Sequence, Expression, and Function of the Gene for the Nonphosphorylating, NADP-Dependent Glyceraldehyde-3-Phosphate Dehydrogenase of Streptococcus mutans

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We report the sequencing of a 2,019-bp region of the Streptococcus mutans NG5 genome which contains a 1,428-bp open reading frame (ORF) whose putative translation product had 50% identity to the amino acid sequence of the nonphosphorylating, NADP-dependent glyceraldehyde-3-phosphate dehydrogenases (GAPN) from maize and pea. This ORF is located approximately 200 bp downstream of the ptsI gene coding for enzyme I of the phosphoenolpyruvate:sugar phosphotransferase transport system. Mutant BCH150, in which the putative gapN gene had been inactivated, lacked GAPN activity that was present in the wild-type strain, thus positively identifying the ORF as the S. mutans gapN gene. Another strain of S. mutans, DC10, which contains an insertionally inactivated ptsI gene, still possessed GAPN activity, as did S. salivarius ATCC 25975, which contains an insertion element between the ptsI and gapN genes. Since the wild-type S. mutans NG5 lacks both glucose-6-phosphate dehydrogenase and NADH:NAD oxidoreductase activities, the NADP-dependent glyceraldehyde-3-phosphate dehydrogenase is important as a means of generating NADPH for biosynthetic reactions.

In many bacteria, the Embden-Meyerhof or glycolytic pathway functions primarily to provide energy in the form of ATP, while the hexose monophosphate pathway (HMP) operates to provide pentose for nucleic acid formation and NADPH for reductive biosynthetic reactions. The hexose monophosphate pathway (HMP) operates to provide energy in the form of ATP, while the hexose monophosphate pathway (HMP) operates to provide pentose for nucleic acid formation and NADPH for reductive biosynthetic reactions. The HMP portion of the HMP (6). As a consequence, members of this species, long implicated as etiological agents of dental caries (2, 23), must use alternative mechanisms to generate NADPH for the use in reductive biosynthetic reactions. These bacteria overcome this metabolic dilemma by possessing two glyceraldehyde-3-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) of the oxidative portion of the HMP (6). As a consequence, members of this species, long implicated as etiological agents of dental caries (2, 23), must use alternative mechanisms to generate NADPH for the use in reductive biosynthetic reactions. These bacteria overcome this metabolic dilemma by possessing two glyceraldehyde-3-phosphate dehydrogenases (GAPDHS) of distinct molecular weights, pH optima, and inhibition responses to glyceraldehyde-3-phosphate and the reduced coenzymes, suggesting differences in regulation. This seems likely in view of the fact that S. mutans does not appear to contain any NADPH:NAD oxidoreductase (EC 1.6.1.1) activity (5).

We have previously cloned and sequenced the genes for HP (ptsH) and enzyme I (ptsI), the general proteins of the phosphoenolpyruvate:sugar phosphotransferase transport system (PTS) from S. mutans (3). Subsequent analysis of the sequence downstream of the ptsI gene has revealed an open reading frame (ORF) of some 1,400 bp, and since we are interested in isolating and characterizing the genes involved in, or closely related to, the PTS in S. mutans, we sought to identify the activity associated with this ORF. Using the putative translation product of this ORF to search the GenBank database, we found that it had about 50% identity to the nonphosphorylating NADP-dependent GAPDHs (GAPNs) of maize and pea (19). We show here that the ORF immediately downstream of ptsI codes for the gene expressing GAPN activity and describe the isolation of a mutant defective in this activity.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth. S. mutans NG5 (A. Biehlweis, University of Florida, Gainesville) and BM71 (G. Bowden, University of Manitoba, Winnipeg, Manitoba, Canada) were maintained on Todd-Hewitt plates (Difco) and grown for DNA isolation, or for preparation of cell extracts, in TYE-glucose broth (1% tryptone, 0.5% yeast extract, 0.5% glucose, 17 mM K2HPO4). S. mutans BCI150 and DC10 were grown as described above with the medium supplemented with 10 μg of tetracycline per ml and 10 μg of erythromycin per ml, respectively. Escherichia coli SURE (Strategene), E. coli DH5α ( Gibco/BRL), and plasmid pBluescript SK (Strategene) were used as previously described (3). Construction of pDB101, consisting of a 3.5-kb EcoRI-SstI fragment of S. mutans NG5 genomic DNA cloned into pBluescript SK, has been described previously (3), while plasmids pDB125 and pDB123 are deletion subclones of pDB101. Plasmid pDB102 is a pBluescript SK that contains a 1.7-kb HindIII-Sau3A fragment isolated from a recombinant αEMBL3 phage containing a 15-kb region of the S. mutans NG5 genome (3). Plasmid pDB123ΔPm-T was constructed by replacing a 600-bp PstI-MunI region from the NG5 DNA insert in pDB123 with the tetracycline resistance gene isolated as a 4.4-kb EcoRI-PstI fragment from pDF1. Plasmid pDPl, which consists of a 4.4-kb fragment from Tnt16 and contains a gene for tetracycline resistance cloned into the BamHI site of pBG8.

