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# Regulation of fructosyltransferase activity by carbohydrates, in solution and immobilized on hydroxyapatite surfaces

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**Abstract**

We tested the effect of several carbohydrates on the activity of cell-free fructosyltransferases (FTF) in solution and immobilized onto hydroxyapatite (HA) and found an inhibitory dose-dependent effect of glucose on FTF activity, both on the surface and in solution. Glucose at 160 mM inhibits FTF activity by 75% both on HA and in solution. Fructose at 160 mM inhibited FTF activity by 25% in solution and by 15% on HA. Levan inhibited FTF activity by 30% in solution, while dextrans and inulin had a limited effect on FTF activity. Circular dichroism and infrared analysis demonstrated no major changes in the chemical structure of fructans synthesized by cell-free FTF on HA and in solution, in the presence or absence of glucose. However, as verified by size-exclusion chromatography, glucose inhibited the synthesis of high molecular-weight fructans. The results indicate that glucose, a byproduct of the FTF enzymatic reaction, is the main carbohydrate affecting FTF activity. Selective inhibition of high molecular-weight fructan production by glucose, may indicate that two mechanisms are involved in the synthesis of fructans, both in solution and on the surface. 

*Keywords:* Fructosyltransferase; Fructans; Biofilm; Hydroxyapatite; Dental plaque

## 1. Introduction

Dental plaque is a biofilm coating tooth surfaces[1] and is composed of bacteria, cells, proteins, enzymes, and their byproducts.[2,3] β-D-Fructosyltransferases (FTF, EC 2.4.1.9) are among the cell-free enzymes found in the oral cavity.[4] FTF produces fructans (polyfructoses) from sucrose, and these play a role in the pathogenicity and formation of the dental biofilm.[5] The origin of these extracellular enzymes are several species of oral bacteria including *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, and *Actinomyces viscosus*.[6–9]

Mutants of *S. mutans* lacking the *ftf* gene, have been shown to be less cariogenic than the native strains.[10,11] Fructans are associated with the progression and pathogenesis of dental caries by serving as carbohydrate reservoirs for bacteria in periods of shortage in the exogenous supply of carbohydrates.[12] The fructanases of several oral bacteria can degrade these fructans into readily metabolized carbohydrates, which serve as the source for acid production by cariogenic bacteria.[13] Recently, it has been suggested that fructans may also serve as binding sites for oral bacteria in the dental plaque biofilm.[14]

The properties of enzymes may change upon adsorption onto surfaces as a result of variation in the microenvironment conditions, changes in molecular conformation, and changes in the molecule charge of the adsorbed enzymes. Immobilization of enzymes induces structural perturbation, resulting in functional changes[15,16] and in the case of bacterial extracellular enzymes, it can alter their degree of virulence. For example, glucosyltransferase (GTF, EC 2.4.1.5) immobilized on hydroxyapatite (HA) synthesizes glucans, which are associated with the formation of the dental biofilm. Theses glucans are structurally distinct from those synthesized by GTF in solution.[17] Cell-free GTF in solution exhibited different kinetics, optimal temper-

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ature, optimal pH range, and different susceptibility to inhibitors as compared to GTF immobilized on HA.[18–24]

Limited information is available on properties of cell-free FTF, both in solution and immobilized on HA surfaces. Rolla et al.[4] and Vacca-Smith and Bowen[25] have shown that cell-free FTF from saliva exhibited enzymatic activity when immobilized on HA. Similarly, Steinberg et al.[26] have demonstrated FTF activity on HA from FTF isolated from oral streptococci. Lately, Rozen et al.[14] have shown that FTF plays an important role in biofilm formation in vitro, while Bromshteym and Steinberg[27] have shown that FTF forms complexes upon immobilization on HA which may result in changes in immobilized FTF physicochemical properties as compared to FTF in solution.

Different models have been used in the literature for exploring dental biofilms.[28] Experimental procedures testing properties of the dental biofilm have often used HA as the hard surface, due to the resemblance of the HA to enamel surfaces.[29]

The purpose of this study was to distinguish the effect of immobilization of FTF on several of its properties as a step in further understanding the role of FTF in the formation of the dental biofilm.

## 2. Results

*The effect of carbohydrates on FTF activity.*—D-Glucose had a marked inhibitory effect on FTF activity immobilized on HA (75% inhibition at 160 mM) (Fig. 1), as recorded both on fructans synthesized on HA and on fructans isolated from the supernatant fluid. Glucose had also a similar significant inhibitory effect on FTF activity in solution. However, the effect of fructose on FTF adsorbed onto HA was minor (15% inhibition at 160 mM) (Fig. 2), while the inhibitory effect of fructose on FTF in solution was $\sim 25\%$.

A 30% inhibition of FTF activity in solution was recorded in the presence of levan (at a concentration of 0.12%), while inulin caused a slight increase (10%) in FTF activity. Both levan and inulin had a minor, insignificant effect on FTF activity on HA. Dextrans, both low MW and high MW, had a minor, insignificant effect on FTF activity in solution and on HA at concentrations of 1.2% (data not shown).

*Circular dichroism–optical rotatory dispersion (CD-ORD) analysis.*—CD scans of fructans synthesized by FTF in solution, in the absence of glucose, displayed one strong band in the range 182–192 nm with a maximum peak at 188 nm (Table 1). This was similar to the maximal peak band of fructans synthesized by FTF on the surface. Small shifts in peak location were recorded, for fructans synthesized by FTF in solution, in supernatant fluid and on the HA surface in the presence of 40 and 160 mM glucose. A shift in wavelength was observed with fructans synthesized in the presence of 40 mM glucose in solution (195 nm), compared with supernatant fluid (190 nm) and on HA surfaces (187 nm). No shifts in peak location were observed in the presence of fructose (data not shown).

