Expression, Purification, and Characterization of a Novel G Protein, SGP, from *Streptococcus mutans*

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The *sgp* gene of *Streptococcus mutans* was recently detected immediately downstream from the *dgk* gene within the same operon. In this study, the *sgp* gene was subcloned into the pMAL-c2 vector and SGP (*S. mutans* G protein) was overexpressed in *Escherichia coli* as a fusion protein with the maltose-binding protein at a level of 40% of total cellular protein. One-step amylose affinity chromatography purification of this fusion protein yielded a product of approximately 95% purity. SGP was purified from this fusion protein following cleavage with protease factor Xa and DEAE-Sephacel chromatography. In nucleotide binding assays, recombinant SGP showed specific binding for GTP and GDP, but not ATP, CTP, and UTP, and also catalyzed efficient hydrolysis of GTP to GDP. Kinetic studies revealed that the SGP K_m value for GTP in this reaction was approximately 5.9 μ M. Mg^{2+} also served as a cofactor of SGP in this reaction. In vivo subcellular localization by immunogold labelling demonstrated that SGP was associated with both membrane and cytoplasmic fractions. SGP not only had structural similarities with other G proteins but also proved to have high-level intrinsic GTPase activity. Therefore, SGP appears to be a new member of the G protein superfamily and may participate in transmembrane signaling in the responses of *S. mutans* cells to environmental stimuli.

The oral gram-positive bacterium *Streptococcus mutans* has been positively associated with dental caries development in experimental animals and humans (7, 12, 16). This organism utilizes the tooth surface as its main habitat and displays great flexibility in its ability to adapt to a wide range of oral environmental conditions. In vitro studies have demonstrated that environmental changes, such as increased acidity, a shift from carbon limitation to carbon excess, and alterations in growth rate, resulted in rapid increases or decreases in the biochemical and metabolic properties of *S. mutans* cells (8). Of particular interest is the observation that these organisms can grow well at pH values as low as 4.8 and metabolize sucrose at pH 4.0 (8, 11). Therefore, this acidurance property of *S. mutans* appears to be an important virulence factor for these organisms. It has been reported that acidic environmental signals induced an increase in the proton-extruding ATPase, lactate dehydrogenase, and glycolytic activities of *S. mutans* cells (12). Such flexibility in the responses of these organisms to environmental changes could play an important role in pathogenicity. Currently, little is known about the molecular mechanisms which initiate and regulate the responses of *S. mutans* cells to environmental signals.

Previous work in this laboratory has indicated that *S. mutans* GS5Tn1, a mutant strain constructed by mutagenesis of strain GS5 with the conjugative transposon Tn916, was defective in acidurance. This mutant grew poorly under low-pH conditions and was also sensitive to high osmolarity and elevated growth temperatures (28). Thus, mutant GS5Tn1 could prove to be an important model in investigating molecular control of the responses of *S. mutans* cells to different environmental signals. Analysis of this mutant indicated that its defects resulted from a single insertion of Tn916 into the GS5 chromosome which

partially inactivated a gene homologous to the *Escherichia coli dgk* (diacylglycerol kinase) gene. Immediately downstream from this gene and within same operon, an open reading frame with extensive homology to the *E. coli era* gene was detected. The latter gene expresses a peripheral membrane-bound protein, Era, which exhibits GTPase activity and appears to be essential for cell growth (5). Recently, Lin et al. suggested that Era is involved in the regulation of transmembrane signaling in bacteria (15). Thus, characterization of the *S. mutans* homolog of the Era protein, SGP (*S. mutans* G protein), may prove to be important in determining how *S. mutans* cells respond to environmental signals in the oral cavity.

The aim of this investigation was to purify and characterize SGP and directly demonstrate that it is a member of the G protein superfamily with characteristic GTP binding and hydrolysis activities. The present results confirm that SGP can be classified as a G protein, and its properties are consistent with a role in signal transduction of environmental changes.

