Inactivation of the ptsI gene encoding enzyme I of the sugar phosphotransferase system of Streptococcus salivarius: effects on growth and urease expression

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The urease genes of Streptococcus salivarius 57.I are tightly repressed in cells growing at neutral pH. When cells are cultivated at acidic pH values, the urease genes become derepressed and transcription is enhanced when cells are growing under carbohydrate-excess conditions. Previously, the authors proposed that the bacterial sugar:phosphotransferase system (PTS) modulated the DNA-binding activity by phosphorylation of the urease repressor when carbohydrate was limiting. The purpose of this study was to assess whether enzyme I (EI) of the PTS could be involved in modulating urease expression in response to carbohydrate availability. An EI-deficient strain (ptsI18-3) of S. salivarius 57.I was constructed by insertional inactivation of the ptsI gene. The mutant had no measurable PTS activity and lacked EI, as assessed by Western analysis. The mutant grew as well as the wild-type strain on the non-PTS sugar lactose, and grew better than the parent when another non-PTS sugar, galactose, was the sole carbohydrate. The mutant was able to grow with glucose as the sole carbohydrate, but displayed a 24 h lag time and had a generation time some threefold longer than strain 57.I. The mean OD₆₀₀ attained after 48 h by ptsI18-3 supplied with fructose was 0.16, with no additional growth observed even after 3 d. Urease expression in the wild-type and mutant strains was assessed in continuous chemostat culture. Repression of urease at neutral pH was seen in both strains under all conditions tested. Growth of wild-type cells on limiting concentrations of lactose resulted in very low levels of urease expression compared with growth on PTS sugars. In contrast, under similar conditions, urease expression in ptsI18-3 was restored to levels seen in the parent growing on PTS sugars. Growth under conditions of lactose excess resulted in further derepression of urease, but ptsI18-3 expressed about threefold higher urease activity than 57.I. The results support a role for EI in urease regulation, but also indicate that additional factors may be important in regulating urease gene expression.

Keywords: sugar transport, urease, oral streptococci, gene regulation

INTRODUCTION

Many species of bacteria produce ureases, which catalyse the hydrolysis of urea to two molecules of ammonia and one of carbon dioxide (Mobley & Hausinger, 1989). Bacterial urease gene clusters usually consist of ureABC, which encode the urease apoenzyme, and ureDEFG, which encode accessory proteins needed for incorporation of nickel into the active site of the enzyme (Collins & D’Orazio, 1993; Mobley et al., 1995). Expression of urease genes by bacteria can be constitutive, but expression levels in these species tend to be low. More commonly, urease gene transcription is activated in the presence of urea or when the bacteria are growing under nitrogen-limiting conditions (Collins & D’Orazio, 1993; Mobley et al., 1995). For example,
urease is produced by some *Proteus* spp. only when urea is present in the growth medium, and expression requires the transcriptional activator UreR (D’Orazio & Collins, 1993). Certain *Klebsiella* spp. activate urease gene transcription through global regulatory circuits controlling gene expression in response to nitrogen availability (Collins et al., 1993; Mobley et al., 1995). In contrast, urease expression in *Streptococcus salivarius* 57.I increases as the growth pH becomes more acidic, and expression of urease at acidic pH values can be significantly enhanced in cells growing under carbohydrate-excess conditions (Chen & Burne, 1996). Results of deletion analysis of the dominant promoter controlling *S. salivarius* urease gene expression revealed that repression of transcription at neutral pH occurs through a cis-element located within 100 bp 5′ of the pH- and carbohydrate-sensitive promoter (Chen et al., 1998b). In addition, mutations in the low-molecular-mass form of enzyme IIA for transport of mannose (EIIA<sub>man</sub>) (Vadeboncoeur & Pelletier, 1997) via the bacterial sugar phosphotransferase system (PTS) affect urease gene expression (Chen et al., 1998a).

