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Sodium Ion-Driven Serine/Threonine Transport in *Porphyromonas gingivalis*

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Porphyromonas gingivalis **is an asaccharolytic, gram-negative bacterium that relies on the fermentation of amino acids for metabolic energy. When grown in continuous culture in complex medium containing 4 mM (each) free serine, threonine, and arginine, *P. gingivalis* assimilated mainly glutamate/glutamine, serine, threonine, aspartate/asparagine, and leucine in free and/or peptide form. Serine and threonine were assimilated in approximately equal amounts in free and peptide form. We characterized serine transport in this bacterium by measuring uptake of the radiolabeled amino acid in washed cells of *P. gingivalis* energized with a tetrapeptide not containing serine. Serine was transported by a single system with an affinity constant for transport (K_t) of 24 μM that was competitively inhibited by threonine. Serine transport was dependent on sodium ion concentration in the suspending buffer, and the addition of the ionophore gramicidin caused the inhibition of serine uptake. Together these data indicate that serine transport was sodium ion-motive force driven. A *P. gingivalis* gene potentially encoding a serine transporter was identified by sequence similarity to an *Escherichia coli* serine transporter (SstT). This *P. gingivalis* gene, designated *sstT*, was inactivated by insertion of a *Bacteroides tetQ* gene, producing the mutant W50ST. The mutant was unable to transport serine, confirming the presence of a single serine transporter in this bacterium under these growth conditions. The transport of serine by *P. gingivalis* was dependent on the presence of free cysteine in the suspension buffer. Other reducing agents were unable to stimulate serine uptake. These data show that *P. gingivalis* assimilates free serine and threonine from culture media via a cysteine-activated, sodium ion-motive force-driven serine/threonine transporter.**

Periodontal diseases are complex, bacterial-associated inflammatory diseases of the supporting tissue of the teeth. The change from a periodontally healthy site to one undergoing destruction is accompanied by a large increase in the relative abundance of a small number of opportunistic pathogens, including *Porphorymonas gingivalis* (9, 31). To understand this shift in the species composition of subgingival plaque during disease initiation and progression, information on the ability of periodontopathogenic bacteria to compete for, acquire, and utilize nutrients, especially energy sources, is crucial. *P. gingivalis* is a black-pigmented, gram-negative, asaccharolytic anaerobe that relies on the fermentation of amino acids for the production of metabolic energy.

To date, the majority of work investigating the utilization of amino acids by *P. gingivalis* has involved growth studies in a range of media supplied with different amino acid sources. While early reports suggested that *P. gingivalis* was capable of at least limited growth in defined media with amino acids supplied only in the free form (25, 36), later studies have indicated that *P. gingivalis* requires a source of peptides for sustained growth (14, 17). The Arg- and Lys-specific extracellular cysteine proteinases of *P. gingivalis*, RgpA, RgpB, and Kgp, have been demonstrated to be essential for the hydrolysis of proteins and the production of peptides of the desired size to support bacterial growth (28).

The lack of growth of *P. gingivalis* in defined media with amino acids supplied only in the free form has been taken to indicate that *P. gingivalis* may not be able to utilize free amino acids due to a lack of suitable transport systems (17). Tang-Larsen et al. (34) have shown, by an indirect method, that *P. gingivalis* does not utilize the sulfur-containing amino acids cysteine and methionine when supplied in the free form but could metabolize these amino acids when supplied in peptide form. Similar results have also been reported by Takahashi et al. (33), who concluded that the amino acids glutamate and aspartate were metabolized when supplied in peptide form but not when supplied as free amino acids. Although Shah and Gharbia (27) reported uptake of free amino acids by *P. gingivalis*, this was by the indirect and nonspecific method of measuring changes in conductance due to the disappearance of metabolites from the suspension buffer.

Here we report on the assimilation of free amino acids by *P. gingivalis* grown in continuous culture and on the first direct study of an amino acid transport system in this bacterium. By characterizing uptake of radiolabeled serine we have identified a sodium ion-driven serine/threonine transporter in *P. gingivalis*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Lyophilized cultures of *P. gingivalis* W50 (ATCC 53978) were obtained from the culture collection of the School of Dental Science, the University of Melbourne. *P. gingivalis* was grown anaerobically and maintained on blood agar (Oxoid Blood Agar Base 2 supplemented

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with 10% defibrinated horse blood) with weekly subculturing. Batch cultures of *P. gingivalis* were grown at 37°C in 3.7% brain heart infusion (BHI) broth (Oxoid) supplemented with 0.5 g of cysteine-HCl per liter and 5 mg of bovine hemin (Sigma, St. Louis, Mo.) per liter. Cells were harvested by centrifugation (5,500 × *g*, 20 min, 4°C) during exponential growth phase, as determined by monitoring optical density at a wavelength of 650 nm. *Escherichia coli* JM109 cells were grown in Luria-Bertani broth or on Luria-Bertani agar plates at 37°C. When required, tetracycline (1.0 μg/ml [for plates] and 0.5 μg/ml [for broth culture]) was added to *P. gingivalis* cultures and ampicillin (100 μg/ml) was added to *E. coli* cultures. Culture purity was assessed regularly by Gram stain and colony morphology.