MATERIALS AND METHODS

Preparation of *sgp* constructs. A 1.2-kb DNA fragment containing the *sgp* gene was excised from plasmid pMCL21(PstI-BstBIdeltaBspHI) (27) following *SwaI* and *XhoI* digestion. The *SwaI* site is located precisely at the 5' end of the second codon of the *sgp* gene. This fragment was blunted with the Klenow fragment of DNA polymerase (Gibco BRL, Grand Island, N.Y.) and ligated into the *XmnI* site of vector pMAL-c2 (18) by T4 ligase (Gibco BRL) (see Fig. 1). In the resultant plasmid, pMAL-SGP10, the *sgp* gene was fused in frame to the upstream maltose-binding protein (MBP) gene and expressed as an MBP-SGP fusion protein under the control of the Ptac promoter.

Expression of MBP-SGP fusion proteins in *E. coli*. Plasmid pMAL-SGP10 was transformed into *E. coli* JM109 cells and grown in tryptone-phosphate medium (2% Bacto Tryptone, 1.5% yeast extract, 0.2% Na_2HPO_4 , 0.1% KH_2PO_4 , 0.8% NaCl, 0.2% glucose) (20) containing 100 μ g of ampicillin per ml. Bacteria were grown at 37°C to an optical density of 0.5 to 0.6 (at 550 nm). Expression of the fusion protein was induced by the addition of isopropyl-1-thio- β -D-galactopyranoside (IPTG) (Amresco, Solon, Ohio) at a concentration of 0.3 mM to the culture medium, after which cultures were grown for 8 h at 25°C with vigorous shaking. Cell pellets were harvested from 1.0-liter cultures, suspended in 50 ml of buffer A (20 mM Tris-HCl [pH 7.2], 200 mM NaCl, 1 mM EDTA) containing 10 mM 2-mercaptoethanol (Sigma, St. Louis, Mo.) and 0.1 mM phenylmethylsulfonyl fluoride (Sigma), and stored at -20°C overnight.

Purification of MBP-SGP fusion protein and SGP. The frozen bacterial sus-

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FIG. 1. Subcloning the *sgp* gene into the pMAL-c2 expression vector. The 1.2-kb DNA fragment containing the *sgp* gene was excised from plasmid pMCL21 and ligated into the dephosphorylated pMAL-c2 vector as described in the text. The resulting construct, designated pMAL-SGP10, contained the IPTG-inducible Ptac promoter and the *malE* translation start site. The *sgp* gene was in frame with the upstream *malE* gene. The junction sequence between these two genes encoded the specific target site for protease factor Xa.

pension was thawed in cold water, placed in an ice bath, and sonicated 10 times with 20-s pulses in a model 450 Sonifier sonic oscillator (Branson Ultrasonics, Danbury, Conn.). Then the cell lysate was centrifuged at $10,000 \times g$ and 4°C for 30 min. Supernatant fluid was diluted with 5 volumes of buffer A and applied to an amylose-resin column (New England Biolabs, Boston, Mass.) pre-equilibrated with buffer A. Bound MBP-SGP fusion protein was eluted with buffer A containing 10 mM maltose (Sigma) and 0.1% polyoxyethylene 9 lauryl ether (Sigma). Subsequently, MBP-SGP fusion protein was cleaved with factor Xa (New England Biolabs) at a ratio of 200:1 (wt/wt) at 4°C overnight. SGP was separated from MBP and factor Xa by chromatography on a DEAE-Sephacel column (Pharmacia, Piscataway, N.J.). Proteins were eluted by using a gradient of 25 to 600 mM NaCl in 20 mM Tris-HCl, pH 8.0. SGP was eluted as a sharp peak at 300 to 350 mM NaCl, while the MBP peak was resolved at 100 to 150 mM NaCl and factor Xa was resolved at approximately 420 mM NaCl. An equal volume of glycerol was added to the SGP preparation and stored at -20°C . All protein samples were subjected to sodium dodecyl sulfate (SDS)-10% polyacrylamide gels for analysis (13).

Protein was measured with a Coomassie plus protein assay kit (Pierce, Rockford, Ill.) as described by the supplier.

