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A Binding-Lipoprotein-Dependent Oligopeptide Transport System in *Streptococcus gordonii* Essential for Uptake of Hexa- and Heptapeptides

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Cells of the oral bacterium *Streptococcus gordonii* express three cytoplasmic membrane-bound lipoproteins with apparent molecular masses of 76 to 78 kDa that are the products of three genes (designated *hppA*, *hppG*, and *hppH*). The lipoproteins are immunologically cross-reactive, contain 60% or more identical amino acid residues, and are highly similar to the AmiA, AliA (PlpA), and AliB substrate-binding protein components of an oligopeptide permease in *Streptococcus pneumoniae*. Insertional inactivation of the *hppA* or *hppH* gene resulted in loss of the ability of *S. gordonii* cells to utilize specific peptides of five to seven amino acid residues for growth. An insertion within the COOH-terminal coding region of *hppG* that caused apparent truncation of the HppG polypeptide had a similar effect; however, *S. gordonii* mutants in which HppG polypeptide production was abolished were still able to grow on all oligopeptides tested. Inactivation of *hppA* gene (but not inactivation of the *hppG* or *hppH* gene) caused reduced growth rate of cells in complex medium, slowed the rate of development of competence for transformation, reduced the efficiency of transformation, and increased the resistance of cells to aminopterin. These results suggest that the formation of a solute-binding-protein complex consisting of at least the HppA and the HppH lipopolypeptides is necessary for binding and subsequent uptake of primarily hexa- or heptapeptides by a Hpp (Hexa-heptapeptide permease) system in *S. gordonii*. In addition, Hpp may play a role in the control of metabolic functions associated with the growth of streptococcal cells on complex nitrogen sources and with the development of competence.

Binding-protein-dependent transport systems for the uptake of nutrients by bacteria are members of the ubiquitous family of ATP-binding-cassette (ABC)-type transporters (20). The binding-protein-dependent systems are responsible for the transmembrane uptake into bacteria of a wide range of substrates including saccharides, amino acids, peptides, vitamins, anions, and cations (52). The systems consist of one or two transmembrane protein components forming the solute-specific channel, one or two ATP-binding proteins associated with the cytoplasmic side of the membrane, and an extracellular ligand-specific binding protein. The last component is located in the periplasm of gram-negative bacteria, while in gram-positive bacteria it is lipid modified and is associated with the external face of the cytoplasmic membrane (51).

Uptake systems of this type in bacteria have a wide range of substrate specificities. Some systems have become evolved to transport specific molecules, while others transport a variety of ligands exhibiting similar chemical structures. For the uptake of peptides by bacteria, essentially three ABC-type permeases have been identified that preferentially transport dipeptides, tripeptides, or larger peptides. The dipeptide permease (Dpp) in *Escherichia coli* and *Salmonella typhimurium* is a binding-protein-dependent transport system that transports peptides consisting of two or three L-amino acids (1, 2). The tripeptide permease (Tpp) in *S. typhimurium* has highest affinity for hydrophobic tripeptides (13) but also transports dipeptides. The oligopeptide permease (Opp) in *E. coli* and in *S. typhimurium* transports essentially any peptide consisting of between two and six L- or D-amino acid residues (14, 15, 42). In gram-positive organisms, the specificities and functions of the various peptide permeases are not yet fully understood. Dipeptides are transported by a Dpp system in *Bacillus subtilis* (37), while tripeptides are taken up by an oligopeptide permease (44). Tetra- and pentapeptides are also transported by the Opp system in *B. subtilis* (44, 46) and by a second system that is designated App (30). *Lactococcus lactis* expresses a di- or tripeptide permease and an oligopeptide permease (32, 59) which is necessary for transport of peptides of four to eight residues. In *Streptococcus pneumoniae*, mutations in the *ami* operon resulted in increased resistance to aminopterin (49) and inability of cells to incorporate di-, tri-, tetra-, and heptapeptides (3, 4). The Ami system is structurally and functionally analogous to the Opp system in *S. typhimurium*, except that three separate ligand-binding proteins, which are highly similar in their primary sequences, appear to be involved in delivering peptides to the membrane channel. These binding proteins, which are lipid modified at the NH_2 terminus, are designated AmiA, AliA, and AliB, and they function in the uptake of peptides consisting of two to seven residues into *S. pneumoniae* (3).

Peptide transport systems in gram-positive bacteria are essential for the uptake of peptides as sources of nutrients, but they are also involved in determining other cellular functions and properties. Mutations in the *opp* genes in *B. subtilis* prevent sporulation (44, 46) and affect the development of competence for transformation (36). In *S. pneumoniae*, mutations in genes encoding components of the Ami oligopeptide transporter affect competence development (3, 43) and reduce the ability of bacteria to adhere to human epithelial and endothe-

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TABLE 1. Bacterial strains and plasmids used

Bacterial strain or plasmid	Genotype and/or phenotype	Reference or source
S. gordonii		
DL1 Challis	Wild type	41
OB285	*hppA1*::*ermAM*	This study
OB300	*hppG1*::*tet*	This study
OB302	*hppG1*::*tet hppA1*::*ermAM* (OB285 DNA→OB300)[a]	This study
OB414	*hppG2*::*aphA-3*	This study
OB427	*hppH1*::*tet*	This study
OB447	*hppA1*::*ermAM hppG2*::*aphA-3* (OB285 DNA→OB414)	This study
OB450	*hppG2*::*aphA-3 hppH1*::*tet* (OB414 DNA→OB427)	This study
OB451	*hppA1*::*ermAM hppH1*::*tet* (OB285 DNA→OB427)	This study
OB457	*hppA1*::*ermAM hppG2*::*aphA-3 hppH1*::*tet* (OB285 DNA→OB450)	This study
E. coli		
DH5α	*recA1 endA1 gyrA96 thi-1 hsdR17*(r_K^- m_K^+) *supE44 relA1 deoR* Δ*(lacZYA-argF)*U169 ϕ80*lacZ*ΔM15	16
JM83	*med* Δ(*lac-proAB*)*rspL* ϕ80*lacZ*ΔM15	61
XL1-Blue	*recA1 endA1 gyrA86 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lac*I^qZΔM15 Tn*10* (*tet*^{r})]	Stratagene
Plasmids		
pUC19	2.68 kb; Ap^r ColE1*ori*	61
pBluescript II SK	2.96 kb; Ap^r ColE1*ori*	Stratagene
pGEM-T	3.00 kb; Ap^r ColE1*ori*	Promega
pVA736	7.60 kb; Em^r (*ermAM*) ColE1*ori*pVA380-1*ori*	35
pVA981	7.10 kb; Tc^r (*tet*) ColE1*ori*	55
pVT838	8.10 kb; Sp^r Kn^r (*aphA-3*) ColE1*ori* pVA380-1*ori*	10
pNL9750	5.23 kb; Ap^r Em^r ColE1*ori*; 750 bp fragment of *hppA*	24
pR244	10.57 kb; Ap^r Em^r ColE1*ori*; $amiA_0CDEF$	4
pHF1002	5.76 kb; Ap^r ColE1*ori*; pUC19 with 3,076-bp *Hin*dIII-*Hin*dIII fragment insert containing $amiA_0'$-*amiC-amiD′* from pR244	This study
pHF1001	4.17 kb; Ap^r ColE1*ori*; pUC19 with 1,498-bp *Hin*dIII-*Eco*RV fragment insert containing *amiD′-amiE-amiF′* from pR244	This study

[a] Donor DNA→recipient strain by transformation.

lial cells (7). A role for peptide permeases in bacterial adherence was first suggested for *Streptococcus gordonii*, an organism that is found to be associated with dental and mucosal surfaces in the human oral cavity (12). Inactivation of a chromosomal gene designated *sarA*, which encoded a protein highly similar in sequence to AmiA of *S. pneumoniae* (24, 25, 43), led to deficiencies in serum- or saliva-mediated aggregation of streptococcal cells and in their adherence to oral *Actinomyces* strains (24, 28). We have now characterized further the *sarA* chromosomal locus in *S. gordonii* and show in the present article that it is genetically similar to the *amiA* locus in *S. pneumoniae*. This Ami-like permease in *S. gordonii* also has three associated peptide-binding proteins, designated HppA (previously SarA), HppG, and HppH, and these appear to be essential for the utilization of peptides containing five to seven amino acid residues.

MATERIALS AND METHODS

Bacteria and media. The bacterial strains and plasmids utilized are listed in Table 1. Streptococci were grown at 37°C on TSBY agar (29) in a GasPak System (BBL Microbiology Systems, Cockeysville, Md.). Liquid cultures were grown without shaking in screw-cap tubes or bottles at 37°C in BHY medium or TY-glucose medium (29) or in a defined medium containing 0.8% (wt/vol) glucose. The defined medium consisted of inorganic salts and vitamins (22) with L-amino acids at the following final concentrations: glutamate, 0.5 g/liter; arginine hydrochloride and cysteine hydrochloride, 0.2 g/liter each; aspartate, 0.1 g/liter; glutamine, asparagine, lysine, leucine, isoleucine, valine, alanine, serine, threonine, and glycine, 67 mg/liter each; histidine, tyrosine, phenylalanine, and proline, 17 mg/liter each; and methionine and tryptophan, 2 mg/liter each.