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reaction mixture as described by Crow and Wittenberger (8). NAD
oxidoreductase (transhydrogenase) was assayed by monitoring
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oxidoreductase (transhydrogenase) was assayed by monitoring

Strain BCH150 was constructed by transformation of S. mutans BM71 with pDB2134PM-T that had been linearized by digestion with XhoI (see below). Transformants were selected on Todd-Hewitt plates containing tetracycline. Genomic DNA was isolated from two tetracycline-resistant colonies, and Southern hybridization was carried out with the insert from pDB2134, the tetracycline resistance cassette from pDP1, and pBluescript. The results indicated that the insert from pDB2134PM-T replaced the corresponding wild-type region by double-crossover allelic exchange in both of the tetracycline-resistant colonies and that no vector sequences were present. One of the colonies was selected for further study and named S. mutans BCH150. Restriction sites: E, EcoRI; H, HindIII; M, ManI; Nc, NcoI; N, NspI; P, PstI; S, SalI.

DNA methodology. S. mutans DNA isolation, plasmid isolation, agarose gel electrophoresis, Southern hybridizations, transformation of E. coli, and sequence analysis were performed as previously described (3). Transformation of streptococci was by electroporation as follows. A single colony was used to inoculate 5 ml of Todd-Hewitt broth containing 10% heat-inactivated horse serum (THBS), and the culture was incubated anaerobically at 37°C. The next day, 125 μl of the overnight culture was used to inoculate 5 ml of prewarmed THBS; this culture was grown anaerobically at 37°C until the optical density at 600 nm reached 0.25. The cells were then collected by centrifugation at 4°C, washed three times with 300 mM sucrose, and finally suspended in 100 μl of 300 mM sucrose. DNA was added to the cell suspension; after 1 min on ice, the suspension was transferred to a 0.1-cm cuvette and pulsed with a Bio-Rad GenePulsar set at 1.25 kV and 25 F, with a pulse controller set at 200 ohms.

Preparation of cell extracts. Cell extracts for enzyme assays were prepared as follows. S. mutans or E. coli grown to late log phase, harvested, washed once with triethanolamine buffer (50 mM triethanolamine, 1 mM EDTA, 1 mM dithiothreitol [pH 8.0]), and the cell pellet suspended in 2 ml of triethanolamine buffer containing 0.1 μM peptatin A and 0.1 mM phenylmethylsulfonyl fluoride (TEDPP buffer). Cells of S. mutans were sonicated on ice (six 30-s bursts) (Heat Systems-Ultrasonics model W375, 50% power, standard microtip) with 1 g of glass beads (0.1 to 0.15 mm) per ml, with the suspension kept on ice for 1 min between each sonication. Cells of E. coli were sonicated without the glass beads. Cell debris and glass beads were removed by centrifugation at 12,000 × g for 10 min at 4°C, and the supernatant was dialyzed overnight against 2 liters of TEDPP buffer with one change of the buffer. The dialysate was collected and centrifuged at 12,000 × g for 10 min at 4°C, and the supernatant was stored at −70°C. Protein concentrations of the cytoplasmic preparations were determined by the method of Lowry et al. (25).