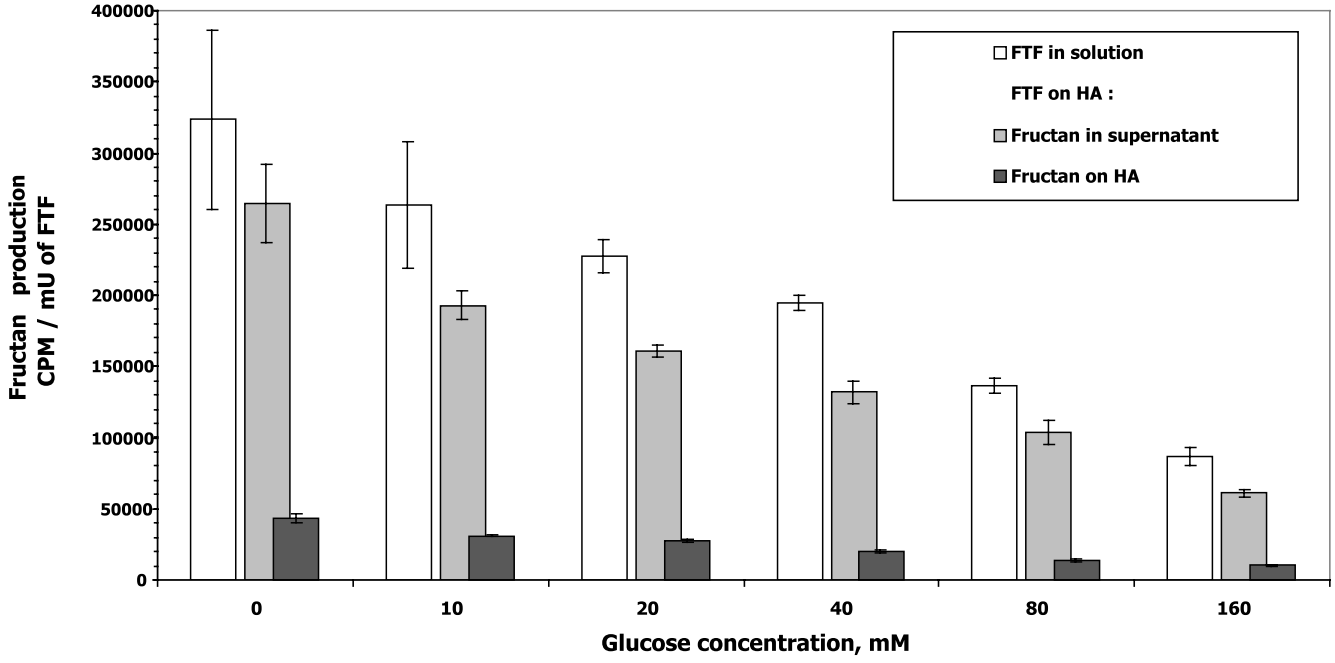


Fig. 1. The effect of glucose on FTF activity in solution and on hydroxyapatite.

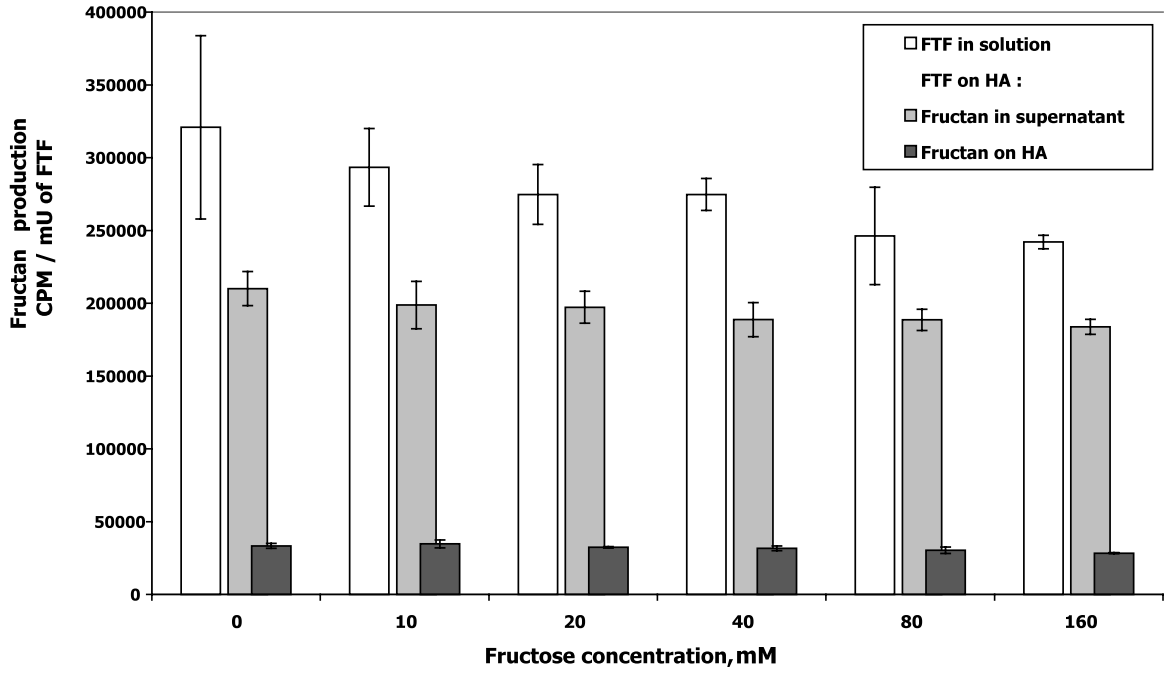


Fig. 2. The effect of fructose on FTF activity in solution and on hydroxyapatite.

Table 1
Effect of glucose on CD-ORD spectrum of fructans synthesized by FTF in solution and on HA

| Sugar tested [a] | FTF in solution | FTF on HA | |
|---|---|---|---|
| | Fructans from solution Peak (max) location (nm) | Fructans from supernatant fluid Peak (max) location (nm) | Fructans from hydroxyapatite surface Peak (max) location (nm) |
| Control [b] | 188 | 189 | 188 |
| Glucose (40 mM) | 195 | 190 | 187 |
| Glucose (160 mM) | 192 | 193 | 192 |

[a] Sucrose (80 mM) was used as substrate in all experiments.
[b] Without addition of tested sugars.