E. coli strains were grown aerobically at 37°C in LB medium (48) or on LB medium supplemented with 15 g of agar per liter and containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (40 μg/ml; Sigma Chemical Co., St. Louis, Mo.) for identifying transformants containing recombinant plasmids. Concentrations of antibiotics used for selection of resistant colonies were as follows: ampicillin, 50 μg/ml (*E. coli*); erythromycin, 50 μg/ml (*E. coli* DH5α) or 1 μg/ml (*S. gordonii*); kanamycin, 25 μg/ml (*E. coli*) or 250 μg/ml (*S. gordonii*); tetracycline, 5 μg/ml (for *E. coli* and *S. gordonii*); and streptomycin, 0.5 mg/ml (*S. gordonii*).

DNA manipulations. Standard molecular biology techniques were performed according to the method described by Sambrook et al. (48). Plasmid DNA was isolated from *E. coli* by using a commercial kit (Promega Corporation, Madison, Wis.). Chromosomal DNA was purified from *S. gordonii* as described elsewhere (23), with the inclusion of 0.1% (vol/vol) diethylpyrocarbonate (BDH, Poole, England) in the cell lysis buffers. Restriction and modifying enzymes (from New England BioLabs, Inc., Beverly, Mass.) were used under the conditions recommended by the manufacturer. Plasmid DNA inserts were sequenced with M13 forward or reverse primers or with custom-synthesized primers (DNA Express; Colorado State University) in an Applied Biosystems model 373A automated DNA sequencer. Sequence data were analyzed with the University of Wisconsin Genetics Computer Group package (9) and the BLAST algorithm (5) for database searches. Conditions for blotting of DNA onto nylon membranes, radioactive labeling of probe DNAs, and hybridization are described elsewhere (29, 38).

PCR amplification. Inverse PCR was utilized to isolate flanking sequences of chromosomal DNA at the *hppGAB* locus. DNA was extracted from *S. gordonii* DL1, digested with *Dra*I or *Hin*dIII, the fragments were diluted to a concentration of 5 μg/ml, and portions (4 μl) were ligated under conditions favoring recircularization of DNA fragments of approximately 2.0 kb (6). PCRs were carried out with 2.5 U of *Taq*I polymerase as follows: 94°C for 4 min (1 cycle); 94°C for 25 s, 56°C for 40 s, and 72°C for 90 s (30 cycles); and 72°C for 4 min (1 cycle). PCR of *Hin*dIII-cut and circularized DNA with primer pair HJ9 (5′GGATGACGGATCTTGGAAGTCAGG; bp 985 to 1008 in Fig. 1) and HJ10 (5′GGAAAATTGTTGGATGAAGCTGCTG; bp 1117 to 1138) generated a fragment of 1,086 bp that was cloned into pGEM-T and led to establishment of the sequence from bp 1 to 1190 in Fig. 1. PCR of *Dra*I-cut and circularized DNA with primer pair 97431R (5′ACCCATTGTTGTTTTTAGAGTT; bp 2307 to 2328) and HJ8 (5′ATAATGTCCATGTGAGCGATG; bp 2491 to 2511) generated a fragment of 1,349 bp that was subsequently blunt-end cloned into *Hin*cII-digested pUC19. This sequence contained the region between bp 2491 (HJ8 oligonucleotide) and bp 3792 (*Dra*I-cut site) in Fig. 1. Lastly, oligonucleotide primers HJ11 (5′GCAGTTTGGTCTACTGGCATATCC; bp 3077 to 4000) and

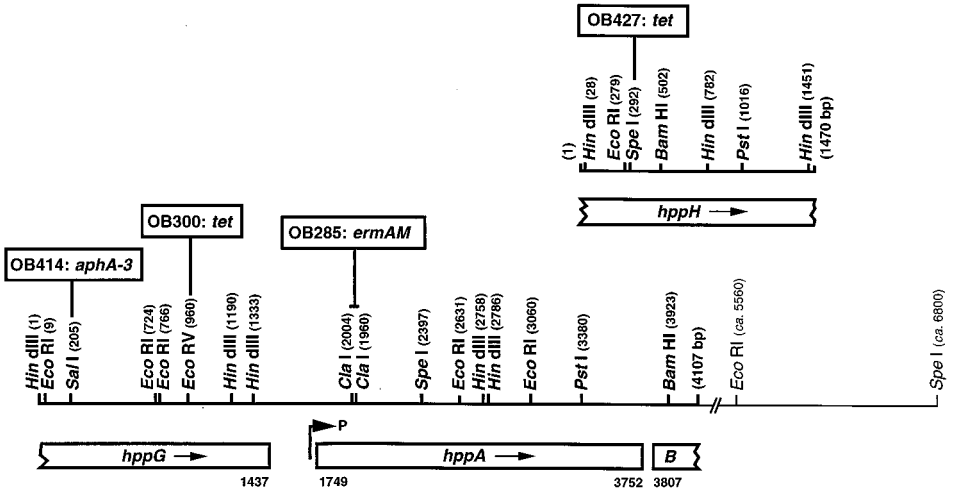

FIG. 1. Map of the *hppGAB* locus in *S. gordonii* and of the *hppH* coding sequence amplified by PCR (upper right). Numbers indicate base pairs from the start of the nucleotide sequences, and those for the restriction enzyme sites correspond to the sites of cleavage. Open reading frames are depicted as boxes, and the start and stop sites of the deduced coding sequences are indicated (inclusive of the stop codons). A putative promoter region (P) is indicated. Arrows indicate directions of transcription of the genes. The sites of insertion of antibiotic resistance markers to generate mutant strains OB414, OB300, OB285, and OB427 are shown above the diagrams. Chromosomal *Eco*RI and *Spe*I sites downstream from the cloned and sequenced *hppGAB* region were mapped by blot hybridization (see text).

HJ17 (5′AACAACAAGTAGGACTGGACGTCC; bp 3512 to 3535) in PCR of *Hin*dII-cut and circularized DNA generated a fragment of 902 bp. This was digested with *Hin*dIII, and the larger (595-bp) fragment corresponding to the region between bp 3512 (HJ17 oligonucleotide) and 4104 (*Hin*dIII site) in Fig. 1 was cloned into pUC19 (digested with a combination of *Hin*dIII and *Hin*cII) and sequenced.

Oligonucleotide primers used to isolate by PCR a fragment (1,470 bp) from the *hppH* gene in *S. gordonii* OB302 were HJ23 (5′TCTAAGGATGGCTTGA CTTATACT; bp 2029 to 2501 in Fig. 1) and HJ24 (5′ACTATCTGTCAACCA AGCTTGAGC; bp 1186 to 1209). PCR was performed under the conditions described above, except with an annealing temperature of 52°C. The fragment was blunt ended and ligated into pUC19 predigested with *Hin*cII, and the insert was sequenced.

DNA-mediated transformation. *E. coli* cells were transformed by a $CaCl_2$ method (8). Competent cells of *S. gordonii* were routinely prepared and transformed as described previously (18). To measure competence development of cells during growth in batch culture, the transformation method was modified as follows. Portions (0.2 ml) of culture in BHY medium containing 2.5% (vol/vol) heat-inactivated fetal bovine serum (Life Technologies, Inc., Gaithersburg, Md.) and 1% (wt/vol) glucose (transformation medium) were removed at intervals and inoculated into 0.8 ml of fresh prewarmed medium, and the medium was incubated at 37°C for 10 min. A portion (10 μl) was removed, diluted, and plated on agar to determine the numbers of CFU per milliliter of culture. Chromosomal DNA (10 ng) from a streptomycin-resistant derivative of *S. gordonii* DL1 was added, the culture was incubated at 37°C for 30 min, and then DNase I solution was added to final concentration 10 μg/ml to stop DNA uptake. The culture was incubated at 37°C for a further 90 min, and suitable dilutions were then plated onto TSBY agar containing streptomycin.