P. gingivalis was grown in continuous culture using a model C-30 BioFlo chemostat (New Brunswick Scientific, Edison, N.J.) with a working volume of 365 ml. Both the culture vessel and medium reservoir were continuously gassed with 10% CO_2 and 90% N_2. The growth temperature was 37°C, and pH was maintained at 7.5 by the automatic addition of either 2 M KOH or 2 M HCl. The dilution rate was 0.07 h^{-1}, giving a mean beneration time of 10 h. The culture medium was 1.75% BHI supplemented with 2% bovine serum albumin (BSA) (Sigma) and 4 mM (each) serine, threonine, and arginine. The medium was filter sterilized, and sterile cysteine-HCl (0.5 g/liter) and hemin (5 mg/liter) were added. The culture was allowed to reach steady state (at least 10 generations) and then was sampled on 3 consecutive days for amino acid analysis. Dry weights were determined by vacuum filtration of a cell suspension through preweighed, 0.2-μm polycarbonate filters (Nuclepore Poretics, Livermore, Calif.). Cell-free culture filtrates were prepared as previously described (22) and were stored at −20°C.

Amino acid analysis. Free and peptide amino acid concentrations in uninoculated and cell-free spent media from *P. gingivalis* continuous culture were determined. Liquid phase hydrolysis was carried out according to the method of Barkholt and Jensen (1), and amino acid analysis was carried out on an Amino Quant II amino acid analyzer (Hewlett-Packard, Palo Alto, Calif.). Amino acids were derivatized according to the methods of Blankenship et al. (3). The derivatized amino acids were then separated on a C_{18} reversed-phase high-performance liquid chromatography column, detected by UV absorbance, and identified by retention time. The amino acid calibration standard used was from Pierce (Rockford, Ill.).

Characterization of serine transport. All transport assays with *P. gingivalis* W50 cells were conducted in an anaerobe chamber (MK3 Anaerobic workstation; Don Whitley Scientific Ltd., Adelaide, South Australia, Australia) in an atmosphere of 5% hydrogen, 10% carbon dioxide, and 85% nitrogen. Working at 4°C, *P. gingivalis* cells grown in batch culture were harvested by centrifugation, washed, and suspended in Pga buffer (10.0 mM NaH_2PO_4, 10.0 mM KCl, 2.0 mM citric acid, 1.25 mM $MgCl_2$, 20.0 μM $CaCl_2$, 25.0 μM $ZnCl_2$, 50.0 μM $MnCl_2$, 5.0 μM $CuCl_2$, 10.0 μM $CoCl_2$, 5.0 μM H_3BO_3, 0.1 μM Na_2MoO_4, 10 mM cysteine-HCl with the pH adjusted to 7.5 with 5 M NaOH at 37°C), which was based on the chemically defined medium of Milner et al. (17). The *P. gingivalis* cell suspension was divided into 2-ml aliquots and stored on ice until use.

To begin the assay the temperature of a stirred cell suspension was raised to 39°C, as preliminary experiments indicated that this was the optimal temperature for transport. Five minutes prior to the addition of radiolabeled serine, a tetrapeptide, Gly-Gly-Tyr-Arg (Sigma), was added to a final concentration of 100 μM as an energy source, as preliminary experiments had indicated that the addition of the tetrapeptide increased the rate of transport. The glutamate and aspartate-containing tetrapeptide Asp-Cys-Glu-Cys also was used as an energy source. Radiolabeled L-[^{14}C(U)]serine (final concentration, 100 μM; specific activity, 5 Ci/mol) was added to the cell suspension followed by the removal of 200-μl samples at indicated time points. Rates of transport were determined from data points obtained up to 20 s after radiolabeled serine addition. Cells were separated from the buffer by centrifugation (16,000 × *g*, 30 s) through 500 μl of silicon oil with a specific gravity of 1.015 g/ml (Dow Corning 556 silicon oil [0.980 g/ml] and 550 silicon oil [1.068 g/ml], 60:40 [vol/vol]). A sample of the supernatant (10 μl) was transferred to a scintillation vial, and the remaining supernatant, along with the silicon oil, was removed from the cell pellet by suction. The radioactivity of the cell pellet and supernatant was determined by liquid scintillation counting as described previously (6, 8).

