molecular oral microbiology

molecular oral microbiology

Effect of starch and amylase on the expression of amylase-binding protein A in *Streptococcus gordonii*

A.E. Nikitkova, E.M. Haase and F.A. Scannapieco

Department of Oral Biology, School of Dental Medicine, University at Buffalo, State University of New York, Buffalo, NY, USA

Correspondence: Frank A. Scannapieco, University at Buffalo, Department of Oral Biology, 109 Foster Hall, 3435 Main St, Buffalo, NY 14214, USA Tel.: +1 716 829 3373; fax: +1 716 829 3942; E-mail: fas1@buffalo.edu

Keywords: amylase-binding protein A; dental plaque; oral cavity; saliva

Accepted 14 February 2012
DOI: 10.1111/j.2041-1014.2012.00644.x

SUMMARY

Streptococcus gordonii **is a common oral commensal bacterial species in tooth biofilm (dental plaque) and specifically binds to salivary amylase through the surface exposed amylase-binding protein A (AbpA). When *S. gordonii* cells are pretreated with amylase, amylase bound to AbpA facilitates growth with starch as a primary nutrition source. The goal of this study was to explore possible regulatory effects of starch, starch metabolites and amylase on the expression of *S. gordonii* AbpA. An amylase ligand-binding assay was used to assess the expression of AbpA in culture supernatants and on bacterial cells from *S. gordonii* grown in defined medium supplemented with 1% starch, 0.5 mg ml^{-1} amylase, with starch and amylase together, or with various linear malto-oligosaccharides. Transcription of *abpA* was determined by reverse transcription quantitative polymerase chain reaction. AbpA was not detectable in culture supernatants containing either starch alone or amylase alone. In contrast, the amount of AbpA was notably increased when starch and amylase were both present in the medium. The expression of *abpA* was significantly increased ($P < 0.05$) following 40 min of incubation in defined medium supplemented with starch and amylase. Similar results were obtained in the presence of maltose and other short-chain malto-oligosacchrides. These results suggest that the products of starch hydrolysis produced from the action of salivary α-amylase, particularly maltose and maltotriose, up-regulate AbpA expression in *S. gordonii*.**

INTRODUCTION

Amylase-binding streptococci are numerous in the supra-gingival plaque of host species that express functional salivary α-amylase (Scannapieco *et al.*, 1992, 1993, 1994). A representative amylase-binding species is *Streptococcus gordonii*, a commensal bacterium typically associated with oral health (Kolenbrander, 2000). *Streptococcus gordonii* binds salivary amylase to its surface with high efficiency and specificity via the surface expressed 20-kDa amylase-binding protein A (AbpA) (Scannapieco *et al.*, 1992). AbpA is attached to the nascent cell wall at sites of cell division, and later, as the cell matures, is shed into the environment (Scannapieco *et al.*, 1992). Though AbpA is the major protein responsible for amylase binding to bacterial cell surface, it is transiently associated with the cell wall and abundantly present in culture supernatants (Rogers *et al.*, 1998). Mutation of *abp*A in *S. gordonii* eliminated amylase binding to bacterial surface, decreased biofilm

formation and reduced adhesion to saliva-coated and amylase-coated hydroxylapatite *in vitro* (Rogers *et al.*, 2001).

Salivary α-amylase is an abundant enzyme in saliva and is best known for its ability to degrade starch by hydrolysing 1,4-glycosidic linkages, with subsequent formation of maltose and various linear maltooligosaccharides. Previous studies found that *in vitro* bound salivary amylase remains active and can be used by *S. gordonii* for carbohydrate metabolism in the presence of starch (Scannapieco *et al.*, 1990; Douglas *et al.*, 1992). In the absence of host amylase in the milieu, *S. gordonii* does not readily use starch and grows very poorly with starch as the sole nutrition source (Douglas *et al.*, 1992; Rogers *et al.*, 2001). Hence, the ability of *S. gordonii* to bind salivary amylase may be important for growth of this and other amylase-binding streptococcal species.

Previous studies have shown that carbohydrates in the milieu could influence the expression of AbpA, particularly by carbon catabolite repression (Rogers & Scannapieco, 2001). Indeed, AbpA was undetectable in cultures of bacteria in defined medium (DM) supplemented with 1% glucose, and greatly reduced in cultures of bacteria in brain–heart infusion medium containing 1% glucose (Rogers & Scannapieco, 2001). A catabolite responsive element was identified in the promoter region of *abp*A that probably regulates AbpA through the action of regulatory protein RegG, a CcpA-like catabolite control protein homologue (Rogers & Scannapieco, 2001). Mutation of *regG* eliminated the repressive effect of glucose on AbpA expression. These findings suggested that AbpA is likely to be a component of the bacterial carbohydrate utilization machinery, and is under the control of regulatory mechanisms governing carbohydrate metabolism.

Considering the fact that starch, as the substrate for bound amylase, is hydrolysed into smaller carbohydrates by the action of amylase, we hypothesized that starch could play a role in the regulation of expression of AbpA. In the presence of a potential food source (starch), it would be advantageous for the bacterium to exploit host amylase by increasing the expression of amylase-binding protein. Alternatively, we could not rule out the possibility of amylase itself exerting a regulatory effect on AbpA expression in the presence of starch. Hence, the goal of this study was to explore the regulatory effects of starch, starch metabolites and amylase on the expression of *S. gordonii* AbpA.

METHODS

Bacterial strains and culture conditions

Streptococcus gordonii CH1 Challis (GenBank, accession number s_gordonii_challis_n CP000725) was cultivated from frozen stocks on Todd–Hewitt (Difco, Detroit, MI) agar and incubated overnight at 37°C in a candle jar. For routine experiments, bacteria were cultured in the DM of Jenkinson, as modified by Loo (Jenkinson, 1986; Loo *et al.*, 2000).