Construction of strains. The streptococcal *ermAM* determinant conferring erythromycin resistance in *E. coli* DH5α or *S. gordonii* was prepared from plasmid pVA736 (35) as a 1.8-kb blunt-end fragment as described previously (29). A blunt-end fragment (3.5 kb) conferring tetracycline resistance (TetM) in both *S. gordonii* and *E. coli* was prepared from plasmid pVA981 by digestion with *Hin*cII (55). The *aphA-3* gene conferring kanamycin resistance (56) was excised as a 1.5-kb fragment by digestion of pVT838 (10) with *Bam*HI and *Sac*I. The fragment was ligated into pUC19 and then excised from the recombinant plasmid by digestion with a combination of *Eco*RI and *Hin*cII and blunt ended with Klenow polymerase. Plasmid constructs containing *ermAM*, *tet*, or *aphA-3* inserted within fragments of streptococcal DNA were prepared in *E. coli* DH5α. To inactivate *S. gordonii* chromosomal genes, plasmids were purified, linearized by digestion with an appropriate restriction enzyme cutting only within the vector sequence, and transformed into *S. gordonii* with selection for appropriate antibiotic resistance. Several transformants from each experiment were purified, and confirmation that the antibiotic resistance markers were inserted at the predicted genetic locations was achieved by blot hybridization with the appropriate streptococcal DNA fragment as a probe and, separately, the antibiotic resistance gene fragment as a probe.

Preparation of cell envelope proteins, electrophoresis, and blotting. Cell envelope fractions of *S. gordonii* cells grown in TY-glucose medium were prepared following breakage of cells with glass beads in a Braun homogenizer as described previously (28). Proteins were solubilized from envelope fractions by incubation at 70°C for 10 min in 0.125 M Tris-hydrochloride (pH 6.8) containing 1% (wt/vol) sodium dodecyl sulfate (SDS) and 0.1% (vol/vol) 2-mercaptoethanol. The suspensions were centrifuged (10,000 × *g* at 20°C for 5 min), and the supernatants were prepared for electrophoresis by adding loading dye (29). Proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) (33) and were stained with Coomassie blue R250 (BDH) or with silver nitrate (39). The molecular masses of proteins were calculated by reference to the mobilities of marker proteins (216- to 16-kDa range, prestained, from Life Technologies). The proteins were transferred to nitrocellulose membranes by electroblotting and were reacted with antiserum diluted 1:500 and with peroxidase-linked secondary antibody as previously described (29).

[^{3}H]palmitate labeling of lipoproteins. The proteins were labeled with [^{3}H]palmitate essentially as described elsewhere (25), except that 150 μCi of [9,10-^{3}H]palmitic acid (40 to 60 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) was added to a final culture volume of 1.2 ml. After incubation of the cultures at 37°C for 3 h, the cells were collected by centrifugation, washed once with distilled water, and disrupted by vortex mixing with glass beads (25). The proteins were solubilized with SDS extraction buffer and separated by electrophoresis, and labeled polypeptides were detected by autoradiography of dried gels as described elsewhere (25).

Amino acid uptake determination. Cells grown to mid-exponential phase in TY-glucose medium or in defined medium containing amino acids and glucose (10 ml) were harvested by centrifugation, washed twice with KPM solution (0.1 M K_2HPO_4 adjusted to pH 6.5 with H_3PO_4 and containing 10 mM $MgSO_4 \cdot 7H_2O$), and suspended in KPM at a density of 10^{10} cells per ml. Portions of this suspension (0.4 ml) were added to KPM solution (1.5 ml) at 37°C, and cells were energized by the addition of 20 mM D-glucose (final concentration) for 7 min or were de-energized by the addition of 20 mM 2-deoxyglucose for 10 min. Uptake was initiated by the addition of 1 μCi of ^{14}C-amino acids (57 mCi/mmol; Amersham Corp.), and samples of cell suspension (0.2 ml) were removed at intervals into ice-cold 0.1 M LiCl (50). Cells were collected on GF/C filters (Whatman International Ltd., Maidstone, England), which were washed and then counted for radioactivity with a liquid scintillation counter.

Growth on peptides. The ability of *S. gordonii* strains to utilize tyrosine- or leucine-containing peptides was tested in defined medium containing glucose and all amino acids except tyrosine or leucine, respectively. Tyrosine-containing peptides were added at a final concentration of 60 nmol/ml, while leucine-containing peptides were added at 70 nmol/ml. Peptides were obtained from Sigma and included Tyr-Tyr, Leu-Leu, Tyr-Gly-Gly, Leu-Leu-Leu, Tyr-Pro-Leu-Gly, Tyr-Gly-Gly-Phe-Leu, Leu-Pro-Pro-Ser-Arg, Tyr-Gly-Gly-Phe-Leu-Lys, Tyr-Gly-Gly-Phe-Met-Arg-Phe, Ser-Ile-Gly-Ser-Leu-Ala-Lys, Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu, and Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu. Microtiter plate 96 wells each containing 90 μl of growth medium were inoculated with 10 μl of washed cell suspension (2×10^7 TY-glucose-grown cells), and the plates were incubated anaerobically at 37°C for 24 h. The optical densities at 490 nm of the cultures were measured with an EA340 Easyreader (SLT Labinstruments, Salzburg, Austria). The extent of culture growth on various peptides was expressed relative to growth of the strain in defined medium containing glucose and all

amino acids by the following scores: ++, 80 to 100% growth; +, growth in the range 30 to 70%; or −, no growth.

Measurement of growth sensitivity to aminopterin. Microtiter plate wells containing 90 μl of defined medium with glucose and amino acids and twofold serial dilutions of 10 μM aminopterin (Boehringer GmbH, Mannheim, Germany) were inoculated with bacterial cells and incubated at 37°C for 24 h, and culture densities were then measured as described above. The MIC for aminopterin for growth was defined as the minimum concentration required to obtain 80% inhibition of growth compared with controls containing no drug.

Nucleotide sequence accession number. The *hppGAB* and the *hppH* sequences have been assigned the GenBank (GSDB) numbers L41358 and L41359, respectively.

RESULTS

Genetic structure of the *hppGAB* locus. Previous work had identified a cloned fragment of *S. gordonii* DL1 DNA of approximately 1.8 kb in λ gt11 as encoding the NH_2-terminal portion of a polypeptide designated SarA (24). Inactivation of the *sarA* gene in *S. gordonii* resulted in the loss from the envelope fraction of cells of a polypeptide with an approximate molecular mass of 76 kDa (24, 28). This polypeptide was subsequently identified as a lipoprotein (25) with an NH_2-terminal leader sequence similar in length and composition to those of the AmiA polypeptide in *S. pneumoniae* and containing the consensus sequence LxyCz (where x = A, S, V, Q or T; y = G or A; and z = S, T, G, A, N, Q, D or F) for signal peptidase cleavage and lipid modification of the NH_2-terminal cysteine residue (60). The original cloned DNA corresponds to the sequence between the *Eco*RI sites at bp 766 and 2631 in Fig. 1. To isolate the entire coding region of the *sarA* gene (which we now designate *hppA*), two inverse PCR amplifications were utilized as described in Materials and Methods to generate clones encompassing the region from bp 2631 to 4107 on the map in Fig. 1. Inverse PCR was also utilized to isolate the chromosomal DNA upstream of the 766-bp *Eco*RI site in Fig. 1.

Analysis of the sequence revealed three open reading frames (shown in Fig. 1). The *hppA* gene encoded a precursor polypeptide of 667 amino acid residues with a predicted molecular mass of 74 kDa. A second open reading frame, designated *hppB*, commenced 55 bp downstream from the translational stop codon of *hppA* (at bp 3752 in Fig. 1) and encoded a COOH-terminal truncated polypeptide of 99 amino acid residues. The third open reading frame, designated *hppG*, extended from bp 1 to 1437 (Fig. 1) and encoded an NH_2-terminal truncated polypeptide of 478 amino acid residues. This portion of the HppG polypeptide and the corresponding region of HppA contained 61% identical amino acids. These sequences were also highly similar to sequences within the AmiA, AliA (PlpA), and AliB polypeptides in *S. pneumoniae* (see below). The HppB polypeptide sequence was 77% identical (86% similarity) to the corresponding NH_2-terminal sequence of AmiC protein, which is one of the two transmembrane subunits of the *S. pneumoniae* Ami (oligopeptide) transporter (4).

The *hppB* sequence was contained within a chromosomal 2.5-kb *Eco*RI fragment and a 4.4-kb *Spe*I fragment, as determined by blot hybridization (Fig. 1). A probe consisting of the *amiC* gene and part of the *amiD* gene cloned from *S. pneumoniae* in plasmid pHF1002 (Table 1) hybridized with both the *Eco*RI and the *Spe*I fragments. A second hybridization probe, cut from plasmid pHF1001 (Table 1) and consisting of the *amiE* gene and part of the *amiF* gene which encode the ATP-binding proteins of the Ami permease, hybridized with the 4.4-kb *Spe*I fragment but not with the 2.5-kb *Eco*RI fragment. Taken together, these data suggest that all of the components of an ABC-type transport system similar to Ami in *S. pneumoniae* are encoded by genes at the *hppGAB* locus.