For each assay the volume of extracellular and intracellular water in the pellet was determined. Tritiated water (specific activity, 0.5 μCi/ml) was used to determine the total pellet water, while [^{14}C(U)]sucrose (final concentration, 1 mM; specific activity, 0.5 Ci/mol) was used to determine the extracellular pellet water volume. A 4-min equilibration time was allowed, and radioactivity was determined as described above. Preliminary experiments demonstrated that sucrose was not transported by *P. gingivalis*. A constant relationship between the intracellular water volume and dry weight of *P. gingivalis* was determined and was found to be 2.13 ± 0.21 μl/mg (dry weight). Dry weights were determined as described above. The rate of serine transport is expressed as nmol/mg (dry weight) of cells/min. All radiolabeled substrates were purchased from DuPont NEN Products (Boston, Mass.).

In order to characterize the kinetics of serine transport, the rate of [^{14}C]serine accumulation was determined over a range of external serine concentrations between 3 and 200 μM (166.7 to 2.5 Ci/mol) as described above. In competition experiments, [^{14}C]serine (final concentration, 25 μM; specific activity, 20 Ci/mol) and the unlabeled competing amino acid, either leucine, arginine, glycine, tyrosine, alanine, asparagine (final concentration, 250 μM), or threonine (final concentration, 25, 125, or 250 μM), were mixed before addition to the cell suspension.

Transmembrane ion gradients were collapsed by the addition of the ionophore gramicidin (13). EDTA (final concentration, 400 μM) was added to 2 ml of a cell suspension 4 min prior to the addition of gramicidin (final concentration, 25 μM). EDTA was included to assist gramicidin to gain access to the inner membrane (13). After 1 min of incubation with gramicidin, [^{14}C]serine (final concentration, 25 μM; specific activity, 20 Ci/mol) was added and uptake was determined as described above. The effect of gramicidin on accumulated serine was determined by the addition of gramicidin to a cell suspension that had been incubated with [^{14}C]serine for approximately 70 s. EDTA was added to the cell suspension 5 min before the addition of serine.

The effect of sodium ion concentration on the rate of serine transport in *P. gingivalis* was investigated. The standard Pga buffer was modified by using KOH to adjust the pH (PgaNaK), KH_2PO_4 replacing NaH_2PO_4 and the pH adjusted with KOH (PgaK), or 80 mM NaCl being added to the standard Pga buffer (Pga + NaCl). The sodium ion concentrations of these buffers were determined by atomic emission spectroscopy (Perkin-Elmer 373; Norwalk, Conn.), and the rate of [^{14}C]serine (25 μM, 20 Ci/mol) transport was determined for each sodium concentration.

Role of cysteine in serine transport. The effect of cysteine concentration on *P. gingivalis* serine transport was determined by varying the cysteine concentration in the Pga buffer (1 to 20 mM). To determine the role of cysteine it was replaced with either an alternative reducing agent (10 mM dithiothreitol [DTT] or 10 mM β-mercaptoethanol) or a combination of both (5 mM cysteine and 5 mM DTT or 10 mM cysteine and 10 mM DTT). The rate of [^{14}C]serine (25 μM, 20 Ci/mol) transport by *P. gingivalis* was determined in triplicate in each buffer. Cysteine transport experiments were conducted by adding [^{14}C]cysteine (1 mM, 5 Ci/mol) {prepared by reducing L-[^{14}C(U)]cystine with 10 mM DTT} to a *P. gingivalis* cell suspension in Pga buffer without cysteine but containing 10 mM DTT, essentially as described above.

Construction of a serine transport mutant. A gene potentially encoding the serine/threonine transporter in *P. gingivalis* was identified by a BLAST search of the unfinished *P. gingivalis* W83 genome on The Institute for Genomic Research website at http://www.tigr.org using the sequence of an *E. coli* serine/ threonine transporter (*sstT*) (open reading frame *ygjU*, accession number AE000391) (4, 18). The TopPred 2 program (35), provided by Stokholm University and accessed through the ExPASy molecular biology server (http://www.expasy.ch), was used for hydropathy analysis. Sequence alignments were prepared using the CLUSTALW program accessed through the European Bioinformatics Institute website at http://www.ebi.ac.uk.

The putative *P. gingivalis* W50 *sstT* coding region was amplified by PCR from genomic DNA, prepared by the method of Chen and Fuo (5) by using Elongase (Life Technologies, Mulgrave, Victoria, Australia) according to the manufacturer's instructions on a PC-960 thermal sequencer (Corbett Research, Brisbane, Queensland, Australia). The PCR was performed using primers based on the *P. gingivalis* W83 sequence (forward primer 5′-ATGCCTACTTCCGCTATATT TCCA-3′ and reverse primer 5′-CTACCTTGCGACTGCCTGCCTCT-3′). A 1.2-kb PCR product was gel purified using the QIAquick gel extraction kit (Qiagen, Clifton Hill, Victoria, Australia) and ligated into pGEM-T Easy (Promega Corporation, Madison, Wis.). *E. coli* JM109 cells were transformed by electroporation, and colonies were selected on Luria-Bertani plates containing ampicillin (100 μg/ml) using standard procedures (24).