*Fourier-transform infrared spectroscopy (FT-IR) analysis.*—The infrared spectra of fructans synthesized by FTF in solution in the absence of either glucose or fructose showed strong absorption at 3200–2900 $cm^{-1}$, typical of O–H bonding, and absorption bands typical of C–H group at 1650–1400 cm (Fig. 3(A)). A similar FT-IR spectrum was obtained for fructans, in supernatant fluid, produced by FTF adsorbed on the HA surface (Fig. 3(B)). Shifts in bands and additional bands were recorded for fructans from HA surface (Fig. 3(C)). These showed an absorption typical of O–H bonding at 3550–3500 $cm^{-1}$ and two absorption bands typical of C–H groups at 1650–1400 $cm^{-1}$ and 1450–1400 $cm^{-1}$. The presence of fructose had no effect (data not shown), while the presence of glucose had a slight effect on the FT-IR spectrum in comparison to the spectrum of sucrose alone (Fig. 4). The only exception was the spectrum of fructans synthesized by FTF in solution in the presence of 160 mM glucose, which exhibited a different pattern than the control, especially in the range of 3200–1500$cm^{-1}$ (Fig. 4(A)).

*Molecular weight distribution (MWD) of fructans.*—Cell-free FTF in solution synthesized both high and low MW fructans (Fig. 5(A)). The low-MW fructans are characterized by a very low polydispersity index (1.16). In contrast, the polydispersity index of the high-MW fructans was high (in the range 9.2–13.6). In the presence of 50 mM glucose, FTF in solution synthesized only low-MW fructans. The polydispersity index of low-MW fructans synthesized in the presence of glucose remained low (1.11).

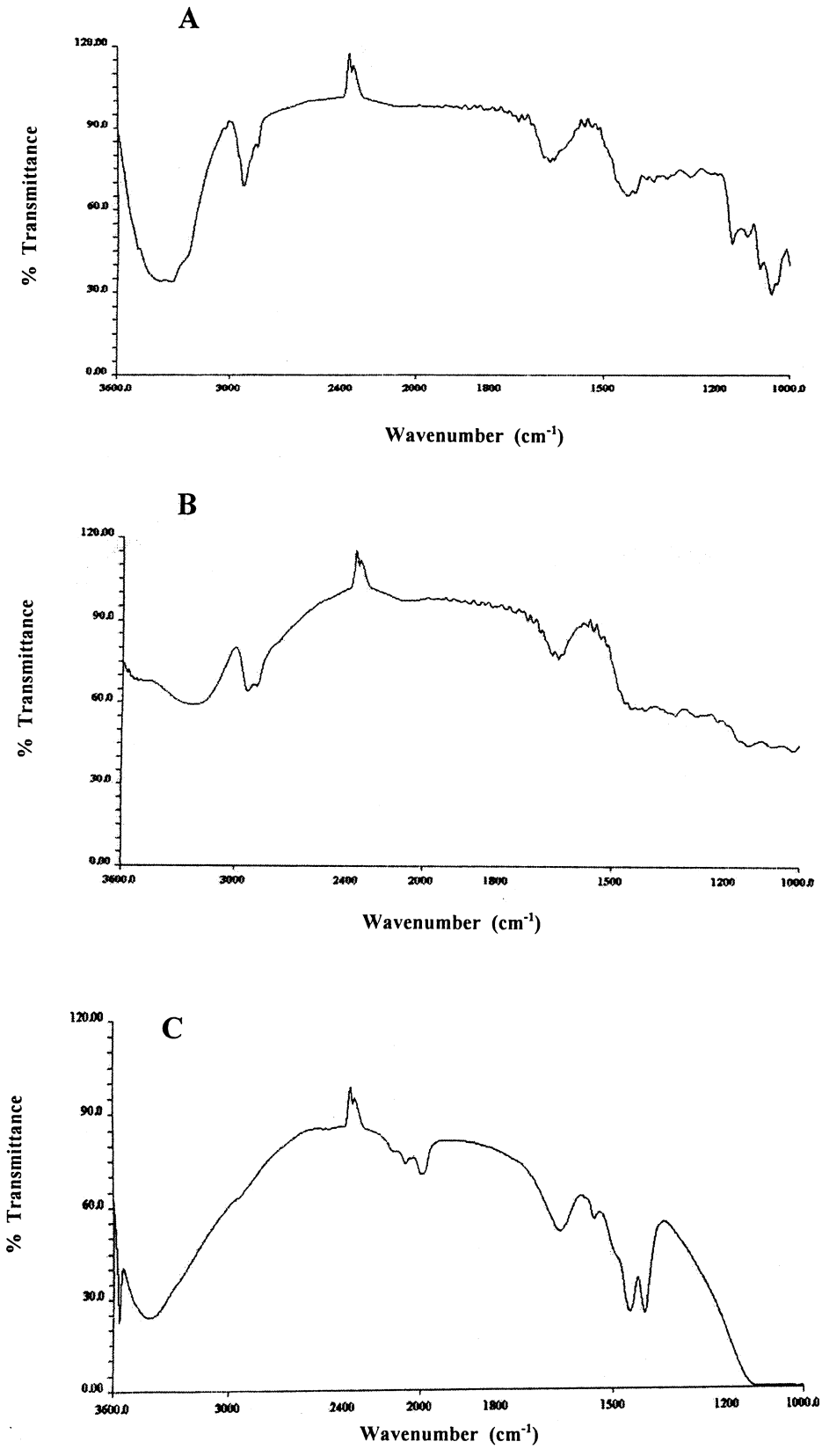


Fig. 3. FT-IR spectra of fructans synthesized by FTF in solution and by FTF immobilized on HA. Fructans synthesized by FTF in solution (A), fructans in supernatant fluid from immobilized FTF on HA (B), fructans from immobilized FTF on HA (C).