The PTS is the major high-affinity carbohydrate-transport system in eubacteria (Postma et al., 1993), but components of the PTS are also involved in regulation of gene expression and allosteric modulation of the activity of enzymes and transcription factors. Well-established examples of involvement of enzyme I (EI), HPr and sugar-specific enzyme II (EII) components of the PTS include modification of the activity of DNA- and RNA-binding proteins, control of catalolite repression and modulation of the activity of sugar permeases (Deutscher et al., 1995; Lindner et al., 1999; Martin-Verstraete et al., 1998; Reizer et al., 1993). In oral streptococci, the PTS is regulated by the same environmental factors that control urease expression (Chen & Burne, 1996), but growth at low pH and in high concentrations of carbohydrate are repressive for PTS-dependent sugar transport (Hamilton, 1987). Consequently, it was hypothesized that the PTS might play a role in regulation of urease expression through phosphorylation of a putative urease regulatory protein (Chen & Burne, 1996). To test this hypothesis, spontaneously arising 2-deoxyglucose-resistant mutants that were defective in the production of EIIA<sub>man</sub> were isolated and shown to have about eightfold less urease activity than the wild-type strain when cells were cultured in a chemostat under sugar-limiting conditions (Chen et al., 1998a). Notably, urease expression in the EIIA<sub>man</sub>-deficient mutant could be restored to wild-type levels when cells were grown under carbohydrate-excess conditions. At that time, a working model was proposed in which a repressor protein that senses cytoplasmic pH binds near the urease promoter in cells growing at neutral pH values and blocks urease gene transcription. Further, it was proposed that the DNA-binding activity of the regulatory protein was enhanced by phosphorylation by a component of the PTS. Specifically, under carbohydrate-limiting conditions, the PTS would preferentially phosphorylate the repressor, whereas under carbohydrate-excess conditions, incoming sugars would receive the phosphate group from the PTS and the repressor would exist in a dephosphorylated state.

EI is involved in the transfer of a phosphate group to a number of different regulatory proteins, including anti-terminators (Lindner et al., 1999) and transcriptional activators (Martin-Verstraete et al., 1998). One possible interpretation for the urease expression patterns in EIIA<sub>man</sub> mutants of *S. salivarius* is that under carbohydrate-limiting conditions, the steady-state rate of sugar transport by the PTS is lower as a result of the EI deficiency. This could result in an accumulation of the phosphorylated form of EI, which is capable of phosphorylation of the urease repressor. When carbohydrate is present in excess, sufficient sugar could flow through the system such that EI preferentially transfers the phosphate to the incoming sugars. Therefore, we hypothesized that EI could play a significant role in regulation of urease gene expression. Although spontaneous EI mutants of *S. salivarius* ATCC 29575 have been selected by growth on 2-deoxyxyglucose (Gauthier et al., 1994), no well-defined, otherwise isogenic EI mutants have been described in *S. salivarius*, which has traditionally been refractory to genetic manipulation. In this work, a mutant of *S. salivarius* 57.I lacking all detectable EI was constructed by insertional inactivation of the ptsI gene, the growth characteristics of the mutant on PTS and non-PTS sugars were assessed, and the effects of EI ablation on the expression of urease in steady-state continuous culture were evaluated.

**METHODS**

**Bacterial strains and plasmids.** *S. salivarius* 57.I and its derivatives were maintained on Brain Heart Infusion Agar (Difco) supplemented, if necessary, with 3 µg tetracycline ml<sup>−1</sup> or 750 µg kanamycin (Km) ml<sup>−1</sup>. *S. salivarius* was grown in tryptone/yeast extract (TY) medium (Burne et al., 1987) containing 25 mM glucose, fructose or galactose, or 12.5 mM lactose, as the growth carbohydrate. Alternatively, *S. salivarius* was grown in the chemically defined medium FMC (Terlecky et al., 1975) formulated with the carbohydrates indicated above. Strains of *Escherichia coli* were grown and maintained as described by Sambrook et al. (1989). When antibiotic supplementation of *E. coli* cultures was required, ampicillin or Km was present at 100 µg ml<sup>−1</sup> or 50 µg ml<sup>−1</sup>, respectively.