The *tetQ* gene from *Bacteroides thetaiotaomicron,* which confers tetracycline resistance to *P. gingivalis,* was excised from pNJR12 (15) (kindly provided by M. Curtis, St. Bartholomew's and Royal London School of Medicine and Dentistry, London, United Kingdom) using *Eco*ICRI and the 2.651-kb fragment ligated into a unique, dephosphorylated *Stu*I site within the putative *sstT* gene contained in the pGEM-T Easy vector. The construct was then transformed into competent *E. coli* JM109 cells by heat shock, and cells were selected on Luria-Bertani plates containing ampicillin (100 μg/ml). Colonies containing *tetQ* were identified by hybridization with an *Eco*ICRI DNA fragment containing *tetQ* labeled with [^{32}P]dATP using the Prime-a-Gene labeling kit (Promega).

TABLE 1. Concentrations of amino acids in *P. gingivalis* W50 continuous culture medium, 1.75% BHI broth containing 2% BSA and 4 mM (each) free serine, threonine, and arginine, before inoculation and in spent, cell-free medium

Residue	Amino acid concentration (mM)[a]			
	Before inoculation		In spent medium	
	Free form	Peptide form	Free form	Peptide form
Ser	5.1	11.3	0.1	5.4
Thr	5.1	12.2	0.0	6.8
Arg	5.3	7.5	0.1	3.6
Asx	1.1	19.9	0.7	10.1
Glx	1.6	33.4	1.3	16.8
His	0.3	5.9	0.2	3.1
Gly	0.5	7.7	0.6	3.5
Cys	3.1	12.1	2.9	8.2
Ala	1.4	21.9	1.2	13.8
Tyr	0.4	6.5	0.5	3.3
Val	1.2	13.4	1.6	6.5
Met	0.6	2.4	0.3	0.9
Trp	0.4	ND[b]	0.0	ND
Phe	1.4	8.6	0.7	4.1
Ile	0.9	6.1	0.6	2.4
Leu	3.3	19.9	2.8	10.4
Lys	1.7	19.8	2.4	10.2
Pro	0.2	15.1	7.2	4.4

[a] Mean of three determinations taken on consecutive days.
[b] ND, not determined.

The pGEM-T Easy vector containing *sstT*::*tetQ* was linearized with *Sca*I and transformed into *P. gingivalis* cells by electroporation. The procedure for preparation and transformation of *P. gingivalis* cells was essentially that of Fletcher et al. (11), except that cells were selected on blood agar plates containing tetracycline (1 μg/ml) after 7 days of incubation at 37°C under anaerobic conditions. Genomic DNA purified from the *P. gingivalis* W50 *sstT* mutant (W50ST) and wild-type W50 was double digested with *Bam*HI and *Pst*I and analyzed by Southern blot as described by Slakeski et al. (29). Probes specific for *tetQ* and putative *sstT* were labeled with [^{32}P]dATP using the Prime-a-Gene labeling kit and were hybridized to Southern blots at 65°C. Membranes were washed down to 0.5× SSC (1× SSC is 0.015 M NaCl plus 0.015 M sodium citrate), 0.1% (wt/vol) sodium dodecyl sulfate. Serine transport analysis of *P. gingivalis* W50ST was conducted with [^{14}C]serine (25 μM, 20 Ci/mol) as described above.

RESULTS

Amino acid assimilation by *P. gingivalis* in continuous culture. Amino acid analysis was performed on nonhydrolyzed and hydrolyzed samples of uninoculated and cell-free spent growth medium from *P. gingivalis* continuous culture (Table 1). The medium contained BHI, BSA, and a 4 mM concentration of (each) free serine, threonine, and arginine. These free amino acids were included because initial experiments revealed the disappearance of serine (95%), threonine (100%), and arginine (82%) from *P. gingivalis* growth medium when these amino acids were present in relatively low concentrations (data not shown). At the higher concentrations effectively all of the free serine (99%), threonine (100%), and arginine (98%) disappeared from the medium (Table 1). The concentration of a number of other free amino acids also decreased, most notably phenylalanine, although the decrease in concentration only represented 53% disappearance of the total available phenylalanine, and the initial concentration was relatively low (Table 1). There was a decrease in the concentration of all amino acids in peptide form from the uninoculated to cell-free spent medium (Table 1). The concentration of free proline markedly increased, even though there was an overall decrease in the amount of proline present in the medium. This could indicate that this residue is removed prior to peptide assimilation or, more likely, that excess proline produced within the cell by peptide hydrolysis is excreted into the medium.