Enzyme assays. GAPN was assayed with either NADP+ or NAD+ in the reaction mixture as described by Crow and Wittenberger (8). NAD+–dependent GAPDH (8) and G6PDH (6) were assayed as previously described. NADPH: NAD oxidoreductase (transhydrogenase) was assayed by monitoring the reduction of the NAD+–analog acetylpyridine adenine dinucleotide at 375 nm as described by Hutton et al. (21). All assays were carried out in a total volume of 1 ml at 37°C. The dl-glyceraldehyde-3-phosphate used in the enzyme assays was prepared from dl-glyceraldehyde-3-phosphate diethyl acetal monobarium salt by conversion to the free acid followed by titration to pH 6.0 with 0.5 M triethanolamine (pH 8.3) just before use in the enzyme assays. All enzyme activities are expressed as nanomoles of NAD+ or NADP+ reduced per milligram of protein per minute.

Reagents. dl-Glyceraldehyde-3-phosphate diethyl acetal monobarium salt and acetylpyridine adenine dinucleotide were obtained from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals and enzymes were obtained either from GIBCO/BRL, Sigma, or Fisher Scientific Limited.

Computer-aided analysis. The BLAST program (1) was used to search the GenBank database. Similarities were assessed by using the ALIGN program (11), multiple sequence alignments were carried out with the CLUSTALV program (20), and the ΔG values and structures of transcriptional terminator regions were analyzed by the MULFOLD program (22).

Nucleotide sequence accession number. The sequence shown in Fig. 2 is available in the GenBank database under accession number L38521.

RESULTS

Nucleotide sequence of the gapN gene. We have previously cloned a 3.5-kb EcoRI-SstI fragment from the S. mutans NG5 genome (pDB101) that contains the ORFs for the ptsH and ptsl genes (3) (Fig. 1). The completed sequence analysis of pDB101 revealed an ORF in the same orientation as the ptsl gene starting with a TTG codon at position 233 and extending 1,428 bp to a stop codon TAA beginning at position 1658 (Fig. 2). There is another in-frame TGT codon at position 323 before the first in-frame ATG codon is encountered at position 341 (Fig. 2).

A search of the GenBank database with the putative translation product of the 1,428-bp ORF revealed that it had about 50% identity to GAPNs (encoded by the gapN gene) of maize and pea identified by Habenicht and coworkers (19) (Fig. 3). As an alignment of the S. mutans NG5 putative GAPN with those from pea and maize (Fig. 3) revealed several shared residues near the N terminus, it is likely that the TGT codon at position 233 is the true start codon for the S. mutans gene. Further indication of this is that the TGT codon (position 233) is preceded 8 bp away from a putative ribosome binding site, AGGAG at positions 220 to 224, while downstream, the TGT

FIG. 1. (A) Restriction map of the S. mutans NG5 genomic ptsH-ptsI region showing locations of the ptsH and ptsl genes as previously determined (3) and the location of gapN gene (this report). (B) Restriction fragments subcloned into plasmids and used for sequencing and activity assays in E. coli and for constructing the gapN mutant S. mutans BCH150. Restriction sites: E, EcoRI; H, HindIII; M, ManI; Nc, NcoI; N, NspI; P, PstI; S, SalI.
codon (position 323) and ATG codon (position 341) are not preceded by sequences resembling ribosome binding sites (Fig. 2). The 1,428-bp putative gapN ORF has a putative translation product of 475 amino acids with a molecular mass of 51,145 Da. Base composition analysis of the 1,428-bp gapN ORF showed it to be 59.8% A + T, which is similar to the values for S. mutans NG5 ptsI and ptsH genes, which are 58.4 and 60.6%, respectively (3).