Preparation of anti-SGP serum and immunoelectron microscope analysis. SGP-specific antiserum was prepared by the Pocono Rabbit Farm and Laboratory (Canadensis, Pa.) following immunization of rabbits with purified MBP-SGP proteins by conventional methods previously described (9). Serum was absorbed twice with MBP protein and showed no reaction with MBP in Western blot (immunoblot) assays. SGP-specific immunoglobulins were partially purified by precipitation with 34% saturated $(\text{NH}_4)_2\text{SO}_4$. Immunoelectron microscope examination was performed as previously described (24). *S. mutans* GS5 cells were cultured in Todd-Hewitt broth (28) and centrifuged at $5,000 \times g$ and 4°C for 15 min. Then the pellet was fixed in 3% paraformaldehyde in 0.1 M phosphate buffer for 30 min at 4°C , cut into small pieces, and dehydrated in a Balzers

FIG. 2. Overexpression and purification of MBP-SGP fusion protein and SGP. Fractions obtained for overexpression in *E. coli* (A) and purification of SGP as a fusion or isolated protein (B) were analyzed by SDS-PAGE analysis. Lanes in panel A: 1, total bacterial protein before IPTG induction; 2, bacterial lysate after IPTG induction; 3, soluble fraction of bacterial lysate; lane 4, MBP-SGP fusion protein purified by amylose-resin affinity chromatography. Lanes in panel B: 1, isolated SGP purified by DEAE-Sephacel ion-exchange chromatography; 2, factor Xa cleavage of MBP-SGP fusion protein; 3, purified MBP-SGP protein (same as lane 4 in panel A). Lanes M, Bio-Rad low-range protein molecular markers.

low-temperature embedding apparatus LTE 020 (BAL-TEC Products, Inc., Hudson, N.H.) as follows: 30% methanol at 0°C for 15 min; 50% methanol at 20°C for 1 h; and 70, 80, and 95% methanol at -35°C for 1 h each. Pieces were infiltrated with a mixture of Lowicryl K4M resin (Polysciences, Warrington, Pa.) and 95% methanol (1:1) at -35°C for 24 h by using a Balzers polymerization unit TTP 010. Then immunogold labelling was performed as follows. Ultrathin sections were cut on a Reichert Jung Ultracut E microtome (Leica, Deerfield, Ill.) and collected on Formvar-coated nickel grids. Sections on grids were incubated with 10% normal goat serum in phosphate-buffered saline (PBS) for 1 h at room temperature and then with rabbit immunoglobulin G (IgG) (50 $\mu\text{g}/\text{ml}$) against SGP at 4°C overnight. Next, grids were washed with PBS and incubated with goat anti-rabbit IgG conjugated with 15-nm gold particles (1:20; E-Y Laboratories, San Mateo, Calif.) for 30 min at room temperature. After being washed with PBS and distilled water, sections were stained with uranyl acetate and lead citrate, examined, and photographed with a Hitachi H600 electron microscope.

Nucleotide binding assays (23). SGP was incubated with either $\alpha\text{-}^{32}\text{P}$ - or $8\text{-}^3\text{H}$ -labelled nucleotides (0.4 Ci/mmol) in 100 μl of binding buffer (50 mM Tris-HCl [pH 7.5], 15 mM MgCl_2 , 0.1% polyoxyethylene 9 lauryl ether [Sigma], 1 mg of bovine serum albumin [BSA] per ml) by shaking at 30°C for 30 min. Aliquots were applied to a nitrocellulose filter presoaked with binding buffer. After being washed four times with TMD buffer (50 mM Tris-HCl [pH 7.5], 10 mM MgCl_2 , 1 mM dithiothreitol [J. T. Baker, Inc., Phillipsburg, N.J.]) at room temperature, the filter was dried; bound radioactivity was measured with an LS5801 liquid scintillation counter (Beckman Instruments, Fullerton, Calif.).

GTPase assay. The GTPase hydrolytic activity of recombinant SGP was measured as previously described (26). Briefly, purified recombinant SGP (50 pmol) was incubated in TMD buffer in the presence of GTP (10 μM ; 0.4 μCi of $[\alpha\text{-}^{32}\text{P}]\text{GTP}$; Du Pont, Boston, Mass.) in a total volume of 100 μl . The reaction was initiated by the addition of protein that had been prewarmed to 37°C . The reaction was kept at 37°C for 30 min and terminated by adding 10 μl of 0.5 M EDTA (pH 8.0). Two microliters of the sample was placed on polyethyleneimine-cellulose plates (Sigma), and thin-layer chromatography was carried out in 0.5 M KH_2PO_4 -1 M NaCl at room temperature. After autoradiography of the chromatogram, the spots corresponding to GTP and GDP (visualized under UV irradiation) were eluted and counted with a Beckman LS5801 liquid scintillation counter. The amount of GTP hydrolyzed was calculated on the basis of the specific activity of the substrate. One unit of GTPase activity was defined as the amount of enzyme catalyzing the formation of 1.0 nmol of GDP at 37°C for 30 min.