Growing in continuous culture in a complex medium, *P. gingivalis* appeared to assimilate all amino acids to some extent in peptide and/or free form. Glutamate/glutamine (13.1 mmol/g [dry weight] of cells) was the most highly consumed amino acid, followed by serine (8.4 mmol/g [dry weight]), threonine (8.1 mmol/g [dry weight]), aspartate/asparagine (7.9 mmol/g [dry weight]), leucine (7.7 mmol/g [dry weight]), arginine (6.9 mmol/g [dry weight]), lysine (6.9 mmol/g [dry weight]), and alanine (6.4 mmol/g [dry weight]). However, due to the complex nature of the medium these amino acids were not present in the uninoculated medium in similar concentrations, and in terms of percentages, serine (67%), threonine (61%), and arginine (71%) were the most highly consumed. These three amino acids disappeared from the medium in approximately equal amounts in both free amino acid and peptide forms (Table 1).

Characterization of serine transport. The disappearance of free serine, threonine, and arginine from the growth medium strongly suggested the presence of transport systems for these amino acids. The transport of the amino acid serine was therefore selected for characterization. *P. gingivalis* W50 grown in complex media (BHI) was washed and suspended in Pga buffer, and transport experiments were conducted under strictly anaerobic conditions in the presence of the tetrapeptide Gly-Gly-Tyr-Arg as an energy source. The addition of the tetrapeptide Asp-Cys-Glu-Cys as an energy source under identical conditions gave a similar rate of serine uptake (data not shown). In the absence of the tetrapeptide, the accumulation of serine by *P. gingivalis* was markedly slower, indicating that these cells are able to transport and utilize tetrapeptides as an energy source (Fig. 1). The kinetics of serine transport in *P. gingivalis* were determined under the same conditions by

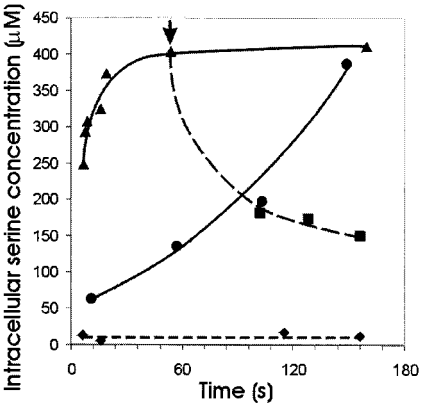

FIG. 1. [^{14}C]serine (25 μM) accumulation by washed cells of *P. gingivalis* W50 (▲) and the serine transport mutant W50ST (♦) that were energized by tetrapeptide Gly-Gly-Tyr-Arg (100 μM) addition 5 min prior to radiolabeled serine addition. The effect of gramicidin addition (↓) on serine accumulation by *P. gingivalis* W50 is shown (■). Serine accumulation by washed *P. gingivalis* W50 cells with no tetrapeptide addition is also shown (●). All experiments included 10 mM cysteine in the assay buffer. The data points represent the mean values from three separate experiments.

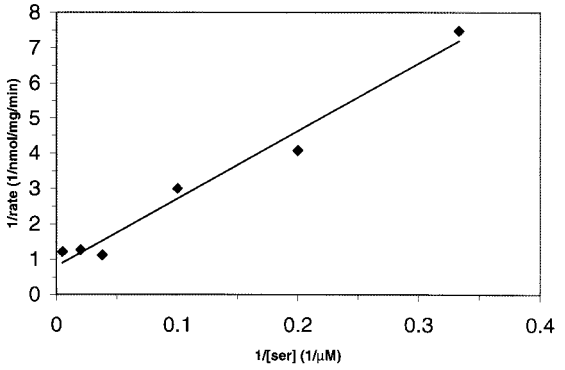

FIG. 2. Lineweaver-Burk double reciprocal plot of initial serine uptake rate versus initial serine concentration. Initial uptake rates were determined in washed *P. gingivalis* cells energized by addition of the tetrapeptide Gly-Gly-Tyr-Arg 5 min prior to [^{14}C]serine addition at pH 7.5, 37°C. Data points represent the average of three to six determinations.

measuring the uptake rate of [^{14}C]serine at initial extracellular concentrations between 3 and 200 μM. A double reciprocal plot gave a straight line ($R = 0.988$) that indicated a single transport system, with an affinity constant for transport (K_t) of 23.8 μM and a V_{max} of 1.24 nmol/mg (dry weight)/min (Fig. 2).

The transport of serine by *P. gingivalis* was not significantly inhibited by a 10-fold excess of glycine (86% ± 11% of control rate), arginine (94% ± 25%), tyrosine (89% ± 29%), leucine (95% ± 24%), or asparagine (96% ± 17%). However, an equimolar level of threonine inhibited serine transport by 19% ± 5% of the control rate, a 5-fold excess of threonine inhibited serine transport by 55% ± 9%, and a 10-fold excess inhibited serine transport by 88% ± 2%. This concentration-dependent competitive inhibition of serine transport indicates that the *P. gingivalis* serine transporter also transports threonine.