We sequenced 359 bp downstream from the stop codon of the putative gapN gene and could not detect any other ORFs of significance. This region does, however, contain at least two regions of dyad symmetry which could play a role in transcriptional termination. The first one, located at bases 1720 to 1745 (Fig. 2), has a \( \Delta G \) of \(-12.1 \text{ kcal mol}^{-1} \) (\(-50.7 \text{ kJ mol}^{-1} \)) and is followed by a short run of T's characteristic of rho-independent terminators (10). The second region may play a role in transcriptional regulation of a downstream gene that is in the opposite orientation to the putative gapN gene.

**Insertional inactivation of GAPN.** To determine if the putative gapN gene was functional, we mutated the gene in vitro by replacing a small internal fragment of it with a tetracycline resistance gene (pDB123 ΔPM-T; Fig. 1) and then used this mutated gene to transform S. mutans BM71 to tetracycline resistance. The mutated gene replaced the wild-type gene in a tetracycline-resistant transformant (BCH150) by double-crossover allelic exchange as determined by Southern analysis (data not shown). We used BM71 to construct the gapN mutant, since this strain could be made competent for DNA uptake whereas NG5 could not. We had previously carried out Southern hybridizations on various restriction digests of BM71 DNA with the cloned NG5 DNA from the ptsI-gapN region and had shown that the genetic organization in this region of the two strains was identical (data not shown).

Growth of the mutant BCH150 was slower than growth of the wild-type strain BM71 on both solid and liquid media. For example, in TYE-glucose broth, anaerobic cultures of BCH150 were characterized by long lag periods, with an averaged doubling time of 200 ± 20 min (data not shown). This doubling time was threefold longer than that for BM71 (60 ± 12 min).

In addition, while the final terminal pH values of the cultures were similar, i.e., 4.1 ± 0.05 for BM71 and 4.2 ± 0.05 for BCH150, the final cell yield of stationary-phase BM71 cultures was only 60% of that of the BM71 (data not shown). BCH150 was also unable to grow in the presence of oxygen.

**FIG. 2.** Nucleotide sequence of the S. mutans NG5 gapN gene and its deduced amino acid sequence. Putative transcriptional terminator-like regions are indicated by inverted arrows. The sequence begins with the first nucleotide of the ptsI stop codon, with the sequence from positions 1 to 140 previously reported as part of GenBank accession number L15191 (3). Gaps are not counted in the numbering scheme. RBS, ribosome binding site.
Enzyme activity. Assays for GAPN activity were carried out with NADP⁺, using cell extracts from S. mutans NG5, BM71, and BCH150. The results showed that NG5 and BM71 had similar levels of GAPN activity, while BCH150 had less than 2% of the wild-type activity of the enzyme (Table 1). The activity of NAD⁺-dependent GAPN in wild-type strains was 1 to 3% of NADP⁺-dependent activity. Consequently, we designated the ORF in S. mutans NG5 as the gapN gene encoding a nonphosphorylating, NADP⁺-dependent GAPDH. Assays for the phosphorylating, NAD⁺-dependent GAPDH showed that NG5 and BM71 had similar levels, while BCH150 had approximately 50% more activity than the wild-type strains (Table 1).

Earlier work has demonstrated that strains of S. mutans and S. salivarius that possess GAPN lacked both the oxidative portion of the HMP and NADPH:NAD⁺ oxidoreductase (transhydrogenase) (5, 6). To determine if the strains used in our study possessed this part of the HMP, we assayed them for the first oxidative enzyme of the pathway, G6PDH. G6PDH activity in the test strains was very low (Table 1), in keeping with earlier results (6), and 22-fold lower than that in S. sanguis (5).

To determine whether NADPH could be generated by a transhydrogenase-catalyzed reaction, BM71 and BCH150 were assayed for this activity. No activity could be detected (data not shown).