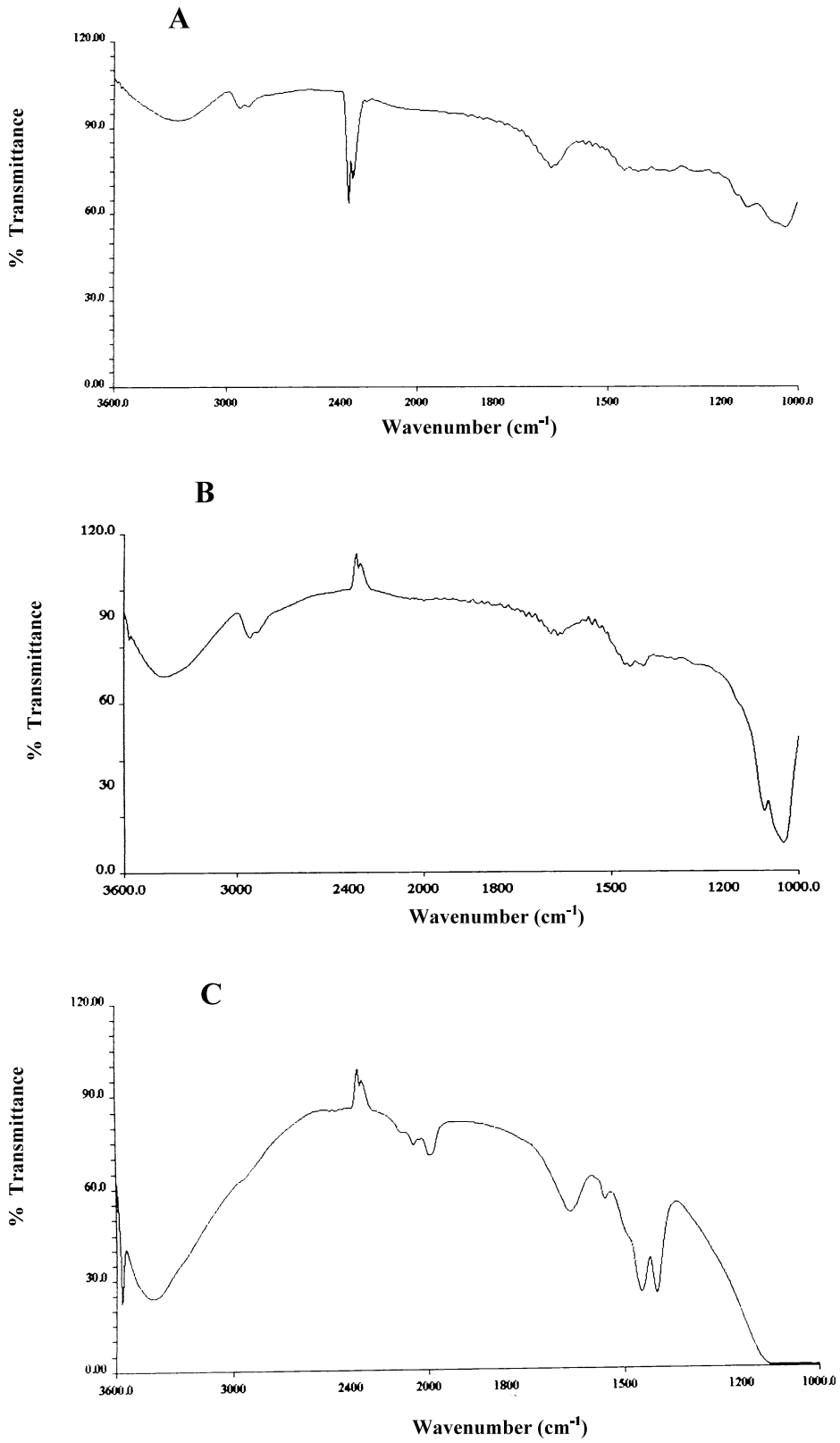


Fig. 4. FT-IR spectra of fructans synthesized, in the presence of 160 mM glucose, by FTF in solution and by FTF immobilized on HA. Fructans synthesized by FTF in solution (A), fructans in supernatant fluid from immobilized FTF on HA (B), fructans on HA from immobilized FTF on HA (C).

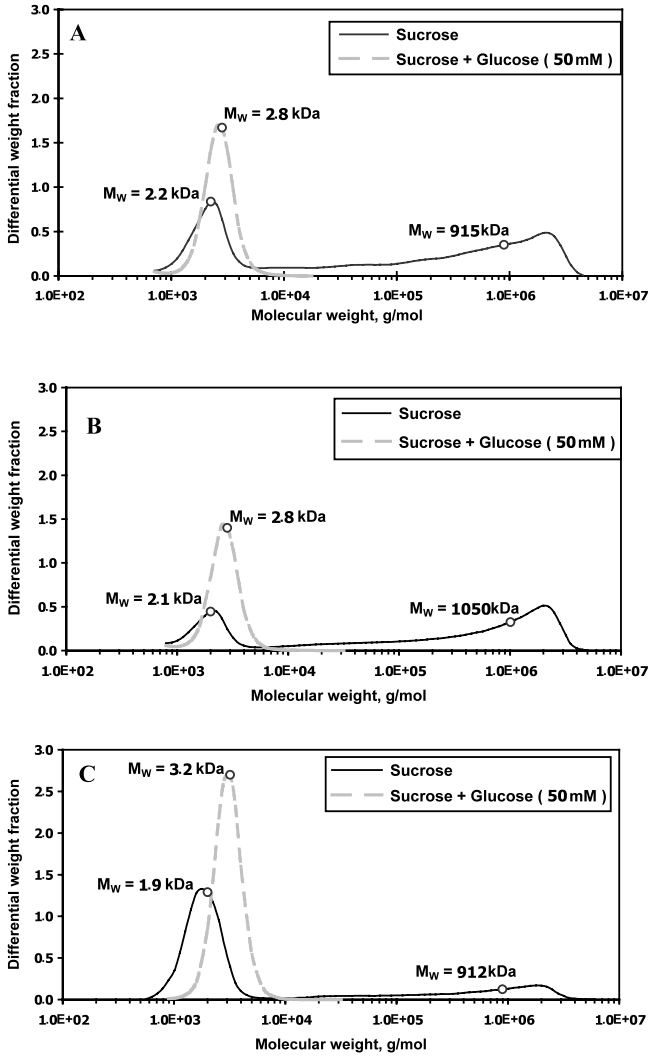


Fig. 5. The effect of glucose on molecular weight distribution of fructans synthesized by FTF. (A) Fructans in solution. (B) Fructans immobilized on HA. (C) Fructans detached from HA.