The ion gradients across the inner membrane of *P. gingivalis* were collapsed using the ionophore gramicidin in the presence of EDTA (13). This disruption of the transmembrane ion gradients inhibited the intracellular accumulation of serine, whereas the addition of EDTA alone had no effect on serine transport (Table 2). The association of *P. gingivalis* serine transport with a transmembrane ion gradient is also consistent with the observed rapid efflux of [^{14}C]serine from *P. gingivalis* cells when gramicidin was added to the cell suspension approximately 70 s after intracellular serine accumulation had commenced (Fig. 1). The intracellular radiolabeled serine concentration decreased from approximately 400 to 150 μM within 100 s of the gramicidin addition.

Sodium ions are commonly involved in solute transport by symporters, so the effect of sodium ion concentration on the rate of serine transport by *P. gingivalis* was investigated. The Pga buffer used for the determination of amino acid transport had a sodium ion concentration of 39 mM, as determined by atomic emission spectroscopy. Reducing the sodium ion concentration to 9.3 mM reduced the rate of serine transport by over 50% (Table 2). When sodium ions were not added to the buffer (although 350 μM sodium was still present), serine transport was detected only at a very low rate. Increasing the sodium ion concentration of the buffer to 112 mM abolished serine transport (Table 2).

Role of cysteine in serine transport. In all of the experimental data presented above, 10 mM cysteine was present in the Pga buffer. Lowering the concentration of cysteine in the buffer markedly decreased the rate of serine transport. At 5 mM cysteine the rate of serine transport was 50% ± 1% of the control value, and a further lowering of the cysteine concentration to 1 mM effectively abolished serine transport (2% ± 1% of control). Raising the cysteine concentration in the buffer from 10 to 20 mM had little effect on the serine uptake rate. When cysteine was replaced in the Pga buffer with the same concentration (10 mM) of an alternative reducing agent, either DTT or β-mercaptoethanol, the rate of serine transport by *P. gingivalis* was less than 5% of that in the presence of cysteine. These alternative reducing agents did not inhibit the rate of serine uptake, as in the presence of 10 mM DTT and 10 mM cysteine the rate was the same as the rate in the presence of 10 mM cysteine alone; furthermore, the rate with 5 mM DTT and 5 mM cysteine was similar to that with 5 mM cysteine alone.

We investigated the transport of cysteine in *P. gingivalis* by adding [^{14}C]cysteine to a *P. gingivalis* cell suspension in the presence of 10 mM DTT. However, cysteine transport was not detected over a 30-min period under these conditions, suggesting that the cysteine in the buffer was not being taken up and used as an energy source by *P. gingivalis*.

Identification and insertional inactivation of the *P. gingivalis* gene, *sstT*, encoding the serine transporter. A BLAST search of the unfinished *P. gingivalis* genome using the amino acid sequence of SstT, an *E. coli* sodium ion-driven serine and threonine transport protein (18), identified a single match. This open reading frame encoded a putative protein containing 412 amino acids with a predicted molecular mass of 44 kDa and an amino acid sequence that was 27% (112 out of 412 amino acids) identical and 64% (262 out of 412 amino acids) similar to the *E. coli* SstT (Fig. 3). Hydropathy plots of the deduced amino acid sequences of the *P. gingivalis* and *E. coli* proteins had similar profiles with nine predicted transmembrane domains. Based on this similarity the *P. gingivalis* gene was identified as potentially encoding a serine/threonine transporter.

TABLE 2. Effects of EDTA, gramicidin, and sodium ion concentration on the rate of [^{14}C]serine uptake by washed cells of *P. gingivalis*

Buffer	Sodium ion concentration (mM)[a]	Rate of serine transport (%)[b]
Pga	39	100 ± 16
Pga + EDTA	39	103 ± 2
Pga + gramicidin + EDTA	39	ND[c]
PgaK[d]	0.35	6 ± 17
PgaNaK[e]	9.3	43 ± 2
Pga + NaCl	112	ND

[a] Determined by atomic emission spectroscopy.
[b] Rates are presented as a percentage of the control treatment. Experiments were conducted in triplicate, and the results are presented as the mean ± standard deviation.
[c] No uptake detected.
[d] PgaK buffer was essentially the same as Pga buffer, except that the buffering capacity was provided by KH_2PO_4 and the pH was adjusted with KOH.
[e] PgaNaK buffer was essentially the same as Pga buffer, except that the pH was adjusted with KOH.