**DNA manipulations.** Primers for amplification of the ptsI gene from *S. salivarius* 57.I were designed based on the previously published sequence of the *S. salivarius* 25975 ptsI (Gagnon et al., 1992, GenBank accession no. M81756). *Spb*1 and *Ban*HI sites (underlined) were incorporated into the sense (5′-ATGCATGCATGCTTTAAAGGAATCGCAGC-3′) and antisense (5′-TTAGGATCCCGTAAACGTATTCTTTTGAG-3′) primers, respectively. PCR reactions were carried out using *Taq* DNA polymerase (Life Technologies) and Perfect Match Enhancer (Stratagene) with *S. salivarius* 57.I chromosomal DNA as template. Reactions were performed for five cycles at low stringency (40 °C annealing) and for 30 cycles at high stringency (55 °C annealing). Amplification was completed with a final extension step of 72 °C for 10 min. The resulting 1.7 kbp product was cloned in the pCRII vector.
Chromosomal DNA was isolated from *S. salivarius* as previously described (Chen *et al.*, 1996), digested with *HpaI*, electrophoresed through a 0.8% agarose gel, and the fragments transferred to Optitran membrane (Schleicher and Schuell). Purified DNA fragments of the *Km* and *ptsI* genes were isolated from agarose gels using the Elu-Quik kit (Schleicher and Schuell) and radiolabelled using the Random Primers DNA Labelling System (LTI) and [α-<sup>32</sup>P]dATP (New England Nuclear). Integration of the *Km* determinant at the *ptsI* locus by allelic exchange was confirmed by Southern hybridizations (Sambrook *et al.*, 1989) under stringent conditions.

**Western blotting.** Cell extracts for Western blot analysis ( Towbin *et al.*, 1979) were prepared by mechanical disruption in the presence of an equal volume of glass beads (0.1 mm mean diameter) in a Bead Beater (Biospec Products) for a total of 2 min at 4 °C. The concentration of protein in each lysate was measured using the Bio-Rad Protein Assay with BSA as standard. Proteins were separated for Western immunoblotting by 12% SDS-PAGE and transferred to Immobilon P membranes (Millipore) as described by Sambrook *et al.* (1989). An anti-*S. salivarius* EI antibody (generously provided by C. Vadeboncoeur, Université Laval, Quebec, Canada) was used at a dilution of 1:500 and immunoreactive proteins were detected by incubation with goat-anti-rabbit IgG (Kirkegaard and Perry Laboratories) followed by disclosure of bound antibody with diaminobenzidine.

**PTS assay.** Phosphoenolpyruvate (PEP)-dependent phosphotransferase activities were assayed by the method of Kornberg & Reeves (1972) as modified by LeBlanc *et al.* (1979). Briefly, cells were grown to an OD<sub>600</sub> of approximately 0.6 in TY broth supplemented with 12.5 mM lactose or 25 mM glucose for strain 57.I, or 12.5 mM lactose and 750 µg Km ml<sup>−1</sup> Km for *S. salivarius* strain ptsI18-3. Cells were washed, permeabilized with a mixture of toluene and acetone (1:9), and assays were carried out with glucose (10 mM) as the PTS substrate. The rate of PEP-dependent oxidation of NADH was followed at 340 nm with a Beckman DU640 spectrophotometer at a temperature of 37 °C. Controls for spontaneous oxidation of NADH consisted of assay mixtures without added PEP. PTS activities were expressed as nmol NADH oxidized in a PEP-dependent manner min<sup>−1</sup> (mg dry weight of cells)<sup>−1</sup>.

**Measurement of growth.** Growth in FMC medium containing glucose or fructose (25 mM) as PTS sugars, or lactose (12.5 mM) or galactose (25 mM) as non-PTS sugars, was monitored as OD<sub>600</sub> using a Spectronic 20 spectrophotometer. Overnight cultures were grown in FMC-lactose, harvested, washed in sterile deionized water, and used to inoculate FMC containing various carbohydrates. Growth at 37 °C in a 5% CO<sub>2</sub> aerobic atmosphere was followed for 48 h.