Isolation of human salivary α-amylase

Human parotid saliva from several healthy donors was duct-collected, as previously described (Miller, 1960; Bradway *et al.*, 1989). This saliva was extensively dialysed against distilled water, lyophilized, resuspended in chromatography buffer (Scannapieco *et al.*, 1989), and subjected to Bio-Gel P60 gel-filtration chromatography (Bradway *et al.*, 1989; Scannapieco *et al.*, 1989). The peaks containing amylase were analysed for purity by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blot; selected fractions were pooled, dialysed, lyophilized and stored at −20°C. Isolated amylase appeared as a single band on SDS–PAGE stained with Coomassie Brilliant Blue as previously described (Scannapieco *et al.*, 1989). Amylase peaks were also proven to be free from sIgA (Nikitkova *et al.*, 2012). For the experiments in this study, amylase was re-suspended in distilled water and used to supplement DM for bacterial growth experiments.

AbpA expression in overnight cultures under various growth conditions

Bacteria were streaked on Todd–Hewitt (Difco) agar from frozen stocks and incubated overnight at 37°C in a candle jar. Several colonies were transferred to 10 ml liquid medium and incubated for 16–18 h (overnight) statically at 37°C in a candle jar until the optical density of the culture at 600 nm (OD_{600}) reached 0.6–0.8. The overnight conditions tested were as follows: DM supplemented with 0.5 mg ml^{-1} purified human

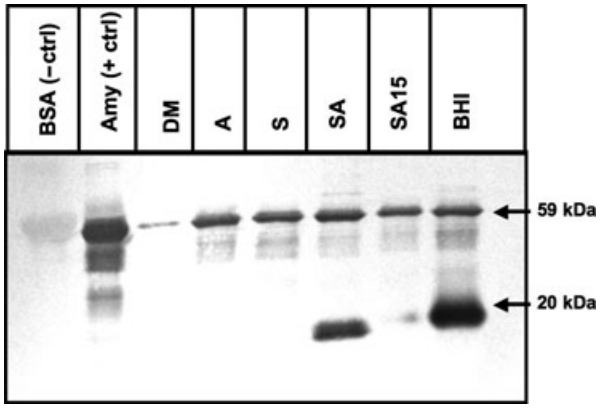

Figure 1 Expression of AbpA protein in culture supernatants of various growth conditions. Amylase ligand-binding assay of overnight culture supernatants precipitated with amylase. Bovine serum albumin as a negative control (BSA –ctrl), and salivary amylase as a positive control (Amy + ctrl). Growth conditions: defined media (DM); DM with 0.5 mg ml^{-1} amylase alone (A); DM supplemented with 1% starch (S); DM 1% starch + 0.5 mg ml^{-1} amylase (SA); DM with 1% starch grown overnight and 0.5 mg ml^{-1} amylase added and incubated for 15 min (SA15); brain–heart infusion (BHI). All supernatants were adjusted to have equal total protein content. AbpA protein (20 kDa band). Salivary amylase (59 kDa) used for precipitation is present in all lanes except negative control.

salivary amylase, which approximates the normal concentration of amylase in saliva (Jacobsen *et al.*, 1972); DM supplemented with 1% (weight/volume; W/V) soluble starch (Fisher Scientific, Fair Lawn, NJ); DM supplemented with 1% (W/V) soluble starch and 0.5 mg ml^{-1} purified human salivary amylase. Brain–heart infusion broth (Bacto™; BD, Sparks, MD) was tested as a positive control, because *S. gordonii* shows high expression of AbpA in this medium (Rogers & Scannapieco, 2001). Additionally, 0.5 mg ml^{-1} salivary amylase was added to the bacterial culture grown overnight in DM 1% soluble starch, and incubated for 15 min (SA15 in Fig. 1). Bacterial cultures were centrifuged at 6000 ***g*** for 5 min; the supernatants were collected and used for precipitation of AbpA and amylase ligand-binding assay.

Precipitation of AbpA from culture supernatants

To precipitate proteins, culture supernatants were filtered through a 0.2-μm filter, adjusted to 1 mg/ml total protein and incubated for 1 h at room temperature with 50 μg ml^{-1} purified salivary amylase in a 1-ml total volume (Li *et al.*, 2002; Chaudhuri *et al.*, 2007). The amylase-precipitated proteins were collected by centrifugation at 10,000 ***g*** at room temperature for 10 min, resuspended in loading buffer (0.06 M Tris–HCl, pH 6.8, 10% glycerol, 2% SDS, 0.05% 2-β-mercaptoethanol and 0.00125 mg bromophenol blue), and boiled for 3 min before loading into 12% SDS–PAGE gels. Purified salivary amylase (1 μg) was loaded as a positive control and bovine serum albumin (1 μg) was loaded as a negative control. SDS–PAGE gels were then transblotted and used in the amylase ligand-binding assay.

Amylase ligand-binding assay

The proteins were electro-transferred from SDS–PAGE gels to Immobilon-P membranes (Millipore, Bedford, MA) and blocked overnight with 5% skim milk in Tris-buffered saline with 0.1% Tween (TBST). After each change of reagent, the membrane was washed three times for 10 min in TSBT. Following blocking, the membrane was incubated with 10 mg ml^{-1} amylase in TBST, washed, exposed to rabbit anti-amylase antibody (Calbiochem; Merck, Rockland, MA) for 30 min, washed and incubated with goat anti-rabbit IgG (Bio-Rad, Hercules, CA) for 30 min. The membrane was then developed using Sigma *FAST* BCIP/NBT (Sigma, St Louis, MO).