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Pg (1)      MPTSAIFPTNNLLDQATPNHMRKLRIG-LLPKIILAIVLGIGFGNLFSFPFIRIFVTFNA
Ec (1)      ----------MTTQRSPGLFRRLAHGSLVKQILVGLVLGILLA-WISKPAAEAVGLLGT

Pg (60)     LFSELLGFSIPLIILGLVTVAIAD--IGKGAG-RMLLITVLIAYMATVLSG-LFAYFAGN
Ec (49)     LFVGALKAVAPILVLMLVMASIANHQHGQKTNIRPILFLYLLGTFSAALAAVVFS-FA--

Pg (116)    AFFPS-L--------ISPGTGMSDVTQSQG-VQPYFSIAIPPMMSVMTALVLSFVLGLGL
Ec (106)    --FPSTLHLSSSAGDISPPSGIVEVMR--GLVMSMVSNPIDALLKGNYIGILVWAIGLGF

Pg (166)    A---GMESRGMKGMMQDFQEIITKLIAKVIIPLLPIYIFGIFLN-MTHQGEVVKVLSVFL
Ec (162)    ALRHGNETT--KNLVNDMSNAVT-FMVKLVIRFAPIGIFGLVSSTLATTG--FSTLWGYA

                                                        Motif A
Pg (222)    SIIGIIFG-LHIILLIFQYSVAGFIAKKNPFRMLWRML-PAYFTRLGTQSSAATIPVTLK
Ec (217)    QLLVVLVGCMLLVALVVNPLLVWWKIRRNPFPLVLLCLRESGVYAFFTRSSAANIPVNMA

                                  Motif B
Pg (280)    SAVKCGVSEEIAGFTIPLCATIHLSGSTLKITCCALALMIMQGMPYDAGLFTGFIFMLGI
Ec (277)    LCEKLNLDRDTYSVSIPLGATINMAGAAITITVLTLAAVNTLGIPVD--LPTALLLSVVA

                                                        Motif D
Pg (340)    TMVA--APGVPGGAIMAALGVLQSMLGF--DESLQALMIALYI--TMDNFGTACNVTGDG
Ec (335)    SLCACGASGVAGGSLLL-IPLACNMFGISNDIAMQVVAVGFIIGVLQDSCETALNSSTDV

Pg (394)    AISLIVDKLMMRKRQAVAR---
Ec (394)    LFTAAACQAE-DDRLANSALRN
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FIG. 3. Alignment of the deduced amino acid sequences of SstT from *P. gingivalis* (Pg) and *E. coli* (Ec). Shading indicates identical residues and bold indicates conservative substitutions. Conserved motifs found in the glutamate transport family (30) are boxed.

The gene identified in *P. gingivalis*, designated *sstT*, was insertionally inactivated with *tetQ*, which confers tetracycline resistance in *P. gingivalis*. Transformed *P. gingivalis* cells (W50ST) capable of growth on plates containing tetracycline were subjected to Southern blot analysis and compared to the wild type. Digested genomic DNA from W50ST contained a single band that hybridized with a *tetQ* probe, while no hybridization occurred with digested DNA from the wild type (Fig. 4). When digested genomic DNA from both the wild type and the mutant were probed with the radiolabeled *sstT* PCR product, a single band was present in each. However, the band in the mutant was approximately 2.6 kb larger than the band in the wild type, which is consistent with the insertion of *tetQ* into *sstT* (Fig. 4).

When [^{14}C]serine (25 μM) was added to a cell suspension of *P. gingivalis* W50ST in Pga buffer, no serine uptake was detected (Fig. 1). This lack of transport is consistent with the putative *sstT* gene encoding the *P. gingivalis* serine/threonine transporter. *P. gingivalis* W50ST grew at a similar rate and to the same final optical density as the wild type when grown in batch culture in BHI, indicating that the transporter is not essential for growth in this complex medium.

DISCUSSION

When *P. gingivalis* was grown in continuous culture in a complex medium containing free amino acids, peptides, and protein, the most highly consumed amino acids (not differentiating between free and peptide forms) were glutamate/glutamine, serine, threonine, aspartate/asparagine, and leucine (Table 1). This is consistent with other studies that collectively show that *P. gingivalis* preferentially utilizes aspartate/asparagine, glutamate/glutamine, threonine, serine, and possibly arginine and leucine from the culture medium (16, 26, 33). The disappearance of small amounts of free serine, threonine, and arginine has previously been shown for *P. gingivalis* incubated with tryptone, although those authors concluded that this bacterium did not transport free amino acids (33). We found that nearly all the free serine, threonine, and arginine, even when initially present at relatively high concentrations, disappeared from the *P. gingivalis* continuous culture growth medium. This led us to investigate the transportation of serine by *P. gingivalis*.

The ability of *P. gingivalis* to transport [^{14}C]serine was determined with washed cells, energized with the tetrapeptide Gly-Gly-Tyr-Arg, under strictly anaerobic conditions. Serine was transported and rapidly accumulated within the cell to levels 8 to 16 times that of the extracellular concentration.