Fructans synthesized by immobilized FTF also demonstrated a bimodal MW distribution profile (Fig. 5(B, C)) as observed for the fructans in solution. Fructans attached to the HA were both low-MW and high-MW fructans, with polydispersity indices of 1.15 and 9.2, respectively. Fructans detached from the immobilized FTF showed a similar MW profile and similar polydispersity index. The addition of 50 mM glucose inhibited the production of the high-MW fructans, both on HA and in the supernatant fluid.

## 3. Discussion

Dental caries is a disease that is closely associated with the presence of carbohydrates.[30] Such polysaccharides as fructans and glucans are associated with bacterial adhesion and accumulation, and may serve as extracellular reservoirs of carbohydrates. One mechanism whereby FTF affects caries progression is in contributing to the acidogenicity of oral bacteria, by supplying fermentable carbohydrates to oral bacteria. Fructans are rapidly synthesized in the plaque[31,32] and can readily be degraded by fructanase to fermentable sugars that induce the cariogenic challenge in the oral cavity.[33] Carbohydrates produced by oral bacteria and dietary carbohydrates are abundant in the oral cavity. Out of the tested carbohydrates, glucose was found to have the greatest effect on FTF activity. Glucose demonstrated a similar inhibitory effect on FTF in solution as on FTF absorbed onto HA. This resemblance in functional dependence inhibition indicates that regulation of FTF activity by glucose is independent of the microenvironment or of FTF conformation.

Inhibition of FTF activity by glucose results in less fructan production, and this may be accompanied by a change in the structure or MWD of the synthesized fructans. A potential method for determining the absolute configuration of sugar molecules is the CD technique.[34] Unlike proteins, carbohydrates have few natural chromophores. The CD method is sensitive to the chirality of carbohydrates, demonstrating a shift in chromophore due to a change in chirality. Our CD results do not indicate a major change in the fructan spectra in the presence of glucose or fructose. The most significant shift in optical activity between the fructans synthesized on the surface and in solution was evident with 40 mM glucose. The presence of fructose did not induce a shift in the chromophore of the fructans synthesized by FTF both in solution and on the surface.

FT-IR analysis is another technique used to investigate structure of polymers.[35,36] Our FT-IR investigations indicated only small shifts in bands of functional groups comparing fructans produced in the presence of glucose or fructose. The shifts in bands were all within the typical range of O–H or C–H bonding. Since the FT-IR spectra of fructans synthesized by FTF in solution and on the surface is similar, it is conceivable that these minor spectral changes do not indicate a major change in the chemical structure, as analyzed by FT-IR. Differences in spectra were observed when comparing fructans synthesized by FTF immobilized on HA to those synthesized by FTF in solution. These differences occurring at 160 mM glucose indicate a change in the synthesized polysaccharide structure, although the degree of inhibition is very similar in both phases.

Various MW distributions of fructans synthesized in solution have been reported.[36–38] We have found that FTF, both in solution and immobilized on HA, synthesized two widely separated types of fructans; low- and high-MW fructans. Glucose, at concentrations of 50 mM, inhibited synthesis of high-MW fructans both in

solution and on HA. Synthesis of high-MW and low-MW fructans may be affected by other environmental factors. For example; Tanaka et al.[39] have found that high ionic strength selectively inhibited synthesis of high-MW fructans, but not low-MW fructans synthesized by levansucrase from *B. subtilis*. This selective inhibition of fructans synthesis further supports the notion that there are two distinct mechanisms involved in the synthesis of fructans by FTF. Additional support for the two mechanisms concept of the high- and low-MW fructans is found in the polydispersity data, which shows two distinct polydispersity values for the high- and low-MW fructans. A low polydispersity value for the low-MW fructans and the absence of intermediate products is typical for a single-chain elongation mechanism.[40] In contrast, a high polydispersity index value, as found for the high-MW fraction of fructans, and availability of fructans with intermediate MW is quite typical for a multiple-chain elongation mechanism.

Our results show a similarity between MW of fructans synthesized by immobilized FTF and FTF in solution. Chambert et al.[41] have found that fructans synthesized by FTF from *B. subtilis* immobilized on HA synthesizes mainly high MW fructans, while the FTF in solution synthesizes low MW fructans.

Glucose is a byproduct in the enzymatic reaction of fructans synthesized by FTF from sucrose. Enzymatic inhibition by means of a byproduct differs from feedback regulation, where the end product regulates enzymatic activity. Glucose, found to be the principal regulatory agent of FTF activity, is readily metabolized by oral bacteria. This results in a constant production of fructans, and consequently an increased concentration of fructans, serving as a carbohydrate reservoir in the dental plaque. As with the inhibition of FTF by glucose discussed here, it has been reported that GTF, which synthesizes glucans from sucrose, is inhibited by fructose, which is the byproduct of the GTF enzymatic reaction.[42]

The mechanism controlling inhibition of FTF from oral bacteria by glucose is not clear, although this effect has been noted in fructosyltransferase enzymes in solution for various types of bacteria.[40,43,44] It has been suggested that, in the presence of glucose, FTF may also catalyze an exchange reaction whereby a fructosyl moiety in the sucrose—or a fructosyl moiety in fructans, in a reverse reaction—was transferred to the glucose while forming sucrose.

In addition to glucose and fructose we have also examined the effect of several other polysaccharides on FTF activity. Chambert et al.[40] found that levans can increase FTF activity from *B. subtilis*. This positive modulation occurred at concentrations less than 5 mM. However, at concentrations $>5$ mM, they did not find a dose-dependent correlation between FTF activity and the amount of levan. Similarly Tanaka et al.[39] have shown that FTF activity (purified also from *B. subtilis*) was dependent on levan only with a polymerization degree of $>10$ and only at concentrations up to 5mg/mL (0.5%), after which the reaction was constant and independent of levan concentrations. The enhanced synthesis of fructans in the presence of low concentrations of levan was explained by the need for fructan (levan) primers for the forward reaction. At high concentrations of levan, the levan hydrolysis reaction was enhanced.