To determine if GAPN activity is dependent on the expression of the ptsI gene, we assayed for GAPN activity in S. mutans DC10, a mutant in which the ptsI gene of BM71 has been inactivated by the insertion of an erythromycin resistance gene (9). The assay revealed that the gapN gene is expressed, although the levels are about 60 to 70% of those of BM71 (Table 1). This finding indicated that the gapN gene can be expressed independently of the presence of a functional enzyme I. DC10 showed levels of GAPDH activity similar to those of BM71 and NG5 (Table 1).

TABLE 1. GAPN, GAPDH, and G6PDH activities in cell extracts of S. salivarius and wild-type and mutant strains of S. mutans

<table>
<thead>
<tr>
<th>Organism</th>
<th>Mean enzyme activity (nmol/min/mg of protein ± SE)</th>
<th>NAD⁺</th>
<th>NAD⁺</th>
<th>GAPDH, NAD⁺</th>
<th>6GPDH, NAD⁺</th>
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<tr>
<td>Streptococci</td>
<td></td>
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<tr>
<td>S. mutans NG5</td>
<td>164 ± 6</td>
<td>4.3 ± 0.7</td>
<td>35 ± 4</td>
<td>2.2 ± 0.3</td>
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<tr>
<td>S. mutans BM71</td>
<td>179 ± 47</td>
<td>7.4 ± 0.7</td>
<td>43 ± 5</td>
<td>2.6 ± 1.0</td>
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<tr>
<td>S. mutans BCH150</td>
<td>2.5 ± 0.4</td>
<td>5.5 ± 0.4</td>
<td>77 ± 6</td>
<td>0.8 ± 0.3</td>
<td></td>
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<tr>
<td>S. mutans DC10</td>
<td>97 ± 24</td>
<td>1.5 ± 0.1</td>
<td>59 ± 11</td>
<td>0.6 ± 0.3</td>
<td></td>
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<tr>
<td>S. salivarius 25975</td>
<td>498 ± 67</td>
<td>5.5 ± 1.5</td>
<td>88 ± 9</td>
<td>3.5 ± 0.6</td>
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<tr>
<td>E. coli SURE with plasmid:</td>
<td></td>
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<tr>
<td>pDB101</td>
<td>7.800 ± 760</td>
<td>2.6 ± 0.4</td>
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<tr>
<td>pDB125</td>
<td>5.400 ± 702</td>
<td>2.4 ± 0.6</td>
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<tr>
<td>pDB123</td>
<td>0.5 ± 0.2</td>
<td>2.5 ± 0.6</td>
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<tr>
<td>pBlueScript</td>
<td>1.5 ± 0.4</td>
<td>4.2 ± 3.4</td>
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—, not done.

FIG. 3. Alignment of the amino acid sequences of S. mutans NG5 GAPN (NG5) with the GAPNs of S. salivarius 25975 (Ssa1), pea (Pisum sativum [Psat]), and maize (Zea mays [Zmays]). The gapN gene of S. salivarius has been only partially sequenced (24). Under the sequences, asterisks indicate identical residues and dots indicate similar residues. Residue numbers are indicated; gaps are not counted in the numbering scheme.
Previously, *S. salivarius* SS2 was shown to possess GAPN activity (5); recently the *ptsH-ptsI* region of *S. salivarius* 25975 was sequenced (15, 17), and an insertion element, IS1139, has been identified downstream of the *ptsI* gene followed by an ORF coding for a putative dehydrogenase (24). By comparison of the putative translation product of the sequenced region of this ORF with the GAPN of *S. mutans* NG5, we showed that this ORF is the gapN gene of *S. salivarius* 25975, as the two proteins have 84% identity in this region (Fig. 3). It has also been shown in *S. salivarius* 25975 that no transcripts originating from *ptsH-ptsI* extend past IS1139 (16). Assays for GAPN activity in *S. salivarius* 25975 revealed levels of activity 40% higher than those of BM71 and NG5 (Table 1), suggesting that the gapN gene can be expressed from its own promoter. *S. salivarius* 25975 exhibited levels of GAPDH activity 50% higher than those of BM71 and NG5 (Table 1). These higher levels of GAPN and GAPDH activities in *S. salivarius* 25975, which has a functional PTS, are mirrored in its growth rate, which is 1.5 to 2 times faster than those of BM71 and NG5 (data not shown).