RESULTS

Expression and purification of SGP. To obtain sufficient amounts of highly purified SGP for characterization of this protein, we used a fusion protein strategy to express and purify SGP in *E. coli*. Initially, plasmid pMAL-SGP10 was constructed (Fig. 1) by subcloning the *sgp* gene into the pMAL-c2 expression vector downstream from and in the same reading frame as the MBP coding gene, *malE*. At the fusion junction,

TABLE 1. Purification of recombinant SGP^a

Step	Total protein (mg)	Total activity ^b	Sp act (total activity units/mg)
Crude extract	2,880	10,080	3.5
Amylose affinity chromatography	85	11,815	139
DEAE-Sephacel chromatography	18	5,760	320

^a From 1 liter of bacterial culture.^b One unit of GTPase activity is defined as the amount of enzyme catalyzing the formation of 1.0 nmol of GDP at 37°C for 30 min.

the vector included a sequence coding for the recognition site of protease factor Xa, which cleaves after amino acids Ile-Glu-Gly-Arg. This allows SGP to be cleaved from MBP following purification without adding any vector-derived residues to the former protein. This fusion protein was expressed by using the strong, IPTG-inducible Ptac promoter and the translation signal of MBP.

High-level expression of MBP-SGP fusion protein in *E. coli* was obtained following induction with IPTG for 8 h at 25°C. A prominent MBP-SGP fusion protein of 75 kDa was apparent following SDS-polyacrylamide gel electrophoresis (PAGE) analysis of bacterial lysates (Fig. 2A, lane 2). The size of this fusion protein is almost identical to that predicted from the amino acid sequences of both MBP and SGP. On the basis of laser densitometric analysis of the Coomassie brilliant blue-stained SDS-polyacrylamide gel, MBP-SGP fusion protein accounted for approximately 40% of cell protein. Moreover, most of this fusion protein in ultrasonic-disrupted cell supernatants was soluble (Fig. 2A, lane 3). Very little fusion protein was detected in inclusion bodies (data not shown). The expression of fusion protein was dependent on IPTG induction since uninduced cells did not produce any detectable MBP-SGP fusion protein (Fig. 2A, lane 1). Highly purified MBP-SGP fusion protein was obtained from bacterial lysates by using an amylose-resin affinity chromatography column (Fig. 2A, lane 4). As much as 85 mg of MBP-SGP fusion protein, with at least 95% purity, per liter of cell culture was obtained (Table 1). This fusion protein was highly susceptible to protease factor Xa cleavage, even at low factor Xa-to-fusion protein ratios, generating both SGP (33 kDa) and MBP (42 kDa) without any apparent secondary cleavage. It had been assumed that cleavage would result in nearly equivalent amounts of MBP and SGP. However, SDS-PAGE analysis revealed that the amount of SGP detected was much smaller than that of MBP (Fig. 2B, lane 2) because of apparent precipitation of SGP following cleavage. SGP was further purified by DEAE-Sephacel chromatography and eluted with a NaCl gradient. Purification, as visualized by SDS-PAGE analysis (Fig. 2B, lane 1), resulted in SGP with a specific activity of 320 units/mg (Table 1).

Subcellular localization of SGP in *S. mutans*. Following purification of SGP, specific antiserum against this protein was prepared. Initially, we demonstrated the existence of SGP in *S. mutans* cells by Western blotting with rabbit anti-SGP serum generated from recombinant SGP. This anti-SGP serum recognized a single 33-kDa band in both *S. mutans* samples of cell membrane and cytoplasmic preparations in Western blot assays (data not shown). The 33-kDa band was not visualized when these duplicate blots were probed with preimmune control serum or anti-SGP serum preabsorbed with SGP.

Then subcellular localization of SGP in *S. mutans* cells was directly determined by immunoelectron microscopy. Colloidal

gold particles were detected with anti-SGP serum in *S. mutans* cells (Fig. 3a) associated both with the cell membrane and cytoplasm. No colloidal gold particles were observed in *S. mutans* cells reacted with control serum (Fig. 3b). Although the number of particles observed were minimal because of the low levels of SGP expressed in cells, these results were consistent with those from Western blot analysis. These data suggested that *S. mutans* cells express SGP which is associated, in part, with the cytoplasmic membrane. Membrane association is a common characteristic for many members of the G protein superfamily (2).