**Chemostat culture and urease assays.** *S. salivarius* strains were grown in a Bio-FloIII chemostat (New Brunswick Scientific) with a working volume of 650 ml in TY base medium supplemented with lactose at 12.5 mM for carbohydrate-limiting conditions, or 100 mM lactose for carbohydrate-excess conditions. Cells were grown for a minimum of ten generations for each set of growth parameters before the culture was considered to be at steady state. Urease activity was determined by measuring the amount of ammonia released from urea by intact cells using Nessler’s Reagent (Aldrich) with ammonium sulfate as the standard. Urease activity was expressed as nmol urea hydrolysed min<sup>−1</sup> (mg cell dry weight)<sup>−1</sup>.

**RESULTS AND DISCUSSION**

**Construction of an EI-deficient strain of *S. salivarius* 57.I**

The *ptsI* gene of *S. salivarius* was inactivated by insertion of a Km<sup>+</sup> determinant into the gene as detailed in Methods. Plasmid pCW58, carrying the interrupted *ptsI* gene, was introduced into *S. salivarius* 57.I by electroporation and two Km<sup>+</sup> transformants were selected after 48 h on TY agar containing the non-PTS sugar lactose. One of the transformants, designated ptsI18-3, was selected for subsequent analysis. To confirm that integration of the Km<sup>+</sup> determinant into the *ptsI* gene of *S. salivarius* 57.I had occurred, Southern blot analysis was performed using probes specific for the Km<sup>+</sup> and *ptsI* genes (Fig. 1). Both probes hybridized to a 3.5 kbp *HpaI* fragment.

![Fig. 1. Confirmation of interruption of the *S. salivarius* *ptsI* gene. Chromosomal DNA from *S. salivarius* 57.I and ptsI18-3 was digested with HpaI, transferred to membranes and probed with radiolabelled DNA fragments from the Km<sup>+</sup> determinant or the *ptsI* gene as detailed in Methods. (a) The hybridization patterns reveal the presence of the Km<sup>+</sup> marker in ptsI18-3 migrating with the correct predicted size (3.5 kbp, lane 2), but not in 57.I (lane 1). (b) Using the *ptsI* probe revealed a 2.0 kbp fragment in 57.I corresponding to an intact *ptsI* gene (lane 2) and a 3.5 kbp fragment representing *ptsI* with the Km<sup>+</sup> determinant inserted by double-crossover recombination (lane 1).](image-url)
fragment in ptsI18-3, corresponding to the predicted size of the DNA fragment containing an inactivated ptsI. No hybridization of the Km' probe was detected with wild-type DNA. The ptsI probe hybridized to a 2.0-kbp fragment in S. salivarius ATCC 25975, corresponding to the predicted size of an intact ptsI. Km' transformants were also analysed by Western blot analysis (Fig. 2). S. salivarius 57.1 contained an immunoreactive protein migrating with an apparent molecular mass of 63 kDa, consistent with the mass predicted for EI. No EI was detected in ptsI18-3.

Characterization of EI-deficient S. salivarius

S. salivarius 57.1 was grown in TY containing 25 mM glucose or 12.5 mM lactose, and ptsI18-3 was grown in TY containing 12.5 mM lactose and 750 µg ml⁻¹ Km. When the OD₆₀₀ reached 0.6, the cells were harvested and assayed for PEP-dependent PTS activity using 10 mM glucose as the substrate. For strain 57.1, no significant differences in PTS activity were observed between cells that were grown with the PTS sugar glucose [1157 ± 57 nmol PEP-dependent NADH oxidation min⁻¹ (mg cell dry weight)⁻¹; mean ± SD of triplicate assays on a minimum of six separate cultures] or the non-PTS sugar lactose [927 ± 18.2 nmol min⁻¹ (mg cell dry weight)⁻¹], whereas ptsI18-3 had no detectable PEP-PTS activity. The growth of 57.1 and ptsI18-3 in FMC containing various sugars was monitored (Fig. 3). Strain ptsI18-3 grew extremely poorly on the PTS sugar fructose and did not reach an OD₆₀₀ greater than 0.22 in fructose after 48 h on any of the eight occasions that the growth curves were recorded. No additional growth was observed for up to 72 h. Interestingly, ptsI18-3 could grow fairly well with glucose as the sole carbohydrate, but only after a lag period of about 24 h. A doubling time of approximately 63 min was observed for the wild-type strain growing on glucose, whereas ptsI18-3, once it exited the lag phase, had a doubling time of around 160 min in glucose, and a lower final OD₆₀₀ was attained.