Expression of GAPN in *E. coli*. To determine if the gapN gene could be expressed in *E. coli*, which has no GAPN activity, we transformed *E. coli*SURE with pDB101 and pDB125 and assayed cell extracts for activity. Both plasmids contain the complete gapN gene, and pDB101 also contains the complete ptsI gene, which we have shown previously is expressed from this construct in *E. coli* (3). The assays revealed high levels of GAPN activity in cells harboring pDB101 or pDB125 compared with the negative control, pBluescript, which showed negligible activity (Table 1). The extremely high levels of activity in these *E. coli* strains is likely due in part to the dosage effect of the cloned gene in the high-copy-number pBluescript vector, as well as more efficient recovery of protein from gram-negative *E. coli* with the extraction procedure used. We were also interested in whether *E. coli* SURE harboring pDB125 had any GAPN activity, as the insert in pDB123 begins at a HindIII site (bases 272 to 277; Fig. 2) 68 bp upstream of the first ATG codon of the gapN ORF (bases 341 to 343; Fig. 2) and continues to the end of the gene as for pDB101 and pDB125 (Fig. 1). This construct conferred no GAPN activity to the extract of the *E. coli* harboring the plasmid (Table 1), showing that the HindIII site either lies within the gapN coding sequence or lies downstream of the gapN promoter, or both. The ability of the *S. mutans* gapN gene to be expressed in *E. coli* could aid in purification of the protein, which could be used in determination of the N-terminal sequence.

**DISCUSSION**

The data reported here show that the putative translation product of an ORF located 233 bp downstream of the *ptsI* gene has 50% identity to the GAPNs of maize and pea reported by Habenicht et al. (19) (Fig. 3). This protein is not related to the classical NAD-specific GAPDH of glycolysis but is a member of the aldehyde dehydrogenase superfamily, which contains specialized and nonspecialized aldehyde dehydrogenases of both prokaryotes and eukaryotes that share between 20 and 40% identity (19, 28). Among eukaryotes, GAPN has been found only among photosynthetic organisms, including algae, in which it is found in the cytosol, catalyzing the irreversible, phosphate-independent oxidation of chloroplast-produced glyceraldehyde-3-phosphate to 3-phosphoglycerate, thereby generating NADPH for biosynthetic processes. In prokaryotes, studies have shown that certain strains of streptococci possess an NADP+ dependent GAPDH (5), and subsequent purification and kinetic studies revealed the enzyme to catalyze a reaction identical to that catalyzed by the GAPN of photosynthetic eukaryotes (8). To show whether the ORF in *S. mutans* was the functional gapN gene, we used an internal region of the cloned ORF to construct a mutant, BCH150, in which the gene was insertionally inactivated (Fig. 1). Assays for GAPN activity clearly showed that BCH150 had negligible activity compared with both BM71 and NG5 (Table 1). Thus, we designated this ORF as the gapN gene of *S. mutans* coding for the protein. To our knowledge, this is the first characterization of this gene in prokaryotes and only the third complete nucleotide sequence to be reported, the other two being the cDNA sequences of the *gapN* genes of maize and pea (19). There has been a report of an NADP+ dependent GAPDH activity in *Pseudomonas aeruginosa* that has been separated from an NAD-specific activity; however, it has not been genetically characterized (29).