Inspection of the *hppGAB* sequence for putative transcriptional and translational control signals suggested that transcription of *hppA* was initiated from −35 and −10 promoter-like sequences in the region from bp 1666 to 1696 (Fig. 1). A Shine-Dalgarno (ribosome recognition) sequence, GGAGA (bp 1737 to 1741), was evident 8 bp upstream from the start ATG codon (bp 1749) of the *hppA* coding sequence. The intercistronic region between *hppA* and *hppB* contained a stem-loop structure (bp 3756 to 3777), suggesting possible control of transcriptional read-through into *hppB*. There were 312 bp between the end of the *hppG* coding sequence and the start of the *hppA* coding sequence. In addition to containing the putative *hppAB* promoter sequences, this region contained a possible rho-independent transcriptional terminator (bp 1463 to 1501). Thus, *hppG* was likely to be transcribed independently from *hppAB*.

Insertional mutagenesis was utilized to confirm the designation of the coding regions. A plasmid clone (pNL9750) containing a 742-bp segment (bp 1811 to 2553 in Fig. 1) of the NH_2-terminal coding region of *hppA* (*sarA*) (24) was digested with *Cla*I, which removed a 44-bp fragment (Fig. 1). The plasmid was blunt ended with Klenow polymerase and ligated with the 1.8-kb *ermAM*-containing fragment obtained from pVA736 (see Materials and Methods), and the antibiotic resistance determinant was transformed onto the *S. gordonii* DL1 chromosome to generate mutant strain OB285. To confirm that insertion of *ermAM* had occurred, DNA was extracted from cells of strain OB285 and digested with *Hin*dIII. Blots of agarose gel-separated fragments were then probed at high stringency with a labeled *hppA* fragment excised from pNL9750 or with a labeled 1.8-kb *ermAM*-containing fragment. Each probe reacted with a single band with an approximate size of 3.2 kb, whereas for *Hin*dIII-digested DNA extracted from wild-type cells, a fragment with an approximate size of 1.4 kb (corresponding to bp 1333 to 2758 in Fig. 1) reacted only with the *hppA* probe.

It was known from previous work (25) that the *hppA* gene product was a lipoprotein. Confirmation that *ermAM* insertion had inactivated expression of *hppA* was therefore sought by growing cells in the presence of [^{3}H]palmitate to detect lipoproteins. The wild-type strain DL1 contained 13 lipopolypeptide bands by SDS-PAGE of [^{3}H]palmitate-labeled whole-cell extracts (25), ranging in apparent molecular mass from 78 to 24 kDa. Autoradiographs of SDS-PAGE-separated proteins in extracts of strain OB285 cells showed that the 76-kDa lipopolypeptide band corresponding to HppA was absent in this strain (Fig. 2, lane 2). A second mutant strain (OB300) was then constructed containing *tet* inserted into the *Eco*RV site within the COOH-terminal coding region of *hppG* (Fig. 1). This insertion was confirmed by probing blots of *Hin*dIII-digested DNA extracted from wild-type or strain OB300 cells with an *hppG* gene fragment (bp 766 to 1190 [Fig. 1]) or a *tet*-containing fragment. In the SDS-PAGE profile of [^{3}H]palmitate-labeled proteins from strain OB300 cells, a novel 56-kDa polypeptide was present (Fig. 2, lane 3). Possibly, this was a truncated form of the HppG polypeptide; however, we could not detect corresponding loss of a higher-molecular-mass lipoprotein in extracts from strain OB300 cells. In a double mutant strain (OB302) carrying both *ermAM* and *tet* insertions, the 56-kDa polypeptide was only faintly visible (Fig. 2, lane 4), while there was a somewhat increased amount of a 78-kDa band present. These data suggested the presence of a third *hpp* gene encoding a 78-kDa lipoprotein that normally comigrated with the *hppG* gene product. This idea of a third gene was supported by the observation that on blots of *Hin*dIII-digested chromosomal DNA, extracted from the wild-type strain and separated by agarose gel electrophoresis, three bands with

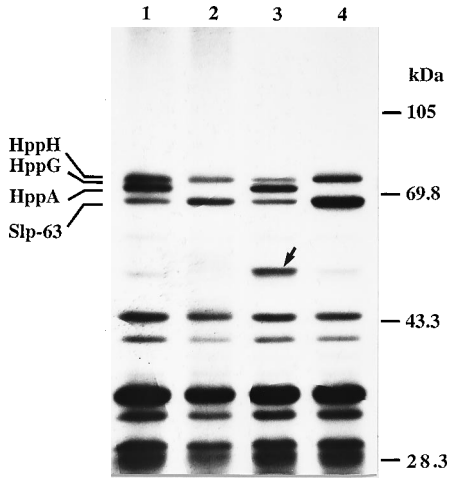

FIG. 2. Autoradiograph of [^{3}H]palmitate-labeled proteins extracted from cells of the wild-type or *hpp* mutant strains of *S. gordonii* and subjected to SDS-PAGE. Lanes: 1, DL1 (wild type); 2, OB285 (*hppA1*); 3, OB300 (*hppG1*); 4, OB302 (*hppA1 hppG1*). The arrow in lane 3 indicates a novel lipoprotein band corresponding possibly to the truncated HppG polypeptide. The positions of molecular mass marker proteins are indicated in kilodaltons. Bands corresponding to HppA, HppG, putative HppH (see Fig. 3), and Slp-63 lipoproteins are indicated.

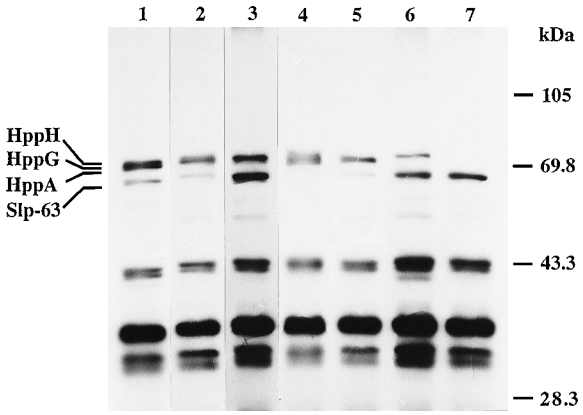

FIG. 3. Autoradiograph of [^{3}H]palmitate-labeled proteins extracted from cells of wild-type or *hpp* mutant strains of *S. gordonii* and subjected to SDS-PAGE. Lanes: 1, DL1 (wild type); 2, OB414 (*hppG2*); 3, OB447 (*hppA1 hppG2*); 4, OB427 (*hppH1*); 5, OB450 (*hppG2 hppH1*); 6, OB451 (*hppA1 hppH1*); 7, OB457 (*hppA1 hppG2 hppH1*). The positions of molecular mass marker proteins (in kilodaltons) are shown. Bands corresponding to HppA, HppG, HppH, and Slp-63 lipoproteins are indicated.

approximate sizes of 1.4, 1.2, and 0.75 kb hybridized at low stringency with a probe consisting of the NH_2-terminal coding sequence of *hppA* (isolated from pNL9750) (results not shown).

Genetic analysis of the *hppH* locus. To isolate the DNA encoding the putative third lipoprotein, two oligonucleotide primers were synthesized on the basis of two well-conserved nucleotide sequences, one present within the COOH-terminal coding regions of *hppA* and *hppG* and the other present within the NH_2-terminal coding regions of *hppA* and *amiA*. To isolate the *hppH* coding sequence, chromosomal DNA prepared from strain OB302 was used as a PCR template, since this strain contained antibiotic resistance markers inserted within the *hppA* and *hppG* genes which would preclude amplification of these gene sequences under the PCR conditions used. A single fragment (1,470 bp) was obtained, cloned into pUC19, and sequenced. To inactivate the *hppH* gene in *S. gordonii*, the 3.5-kb blunt-ended fragment of DNA carrying *tet* was cloned into the unique *Spe*I site (filled using Klenow polymerase) within *hppH* (Fig. 1) and was transformed onto the *S. gordonii* chromosome to generate mutant strain OB427.

It was now possible, then, to construct a series of mutants lacking one, two, or all three of these lipoproteins and to analyze their respective phenotypes. Since *tet* insertion into *hppG* resulted in the formation of a truncated polypeptide, we made an HppG$^-$ mutant (*hppG2*) by insertion of a 1.5-kb fragment of DNA containing the *aphA-3* gene encoding kanamycin resistance at a site (*Sal*I) closer to the NH_2-terminal coding region of the *hppG* gene (Fig. 1). The mutant strain OB414 that was thus generated did not produce HppG polypeptide (as shown below), and double mutants and a triple mutant in the *hpp* genes were subsequently constructed by transformation (Table 1).