Characterization of recombinant SGP as a G protein. Previous deduced amino acid sequence data revealed that SGP displayed GTP binding motifs characteristic of G proteins, as well as sharing 44.3% homology with Era, a GTPase from *E. coli* (28). As the first step in characterizing the biochemical properties of SGP, it was important to directly demonstrate GTP binding and hydrolysis by the purified protein. Nucleotide binding experiments with ³²P- and ³H-labeled substrates indicated that SGP binds both GTP and GDP, although it binds GDP slightly better than it does GTP (Table 2). It does not bind UTP, CTP, or ATP. This specific guanine nucleotide binding activity is typical of the superfamily of G proteins.

Purified soluble SGP (Fig. 4, lane 1) exhibited significant GTPase activity, which was not detected for refolded SGP from insoluble inclusion bodies (Fig. 4, lane 4). MBP-SGP fusion protein also exhibited considerable GTP hydrolytic activity (Fig. 4, lane 2). However, the level of this activity was not as high as that of purified SGP alone. This may result from the influence of MBP domains on SGP domains in this fusion protein. In these experiments, SGP exhibited GTPase activity similar to that of Era (kindly donated by B. Johnstone, University of California at Los Angeles) (Fig. 4, lane 3). It was unclear why SGP renatured from inclusion bodies after solubilization in urea exhibited no detectable GTPase activity.

To further characterize SGP substrate specificity, several related nucleotides were examined as potential competitors of GTP binding by GTPase assay (Fig. 5). Hydrolysis of [α -³²P] GTP to [α -³²P]GDP was significantly inhibited by adding unlabeled GDP (Fig. 5, lane 8), but not UTP, CTP, ATP, GMP, or cyclic GMP (cGMP) (lanes 3 through 7, respectively). In another experiment, we directly incubated SGP with [α -³²P] ATP and no detectable ATP hydrolysis was detected by thin-layer chromatography analysis (data not shown). These data strongly suggested that SGP is a member of the G protein superfamily since it can specifically bind GTP and GDP and catalyze hydrolysis of GTP.

It was also of interest to characterize the cofactor requirements for the GTPase activity of SGP. Similar to most other GTPases, SGP displayed a preference for Mg²⁺ as a divalent metal cofactor. Although SGP exhibited GTPase activity in the absence of Mg²⁺, the addition of this cation significantly increased its GTPase activity. The optimal concentration of free Mg²⁺ for activation was 10 to 20 mM, with higher concentrations inhibiting activity. These observations were similar to those made for Era (3) and other G proteins (10). To examine SGP binding of GTP in greater detail, a kinetic analysis of GTPase activity was initiated. The GTPase activity of SGP exhibited a linear increase during a 30-min incubation. The hydrolysis rates of GTP catalyzed by SGP were determined at various GTP concentrations in the presence and absence of GDP. Then the data obtained were analyzed by the double-reciprocal plot method of Michaelis-Menten (Fig. 6). Purified SGP exhibited an apparent *K_m* value of 5.9 μ M for GTP. Furthermore, it was suggested that the inhibitory effect of

FIG. 3. Subcellular localization of SGP protein by immunogold labelling. Fresh cultured *S. mutans* cells were fixed and sectioned as described in Materials and Methods. Samples were blocked by 10% normal goat serum and subsequently incubated with SGP-specific antibody (IgG) (a) or nonimmunized control IgG (b). Secondary anti-rabbit IgG conjugated with gold particles was used to detect bound rabbit IgG. Arrowheads indicate colloidal gold particles. CW, cell wall; CM, cytoplasmic membrane.

GDP on the GTPase activity of SGP was competitive and that SGP bound GTP and GDP at the same active site.