Gauthier et al. (1994) reported the isolation of spontaneously occurring 2-deoxyglucose-resistant derivatives of S. salivarius ATCC 25975 which either produced a truncated EI or had four- to fivefold less EI than the wild-type strain. Similar to the results obtained with ptsI18-3, the spontaneous ptsI mutants grew well on lactose, but did not grow within 24 h on glucose or fructose. Longer incubation periods were not reported. An EI mutant of S. mutans, which was constructed by allelic exchange (Cvitkovitch et al., 1995), lacked all PTS activity, although the mutant did produce a truncated form of EI. Similar to ptsI18-3, the EI-deficient S. mutans strain could grow with glucose as the sole carbohydrate source after a long lag phase. It was suggested that S. mutans produces a H⁺-glucose symporter (Cvitkovitch et al., 1995), and thus S. salivarius may also transport glucose by this PTS-independent route. On the other hand, some sort of compensatory mutation which allows a small subpopulation to grow, albeit poorly, on PTS sugars cannot be excluded without further study.

Fig. 2. Western blot with anti-S. salivarius EI antiserum. Proteins prepared from S. salivarius ptsI18-3 (lane 1) and 57.1 (lane 2) were separated in 12% SDS-PAGE, transferred to filter membrane and probed with a 1:500 dilution of an anti-S. salivarius EI antibody as detailed in Methods.

Fig. 3. Growth of S. salivarius 57.1 and ptsI18-3 in FMC minimal medium. Overnight cultures were washed and diluted into FMC containing PTS (glucose and fructose) or non-PTS (lactose and galactose) sugars. Growth was monitored as OD₆₀₀ as described in Methods. (a) 57.1, glucose (○) or fructose (△); ptsI18-3, glucose (□) or fructose (○). (b) 57.1, lactose (○) or galactose (△); ptsI18-3, lactose (□) or galactose (○).
Table 1. Urease specific activities in *S. salivarius* 57.1 and ptsI18-3 growing in steady-state continuous chemostat culture on limiting or excess concentrations of the indicated carbohydrate sources

Bacterial strains were cultivated for a minimum of ten generations in TY medium with glucose or lactose at the indicated concentrations.

<table>
<thead>
<tr>
<th>Growth pH</th>
<th>Carbohydrate limiting</th>
<th>Urease specific activity</th>
<th>Carbohydrate excess</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>57.1/Glucose† (25 mM)</td>
<td>57.1/Lactose (12.5 mM)</td>
<td>ptsI18-3/Lactose (12.5 mM)</td>
</tr>
<tr>
<td>7.0</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
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<tr>
<td>6.0</td>
<td>2.4 ± 3.2</td>
<td>4.8 ± 0.4</td>
<td>10.4 ± 20.9</td>
</tr>
<tr>
<td>5.5</td>
<td>1.19 ± 1.5</td>
<td>7.7 ± 1.7</td>
<td>8.6 ± 12.7</td>
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<tr>
<td></td>
<td>1.26 ± 0.4</td>
<td>0.7 ± 0.3</td>
<td>1.7 ± 0.7</td>
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<td></td>
<td>1.26 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>3.4 ± 2.8</td>
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<tr>
<td></td>
<td>1.32 ± 0.8</td>
<td>1.2 ± 0.8</td>
<td>3.1 ± 1.2</td>
</tr>
</tbody>
</table>

*Urease specific activity [nmol urea hydrolysed min\(^{-1}\) (mg cell dry weight\(^{-1}\)] was measured in intact cells as detailed in Methods. The values shown are means ± s.d from multiple reaction sets obtained from a minimum of three separate chemostat runs at pH 7.0, 6.0 and 5.0 for the mutant and wild-type at each of the various sugar concentrations. For each of the values reported, multiple reaction sets were performed to obtain data which fell within the linear range of the standard curve. All reaction sets were performed in triplicate and new standard curves were prepared for each assay.