RNA isolation and reverse transcription

Streptococcus gordonii was cultured in 120 ml DM with 0.2% glucose to mid-log phase (0.5–0.6 OD_{600}). The culture was then divided into 12 aliquots and centrifuged at 6000 ***g*** for 5 min at 19°C to pellet bacterial cells. The supernatant was removed and the bacteria were resuspended in fresh DM (2 ml) supplemented with one of the following: 1% (W/V) D-glucose (Fisher Scientific), 1% soluble starch (Fisher Scientific), 1% starch + 0.5 mg ml^{-1} purified salivary amylase, 1% maltose, 1% maltotriose, 1% maltotetraose, 1% maltopentaose 1% maltohexaose, 1% maltoheptaose, 1% maltoheptaose + 0.5 mg ml^{-1} amylase, 0.5 mg ml^{-1} amylase, or DM alone, and incubated in the candle jar at 37°C for 40 min. The malto-oligosaccharides were obtained from Sigma. After the bacterial suspensions were centrifuged at 6000 ***g*** for 5 min at 19°C, the supernatant was removed and stored at −20°C until analysed for

proteins, and the bacterial pellets were used to isolate RNA.

For RNA isolation, the bacterial pellet from each test condition described above was resuspended in salivary buffer (5 ml) followed by the addition of hot acid phenol as previously described (Vickerman *et al.*, 2007). The RNA was then treated with TurboDNase (Applied Biosystems/Ambion, Austin, TX) according to manufacturer's protocol and purified with the RNeasy minikit columns (Qiagen, Valencia, CA) using the manufacturer's cleanup protocol. Total RNA was quantified using the Nanodrop 2000 spectrophotometer and RNA integrity was determined by agarose gel electrophoresis. Total RNA was used immediately for cDNA synthesis. The *S. gordonii* total RNA was reverse transcribed to cDNA using a modified protocol from the Pathogen Functional Genomics Resource Center (PFGRC) at the J. Craig Venter Institute http://pfgrc.jcvi.org/index.php/microarray.html. Briefly, 4 μg total RNA was reverse transcribed using Superscript II or III (Invitrogen, Carlsbad, CA) reverse transcriptase in a deoxynucleotide mixture. After alkaline hydrolysis to remove the RNA template, cDNA was purified with QIAquick PCR columns (Qiagen) and used in the quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay.

RT-qPCR

The concentration of cDNA was determined by Nanodrop 2000 spectrophotometer, and each sample was normalized to 2 ng μl^{-1} for RT-qPCR. Gene-specific primers were designed using Primer BLAST (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov), and produced by Invitrogen. Amplification of the gyrase A (*gyrA*) gene was used as an endogenous control, and the non-supplemented DM condition was used as the calibrator. For *gyrA*: forward primer GyrA-F 5′-GCGGATTGTTGTAACCGAGT-3′, reverse primer GyrA-R 5′-ACGGACACCCTCACGATTAG-3′ was used in the reaction. For the *abpA*: forward primer AbpA-F 5′-GCTGCAGCACCAGTTTTCTCTGCT-3′, reverse primer AbpA-R 5′- GCGCCGTCGTTGT TACGAGC-3′. The efficiency of the assay, tested using serial dilutions of cDNA template, was between 90 and 100%. Dissociation curve analysis was performed to verify amplification of a single product. Each reaction mixture (total volume, 25 μl) contained 1 μl template, 10 μl diethyl pyrocarbonate-treated water, 12 μl Power SYBR green PCR master mixture (Applied Biosystems, Foster City, CA) and 2 μl primer mix (100 nM). Relative RT-qPCR was performed on the ABI 7500 thermal cycler (Applied Biosystems), using uniform cycling conditions (95°C for 10 min and then 40 cycles of 30 s at 95°C and 1 min at 56°C) for Power SYBR Green (Applied Biosystems). Each reaction was run in triplicate, and non-template controls were run for each primer set. The RT-qPCR was performed on samples from the five independent experiments conducted on distinct days.

Statistical analysis

The log2 relative quantity values of the RT-qPCR data were analysed using Student's *t*-test to compare expression of *abpA* in cells grown in one of the supplemented conditions with the expression of the *abpA* in cells of non-supplemented DM ($n > 3$). Significant values showed difference in *abpA* expression in tested growth condition compared with control condition at $P < 0.05$.

RESULTS

To investigate the role of starch and amylase in the regulation of expression of AbpA, we assessed the amounts of AbpA protein in the supernatants of overnight cultures of *S. gordonii* grown in the presence of starch alone, amylase alone, or starch and amylase together. All supernatants were adjusted with DM to contain an equal amount of total protein. DM has a low concentration of glucose (0.2%) and so was used to minimize catabolite repression of AbpA expression (Rogers & Scannapieco, 2001) and to avoid interference with complex carbohydrates that might be present in complex media. AbpA was abundantly present in overnight culture in the presence of both starch and bound amylase (Fig. 1). When bacteria were grown in DM with 1% starch, the appearance of AbpA was only observed after addition of amylase and 15 min of incubation, which suggests that time is required for the bacteria to express AbpA after exposure of the cell to starch and amylase or the products of their interaction. AbpA was not detectable in overnight cultures with starch alone, or with amylase alone. Together, these results suggest that