Interestingly, on the basis of the sequence analysis of *gapN* (Fig. 2) and alignment of the translation product with the plant GAPNs (Fig. 3), we postulated that the start codon is not the normal ATG but rather a rare TTG. TTG has previously been shown to be used as an initiation codon in gram-positive bacteria, e.g., for the β-lactamase genes of *S. aureus* (26) and *Bacillus cereus* (27), and for the L-(-)-lactate dehydrogenase gene of *Lactobacillus plantarum* (13). Assuming that the gene begins with the TTG codon, the subunit size of the *S. mutans* GAPN would be 51 kDa, which is similar to the size of eukaryotic GAPN subunits (19). However, where the eukaryotic GAPNs have been reported to be homotetramers with the native enzyme thus being about 200 kDa, the *S. mutans* enzyme has been reported to have a size of 350 kDa in its native state (8). If the *S. mutans* native enzyme structure consists of identical subunits, it would have to contain at least six of these subunits. N-terminal sequencing of purified *S. mutans* GAPN will be needed to determine if the TGG codon described above is the true start codon.

The spacing between the *psl* and *gapN* genes (230 bp) and the fact that GAPN function is unrelated to the PTS indicate that the gapN gene is not part of the *pslH-ptsI* transcriptional unit, a fact confirmed by the presence of GAPN activity in *S. salivarius* 25975. Transcription analysis of strains of *S. salivarius* without IS1139 in this region will be needed to determine if gapN can be transcribed with *psl* in those strains. *S. mutans* DC10 also possesses GAPN activity (Table 1) despite a 1.8-kb insert in the *psl* gene (9). In *S. mutans* NG5, we have identified a putative *psl* terminator (Fig. 2) with a ΔG of −22.6 kcal mol⁻¹ (−94.7 kJ mol⁻¹), a more stable structure than the *psl* terminator in IS1139 of *S. salivarius*, which terminates all transcripts reaching it (16). These data taken together indicate that like the *S. salivarius* 25975 GAPN, the *S. mutans* GAPN can be transcribed from its own promoter and is not part of the *pslH-ptsI* transcriptional unit. As we have detected no obvious ORFs within 359 bp downstream of *gapN*, the gene appears to be monocistronic; however, transcriptional analysis of this region in *S. mutans* will be needed to determine if *gapN* can be transcribed with *psl* under some conditions.

Brown and Wittenberger (5, 6) have previously shown that several strains of *S. mutans* and a strain of *S. salivarius* which possess GAPN lack the two oxidative enzymes of the HMP, G6PDH and 6PGDH. In addition, they showed that several related bacteria, *E. faecalis*, *E. faecium*, *S. sanguis*, and *L. lactis*, which possess G6PDH and 6PGDH lack GAPN activity. We have been unable to detect NADH:NADP oxidoreductase activity (transhydrogenase) in *S. mutans*, confirming earlier work by Brown and Wittenberger (5). It is apparent that the physiological role of GAPN is to provide NADPH for biosynthetic reactions, which in other bacteria would be provided by the
HMP. We have shown that GAPN is not essential for cell survival by isolating a mutant strain, \textit{S. mutans} BCH150, in which the \textit{gapN} gene has been inactivated and in which no G6PDH could be detected. The isolation of BCH150 indicates that \textit{S. mutans} possesses alternate means of producing NADPH or that the cell has no absolute requirement for the reduced coenzyme. In \textit{S. salivarius} 25975, genes for the first three enzymes of the tricarboxylic acid cycle, citrate synthase, aconitase, and isocitrate dehydrogenase, have been identified upstream of the \textit{ptsH} gene (12, 14). Isocitrate dehydrogenase, encoded by a functional \textit{idc} gene 200 bp upstream of the \textit{ptsH} gene (14), requires NADP\(^+\), and thus NADPH is produced during the oxidation of isocitrate to 2-ketoglutarate. It is likely that the genetic organization upstream of the \textit{ptsH} in \textit{S. mutans} is the same as that in \textit{S. salivarius} so that a functional \textit{idc} gene would provide a source of NADPH in the GAPN mutant, BCH150. If this alternate means of generating NADPH is functional in \textit{S. mutans}, it would appear to be less efficient than GAPN, as BCH150 exhibited a slower growth rate and achieved lower cell yields compared with similarly grown BM71.

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