Lipoprotein profiles of wild-type and mutant strains. SDS-PAGE profiles of [^{3}H]palmitate-labeled proteins extracted from cells of strains OB414 (*hppG2*) and OB427 (*hppH1*) confirmed that the HppG and HppH lipoproteins comigrated in the 78-kDa band (Fig. 3, lanes 2 and 3). Only in cell extracts from strain OB450 (*hppG2 hppH1*) was the 78-kDa band absent (Fig. 3, lane 5). No labeled proteins were visible in the 76- to 78-kDa region of the gel after SDS-PAGE of cell extracts of the triple mutant strain OB457 (Fig. 3, lane 7), thus confirming that all three genes in this strain were inactivated. These experiments also indicated that the three lipoproteins were expressed more or less independently of one another, although it was possible that in strain OB302 *hppA1 hppG1* (Fig. 2, lane 4) and strain OB447 *hppA1 hppG2* (Fig. 3, lane 3), expression of the *hppH* gene product was elevated. Furthermore, insertional inactivation of *hppA*, but not of *hppG* or *hppH*, resulted in increased intensity of a 63-kDa lipopolypeptide band, designated Slp-63 (Fig. 2 and 3).

Cell envelope proteins prepared from wild-type or mutant strains were separated by SDS-PAGE and reacted with antibodies raised to a partially purified preparation of the HppA polypeptide (24). These antibodies reacted most strongly with bands corresponding to the HppA, HppG, and HppH polypeptides in the wild-type strain and with the Slp-63 polypeptide (Fig. 4). The antibodies also reacted with the novel 56-kDa lipopolypeptide prominent in strain OB300 (Fig. 2), which we suggested might be truncated HppG. The immunoblot profiles obtained for the various mutant strains corroborated entirely the previous results from [^{3}H]palmitate labeling (profiles for strain OB300 and strain OB302 extracts are not shown in Fig.

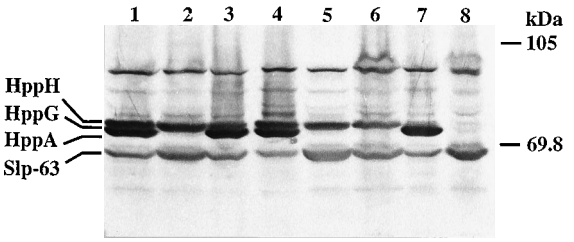

FIG. 4. Western immunoblot of SDS-PAGE-separated cell envelope proteins from *S. gordonii* wild-type or *hpp* mutant strains reacted with antiserum to partially purified HppA polypeptide. Lanes: 1, DL1 (wild type); 2, OB285 (*hppA1*); 3, OB414 (*hppG2*); 4, OB427 (*hppH1*); 5, OB447 (*hppA1 hppG2*); 6, OB451 (*hppA1 hppH1*); 7, OB450 (*hppG2 hppH1*); 8, OB457 (*hppA1 hppG2 hppH1*). The positions of molecular mass marker proteins (in kilodaltons) and the bands corresponding to HppA, HppG, HppH, and Slp-63 polypeptides are indicated.

4). This demonstrated, therefore, that [^{3}H]palmitate-labeling experiments truly revealed differences in the levels of proteins synthesized and not simply differences in the levels of lipid modification.

Relatedness of HppA, HppG, and HppH polypeptides. The inferred amino acid sequence of the HppA precursor polypeptide and the partial sequences of the HppG and HppA polypeptides are aligned in Fig. 5. The sequences had approximately 60% identical amino acids (over the various lengths), and no one of the sequences was more similar to either of the other two. However, of the three Hpp lipoproteins, HppA clearly had greatest similarity (71% identical amino acids) to AliA (3) and PlpA (43) polypeptides from *S. pneumoniae* (AliA and PlpA have 99% identical amino acids). The sequence comparisons in Fig. 5 show that the Hpp group and the AmiA, AliA, and AliB group constitute a family of closely related proteins. Each of these polypeptides (except HppG, for which sufficient sequence has not been obtained) carries the signature sequence for the cluster 5 solute-binding proteins specific for peptides and nickel (52). The HppA, AliA, and AmiA polypeptides all contain the sequence LAACS (LSACG in AliB) at the COOH-terminal end of the leader peptide that corresponds to the consensus sequence for precursor cleavage and lipid modification of prokaryotic polypeptides (60). In addition to showing extensive segments of identical sequence throughout, the six polypeptides all contain regions of sequence similarity (in roughly descending order of similarity) to DciAE dipeptide-binding protein (37) and OppA (44, 46) in *B. subtilis*, OppA in *S. typhimurium* (21), PrgZ (47) and TraC (54) pheromone (peptide)-binding proteins in *Enterococcus faecalis*, Has hyaluronate synthase precursor in *Streptococcus equisimalis* (34), HbpA heme-binding protein in *Haemophilus influenzae* (19), and DppA in *E. coli* (40).

Effects of *hpp* mutations on growth and competence development. Batch cultures of all *S. gordonii* mutant strains carrying *hppA1*::*ermAM* exhibited lower growth rates and lower final growth yields of cells in complex medium (BHY or TY-glucose) than those of the wild-type strain. For example, the doubling time of strain OB285 cells in exponential phase in TY-glucose medium was about 110 min, compared with 60 min for the wild-type strain DL1 (data not shown). Conversely, mutations in the *hppG* or *hppH* gene, either alone or in combination, did not markedly affect growth of cells in this medium. Growth rates of wild-type and mutant strain cells were similar (doubling times approximately 100 min) in defined medium containing amino acids and glucose (not shown).

To determine if *hpp* mutants were impaired in their ability to take up amino acids, the rates of incorporation of ^{14}C-amino acids into energized cells grown in either TY-glucose medium or defined medium containing amino acids and glucose were measured. Amino acid uptake occurred rapidly into energized cells of the wild-type or OB285 (*hppA1*) strain grown in glucose-defined medium (not shown). However, virtually no amino acid uptake was shown by energized cells grown in TY-glucose medium or, as expected, by de-energized cells. Cells of mutant strains OB300 (*hppG1*) and OB427 (*hppH1*) were similar in their responses (not shown).

In *S. gordonii*, competence for DNA uptake and transformation develops in the early exponential phase of growth (41) and is associated with the production of a protease-sensitive extracellular competence factor (45). To test the effect of *hpp* mutations on the development of competence, cells of wild-type or mutant strains were harvested at intervals during growth in transformation (complex) medium (see Materials and Methods) and transformed to streptomycin resistance. In early exponential growth phase at a culture density of 5×10^6

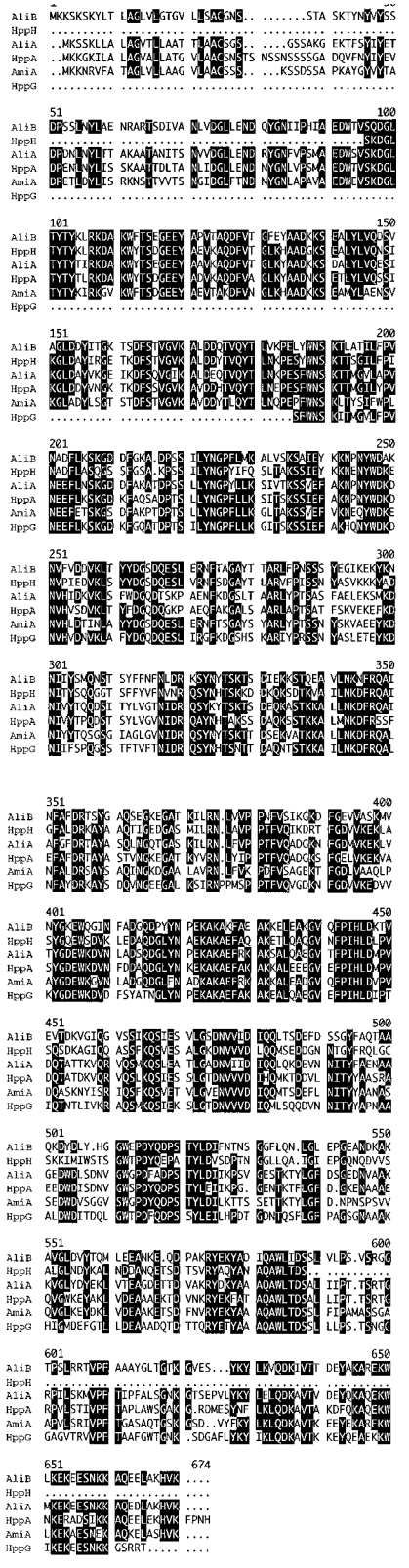

FIG. 5. Alignment of amino acid sequences of streptococcal and pneumococcal peptide-binding lipoproteins obtained with the PILEUP program (GapWeight, 3.0; Gap LengthWeight, 0.1). Partial sequences for HppG and HppH are shown. Identical amino acids are boxed when these are present in four of the sequences. Sequence database accession numbers for the pneumococcal sequences are GenBank Z35135 (AliA), Pir 42857 (AliB), and SwissProt P18791 (AmiA). The sequence of PlpA (GenBank accession number L20556) is 99% identical to AliA and is not included.