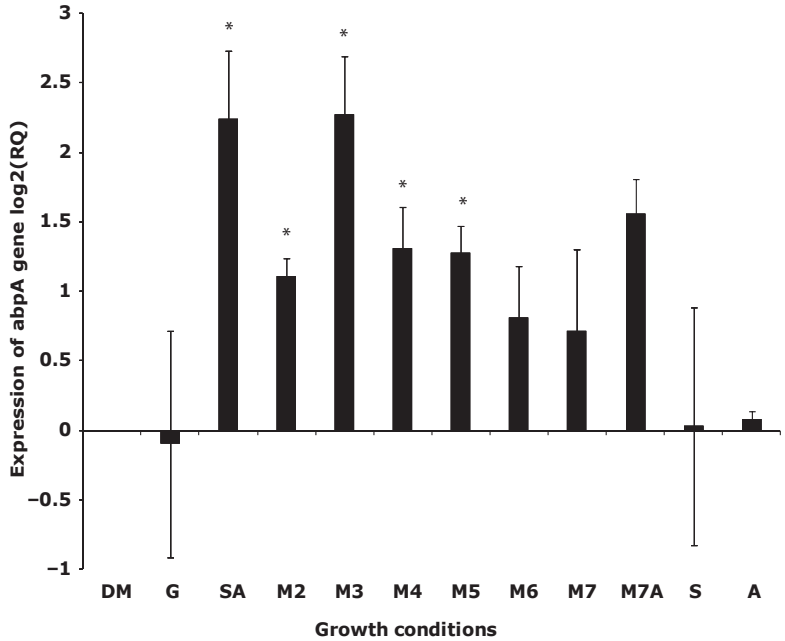

Figure 2 Expression of *abpA* gene in *Streptococcus gordonii* by quantitative reverse transcription–polymerase chain reaction. Bacteria were cultured to mid-log phase and pelleted. To the pellet one of the following was added and the mixture was incubated for 40 min in a candle jar at 37°C: fresh defined medium (DM); DM supplemented with 1% glucose (G); 1% starch and 0.5 mg ml^{-1} amylase (SA); 1% maltose (M2); 1% maltotriose (M3); 1% maltotetraose (M4); 1% maltopentaose (M5); 1% maltohexaose (M6); 1% maltoheptaose (M7); 1% maltoheptaose with 0.5 mg ml^{-1} amylase (M7A); 1% starch (S); 0.5 mg ml^{-1} amylase (A). The *gyrA* gene was used as endogenous control. Incubation in minimal fresh DM was used as the calibrator; log2 of relative quantity (RQ) is presented.

expression of AbpA was influenced by the presence of both starch and amylase in the milieu, and that it takes time to express the protein in the supernatant after exposure.

Considering the fact that starch is digested by salivary amylase to form abundant malto-oligosaccharides, with maltose as the final end product (Ramasubbu *et al.*, 2004), it was not clear if the observed up-regulation of AbpA was the result of the presence of the amylase–starch complex or of the effect of malto-oligosaccharides derived from starch digestion by amylase. Therefore, we further investigated the influence of linear starch-derived malto-oligosaccharides on the expression of *abpA*. Bacteria were grown to mid-log phase, where maximal AbpA protein expression was observed previously (Scannapieco *et al.*, 1992; Gwynn & Douglas, 1994). At mid-log phase the bacteria were exposed to different substrates for 40 min before isolation of total RNA from bacterial cells, and collection of culture supernatant for protein analysis. The expression of *abpA* was elevated sixfold ($P < 0.05$) in the presence of starch and amylase together (Fig. 2), but not in the presence of starch or amylase alone. Expression of *abpA* was also increased in the presence of short-chain malto-oligosaccharides and maltose. Up to sixfold ($P < 0.05$) greater expression of *abpA* was observed in cells incubated with maltotriose, and greater than twofold increased expression was found in the presence of other malto-oligosaccharides (Fig. 2). Hence, the presence of the products of starch degradation in the milieu influences the expression of the *abpA* gene by *S. gordonii*.

We also analysed the amount of AbpA protein in the supernatants after 40 min exposure of bacterial cells to various substrates by using amylase precipitation and the amylase ligand-binding assay. As expected, more AbpA protein was detected in the supernatants in the presence of starch and amylase together compared with cells incubated with un-supplemented DM or DM supplemented with glucose (Fig. 3). AbpA protein was minimal in the supernatants of cells incubated with starch or amylase alone. However, AbpA protein levels were elevated in the supernatants of cells incubated with short-chain malto-oligosaccharides (Fig. 3).

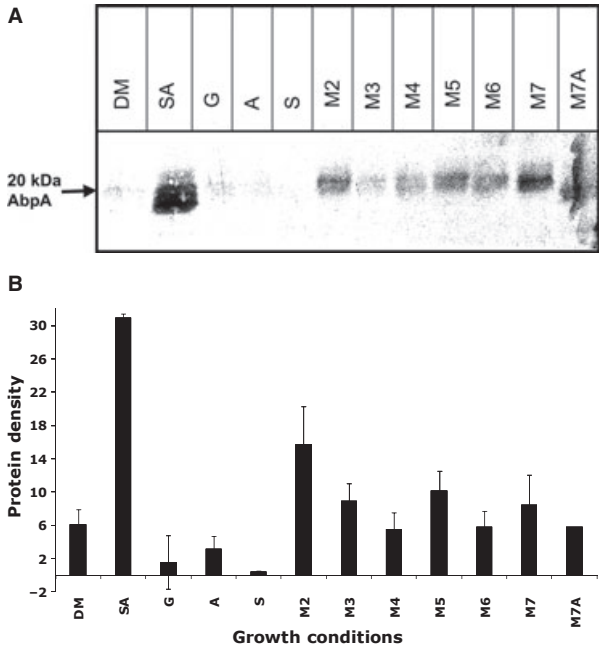

Figure 3 Expression of AbpA protein in culture supernatants grown with different malto-oligosaccharides. (A) Bacterial cells derived from the same culture were incubated for 40 min in DM supplemented with one of the following: 1% starch and 0.5 mg ml^{-1} amylase (SA); 1% glucose (G); 0.5 mg ml^{-1} amylase (A); 1% starch (S); 1% maltose (M2); 1% maltotriose (M3); 1% maltotetraose (M4); 1% maltopentaose (M5); 1% maltohexaose (M6); 1% maltoheptaose (M7); maltoheptaose with 0.5 mg ml^{-1} amylase (M7A). Total protein precipitated from 1 ml supernatant was loaded onto a sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel, electro-transferred to polyvinylidene difluoride (PVDF) membrane, and the amylase ligand-binding assay was performed. (B) Densitometry analysis of AbpA protein on the same PVDF membranes described above.