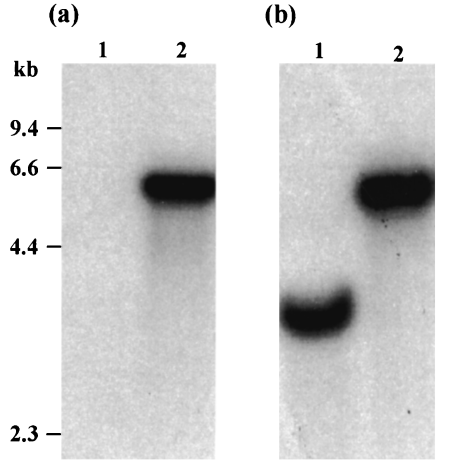

FIG. 4. Southern blot analysis of *P. gingivalis* genomic DNA. The DNA of the wild-type W50 (lane 1) and W50ST (lane 2) were digested with *Bam*HI and *Pst*I, and the resulting DNA fragments were subjected to agarose gel electrophoresis and Southern blot hybridization with a *tetQ* (a) or *sstT* (b) probe.

Competition assays indicated that the *P. gingivalis* serine transporter also transported threonine, but none of the other amino acids that we tested were transported by the serine transporter. The transport of these two free amino acids by washed cells of *P. gingivalis* is consistent with their disappearance from the continuous culture medium. The removal of relatively high concentrations of serine and threonine from the *P. gingivalis* continuous culture medium indicates that these amino acids may also be being fermented for energy production.

The abolition of serine transport by *P. gingivalis* in the presence of the ionophore gramicidin indicated that the transport is driven by a transmembrane ion gradient. This was confirmed by the efflux of accumulated serine from *P. gingivalis* upon the addition of gramicidin. Ion-motive force-driven transporters are reversible and facilitate the transmembrane movement of substrate in either direction dependent on the transmembrane ion-motive force and the transmembrane substrate concentration gradient, as opposed to ATP-driven transport systems that are essentially unidirectional (21). The relatively low affinity of the transporter for serine (K_t = 24 μM) and the low level of intracellular accumulation are both consistent with an ion-motive force-driven transport system. The accumulation of serine within the cell indicates that the magnitude of the ion-motive force driving uptake was 56 to 77 mV under the experimental conditions (7).

A reduction of sodium ion concentration in the cell suspension caused a decrease in the rate of serine transport, indicating that transport was driven by a sodium ion-motive force (Table 2). The transport of amino acids in a number of gram-negative bacteria has been shown to be dependent on a transmembrane sodium gradient (21, 23). Like some amino acid transporters in *E. coli* and *Clostridium fervidum*, the serine/threonine transporter in *P. gingivalis* is inhibited by high concentrations of sodium under experimental conditions (12, 32).

Insertional inactivation of the *P. gingivalis sstT* gene prevented serine uptake, confirming the identity of SstT as the *P. gingivalis* serine transporter. The *P. gingivalis* SstT appears to belong to the glutamate transport family (30), also known as the DAACS (dicarboxylate/amino acid-Na^+/H^+ symporter) family of amino acid transporters (23). This is based on the size of the putative protein, hydropathy profile, sequence similarity, and presence of some of the conserved sequence motifs. Four conserved motifs have been recognized in the glutamate transport family (30) (Fig. 3). Motif A [(ST)(STARK)S(ST)] and motif B [PxGx(TS)xN(ML)DGxx(LI)(FY)] are found in SstT from *P. gingivalis*, although in motif A the second amino acid is Q in the *P. gingivalis* protein. There are also slight differences in motif B found across the bacterial serine transporters. A third motif found in many members of the glutamate transport family, motif C, is not present in any of the putative bacterial serine transporters. Motif D is believed to be part of the translocation channel and is generally conserved throughout the family of glutamate transporters, but the exact amino acid sequence depends on substrate specificity (30). Motif D GhhQDSxETALNSSTD (where h is any hydrophobic residue) is found in SstT from *E. coli*, along with the putative serine transporters (YgjU) from *Haemophilus influenzae*, *Neisseria meningitidis,* and *Campylobacter jejuni* (10, 19, 20). However, in *P. gingivalis* SstT, the sequence in this region (TMDNFGTACNVTGD) is considerably different from the proposed motif, with only 5 of the proposed 13 amino acyl residues conserved (Fig. 3). Therefore a more generalized consensus sequence may be required to describe this motif across a broader range of bacterial ion-motive force-driven serine transporters.

The transportation of free serine by *P. gingivalis* was dependent on the concentration of cysteine in the cell suspension buffer. The lack of serine transport in the presence of alternative reducing agents indicates that the effect of cysteine is specific and dependent on the free amino acid. *P. gingivalis* did not transport free cysteine, indicating that its effects were extracellular. This is consistent with the work of Tang-Larsen et al. (34), who found that *P. gingivalis* did not metabolize free cysteine. Interestingly, free cysteine has been shown to specifically activate the extracellular proteinases RgpA, RgpB, and Kgp of *P. gingivalis,* and this activation could not be replicated using alternative reducing agents (2). Serine transport by *E. coli* SstT has not been reported to be cysteine dependent (18).

In conclusion, we have characterized a cysteine-activated, sodium ion-motive force-driven serine/threonine transporter in *P. gingivalis*. This is the first direct demonstration of amino acid transport in this asaccharolytic bacterium.

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