Sullan et al. 2015). However, SMFS has not yet been extensively used to study the binding properties/mechanics between extracellular proteins and oral microbial surfaces.

We previously identified a fascinating interaction between *S. mutans* and *C. albicans* that is mediated primarily by a bacterially derived exoenzyme (GtfB) that binds to the fungal surface (Gregoire et al. 2011; Falsetta et al. 2014). However, direct measurements of forces and dynamics of GtfB binding to the fungal cell surfaces have not been reported. Thus, we have optimized and applied SMFS-AFM to understand the biophysical properties mediating the observed GtfB–*C. albicans* interaction.

Our data reveal that bonds between GtfB and *C. albicans* have characteristic forces of separation on the order of nanonewtons and low dissociation rates, indicating that the binding is strong and stable, roughly equivalent to the strength of a covalent bond (Grandbois et al. 1999). Accordingly, the GtfB–*C. albicans* complex is strongly stabilized possibly via a “dock, lock, and latch” mechanism (Ponnuraj et al. 2003) between GtfB and cell wall components of *C. albicans*. Surprisingly, GtfB binding force and stability are greater on *C. albicans* surfaces than on *S. mutans*. Interestingly, the spatial distribution of GtfB adhesion strength on the *Candida* surface reveals a highly heterogeneous pattern, indicating that the exoenzyme may bind with more affinity to specific areas on the *C. albicans* cell wall surface. Indeed, fluorescence imaging of glucans produced in situ by GtfB bound to the *C. albicans* surface showed localized areas of glucan accumulation rather than homogeneous coating by the exopolysaccharides (Gregoire et al. 2011). This agrees well with the GtfB binding pattern observed here.

Importantly, the *C. albicans*–adsorbed GtfB not only retains its enzymatic activity but also affects glucan structure that the enzyme synthesizes on the fungal surface (Gregoire et al. 2011). The tight and spatially heterogeneous binding of GtfB and changes in the glucan products (elevated α -1,6-linked glucose) suggest highly specific interactions with cell wall components of *C. albicans*. The exact identities of the GtfB binding sites on the fungal surface are unknown. It was previously found that GtfB binds in an active form to purified mannans and β -1,3 glucans (Falsetta et al. 2014), which are found in the *C. albicans* cell wall (Gow et al. 2012). Among them, mannans could be one of the potential surface ligands because these polysaccharides are particularly abundant at the outermost layer (Gow et al. 2012). However, the composition and molecular architecture of the cell wall of *C. albicans* are quite complex (Klotz et al. 2007; Li and Palecek 2008), and other surface-associated glycoproteins and adhesins (e.g., Als and Eap1) potentially interact with GtfB. Clearly, further studies are needed to identify the binding partners as well as their spatial localization on the fungal surface that mediates GtfB–*C. albicans* adhesion.

The data presented here provide new details about the biophysical properties of GtfB–microbe interactions and the first insights about the mechanisms by which GtfB binds to *C. albicans* surfaces. The exact reasons why *S. mutans* releases GtfB to bind to their own and other microbial surfaces remain to be