DISCUSSION

Streptococcus gordonii is not able to use starch as a nutritional source unless extracellular amylase is present (Douglas, 1990; Scannapieco *et al.*, 1990; Rogers *et al.*, 2001). Salivary amylase binds to a specific receptor, AbpA, on the surface of *S. gordonii*, and retains its activity to degrade starch to provide a source of nutrition for the bacteria (Douglas, 1990; Douglas *et al.*, 1992). The results of this study indicate that expression of the *abpA* gene is induced by both amylase and starch in the growth medium, whereas the presence of either component alone did not significantly alter expression of *abpA.* In the oral cavity, where amylase is always present, *S. gordonii* probably expresses only a basal level of AbpA. The presence of dietary starch together with available amylase increases AbpA expression enabling it to capture the enzyme, so that the available starch can be hydrolysed into a usable form. Amylase quickly hydrolyses starch at 1,4-glycosidic linkages to yield malto-oligosaccharides. Hence, it appears advantageous for the bacterium to be able to maximally capture the exogenous enzyme when starch is present by increasing the expression of the surface-associated AbpA. This would maximize the binding of the host enzyme to increase the amount of usable malto-oligosaccharides in close proximity to the bacterium.

We observed that short-chain malto-oligosaccharides and maltose induced expression of *abpA* in a manner similar to starch and amylase together. The greatest expression was noted in the presence of maltotriose, the linear oligosaccharide starch metabolite consisting of three glucose moieties. These results suggest that products of starch degradation play a regulatory role in *abpA* gene expression.

Our previous work found that *abpA* is regulated by a catabolite repression mechanism through the effect of a catabolite control protein A (CcpA) homologue, the transcriptional regulator known in *S. gordonii* as RegG (Rogers & Scannapieco, 2001). A catabolite-responsive element (*cre*) was identified in the promoter region of *abpA* and mutation of *regG* eliminated the catabolite repression effect of glucose on the expression of *abpA* (Rogers & Scannapieco, 2001). This mechanism of catabolite repression is probably similar to the ATP-dependent protein–protein interaction previously described in other gram-positive bacteria (Saier *et al.*, 1996), where one of the elements of the glucose transport phosphotransferase system, the HPr protein, is phosphorylated on Ser-46 during glucose transport through phosphotransferase, and together with the phosphorylated glucose metabolite binds to a CcpA homologue, RegG (Rogers & Scannapieco, 2001). The latter changes conformation to acquire a high affinity toward *cre* (Saier *et al.*, 1996), which in this case was positioned in the promoter region of *abpA* (Rogers & Scannapieco, 2001). The binding of RegG to *cre* likely prevents transcription of *abpA* (Fig. 4A). The present results support the previous findings, as we did not observe increased expression of *abpA* in the presence of glucose in the milieu. The present work provides further evidence for upregulation of *abpA* through maltose/maltodextrin