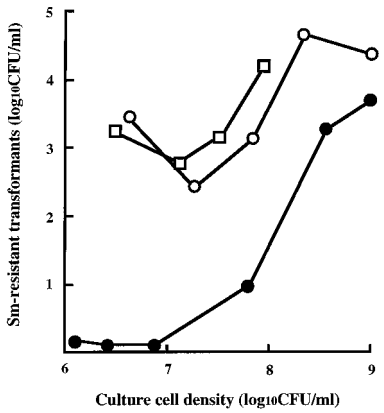

FIG. 6. Development of competence and efficiency of transformation of *S. gordonii* DL1 or *hpp* mutant strains during growth in transformation medium in batch culture. Samples of cultures were removed at intervals, and cells were transformed to streptomycin resistance (see Materials and Methods). The numbers of Sm^r transformant colonies were subsequently determined, together with total viable cell counts at times of sampling (CFU per milliliter). ○, DL1; ●, OB285 (*hppA1*); □, OB300 (*hppG1*). The results are from a typical experiment that was repeated four times. Transformation curves for strains OB302 (*hppA1 hppG1*) and OB457 (*hppA1 hppG2 hppH1*) were similar to those for strain OB285.

CFU per ml, approximately 0.1% wild-type cells were transformed (Fig. 6). The efficiency of transformation decreased for cells in mid- to late-exponential phase; however, high transformability was maintained up to a density of 2×10^8 CFU per ml (Fig. 6). Mutations in *hppG* (Fig. 6) or in *hppH* (not shown) did not affect the development of competence or the transformability of cells. However, development of competence was considerably slower in cultures of OB285 (*hppA1*) (Fig. 6) and in cultures of strains OB447 and OB457 carrying *hppA1*::*ermAM* (not shown). These cultures developed competence only at higher cell densities, and the maximum transformation efficiency of strain OB285 cells was only 30% of that of the wild-type cells (Fig. 6).

Sensitivity to aminopterin and growth on peptides. Since mutations at the *ami* locus in *S. pneumoniae* cause increased resistance to the folate analog aminopterin (4, 49), we determined the effect of *hpp* mutations on the sensitivity of *S. gordonii* to aminopterin (strain DL1 [MIC, 0.62 μM]). Cells of strains carrying mutations in *hppG*, *hppH*, or in both genes were not altered markedly in resistance to aminopterin. Cells of strains OB285, OB447, OB451, and OB457, all carrying the *hppA*::*ermAM* mutation, exhibited a four- to eightfold increase in resistance to aminopterin (MIC, 2.5 to 5.0 μM).

To demonstrate that Hpp proteins were involved in peptide uptake, the growth rates of wild-type or mutant strain cultures were compared in defined medium containing glucose and amino acids except for tyrosine. The latter was replaced by various tyrosine-containing peptides, each of which had Tyr at the NH_2 terminus. As shown in Table 2, all strains were able to utilize for growth peptides consisting of five or fewer amino acid residues. $HppA^-$ and $HppH^-$ mutants were unable to grow on hexa- or heptapeptides, suggesting that HppA and HppH proteins were essential for transport of these peptides. The HppG protein also appeared to be necessary for growth of cells on these peptides; however, this was deduced only for mutant strain OB300 (*hppG1*), in which the HppG polypeptide was apparently truncated (Table 2). Cells of strain OB414 (*hppG2*::*aphA-3*), in which production of the HppG polypeptide was abolished, were apparently able to grow normally on the hexa- and heptapeptides (Table 2). The observation that $HppA^-$ and $HppH^-$ mutant strains were able to grow on Tyr-containing peptides consisting of five or fewer amino acid residues and on peptides consisting of eight or nine amino acid residues supports the idea that growth does not result from extracellular breakdown of larger peptides to smaller peptides and their subsequent uptake. Similar experiments were performed with Leu-containing peptides as a source of leucine for growth. As before, the wild-type strain was able to grow on all of these peptides, whereas all of the mutant strains, with the exception of OB414 (*hppG2*), were deficient in growth on hexa- and heptapeptides (Table 2). These experiments also revealed that the Hpp system was necessary for growth of *S. gordonii* cells on the pentapeptide Leu-Pro-Pro-Ser-Arg.

DISCUSSION

Growth of lactococci, streptococci, and enterococci in their natural environments almost certainly requires that the cells are able to efficiently utilize complex sources of nitrogen such as peptides, proteins, and glycoproteins. The extracellular proteolytic machinery and oligopeptide uptake systems are well-characterized for *L. lactis*, and their concomitant activities are

TABLE 2. Growth of *S. gordonii* wild-type and *hpp* mutant strains in defined medium containing glucose, amino acids, and peptides as a source of either tyrosine (60 μM) or leucine (70 μM)

Peptide	Amino acid source	Growth yield of *S. gordonii* strain (phenotype)[a]:									
		DL1 (A^+ G^+ H^+)	OB285 (A^-)	OB414 (G^-)	OB300 (G')[b]	OB427 (H^-)	OB447 (A^- G^-)	OB302 (A^- G')	OB450 (G^- H^-)	OB451 (A^- H^-)	OB457 (A^- G^- H^-)
YV	Tyr	++	++	+	++	++	+	+	++	++	++
LL	Leu	++	++	++	++	++	++	+	++	++	+
YGG	Tyr	++	++	++	++	++	+	+	++	++	++
LLL	Leu	++	++	++	++	++	++	++	++	++	++
YPLG	Tyr/Leu	++	++/+	++	++/+	++	++/+	+	++	++/+	++/+
YGGFL	Tyr/Leu	++	++	++	++	++	++	+/++	++	++	++
LPPSR	Leu	++	−	++	−	−	−	−	−	−	−
YGGFLK	Tyr/Leu	++	−	+/++	−	−	−	−	−	−	−
YGGFMRF	Tyr	++	−	++	−	−	−	−	−	−	−
SIGSLAK	Leu	++	−	++	−	−	−	−	−	−	−
YGGFMRGL	Tyr/Leu	++	++	++	++/+	++	++	++	+/++	+/++	++
YAGAVVNDL	Tyr	++	++	++	++	++	++	+	++	+	++

[a] Extent of growth relative to that of wild-type (A^+ G^+ H^+) cells in medium with all amino acids. ++, 80 to 100%; +, 30 to 70%; −, no growth.
[b] COOH-terminally truncated HppG polypeptide.

essential for the growth of these bacteria in milk (59). Although the proteolytic and peptide uptake systems operating in the streptococci and enterococci are not so well characterized, it is probable that such systems are important for these organisms to degrade serous and salivary proteins and to utilize the peptide products for growth in the animal host. *L. lactis* contains an Opp system (ABC-type transporter) for peptides, as well as a non-ABC-type transporter, DtpT (17). The lactococcal Opp transport system has broad substrate specificity, and mutations in the *opp* genes prevent uptake and utilization of peptides from four to eight amino acid residues in length (59). The Ami system in *S. pneumoniae* appears to be necessary for growth of pneumococcal bacteria on peptides of two to seven residues (3). We now show that the Hpp system of *S. gordonii*, a permease that seems to be genetically and structurally similar to the Ami permease in *S. pneumoniae*, is essential for the uptake and utilization of primarily hexa- and heptapeptides.

However, the substrate specificity of the Hpp system is not clearcut. The HppA, HppG, and HppH proteins were also essential for the growth of cells on the pentapeptide LPPSR but not for growth on YGGFL. The proteins were not essential for growth of cells on selected di-, tri-, and tetrapeptides, although consistently reduced growth of Hpp^- mutants on YPLG as a source of leucine (but not as a source of tyrosine) might suggest that the Hpp system is at least partly involved in transport of this peptide to satisfy the leucine requirement for growth. The observation that strain OB457 ($HppA^-$ $HppG^-$ $HppH^-$) generally grew normally on di- and tripeptides and on peptides consisting of eight or nine amino acid residues suggests that these substrates are taken up by other peptide transport systems. To determine the precise substrate binding specificities of the Hpp proteins utilizing uptake assays, it will be necessary to first genetically inactivate these other peptide uptake systems.

The evidence presented in this paper indicates that, like the Ami permease in *S. pneumoniae*, the Hpp uptake system in *S. gordonii* has three associated peptide-binding lipoproteins. For the Ami system, it was suggested that the three peptide-binding proteins had overlapping substrate specificities (3) because mutations in either the *amiA* or *aliA* gene did not affect the ability of bacteria to grow on peptides. Only when all three binding-protein genes were inactivated or when the genes encoding the integral membrane proteins or ATPase subunits of the permease were mutated did the bacteria fail to grow on all peptides (3, 4). However, for the Hpp system in *S. gordonii*, the binding-protein requirements for peptide uptake appear to be different. Both HppA and HppH proteins were essential for oligopeptide uptake. This suggests that rather than HppA and HppH proteins acting independently to bind peptides, interaction of these proteins with each other and/or with the integral membrane components of the permease was necessary for peptide binding and uptake to occur. Indeed, if all three lipoproteins normally formed a ligand-binding complex on the cell surface, this could explain the differential phenotypic effects of the two insertional mutations in *hppG*. While loss of HppG protein did not affect the ability of cells of strain OB414 to utilize peptides, the *hppG1* insertion, which was predicted to result in production of truncated HppG protein, prevented growth of strain OB300 cells on peptides. Therefore, we could speculate that a binding-protein complex consisting of HppA and HppH proteins can function adequately in the absence of HppG protein, whereas when a truncated HppG protein is present in the complex, ligand binding and/or uptake is blocked. The formation of a multireceptor cell surface complex would be an efficient way of increasing the affinity of the permease for peptides.