DISCUSSION

Recently, a putative *S. mutans* protein, SGP, was reported to have sequence homology with the *E. coli* Era protein (28). Furthermore, the functional motifs observed in most other proteins with GTP binding or hydrolyzing activity are well conserved in SGP. Therefore, SGP appeared to be a G protein. To directly verify this hypothesis, we initially expressed this protein in *E. coli* by using a fusion protein expression system. This system was capable of expressing SGP to a level of nearly 40% of total cellular protein. To prevent the formation of insoluble and inactive inclusion bodies, which is a common

problem with some high-level expression systems, we adopted a method that uses a relatively low growth temperature and enriched growth medium. Most of the SGP induced in *E. coli* under these growth conditions remained in an active soluble form (Fig. 2). SGP purification was facilitated in this system not only because of high-level synthesis of SGP but also because of utilization of an MBP tag which bound specifically to amylose-resin columns. This one-step affinity chromatography purification produced highly purified MBP-SGP fusion protein. SGP was further cleaved from this fusion protein and purified by DEAE-Sephacel ion-exchange chromatography. However, like most membrane-associated G proteins (17), SGP exhibited a tendency to form aggregates in aqueous solvents and therefore spontaneously precipitated. We utilized soluble active SGP for these studies, and interestingly, it was observed that the addition of GTP prevented this aggregation process (data not shown). This suggested that GTP influences the conformation of SGP and its physical chemical properties.

TABLE 2. Nucleotide binding by recombinant SGP

Substrate	Bound NTP/ bound GTP (cpm) ^a
α - ³² P-NTP	
GTP	1
ATP	0.17
UTP	0.15
CTP	0.18
³ H-NTP	
GTP	1
GDP	1.14

^a Each 100- μ l reaction mixture contained 500 pmol of purified recombinant SGP and 1 nmol of nucleoside triphosphate (NTP) (0.4 μ Ci). The reagent blank value containing BSA (500 pmol) was subtracted from all experimental counts. The net counts per minute of bound SGP for [α -³²P]GTP and [³H]GTP are 12,248 and 25,167 cpm, respectively. Data are means of three independent assays.

FIG. 4. GTPase activity of recombinant SGP. Purified recombinant SGP (50 pmol) (lane 1), MBP-SGP fusion protein (50 pmol) (lane 2), Era (50 pmol) (lane 3), refolded MBP-SGP fusion protein (50 pmol) from inclusion bodies (lane 4), BSA (50 pmol) (lane 5), and control buffer containing no SGP (lane 6) were incubated with [α -³²P]GTP for the standard GTPase assay.

FIG. 5. Inhibition of the GTPase activity of recombinant SGP by nucleotides. Reaction mixtures (100 μ l) contained 50 pmol of purified recombinant SGP and 10 μ M GTP (0.4 μ Ci of [α - 32 P]GTP) (lane 2), with 50 μ M UTP, CTP, ATP, GMP, cGMP, or GDP (lane 3 through 8, respectively), at 37°C for 30 min. Lane 1, control lacking SGP.

Recently, Soldati et al. showed that membrane targeting by a small GTPase is dependent on its GTP binding conformation (25). Therefore, it will be of interest to determine if GTP affects SGP binding to the *S. mutans* cell membrane in subsequent investigations.

Direct evidence that SGP is a G protein came from guanine nucleotide binding and GTP hydrolysis assays. SGP not only specifically binds guanine nucleotides but also catalyzes hydrolysis of GTP to GDP. It cannot bind other nucleotides nor catalyze their hydrolysis. Only GTP can serve as the specific hydrolyzable substrate of SGP. In this study, we have also demonstrated that unlike heterotrimeric G proteins, SGP-mediated GTP hydrolysis does not require any accessory protein for activation. Therefore, SGP appears to be a low-molecular-weight G protein that is capable of expressing high-level intrinsic GTPase activity. Moreover, SGP is similar to many other GTPases in its preference for Mg^{2+} as a divalent cofactor. Kinetic studies further revealed that the biochemical parameters of SGP-mediated hydrolysis of GTP were different from those of high-molecular-weight GTPases, with characteristically high intrinsic K_m values for GTP. The K_m value of SGP

FIG. 6. Kinetics of the GTPase activity of recombinant SGP. Purified recombinant SGP (50 pmol) was incubated with increasing concentrations of GTP in the presence and absence of GDP (2.5 μ M) in 100 μ l of TMD buffer at 37°C for 30 min. Samples were assayed as described in the text. A double-reciprocal plot of the Michaelis-Menten equation was used to analyze the kinetics of GTPase activity.

for this reaction was 5.9 μ M, nearly 75-fold lower than those of high-molecular-weight GTPases, such as Mx proteins (21) and dynamin (6), whose activities are regulated by GTPase-activating proteins. The low K_m value of SGP suggests that its cellular function does not require interactions with other activator proteins. Notably, the K_m value of SGP for GTP is almost identical to that of *E. coli* Era protein (3). This may reflect some functional similarity between these two proteins.