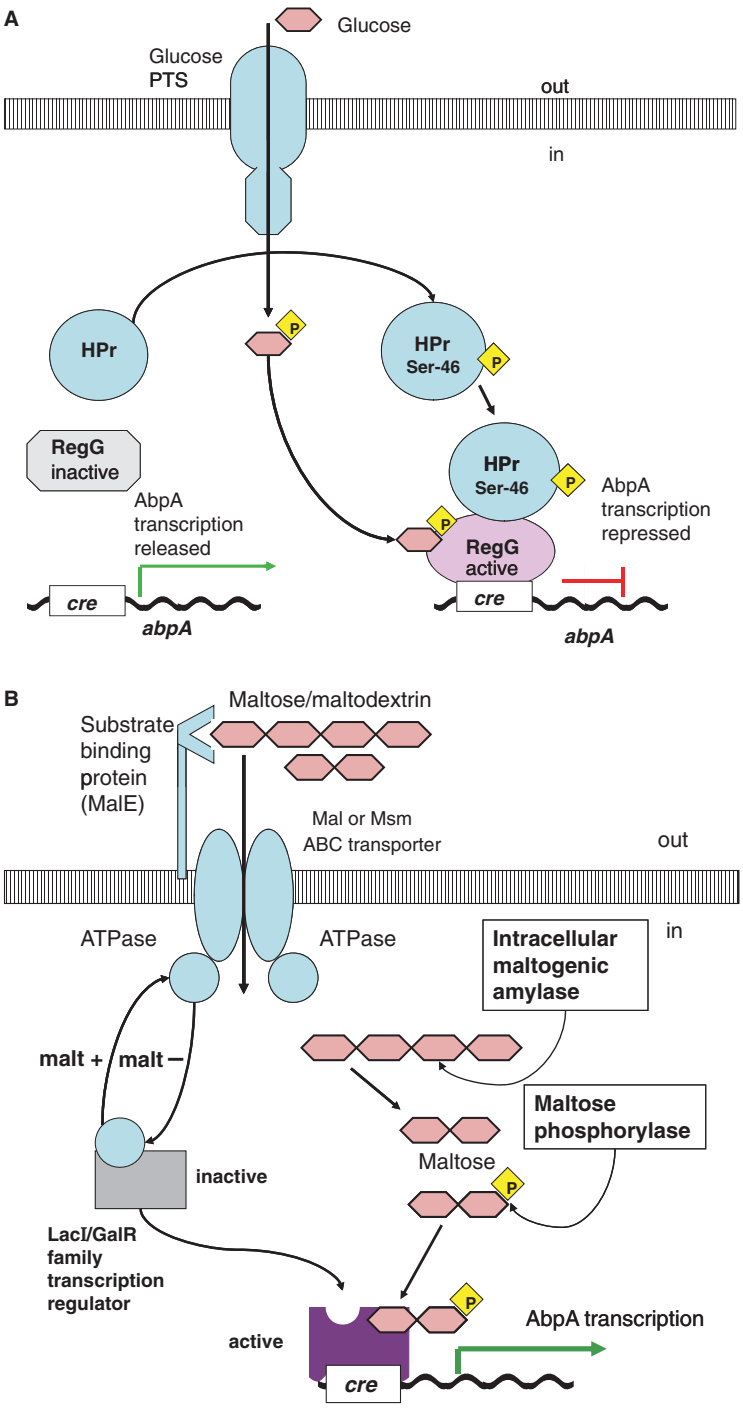

Figure 4 Theoretical model of regulation of *abpA* gene through catabolite repression by glucose (A) and substrate induction by maltose (B). The proposed model for *abpA* is adapted from regulatory pathways of carbohydrate utilization machinery described in various microorganisms (Saier *et al.*, 1996; Boos & Shuman, 1998; Rogers & Scannapieco, 2001; Shelburne *et al.*, 2006, 2008a,b; Gorke & Stulke, 2008; Webb *et al.*, 2008). (A) HPr protein becomes phosphorylated on Ser-46 during glucose transport through glucose phosphotransferase, and together with the phosphorylated glucose metabolite binds to RegG; the latter changes conformation and acquires high affinity for *cre*, which prevents transcription of *abpA*. (B) LacI/GalR transcriptional regulator is inactive while bonded to ATPase protein, a component of a Mal or Msm ABC transporter; during maltose/maltodextrin transport ATPase releases a LacI/GalR transcriptional regulator; latter binds internalized phosphorylated maltose to its phosphosugar site and becomes active; active LacI/GalR transcriptional regulator acquires high affinity for *cre* and initiates transcription of *abpA*.

induction. We observed that the levels of AbpA were elevated in cells exposed to 1% maltose and maltooligosaccharides as determined by transcript levels and protein levels. These results differ from our previous observation where growth in chemically defined medium (FMC) (Terleckyj *et al.*, 1975) supplemented with maltose did not upregulate AbpA expression (Rogers & Scannapieco, 2001). This discrepancy, however, is probably the result of differences in experimental design between the two studies. In the previous studies, the bacteria were grown in the maltose-supplemented medium to mid-log phase for 10 h. In this case, the concentration of maltose diminished with time as the bacteria metabolized maltose to glucose. Hence, the expression of AbpA was probably repressed. In the present experiments, however, a minimal (0.2%) concentration of glucose in the DM was used to minimize catabolite repressive effects, and the time of bacterial exposure to 1% maltose was limited to 40 min. Hence, in the present experiments, the relative concentration of maltose was obviously high enough to induce AbpA expression (Fig. 3).

Maltose/maltodextrin gene regulation has been previously described in various microorganisms (Nieto *et al.*, 1997; Boos & Shuman, 1998; Boos & Bohm, 2000; Shelburne *et al.*, 2006). In *Listeria monocytogenes*, the maltodextrin ABC transporter system is induced by maltose/maltodextrin and repressed by glucose, which seems to be similar to what is observed for regulation of *abpA* in *S. gordonii*. Interestingly, *L. monocytogenes* does not have extracellular amylase and depends on neighboring organisms that are able to hydrolyse starch to provide this function (Gopal *et al.*, 2010). Previous findings suggest that maltose/maltodextrin induces the expression of the maltose ABC transporting system where maltose has the major role of inducer in *L. monocytogenes* (Gopal *et al.*, 2010). Intracellular amylase generates maltose from maltodextrins in the cytoplasm, which become phosphorylated by the action of maltose phosphorylase. Phosphorylated maltose binds to and activates a LacI/GalR family transcriptional regulator, which in turn acts as a transcriptional activator (Gopal *et al.*, 2010).

A similar mechanism of maltose-induced transcriptional activation is present in *Lactococcus lactis* where MalR, the LacI/GalR family regulator, activates expression of the maltose operon (Andersson & Radstrom, 2002).

Maltodextrin use is linked to virulence factor production in selected species of streptococci (Shelburne *et al.*, 2008a,b). In *Streptococcus mutans*, two maltose permease ABC transporter systems for maltose/maltodextrin uptake have been described (Webb *et al.*, 2007, 2008). One system is encoded by *malE*, *malF*, *malG* and *malK* in the *mal* operon and the other system, the multiple sugar metabolism (Msm) transporter, is encoded by the *msm* operon. Interestingly, *S. mutans* UA159 isogenic double mutant strain (KCL82), although lacking these two functional operons was still able to ferment maltose as a primary carbon source (Webb *et al.*, 2007). The principal maltose uptake system in this *S. mutans* strain was a phosphoenolpyruvate-dependent phosphotransferase system *ptsG*, which has sequence similarity with *Enterococcus faecalis* MalT (Webb *et al.*, 2007, 2008).

To be able to better understand the observed regulation of *abpA* through the maltose/maltodextrin transport system, we searched the *S. gordonii* genome for oligosaccharide transporter gene candidates. Using BLAST search and the KEGG database we identified several genes as potential oligosaccharide transporters with sequence similarity with Msm and Mal-like transporters of *S. mutans*. Potential Mal-like ABC transporters in *S. gordonii* are represented by annotated genes *malD* SGO_0102, malC SGO_0103, maltose/maltodextrin-binding protein precursor SGO_0104, and ATP-binding protein SGO_0352. A potential transcriptional regulator for this operon is the upstream positioned transcriptional repressor SGO_0100, which is a LacI/GalR family protein with a sugar-binding domain and a DNA-binding domain. Considering the fact that the promoter region of *abpA* possesses a *cre* element known to be a binding site of LacI/GalR regulators (Rogers & Scannapieco, 2001; Francke *et al.*, 2008), the proposed maltose/maltodextrin transporting system could possibly play a role in activation of *abpA* expression. Figure 4B shows a hypothetical model adapted for *abpA* from previously described maltose/maltodextrin regulation pathways of other microorganisms (Saier *et al.*, 1996; Boos & Bohm, 2000; Shelburne *et al.*, 2006, 2008a,b; Gorke & Stulke, 2008; Webb *et al.*, 2008). However, it is not certain if the transcriptional repressor SGO_0100 would play the role of activator of a *mal* operon as in the case of the *L. monocytogenes* LacI/GalR regulator (Gopal *et al.*, 2010), or is it a

repressor in *S. gordonii*. It is also not yet clear if this annotated maltose transport system is in fact a maltose/maltodextrin transporter, and if it is the role it plays in AbpA induction.