† Values reported for 57.1 grown in limiting (25 mM) glucose are those previously described by Chen & Burne (1996).

The generation times for ptsI18-3 and 57.1 did not differ significantly for growth on lactose (50–55 min), which can be transported by a non-PTS route. In contrast, growth of ptsI18-3 on galactose, also a sugar which can be taken up by a non-PTS permease (Gauthier et al., 1994), was consistently better than that of the parent strain, and strain 57.1 reached a lower final OD\(_{580}\) in galactose than did ptsI18-3 in an equivalent time period. One possible explanation for the differences in the characteristics of the mutant and the parent growing on galactose is that EI, or a factor modified by EI, participates in repression or allosteric regulation of galactose transport or the catabolic pathway(s), which are inducible (Vadeboncoeur & Pelletier, 1997).

Urease activity in *S. salivarius* 57.1 and ptsI18-3

Urease activity was measured in cells growing at steady state in continuous chemostat culture at pH values of 7.0, 6.0 and 5.5, under carbohydrate-limiting or carbohydrate-excess conditions, with lactose as the carbohydrate source (Table 1). Regardless of the growth conditions, urease expression was almost completely repressed at pH 7.0 and urease activity increased in both the mutant and the parent as the pH became more acidic. This result is consistent with previous observations that pH is the dominant influence governing urease expression in *S. salivarius* 57.1 (Chen & Burne, 1996; Chen et al., 1998a). When lactose was supplied to strain 57.1 in carbohydrate-limiting conditions, urease activities were markedly lower than in cells growing on glucose. In contrast, urease levels in ptsI18-3 were about the same at pH 7.0, but were 22-fold higher at pH 6.0, and 11-fold higher at pH 5.5 than in the wild-type growing under the same conditions. Furthermore, under carbohydrate-excess conditions (100 mM lactose), urease activities were approximately twofold higher at pH 7.0 and pH 6.0, and threefold higher at pH 5.5 in the mutant as compared with the parent. Of note, urease activity in 57.1 growing on lactose was always less than that in 57.1 growing on either glucose or fructose at equivalent concentrations when carbohydrate was the limiting growth substrate. Urease activity in the mutant, growing on 12.5 mM lactose at pH values of 6.0 or 5.5, was significantly higher than the urease activity of 57.1 in either glucose or fructose at equivalent concentrations of total carbohydrate. Urease activity in ptsI18-3 growing on 100 mM lactose was also significantly higher than that found in 57.1 growing on glucose or fructose at equivalent concentrations of total carbohydrate at pH 5.5.

Urea can serve as a source of nitrogen for *S. salivarius* and ureolysis can also protect the organisms from lethal environmental acidification (Y. M. Chen and others, unpublished). It is clear from previous work (Chen & Burne, 1996; Chen et al., 1998b) and from data presented in Table 1 that pH is the dominant influence on urease transcription, and that urease expression is tightly repressed at neutral pH values regardless of the concentration of carbohydrate in the growth medium. However, at pH values of about 6 and below, urease expression is strongly dependent on the amount of carbohydrate available to the organisms (Chen & Burne, 1996), and it appears that the PTS exerts its influence on urease gene transcription once the urease operon is derepressed by growth in acidic conditions (Chen et al., 1998a). The PTS primarily governs carbon metabolism, but regulation of the catabolism of nitrogen sources by the PTS is not unprecedented (Postma et al., 1993). In contrast, participation of the PTS in regulation of urease gene expression has not been documented in organisms.
other than \textit{S. salivarius}. In the oral cavity, induction of urease expression at low pH and in the presence of high concentrations of carbohydrate may provide a selective advantage to \textit{S. salivarius}, because ureolysis can protect the organisms from lethal acidification and can provide a source of nitrogen (Y. M. Chen & R. A. Burne, unpublished). Whether this regulatory pathway is peculiar to the homofermentative, non-respiring Gram-positive cocci remains to be determined.