Localizing the subcellular compartments of SGP in *S. mutans* cells could provide information about its *in vivo* biological functions. These immunoelectron microscopy studies revealed that SGP is both located in the cytoplasm and associated with the cell membranes of bacterial cells. For most membrane-associated G proteins, membrane attachment is directed by C-terminal modifications (1, 4). Most frequently, these proteins contain at least one cysteine residue near the C terminus. In Ras, Ras-related, and other G proteins such as hGBP1 (4, 23). This cysteine forms part of a characteristic tetrapeptide motif, CAAX (with A representing an aliphatic residue and X representing any residue). CAAX serves as a signal sequence for isoprenylation, which anchors these G proteins to cell membranes. However, SGP does not contain a C-terminal CAAX sequence. There may be other mechanisms, such as carboxymethylation (22), that are responsible for anchoring G proteins to cell membranes. It is not clear whether SGP undergoes any modification *in vivo* and whether such modifications mediate membrane attachment. The relationship between the cytoplasmic and membrane-associated forms of SGP is also unknown. Recent studies (15, 19) have indicated that homologous Era protein specifically binds to *E. coli* membranes by a saturable mechanism. However, the identity of the receptor(s) is unknown.

In the past decade, considerable information about the roles of GTPases in cell physiology has been obtained (1, 2, 14). GTPases from bacteria as well as eukaryotic cells can be grouped into a G protein superfamily. They have similar structures and appear to act as molecular switches in different biological processes. Each member of the G protein superfamily is activated by binding GTP and deactivated following hydrolysis of GTP to GDP. The switch mechanism is remarkably versatile, enabling different G proteins to sort and amplify transmembrane signals, direct the synthesis and translocation of proteins, guide vesicular traffic through the cytoplasm, and control cell proliferation and differentiation. Therefore, membrane-associated G proteins are important regulators of membrane signaling in eukaryotic cells. As a new member of the G protein superfamily, SGP may function in a similar manner in *S. mutans*. However, the role of SGP in the physiology of *S. mutans* remains to be demonstrated. Recent data suggest that this protein is required for normal growth of this microorganism (28). Taking into consideration that SGP is a membrane-associated GTPase and that the *sgp* gene is present in the same operon as the *dgk* gene coding for a putative signal transduction component involved in aciduricity (28), it is possible that SGP also plays an important role in transmitting environmental signals into the cell cytoplasm. Investigations to confirm this intriguing hypothesis are in progress.