We cannot rule out the possibility that *abpA* regulation could be achieved through one of the Msm transporters (Webb *et al.*, 2008). One Msm transporter candidate in *S. gordonii* is annotated as: *msmE* SGO_1305, *msmF* SGO_1304, and *msmG* SGO_1303 with transcriptional regulator *msmR* SGO_1306; another Msm-like ABC transporter SGO_0120, SGO_0121, SGO_0122 with each gene having up to 54% amino acid identity with Msm proteins of *S. mutans* and closely positioned on the chromosome phosphosugar-binding transcriptional regulator SGO_0127; and yet another sugar transporter SGO_1765, having 41% protein identity with the Msm permease of *S. mutans*. These hypothetical transporting systems are good candidates for a proposed model of positive regulation of *abpA* (Fig. 4B). However, though the function of these genes is annotated as oligosaccharide-transporting systems based on their similarity to the known proteins and conserved domains, their function in *S. gordonii* has not yet been established experimentally and requires further study.

We also searched for the homologue of MalT in the *S. gordonii* identified phosphotransferase system IIBC component SGO_0505 with 70% protein identity to *S. mutans* MalT (*ptsG*), which has been suggested to play a major role in maltose transport in *S. mutans* (Webb *et al.*, 2007). It is possible, but not likely, that MalT is involved in *abp*A induction, because in *S. mutans* MalT is responsible solely for maltose transport (Webb *et al.*, 2007). In our study, however, we observed upregulation of *abpA* not only in the presence of maltose, but also in the presence of other malto-oligosaccharides. Future studies will investigate and determine experimentally the role of SGO_0100, SGO_0127, SGO_1306 and other potentially promising LacI/GalR transcriptional activators on *abpA* gene expression.

Because AbpA is regulated in part through catabolite repression and maltose/maltodextrin induction, it probably plays a role in carbohydrate utilization systems. It is expected that one of the maltose/maltodextrin transporting systems in *S. gordonii* plays a regulatory role for AbpA in the presence of products of dietary starch degradation by salivary amylase.

Now that we have established that AbpA is positively regulated by maltose/maltodextrin, which are abundant substrates in the oral cavity during food consumption, several questions remain. First, it is necessary to determine which of the hypothetical maltose/maltodextrin transporting systems and transcription regulators play a role in AbpA induction. Second, the consequence of upregulation of AbpA for *S. gordonii* colonization remains to be determined. Though *in vitro* studies have shown that AbpA plays a role in biofilm formation on saliva-coated hydroxylapatite (Loo *et al.*, 2000; Rogers *et al.*, 2001), *in vivo* studies have argued against such a role (Tanzer *et al.*, 2003). Our recent studies suggest that the binding of salivary amylase to AbpA on *S. gordonii* results in differential gene expression with a concomitant increase in bacterial growth and fitness (Nikitkova *et al.*, 2012). Third, it is of interest to determine if the observed phenomenon of *S. gordonii* AbpA regulation affects host oral health in a positive or negative way. The intake of foods with higher starch content versus glucose content may increase the expression of AbpA, and alter the course of *S. gordonii* colonization. This in turn may influence the subsequent colonization of the oral cavity by potential pathogenic bacteria. The mechanisms of AbpA regulation and the role of AbpA in the metabolic processes of *S. gordonii* require further study to better understand *S. gordonii* colonization and interaction with the host environment.

ACKNOWLEDGEMENTS

This work was supported by the National Institute of Dental and Craniofacial Research and National Institutes of Health grants DE09838 and DE007034.

REFERENCES

Andersson, U. and Radstrom, P. (2002) Physiological function of the maltose operon regulator, MalR, in *Lactococcus lactis*. *BMC Microbiol* **2**: 28.

Boos, W. and Bohm, A. (2000) Learning new tricks from an old dog: MalT of the *Escherichia coli* maltose system is part of a complex regulatory network. *Trends Genet* **16**: 404–409.

Boos, W. and Shuman, H. (1998) Maltose/maltodextrin system of *richia coli*: transport, metabolism, and regulation. *Microbiol Mol Biol Rev* **62**: 204–229.

Bradway, S.D., Bergey, E.J., Jones, P.C. and Levine, M.J. (1989) Oral mucosal pellicle. Adsorption and transpeptidation of salivary components to buccal epithelial cells. *Biochem J* **261**: 887–896.

Chaudhuri, B., Rojek, J., Vickerman, M.M., Tanzer, J.M. and Scannapieco, F.A. (2007) Interaction of salivary alpha-amylase and amylase-binding-protein A (AbpA) of *Streptococcus gordonii* with glucosyltransferase of *S. gordonii* and *Streptococcus mutans*. *BMC Microbiol* **7**: 60.

Douglas, C.W. (1990) Characterization of the alpha-amylase receptor of *Streptococcus gordonii* NCTC 7868. *J Dent Res* **69**: 1746–1752.

Douglas, C.W., Heath, J. and Gwynn, J.P. (1992) Enzymic activity of salivary amylase when bound to the surface of oral streptococci. *FEMS Microbiol Lett* **71**: 193–197.

Francke, C., Kerkhoven, R., Wels, M. and Siezen, R.J. (2008) A generic approach to identify Transcription Factor-specific operator motifs; Inferences for LacI-family mediated regulation in *Lactobacillus plantarum* WCFS1. *BMC Genomics* **9**: 145.

Gopal, S., Berg, D., Hagen, N. *et al.* (2010) Maltose and maltodextrin utilization by *Listeria monocytogenes* depend on an inducible ABC transporter which is repressed by glucose. *PLoS ONE* **5**: e10349.

Gorke, B. and Stulke, J. (2008) Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. *Nat Rev Microbiol* **6**: 613–624.

Gwynn, J.P. and Douglas, C.W. (1994) Comparison of amylase-binding proteins in oral streptococci. *FEMS Microbiol Lett* **124**: 373–379.

Jacobsen, N., Melvaer, K.L. and Hensten-Pettersen, A. (1972) Some properties of salivary amylase: a survey of the literature and some observations. *J Dent Res* **51**: 381–388.