Collectively, our work and work of others studying FTF activity adds to the growing evidence that FTF plays an important role in the ecology of the oral cavity. Our study has indicated that glucose is the main carbohydrate regulator of FTF activity in solution and on the HA surface. This regulation by inhibition is accompanied by a dramatic change in the MWD of fructans synthesized by FTF in solution and on surfaces.

## 4. Experimental

*Tested carbohydrates.*—These were: D-glucose (E. Merck, Darmstadt, Germany); D-fructose (E. Merck); dextran MW 9600 (Sigma, St. Louis, MO, USA); dextran MW 300,000 (Sigma); levan from *Erwinia herbicola* (Sigma) and inulin (Sigma).

*Purification of FTF.*—Fructosyltransferases (FTF, EC 2.4.1.9) was prepared from a culture fluid of *S. mutans* V-1995, FTF hyperproducing strain V-403 with inactivated *gtf* genes coding for GTF[11] using a modified method of Rozen et al.[14] Bacteria were grown to a stationary phase in BHI medium (Difco Laboratories, Detroit, MI, USA) under 5% $CO_2$ at 37 °C. At the end of the incubation period, the culture fluid was harvested by centrifugation at 9000*g* for 20 min at 4 °C and supplemented with 1 mM of phenymethylsulfonyl fluoride (PMSF) (Sigma) and 0.02% $NaN_3$. The supernatant fluid was concentrated and washed with 5 mM phosphate buffer (pH 6.5) containing PMSF and azide, using ultrafiltration techniques with a 30-kDa cut-off membrane (YM30, Amicon Inc., Danvers, MA) in a stirred-cell ultrafiltration device (Amicon) at 4 °C. The proteins from the prepared concentrate were precipitated stepwise by means of different concentrations of $(NH_4)_2SO_4$ (BDH, Poole, UK). The precipitates were collected by centrifugation at 9000*g* and redissolved in the same phosphate buffer. The fractions demonstrating FTF activity (1.4–1.8 M range of $(NH_4)_2SO_4$ concentration) were pooled, dialyzed against the same buffer and loaded onto a column ($1.5 \times 70$ cm) of DEAE-cellulose (Bio-Rad laboratories, Hercules, CA). The column was developed with a linear concentration gradient of NaCl (0.25 mM/mL in the range 0–500

mM) in the same buffer (flow rate 25–30 mL/h). The eluted fractions were tested for FTF activity using the method described below.

The fractions demonstrating FTF activity but without fructanase activity were collected and dialyzed against phosphate buffer. The enzymatic preparation obtained was used for the surface and solution experiments.

The specific activity of FTF preparations was 45 U/mg protein (U = μmol of fructosyl incorporated into fructans/min).

*FTF activity on hydroxyapatite (HA) surface.*—hydroxyapatite (HA) beads (Type I: 80 μm in diameter, surface area of 50 $m^2$/g, Bio-Rad, Hercules, CA) were equilibrated in two washes with phosphate buffer. The washed HA beads were incubated with FTF (13.3 mU/40 mg HA) for 2 h at 37 °C, while being rotated on a shaker. Excess FTF was washed with phosphate buffer. The remaining FTF was equivalent to 4.8 mU, of which about 95% of the FTF was bound to HA after washings. FTF activity was measured as the incorporation of fructosyl moiety from the radiolabeled sucrose into fructans in a similar method to that described by Germaine et al.[45] and in a modified method for FTF surface activity[21] as follows: FTF adsorbed on HA was incubated with 80 mM sucrose, supplemented with 0.01 mCi of [$^3$H-fructose]sucrose (NEN Products, Boston, MA) per 1 mmol of sucrose. The reaction was carried out in 5 mM phosphate buffer (pH 6.5) in the presence or absence of different concentrations of the tested carbohydrates. After 4 h of incubation at 37 °C, the enzymatic reaction was stopped by removing the HA beads from the supernatant fluid. The amount of fructans from the two different compartments, on HA and in the supernatant fluid, were determined as described by Steinberg et al.[21,22] Briefly, fructans on the HA beads were measured after five washes of the HA with phosphate buffer. The beads were then washed with 0.2 mL of EtOH into a scintillation vial containing Ecosint A scintillation fluid (National Diagnostics, Atlanta, GA, USA). The amount of radioactive labeled fructans was measured using a scintillation counter (Beta-counter, Kontron Basel, Switzerland). Data are presented as radioactive counts of the adsorbed fructans per min per mU of FTF.

Fructans in supernatant fluid were determined using a modified method of Germaine et al.[45] In brief: all HA washes were collected and supplemented with ice-cold EtOH to a final concentration of 75%. Fructans were precipitated at 4 °C for 18 h. The precipitated fructans were than placed over 25-mm glass fiber filters (GF/C Whatman, Maidstone, UK) in a vacuum manifold (Millipore, Bedford, MA, USA). After three washes with ice-cold EtOH, of 4 mL each, the filters were dried and immersed in scintillation fluid Ecosint A. The amount of radioactive EtOH-insoluble fructans was measured in a scintillation counter (Kontron), and the amount of labeled fructans was measured as already described. Preliminary experiments indicated that the concentration of the tested carbohydrate has an impact on the precipitation of fructans. Therefore, before the addition of EtOH, all samples with different concentrations of a tested carbohydrate were adjusted to the same concentration.

*FTF activity in solution.*—The influence of carbohydrates on the activity of FTF in solution was examined using methods similar to those already described for fructans in supernatant fluid. In brief: FTF (4.5 mU) was supplemented with 80 mM of sucrose and [$^3$H-fructose]sucrose. Carbohydrates were added to the enzymatic reaction in the same concentrations that were used for testing the surface activity of FTF. After 4 h, the reaction was stopped by the addition of ice-cold EtOH; radioactive fructans were measured as already described.

*Preparation of fructans for chemical analysis.*—The effect of glucose and fructose on the structure and size of fructans was analyzed using circular dichroism–optical rotatory dispersion (CD-ORD), Fourier-transform infrared spectroscopy (FT-IR) and size-exclusion chromatography (SEC).