The contrasting effects of the various *hpp* mutations on growth of cells in different media underline how closely linked expression of these genes is to general metabolic control functions. The marked effect of *hppA* gene inactivation on the growth rate of cells in TY-glucose medium, but not in defined medium with glucose and amino acids, indicates that expression of this gene is important for efficient growth of *S. gordonii* cells on complex nitrogen sources. More especially, the necessity for peptide transport systems for growth of *S. gordonii* cells on complex nitrogen sources was demonstrated by the data showing that amino acid uptake was shut down in cells grown in TY-glucose medium which contained both peptides and amino acids.

Inactivation of *hppA* resulted in a number of other phenotypic effects including reduced transformation efficiency and increased resistance of cells to aminopterin. Furthermore, all mutants containing the *hppA*::*ermAM* insertion showed increased production of a 63-kDa lipoprotein that was antigenically related to HppA. This protein seems most likely to be another oligopeptide-binding lipoprotein, the product of a gene transcriptionally upregulated in response to inactivation of *hppA*, and associated with either the Hpp system or an alternative peptide transporter. The delay in development of competence by *hppA* mutants might be related to the lower growth rate of these cells and concomitantly slower response to, or production of, a critical concentration of some factor (intracellular or extracellular) necessary for competence. Oligopeptide transport is crucial to the development of competence by other bacteria. Thus, *plpA* mutants of *S. pneumoniae* showed a delayed onset of competence (43), and *opp* mutants of *B. subtilis* had much-reduced transformation efficiency (46). In *B. subtilis*, the development of competence depends on both the stimulation of a sensor-regulator phosphorelay by an extracellular competence pheromone and the uptake by cells of a competence-stimulating peptide via the Opp system (36). The increased resistance of cells to aminopterin caused by insertional inactivation of *hppA* is at present unexplained. Mutations in the *ami* locus of *S. pneumoniae* result in increased resistance to aminopterin and methotrexate; however, it is not yet clear if these drugs are transported directly by the Ami permease (57, 58). The possibility that the multiple effects arising from *ermAM* insertion into *hppA* were owing to aberrant transcription of downstream genes cannot be completely ruled out. However, the effects of *hppA* gene inactivation on transformation efficiency and aminopterin sensitivity in *S. gordonii* are consistent with similar effects observed following inactivation of *ami* or *ami*-like genes in *S. pneumoniae* (3, 4, 43).

The Hpp and Ami-like binding proteins are the largest solute-binding proteins so far identified. Their sequence similarities to the sequences of peptide-binding proteins from different bacterial species reside chiefly within the NH_2-terminal domain II as defined for OppA from *S. typhimurium* by Tame et al. (53). This domain is not predicted to be involved in substrate binding (53). The larger size of the HppA protein in comparison with OppA in *S. typhimurium* is wholly accounted for by an extended COOH-terminal sequence in HppA, corresponding to domain III in OppA (53). Amino acid residues within domain III of OppA are important for the formation of the oligopeptide-binding pocket and contribute to the versatility in substrate-binding (53). The extended COOH-terminal sequence in the HppA polypeptide might be related to different substrate-binding specificity and/or might be significant in protein-protein interactions between the Hpp binding proteins and membrane channel.

The *hppA* gene was identified originally following insertional

mutagenesis experiments. HppA$^-$ mutant cells of *S. gordonii* were reduced in their ability to be aggregated in human saliva or serum and were deficient in adherence to strains of *Actinomyces naslundii* (28). These deficiencies in the adherence-related properties of cells may have arisen as a result of the pleiotropic effects of mutation in *hppA*. Recently, however, mutations in the *amiA* and *plpA* (*aliA*) genes have been shown to affect adherence of pneumococcal cells to epithelial and endothelial cells (7). There is also now good evidence that other binding-protein-dependent permeases may act as adhesins in streptococci (11, 26, 27, 31). It is not yet possible, then, to rule out the idea that the Hpp system in *S. gordonii* acts not only as a peptide transporter but also as an adhesin complex.