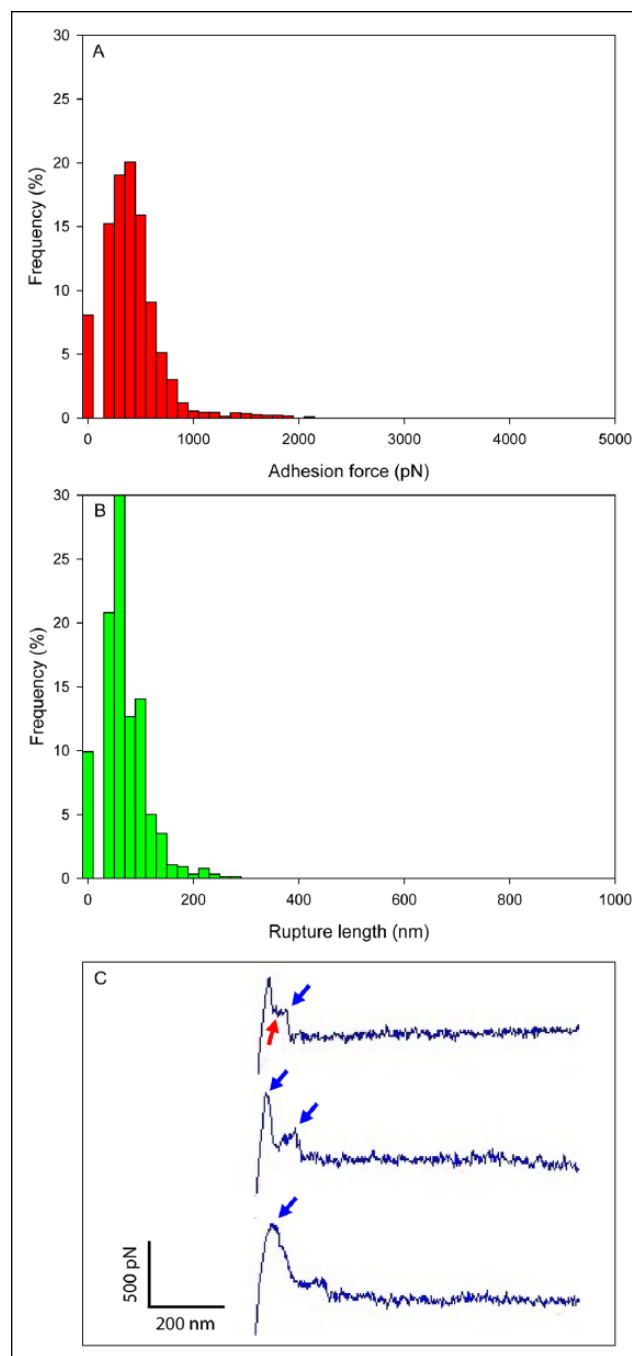


Figure 4. Binding force and rupture length of glucosyltransferase B (GtfB) on *Streptococcus mutans* surfaces. (A) Adhesion force histogram, (B) rupture length histogram, and (C) representative retraction force curves. Blue arrows indicate force plateau, while red arrows indicate sawtooth pattern of force curves. The maximum adhesion force and the rupture length from last peak were used to generate the histograms. This figure is available in color online at <http://jdr.sagepub.com>.

elucidated. One possibility is that *S. mutans* may export GtfB to facilitate biofilm formation as the exoenzyme binds to other microbes and to the saliva-coated apatitic surface in active form (Bowen and Koo 2011). When sucrose is available, the

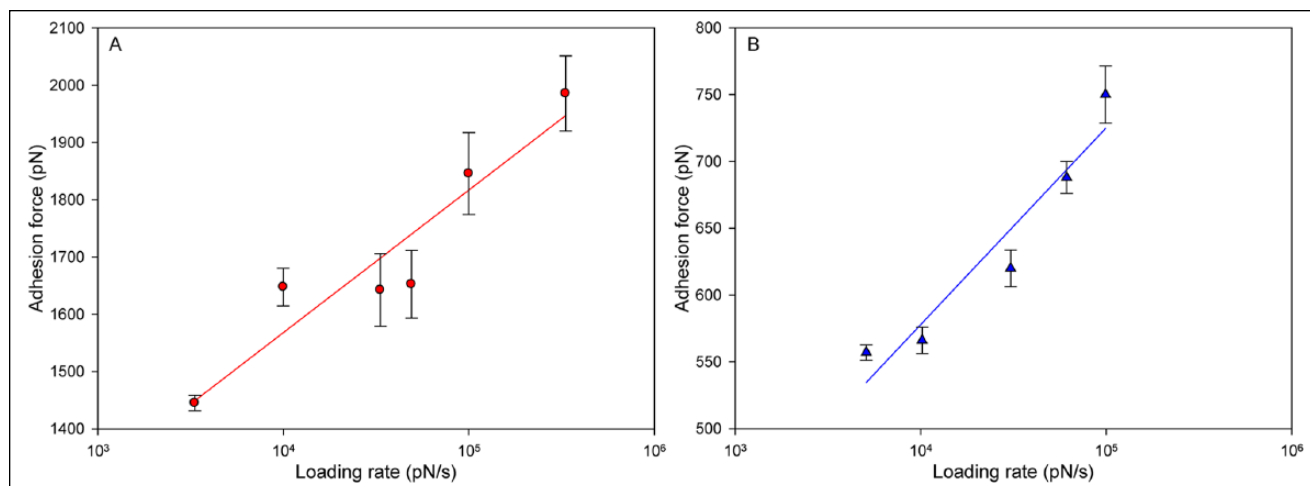


Figure 5. Dynamic adhesion force spectra depending on the loading rate. **(A)** *Candida albicans* and **(B)** *Streptococcus mutans*. The mean adhesion forces vs. the logarithm of the loading rate were straight lines for both *C. albicans* and *S. mutans*. The kinetic off rate constant of dissociation at zero force of *C. albicans* was significantly lower (~20-fold) than the one of *S. mutans*, indicating that bindings of glucosyltransferase B on *C. albicans* are substantially more stable than they are on *S. mutans* surfaces.

surface-bound GtfB produces glucans locally that mediate bacterial binding to teeth and coadherence with *C. albicans* and other bacteria, promoting the initiation of cariogenic biofilm (the preferred environment of *S. mutans*, where it can thrive by creating highly acidic niches).

The fascinating observation of stronger and highly stable GtfB binding to *C. albicans* than to the surface of its own bacterial producer provides a mechanistic rationale for why GtfB binds so effectively and functions onto the *C. albicans* surface (Gregoire et al. 2011), which plays a key role in modulating the exopolysaccharide synthesis in situ and the development of virulent cospecies biofilms (Falsetta et al. 2014). We are currently identifying the molecular ligands of GtfB on the *C. albicans* cell wall using our developed SMFS-AFM combined with genetic approaches (e.g., mutant strains defective of cell wall components). At the same time, further understanding is worthy of detailed exploration regarding how the conformation of the GtfB structure changes upon binding to the fungal surface and what effects it has on the catalytic activity of the enzyme.

Author Contributions

G. Hwang, H. Koo, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; G. Marsh, contributed to data acquisition and analysis, critically revised the manuscript; L. Gao, contributed to data acquisition, drafted the manuscript; R. Waugh, contributed to data analysis and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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