Jenkinson, H.F. (1986) Cell-surface proteins of *Streptococcus sanguis* associated with cell hydrophobicity and coaggregation properties. *J Gen Microbiol* **132**: 1575–1589.

Kolenbrander, P.E. (2000) Oral microbial communities: biofilms, interactions, and genetic systems. *Annu Rev Microbiol* **54**: 413–437.

Li, L., Tanzer, J.M. and Scannapieco, F.A. (2002) Identification and analysis of the amylase-binding protein B (AbpB) and gene (abpB) from *Streptococcus gordonii*. *FEMS Microbiol Lett* **212**: 151–157.

Loo, C.Y., Corliss, D.A. and Ganeshkumar, N. (2000) *Streptococcus gordonii* biofilm formation: identification of genes that code for biofilm phenotypes. *J Bacteriol* **182**: 1374–1382.

Miller, J.L. (1960) Method of pure parotid saliva collection without cannulization. *J Dent Res* **39**: 1075.

Nieto, C., Espinosa, M. and Puyet, A. (1997) The maltose/maltodextrin regulon of *Streptococcus pneumoniae.* Differential promoter regulation by the transcriptional repressor MalR. *J Biol Chem* **272**: 30860–30865.

Nikitkova, A.E., Haase, E.M., Vickerman, M.M., Gill, S.R. and Scannapieco, F.A. (2012) Response of fatty acid synthesis genes to the binding of human salivary amylase by *Streptococcus gordonii*. *Appl Environ Microbiol* **78**: 1865–1875.

Ramasubbu, N., Ragunath, C., Mishra, P.J., Thomas, L.M., Gyemant, G. and Kandra, L. (2004) Human salivary alpha-amylase Trp58 situated at subsite -2 is critical for enzyme activity. *Eur J Biochem* **271**: 2517–2529.

Rogers, J.D., Haase, E.M., Brown, A.E., Douglas, C.W., Gwynn, J.P. and Scannapieco, F.A. (1998) Identification and analysis of a gene (abpA) encoding a major amylase-binding protein in *Streptococcus gordonii*. *Microbiology* **144**(Pt 5): 1223–1233.

Rogers, J.D. and Scannapieco, F.A. (2001) RegG, a CcpA homolog, participates in regulation of amylase-binding protein A gene (abpA) expression in *Streptococcus gordonii*. *J Bacteriol* **183**: 3521–3525.

Rogers, J.D., Palmer, R.J. Jr, Kolenbrander, P.E. and Scannapieco, F.A. (2001) Role of *Streptococcus gordonii* amylase-binding protein A in adhesion to hydroxyapatite, starch metabolism, and biofilm formation. *Infect Immun* **69**: 7046–7056.

Saier, M.H. Jr, Chauvaux, S., Cook, G.M. *et al.* (1996) Catabolite repression and inducer control in Gram-positive bacteria. *Microbiology* **142**(Pt 2): 217–230.

Scannapieco, F.A., Bergey, E.J., Reddy, M.S. and Levine, M.J. (1989) Characterization of salivary alpha-amylase binding to *Streptococcus sanguis*. *Infect Immun* **57**: 2853–2863.

Scannapieco, F.A., Bhandary, K., Ramasubbu, N. and Levine, M.J. (1990) Structural relationship between the enzymatic and streptococcal binding sites of human salivary alpha-amylase. *Biochem Biophys Res Commun* **173**: 1109–1115.

Scannapieco, F.A., Haraszthy, G.G., Cho, M.I. and Levine, M.J. (1992) Characterization of an amylase-binding component of *Streptococcus gordonii* G9B. *Infect Immun* **60**: 4726–4733.

Scannapieco, F.A., Torres, G. and Levine, M.J. (1993) Salivary alpha-amylase: role in dental plaque and caries formation. *Crit Rev Oral Biol Med* **4**: 301–307.

Scannapieco, F.A., Solomon, L. and Wadenya, R.O. (1994) Emergence in human dental plaque and host

distribution of amylase-binding streptococci. *J Dent Res* **73**: 1627–1635.

Shelburne, S.A. 3rd, Sumby, P., Sitkiewicz, I. *et al.* (2006) Maltodextrin utilization plays a key role in the ability of group A Streptococcus to colonize the oropharynx. *Infect Immun* **74**: 4605–4614.

Shelburne, S.A. 3rd, Keith, D., Horstmann, N. *et al.* (2008a) A direct link between carbohydrate utilization and virulence in the major human pathogen group A Streptococcus. *Proc Natl Acad Sci U S A* **105**: 1698–1703.

Shelburne, S.A., Davenport, M.T., Keith, D.B. and Musser, J.M. (2008b) The role of complex carbohydrate catabolism in the pathogenesis of invasive streptococci. *Trends Microbiol* **16**: 318–325.

Tanzer, J.M., Grant, L., Thompson, A. *et al.* (2003) Amylase-binding proteins A (AbpA) and B (AbpB) differentially affect colonization of rats' teeth by *Streptococcus gordonii*. *Microbiology* **149**: 2653–2660.

Terleckyj, B., Willett, N.P. and Shockman, G.D. (1975) Growth of several cariogenic strains of oral streptococci in a chemically defined medium. *Infect Immun* **11**: 649–655.

Vickerman, M.M., Iobst, S., Jesionowski, A.M. and Gill, S.R. (2007) Genome-wide transcriptional changes in *Streptococcus gordonii* in response to competence signaling peptide. *J Bacteriol* **189**: 7799–7807.

Webb, A.J., Homer, K.A. and Hosie, A.H. (2007) A phosphoenolpyruvate-dependent phosphotransferase system is the principal maltose transporter in *Streptococcus mutans*. *J Bacteriol* **189**: 3322–3327.

Webb, A.J., Homer, K.A. and Hosie, A.H. (2008) Two closely related ABC transporters in *Streptococcus mutans* are involved in disaccharide and/or oligosaccharide uptake. *J Bacteriol* **190**: 168–178.