It is possible that EI, perhaps in conjunction with HPr, plays a direct role in governing the activity of the urease repressor. First, it is generally agreed that lactose is a non-PTS sugar for \textit{S. salivarius} (Vadeboncoeur & Pelletier, 1997). Supporting this notion is the observation that insertional inactivation of the endogenous β-galactosidase in \textit{S. salivarius} 57.I eliminates the ability of the organism to grow with lactose as the sole carbohydrate (Y. M. Chen & R. A. Burne, unpublished). Consequently, as proposed in the model of urease regulation and consistent with data presented in Table 1, growth on a non-PTS sugar resulted in lower levels of urease expression, presumably because no sugar is coming in through the PTS. In turn, the accumulation of phosphorylated EI and subsequent transfer of a phosphate group to the urease regulatory element, perhaps via HPr, causes diminished urease expression. Secondly, mutants of \textit{S. salivarius} lacking EI have much higher levels of urease expression at low pH when carbohydrate is limiting, suggesting that EI, particularly at low carbohydrate concentrations, may be an important modulator of urease gene transcription.

It has also been demonstrated that lack of phosphorylation of HPr at histidine residue 15 in bacteria with mutations in \textit{ptsI} could lead to accumulation of the form of HPr which is phosphorylated at serine 46 (Deutscher & Engelmann, 1984; Reizer et al., 1984). Seryl-phosphorylated HPr has multiple regulatory roles, including allosteric modulation of the global regulator of carbon catabolite repression CcpA (Deutscher et al., 1995; Reizer et al., 1993). A CcpA-like protein has been detected by immunological means in \textit{S. salivarius} using antibodies raised to \textit{Bacillus megaterium} CcpA (Küster et al., 1996) and specifically in \textit{S. salivarius} 57.I using an antibody raised to histidine-tagged, purified \textit{S. mutans} CcpA (Y. M. Chen & R. A. Burne, unpublished). Although there are no identifiable consensus catabolite-response elements near the \textit{ure} gene promoter, it cannot be excluded that the effects of EI ablation may be exerted, directly or more likely indirectly, through HPr- or CcpA-dependent regulatory circuits.

There is also reason to believe that factors other than EI may exert control over carbohydrate-concentration-dependent expression of \textit{S. salivarius} urease. Specifically, if EI were entirely responsible for the observed effects, the concentration of lactose on which the wild-type cells were grown should not have had a dramatic influence on urease levels (Table 1), and in particular, the amount of urease expressed in the EI mutant should not have varied at all as carbohydrate concentrations were altered. As suggested above, enhancements in urease expression could occur indirectly through the HPr/CcpA pathway at high carbohydrate concentrations, such as CcpA-dependent repression of the urease regulatory gene. Given the rapidity of induction of urease at low pH (Y. H. Li & R. A. Burne, unpublished), it is more likely that the level of glycolytic intermediates, perhaps fructose 1,6-bisphosphate or glucose 6-phosphate, may also affect the DNA-binding activity of the urease repressor. It also cannot yet be excluded that enhanced urease expression in cells growing in excess carbohydrate may occur through a regulatory pathway involving transcriptional activation once the repressor is dissociated from the promoter region. Preliminary data from deletion analyses indicate the potential for a region upstream of the urease promoter which may be required for optimal expression (Chen et al., 1999), although there is no evidence yet to suggest that the regulation by pH and carbohydrate occur through separate entities.

\textbf{Concluding remarks}

In conclusion, the PTS plays an intimate role in the regulation of expression of the urease gene cluster of \textit{S. salivarius}. Although pH is the dominant influence and repression occurs at neutrality regardless of the sugar concentration in which the cells are growing, transcription of the urease operon becomes very sensitive to carbohydrate availability once the operon is de-repressed. Whether this occurs through the urease repressor as proposed in our working model (Chen et al., 1998b) or through separate pathways can only be determined once the regulatory protein is isolated. Efforts to do so are currently under way.

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\textbf{References}


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