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REFERENCES

1. Bourne, H. R., D. A. Sanders, and F. McCormick. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature (London)* **348**: 125–132.
2. Bourne, H. R., D. A. Sanders, and F. McCormick. 1991. The GTPase superfamily: conserved structure and molecular mechanism. *Nature (London)* **349**:117–127.
3. Chen, S. M., H. E. Takiff, A. M. Barker, G. C. Dubois, J. C. A. Bardwell, and D. L. Court. 1990. Expression and characterization of RNase III and Era proteins. *J. Biol. Chem.* **265**:2888–2895.
4. Clerke, S. 1992. Protein isoprenylation and methylation of carboxy-terminal cysteine residues. *Annu. Rev. Biochem.* **61**:355–386.
5. Gollop, N., and P. E. March. 1991. A GTP-binding protein (Era) has an essential role in growth rate and cell cycle control in *Escherichia coli*. *J. Bacteriol.* **173**:2265–2270.
6. Gout, I., R. Dhand, I. D. Hiles, M. J. Fry, G. Panayotou, P. Das, O. Trounny, N. F. Totty, J. Hsuan, G. W. Booker, I. D. Campbell, and M. D. Waterfield. 1993. The GTPase dynamin binds to and is activated by a subset of SH3 domains. *Cell* **75**:25–36.
7. Hamada, S., and H. D. Slade. 1980. Biology, immunology, and cariogenicity of *Streptococcus mutans*. *Microbiol. Rev.* **44**:331–384.
8. Hamilton, I. R. 1986. Growth, metabolism and acid production by *Streptococcus mutans*, p. 145–155. In S. Hamada, S. M. Michalek, H. Kiyono, L. Menaker, and J. R. McGhee (ed.), *Molecular microbiology and immunology of Streptococcus mutans*. Elsevier, Amsterdam.
9. Harlow, E., and D. Lane. 1988. *Antibodies: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. Higashijima, T., K. M. Ferguson, M. D. Smigel, and A. G. Gilman. 1987. The effect of GTP and Mg^{2+} on the GTPase activity and fluorescent properties of G_0 . *J. Biol. Chem.* **262**:751–761.
11. Iwami, Y., K. Abbe, S. Takahashi-Abbe, and T. Yamada. 1992. Acid production by streptococci growing at low pH in a chemostat under anaerobic conditions. *Oral Microbiol. Immunol.* **7**:304–308.
12. Kuramitsu, H. K. 1993. Virulence factors of mutans streptococci: role of molecular genetics. *Crit. Rev. Oral Biol. Med.* **4**:159–176.
13. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680–685.
14. Lambright, D. G., J. P. Noel, H. E. Hamm, and P. B. Sigler. 1994. Structural determinants for activation of the α -subunit of a heterotrimeric G protein. *Nature (London)* **369**:621–628.
15. Lin, Y.-P., J. D. Sharer, and P. E. March. 1994. GTPase-dependent signaling in bacteria: characterization of a membrane-binding site for Era in *Escherichia coli*. *J. Bacteriol.* **176**:44–49.
16. Loesche, W. J. 1986. Role of *Streptococcus mutans* in human dental decay. *Microbiol. Rev.* **50**:353–380.
17. Lounshury, K. M., A. L. Beddow, and I. G. Macara. 1994. A family of proteins that stabilize the Ran/TC4 GTPase in its GTP-bound conformation. *J. Biol. Chem.* **269**:11285–11290.
18. Manina, C. V., P. D. Riggs, A. G. Grandea, B. E. Slatko, L. S. Moran, J. A. Tagliamonte, L. A. McReynolds, and C. Guan. 1988. A vector to express and purify foreign proteins in *E. coli* by fusion to, and separation from, maltose-binding protein. *Gene* **74**:365–373.
19. March, P. E. 1994. Personal communication.
20. Moore, J. J., A. Uppal, F. Maley, and G. F. Maley. 1993. Overcoming inclusion body formation in a high-level expression system. *Protein Exp. Purif.* **4**:160–163.
21. Pavlovic, J., A. Schroder, A. Blank, F. Pitossi, and P. Staeheli. 1993. Mx proteins: GTPase involved in the interferon-induced antiviral state. *Ciba Found. Symp.* **176**:233–247.
22. Ridley, A. J. 1994. Membrane-ruffling and signal transduction. *Bioessays* **16**:321–327.
23. Schwemmle, M., and P. Staeheli. 1994. The interferon-induced 67-kDa guanylate-binding protein (hGBP1) is a GTPase that converts GTP to GMP. *J. Biol. Chem.* **269**:11299–11305.
24. Sojar, H. T., J. Lee, G. S. Bedi, M. I. Cho, and R. J. Genco. 1991. Purification, characterization, and localization of a major membranous protein antigen from *Porphyromonas (Bacteroides) gingivalis*. *Biochem. Int.* **25**:437–446.
25. Soldati, T., A. D. Shiparo, A. B. D. Svejstrup, and S. R. Pfeffer. 1994. Membrane-targeting of the small GTPase Rab9 is accompanied by nucleotide exchange. *Nature (London)* **369**:76–78.
26. Sood, P., C. G. Lerner, T. Shimamoto, Q. Lu, and M. Inouye. 1994. Characterization of the autophosphorylation of Era, an essential *Escherichia coli* GTPase. *Mol. Microbiol.* **12**:201–208.
27. Yamashita, Y. 1993. Unpublished results.
28. Yamashita, Y., T. Takehara, and H. K. Kuramitsu. 1993. Molecular characterization of a *Streptococcus mutans* mutant altered in environmental stress responses. *J. Bacteriol.* **175**:6220–6228.