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REFERENCES

1. **Abouhamad, W. N., and M. D. Manson.** 1994. The dipeptide permease of *Escherichia coli* closely resembles other bacterial transport systems and shows growth-phase-dependent expression. Mol. Microbiol. **14:**1077–1092.
2. **Abouhamad, W. N., M. D. Manson, M. M. Gibson, and C. F. Higgins.** 1991. Peptide transport and chemotaxis in *Escherichia coli* and *Salmonella typhimurium*: characterization of the dipeptide permease and the dipeptide-binding protein. Mol. Microbiol. **5:**1035–1047.
3. **Alloing, G., P. de Philip, and J.-P. Claverys.** 1994. Three highly homologous membrane-bound lipoproteins participate in oligopeptide transport by the ami system of gram-positive *Streptococcus pneumoniae*. J. Mol. Biol. **241:** 44–58.
4. **Alloing, G., M.-C. Trombe, and J.-P. Claverys.** 1990. The *ami* locus of the gram-positive bacterium *Streptococcus pneumoniae* is similar to binding protein-dependent transport operons of gram-negative bacteria. Mol. Microbiol. **4:**633–644.
5. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
6. **Collins, F. S., and S. M. Weissman.** 1984. Directional cloning of DNA fragments at a large distance from initial probe: a circularization method. Proc. Natl. Acad. Sci. USA **81:**6812–6816.
7. **Cundell, D. R., B. J. Pearce, J. Sandros, A. M. Naughton, and H. R. Masure.** 1995. Peptide permeases from *Streptococcus pneumoniae* affect adherence to eukaryotic cells. Infect. Immun. **63:**2493–2498.
8. **Dagert, M., and S. D. Ehrlich.** 1979. Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. Gene **6:**23–28.
9. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
10. **Fenno, F. C., A. Shaikh, and P. Fives-Taylor.** 1993. Characterization of allelic replacement in *Streptococcus parasanguis*: transformation and homologous recombination in a 'nontransformable' streptococcus. Gene **130:**81–90.
11. **Fenno, J. C., A. Shaikh, G. Spatafora, and P. Fives-Taylor.** 1995. The *fimA* locus of *Streptococcus parasanguis* encodes an ATP-binding membrane transport system. Mol. Microbiol. **15:**849–863.
12. **Frandsen, E. V. G., V. Pedrazzoli, and M. Kilian.** 1991. Ecology of viridans streptococci in the oral cavity and pharynx. Oral Microbiol. Immunol. **6:** 129–133.
13. **Gibson, M. M., M. Price, and C. F. Higgins.** 1984. Genetic characterisation and molecular cloning of the tripeptide permease (*tpp*) genes of *Salmonella typhimurium*. J. Bacteriol. **160:**122–130.
14. **Goodell, E. W., and C. F. Higgins.** 1987. Uptake of cell wall peptides by *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. **169:**3861–3865.
15. **Guyer, C. A., D. G. Morgan, and J. V. Staros.** 1986. Binding specificity of the periplasmic oligopeptide-binding protein from *Escherichia coli*. J. Bacteriol. **168:**775–779.
16. **Hanahan, D.** 1985. Techniques for transformation of *E. coli*, p. 109–135. *In* D. M. Glover (ed.), DNA cloning, vol. 1. IRL Press, Oxford.
17. **Hagtang, A., E. R. S. Kunji, K. J. Leenhouts, B. Poolman, and W. N. Konings.** 1994. The di- and tripeptide transporter of *Lactococcus lactis*. J. Biol. Chem. **269:**11391–11399.
18. **Haisman, R. J., and H. F. Jenkinson.** 1991. Mutants of *Streptococcus gordonii* Challis over-producing glucosyltransferase. J. Gen. Microbiol. **137:**483–489.
19. **Hanson, M. S., C. Slaughter, and E. J. Hansen.** 1992. The *hbpA* gene of *Haemophilus influenzae* type b encodes a heme-binding lipoprotein conserved among heme-dependent *Haemophilus* species. Infect. Immun. **60:** 2257–2266.
20. **Higgins, C. F.** 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. **8:**67–113.
21. **Hiles, I. D., M. P. Gallagher, D. J. Jamieson, and C. F. Higgins.** 1987. Molecular characterization of the oligopeptide permease of *Salmonella typhimurium*. J. Mol. Biol. **195:**125–142.
22. **Jenkinson, H. F.** 1986. Cell-surface proteins of *Streptococcus sanguis* associated with cell hydrophobicity and coaggregation properties. J. Gen. Microbiol. **132:**1575–1589.
23. **Jenkinson, H. F.** 1987. Novobiocin-resistant mutants of *Streptococcus sanguis* with reduced cell hydrophobicity and defective in coaggregation. J. Gen. Microbiol. **133:**1909–1918.
24. **Jenkinson, H. F.** 1991. Phenotypic effects of inactivating the gene encoding a cell-surface binding-protein in *Streptococcus gordonii* Challis, p. 284–288. *In* G. M. Dunny, P. P. Cleary, and L. L. McKay (ed.), Genetics and molecular biology of streptococci, lactococci, and enterococci. American Society for Microbiology, Washington, D.C.
25. **Jenkinson, H. F.** 1992. Adherence, coaggregation, and hydrophobicity of *Streptococcus gordonii* associated with expression of cell-surface lipoproteins. Infect. Immun. **60:**1225–1228.
26. **Jenkinson, H. F.** 1994. Adherence and accumulation of oral streptococci. Trends Microbiol. **2:**209–212.
27. **Jenkinson, H. F.** 1994. Cell-surface protein receptors of oral streptococci. FEMS Microbiol. Lett. **121:**133–140.
28. **Jenkinson, H. F., and R. A. Easingwood.** 1990. Insertional inactivation of the gene encoding a 76-kilodalton cell-surface polypeptide in *Streptococcus gordonii* Challis has a pleiotropic effect on cell surface composition and properties. Infect. Immun. **58:**3689–3697.
29. **Jenkinson, H. F., S. D. Terry, R. McNab, and G. W. Tannock.** 1993. Inactivation of the gene encoding surface protein SspA in *Streptococcus gordonii* DL1 affects cell interactions with human salivary agglutinin and oral actinomyces. Infect. Immun. **61:**3199–3208.
30. **Koide, A., and J. A. Hoch.** 1994. Identification of a second oligopeptide transport system in *Bacillus subtilis* and determination of its role in sporulation. Mol. Microbiol. **13:**417–426.
31. **Kolenbrander, P. E., R. N. Andersen, and N. Ganeshkumar.** 1994. Nucleotide sequence of the *Streptococcus gordonii* PK488 coaggregation adhesin gene, *scaA*, and ATP-binding cassette. Infect. Immun. **62:**4469–4480.
32. **Kunji, E. R. S., E. J. Smid, R. Plapp, B. Poolman, and W. N. Konings.** 1993. Di-tripeptides and oligopeptides are taken up via distinct transport mechanisms in *Lactococcus lactis*. J. Bacteriol. **175:**2052–2059.
33. **Laemmli, U. K., and M. Favre.** 1973. Maturation of the head of bacteriophage T4. I. DNA packaging events. J. Mol. Biol. **80:**575–599.
34. **Lansing, M., S. Lellig, A. Mausolf, I. Martini, F. Crescenzi, M. O'Regan, and P. Prehm.** 1993. Hyaluronate synthase: cloning and sequencing of the gene from *Streptococcus* sp. Biochem. J. **289:**179–184.
35. **Macrina, F. L., K. R. Jones, and P. H. Wood.** 1980. Chimeric streptococcal plasmids and their use as molecular cloning vehicles in *Streptococcus sanguis* (Challis). J. Bacteriol. **143:**1425–1435.
36. **Magnuson, R., J. Solomon, and A. D. Grossman.** 1994. Biochemical and genetic characterization of a competence pheromone from *Bacillus subtilis*. Cell **77:**207–216.
37. **Mathiopoulos, C., J. P. Mueller, F. J. Slack, C. G. Murphy, S. Patankar, G. Bukusoglu, and A. L. Sonenshein.** 1991. A *Bacillus subtilis* dipeptide transport system expressed early during sporulation. Mol. Microbiol. **5:**1903–1913.
38. **McNab, R., and H. F. Jenkinson.** 1992. Gene disruption identifies a 290 kilodalton cell-surface polypeptide conferring hydrophobicity and coaggregation properties in *Streptococcus gordonii*. Mol. Microbiol. **6:**2939–2949.
39. **Morrissey, J. H.** 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. Anal. Biochem. **117:** 307–310.
40. **Olson, E. R., D. S. Dunyak, L. M. Jurss, and R. A. Poorman.** 1991. Identification and characterization of *dppA*, an *Escherichia coli* gene encoding a periplasmic dipeptide transport protein. J. Bacteriol. **173:**234–244.
41. **Pakula, R., and W. Walczak.** 1963. On the nature of competence of transformable streptococci. J. Gen. Microbiol. **31:**125–133.
42. **Payne, J. W., and C. Gilvarg.** 1968. Size restriction on peptide utilization in *Escherichia coli*. J. Biol. Chem. **243:**6292–6299.
43. **Pearce, B. J., A. M. Naughton, and H. R. Masure.** 1994. Peptide permeases modulate transformation in *Streptococcus pneumoniae*. Mol. Microbiol. **12:** 881–892.
44. **Perego, M., C. F. Higgins, S. R. Pearce, M. P. Gallagher, and J. A. Hoch.** 1991. The oligopeptide transport system of *Bacillus subtilis* plays a role in the initiation of sporulation. Mol. Microbiol. **5:**173–185.
45. **Ranhand, J. M., R. M. Cole, and C. G. Leonard.** 1970. A comparison of streptococcal competence factors produced by strain Challis in chemically defined and complex media. J. Gen. Microbiol. **65:**131–138.
46. **Rudner, D. Z., J. R. LeDeaux, K. Ireton, and A. D. Grossman.** 1991. The

spoOK locus of *Bacillus subtilis* is homologous to the oligopeptide permease locus and is required for sporulation and competence. J. Bacteriol. **173:** 1388–1398.

47. **Ruhfel, R. E., D. A. Manias, and G. M. Dunny.** 1993. Cloning and characterization of a region of the *Enterococcus faecalis* conjugative plasmid, pCF10, encoding a sex pheromone-binding function. J. Bacteriol. **175:**5253–5259.
48. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
49. **Sicard, A. M., and H. Ephrussi-Taylor.** 1965. Genetic recombination in DNA-induced transformation of pneumococcus. II. Mapping the *amiA* locus. Genetics **52:**1207–1227.
50. **Smid, E., A. J. M. Driessen, and W. N. Konings.** 1989. Mechanism and energetics of dipeptide transport in membrane vesicles of *Lactococcus lactis*. J. Bacteriol. **171:**292–298.
51. **Sutcliffe, I. C., and R. R. B. Russell.** 1995. Lipoproteins of gram-positive bacteria. J. Bacteriol. **177:**1123–1128.
52. **Tam, R., and M. H. Saier, Jr.** 1993. Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. Microbiol. Rev. **57:**320–346.
53. **Tame, J. R. H., G. N. Murshudov, E. J. Dodson, T. K. Neil, G. G. Dodson, C. F. Higgins, and A. J. Wilkinson.** 1994. The structural basis of sequence-independent peptide binding by OppA protein. Science **264:**1578–1581.
54. **Taniomoto, K., F. Y. An, and D. B. Clewell.** 1993. Characterization of the *traC* determinant of the *Enterococcus faecalis* hemolysin-bacteriocin plasmid pAD1: binding of sex pheromone. J. Bacteriol. **175:**5260–5264.
55. **Tobian, J. A., M. L. Cline, and F. L. Macrina.** 1984. Characterization and expression of a cloned tetracycline resistance determinant from the chromosome of *Streptococcus mutans*. J. Bacteriol. **160:**556–563.
56. **Trieu-Cuot, P., and P. Courvalin.** 1983. Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3′5′-aminoglycoside phosphotransferase type III. Gene **23:**331–341.
57. **Trombe, M. C.** 1985. Entry of methotrexate into *Streptococcus pneumoniae*: a study on a wild-type strain and a methotrexate resistant mutant. J. Gen. Microbiol. **131:**1273–1278.
58. **Trombe, M. C., G. Laneelle, and A. M. Sicard.** 1984. Characterization of a *Streptococcus pneumoniae* mutant with altered electric transmembrane potential. J. Bacteriol. **158:**1109–1114.
59. **Tynkkynen, S., G. Buist, E. Kunji, J. Kok, B. Poolman, G. Venema, and A. Haandrikman.** 1993. Genetic and biochemical characterization of the oligopeptide transport system of *Lactococcus lactis*. J. Bacteriol. **175:**7523–7532.
60. **Yamaguchi, K., F. Yu, and M. Inouye.** 1988. A single amino acid determinant of the membrane localization of lipoproteins in *E. coli*. Cell **53:**423–432.
61. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.