Fructans synthesized by FTF in solution and the two fractions of fructans synthesized by FTF on the HA surface (fructans bound to the HA surface and fructans released from HA into the supernatant fluid) were prepared a follows: In order to obtain a sufficient amount of fructans for the chemical analysis, we used higher amounts of FTF than in the radioactive experiments. FTF (200 mU) in solution or FTF on HA (200 mU on 500 mg of HA) were incubated for 24 h at 37 °C with 80 mM unlabeled sucrose in 10 mL of phosphate buffer, in the presence or absence of glucose or fructose.

*Fructans on HA*. FTF adsorbed onto HA were incubated in the presence or absence of the tested carbohydrate. Fructans bound to HA were detached from the surface by sonication at a fixed vibration frequency of 23 kHz (Microsom, Misonix Ind., USA) for 3-min intervals in an ice bath, by means of a method similar to that of Kopec et al.[17] The fructans obtained were precipitated by addition of ice-cold EtOH to a final concentration of 75% for 18 h at 4 °C. The precipitated fructans were recovered by centrifugation at 3500*g* for 10 min, washed with ice-cold EtOH, centrifuged at 3500*g* for 10 min and air-dried at rt.

*Fructans in the supernatant fluid.* The fructans from the supernatant fluid were isolated using the same precipitation procedure described.

*Fructans synthesized by FTF in solution*. FTF was incubated in solution with the tested carbohydrates being isolated as already described for the fructans in the supernatant fluid.

*Circular dichroism–optical rotatory dispersion.*—The CD-ORD spectral measurements were performed on a CD analyzer (Jobin-Yvon apparatus, France) interfaced with an IBM computer equipped with a DICHROGRAPH software, by a modified method of Kaluarachchi and Bush.[33] The instrument was calibrated with deionized water. Spectral data was recorded at 1-mm intervals in the wavelength region from 185 to 230 nm at 25 °C, under a nitrogen environment, using a cell pathway of 0.5 cm. The sample concentration was 0.25 mg polysaccharide/mL in distilled water. All spectra were corrected for solvent refractive index and represent the average of three different scans.

*Fourier-transform infrared spectroscopy.*—The fructans samples obtained as already described were also analyzed by the Fourier-transform infrared spectroscopy (FT-IR), as described by Lijour et al.[46] Pellets for FT-IR analysis were obtained by carefully grinding a mixture of 3–4 mg of fructans with 100 mg of dry KBr powder, and then pressing the mixture into a 12-mm diameter mold. Spectra of fructans were recorded in the 1000–3600 $cm^{-1}$ on an FT-IR spectrometer (Perkin–Elmer, UK) interfaced with an IBM computer equipped with SPECTRUM V2.00 software. The results presented herein are an average of 16 scans.

*Size-exclusion chromatography measurement.*—Fructans for the size-exclusion chromatography (SEC) analysis were prepared in the same manner already described. As a large quantity of fructans was needed, we used the dialysis tube technique to obtain a high concentration of fructans. FTF was incubated in 12,000–14,000 dialysis tubing (Spectrum) containing 80 mM of sucrose solution. The dialysis tube was immersed in 80 mM sucrose solution. Following incubation for 24 h, the fructans in solution and supernatant fluid were isolated as just described. Fructans synthesized on HA were detached from the surface by mild extraction of the HA beads with 100 mM EDTA.

The molecular weight distribution (MWD) of the fructans was determined using aqueous SEC with a multi-angle laser light-scattering detection (MALLS) system coupled with differential refractive index as described by Heyer et al.[38] with minor modifications. The SEC system consisted of a Spectra-Physics-IsoChrom HPLC-pump, an argon-ion laser (488 nm) operating the MALLS detector with a detector angle of 90°, a DAWN-F-DSP laser photometer connected to a Shodex RI-71 differential refractometer (DRI). Fructan samples were dissolved in 0.1 M $NaNO_3$ and 100 μL of the sample was run over PSS-Suprema columns (size 100, 1000, and 10,000), 300 × 8.0 mm, calibrated by dextran and pullulan standards. The elution was carried out at a flow rate of 1.0 mL/min at 65 °C. During a sample run on the SEC-MALLS system, data from the RI and MALLS detectors were collected and processed using PSS-WINGPC Ver. 6.1 software. The weight-average molecular weight ($M_W$), number-average molecular weight ($M_N$), and polydispersity ($M_W/M_N$) for each fructan sample were computed from their chromatograms.

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## References

1. Marsh, P. D.; Bradshaw, D. J. *J. Ind. Microbiol.* **1995**, *15*, 169–175.
2. Listgarten, M. A. *Periodontal 2000* **1994**, *5*, 52–65.
3. Marsh, P. D.; Bradshaw, D. J. In *Dental Plaque Revisited—Oral Biofilms in Health and Disease*; *Microbial Community Aspects of Dental Plaque*; Newman, H. N.; Wilson, M., Eds.; Bioline Press: UK, 1999; pp. 237–253.
4. Rolla, G.; Ciardi, J. E.; Eggen, K. H.; Bowen, W. H.; Afseth, J. In *Glucosyltransferase*, *Glucans*, *Sucrose and Dental Caries*; Doyle, R. J.; Ciardi, J. E., Eds. Free Glucosyl- and Fructosyl-Transferase in Human Saliva and Adsorption of These Enzymes to Teeth In Vivo; Chemical Senses IRL Press: Washington, 1983; pp. 21–30.
5. Burne, R. A. In *Cariology for the Nineties*; Bowen, W. H.; Tabak, L. A., Eds.; Oral Ecology Disasters: The Role of Short-Term Extracellular Storage Polysaccharides; University of Rochester Press: Rochester, NY, 1993; pp. 351–364.
6. Townsend-Lawman, P.; Bleiweis, A. S. *J. Gen. Microb.* **1991**, *137*, 5–13.
7. Keevil, C. W.; West, A. A.; Bourne, N.; Marsh, P. D. *FEMS Microb. Lett.* **1983**, *20*, 155–157.
8. Allen, P. Z.; Bowen, W. H. *Arch. Oral Biol.* **1990**, *35*, 55–62.
9. Okahashi, N.; Asakawa, H.; Koga, T.; Masuda, N.; Hamada, S. *Infect. Immun.* **1984**, *44*, 617–622.
10. Schroeder, V. A.; Michalek, S. M.; Macrina, F. L. *Infec. Immun.* **1989**, *57*, 3560–3569.
11. Munro, C.; Michalek, S. M.; Macrina, F. L. *Infec. Immun.* **1991**, *59*, 2316–2323.
12. Colby, S. M.; Russell, R. R. B. *J. Appl. Microb.* **1997**, *83*, 80s–88s.
13. Burne, R. A.; Wen, Z. T.; Chen, Y. W. M.; Penders, J. A. E. C. *J. Bacteriol.* **1999**, *181*, 2863–2871.
14. Rozen, R.; Bachrach, G.; Bronshteyn, M.; Gedalia, I.; Steinberg, D. *FEMS Microbiol. Lett.* **2001**, *195*, 205–210.
15. Wahlgren, M.; Arnebrant, T. *Trends Biotechnol.* **1991**, *9*, 201–208.
16. Hlady, V. V.; Buijs, J. *Curr. Opin. Biotechnol.* **1996**, *7*, 72–77.
17. Kopec, L. K.; Vacca-Smith, A. M.; Bowen, W. H. *Glycobiology* **1997**, *7*, 929–934.
18. Schilling, K. M.; Bowen, W. H. *J. Dent. Res.* **1988**, *67*, 2–8.

19. Venkitaraman, A. R.; Vacca-Smith, A. M.; Kopec, L. K.; Bowen, W. H. *J. Dent. Res.* **1995**, *74*, 695–701.
20. Steinberg, D.; Beeman, D.; Bowen, W. H. *Art. Cells Blood Subst. Immob. Biotechnol.* **1996**, *24*, 553–566.
21. Steinberg, D.; Beeman, D.; Bowen, W. H. *J. Dent. Res.* **1992**, *71*, 1797–1802.
22. Steinberg, D.; Beeman, D.; Bowen, W. H. *Arch. Oral Biol.* **1992**, *37*, 33–38.
23. Vacca-Smith, A. M.; Bowen, W. H. *Biofilm* **1996**, *1*, paper no. 2.
24. Wunder, D.; Bowen, W. H. *Arch. Oral Biol.* **1999**, *44*, 203–214.
25. Vacca-Smith, A. M.; Bowen, W. H. *Arch. Oral Biol.* **2000**, *45*, 277–291.
26. Steinberg, D.; Kopec, L. K.; Bowen, W. H. *J. Dent. Res.* **1993**, *72*, 1015–1020.
27. Bromshteym, M.; Steinberg, D. *Lett. Appl. Microbiol.* **2001**, in press.
28. Wimpenny, J. In *Dental Plaque Revisited—Oral Biofilms in Health and Disease*; Newman, H. N.; Wilson, M., Eds. Microbial Community Aspects of Dental Plaque; Bioline Press: UK, 1999; pp. 89–110.
29. Steinberg, D. In *Handbook of Bacterial Adhesion*: *Principles*, *Methods*, *and Applications*; An, Y. H.; Friedman, R. J., Eds. Studying Plaque Biofilms on Various Dental Surfaces; Humana Press: Totowa, NJ, 2000; pp. 353–370.
30. Bowden, G. H.; Li, Y. H. *Adv. Dent. Res.* **1997**, *11*, 81–99.
31. Higuchi, M.; Iwami, Y.; Yamada, T.; Araya, S. *Arch. Oral Biol.* **1970**, *15*, 563–567.
32. Gold, W.; Preston, F. B.; Lache, M. C.; Blechman, H. *J. Dent. Res.* **1974**, *53*, 442–446.
33. Kaluarachchi, K.; Bush, A. *Anal. Biol.* **1989**, *179*, 209–215.
34. Embery, G.; Green, D. R.; Rolla, G *Caries Res.* **1989**, *23*, 247–251.
35. Embery, G.; Rolla, G. *Acta Odontol. Scand.* **1980**, *38*, 105–108.
36. Ebisu, S.; Kato, K.; Kotani, S.; Misaki, A. *J. Biochem.* **1975**, *78*, 879–887.
37. Birkhed, D.; Rosell, K. G.; Granath, K. *Arch. Oral Biol.* **1979**, *24*, 53–61.
38. Heyer, A. G.; Schroeer, B.; Radosta, S.; Wolff, D.; Cazpla, S.; Springer, J. *Carbohydr. Res.* **1998**, *313*, 165–174.
39. Tanaka, T.; Oi, S.; Yamamoto, T. *Biochemistry* **1979**, *85*, 287–293.
40. Chambert, R.; Treboul, G.; Dedonder, R. *J. Biochem.* **1974**, *41*, 285–300.
41. Chambert, R.; Petit-Glatron, M. F. *Carbohydr. Res.* **1993**, *244*, 129–136.
42. Ono, K.; Smith, E. E. In *Glucosyltransferases*, *Glucans*, *Sucrose and Dental Caries*; Doyle, R. J.; Ciardi, J. E., Eds. Acceptor Reaction of *Streptococcus* Mutans 6715 Glucosyltransferase; IRL Press, Chemical Senses: Washington, DC, 1983; pp. 145–154.
43. Song, D. D.; Jacques, N. A. *Biochem. J.* **1999**, *341*, 285–291.
44. Hernandez, L.; Arrieta, J.; Menendez, C.; Vazquez, R.; Coego, A.; Suarez, V.; Selman, G.; Petit-Glatron, M. F.; Chambert, R. B. *Biochem. J.* **1995**, *309*, 113–118.
45. Germaine, G. R.; Schachtele, C. F.; Chludzinski, A. M. *J. Dent. Res.* **1974**, *53*, 1355–1360.
46. Lijour, Y.; Gentric, E.; Deslandes, E.; Guezennec, J. *Anal. Biochem.* **1994**, *220*, 244–248.