Nucleotide sequence and molecular characterization of a gene encoding GTP-binding protein from Streptococcus gordonii

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Received 3 September 1997; accepted 7 September 1997

Abstract

A 1286-bp fragment of chromosomal DNA from Streptococcus gordonii strain Challis was cloned and sequenced. The gene sgg consisted of 897-bp nucleotides encoding a 299-amino acid polypeptide (33 200 Da). The deduced amino acid sequence exhibited significant similarity to Era, G protein of Escherichia coli. The nucleotide binding assay demonstrated that recombinant Sgg bound $[^{32}]$P-GTP but not $[^{32}]$P-ATP, $[^{32}]$P-CTP, or $[^{32}]$P-UTP. These findings indicate that Sgg is a member of the G protein superfamily in the genus Streptococcus.

Keywords: GTP-binding protein; Streptococcus gordonii; Recombinant protein

1. Introduction

"Streptococcus sanguis" is known to be one of the first colonizers on newly erupted teeth or freshly cleaned tooth surfaces and plays an important role in the establishment of oral microbial flora [1-3]. 'S. sanguis' has been noticed because of its etiological significance in subacute bacterial endocarditis [4,5]. Kilian et al. [6] proposed that strains classified as 'S. sanguis' were divided into three distinct species, S. sanguis, Streptococcus oralis and Streptococcus gordonii. These oral streptococcal species can metabolize various sugars, mainly produce lactic acid, and survive an acidic environment [7]. Ability to adapt to various environmental changes such as pH, salt, and temperature should contribute to the pathogenicity of the organism; however, only little is known about the molecular mechanisms.

In this study, we focused on GTP-binding proteins (G proteins), which have been reported to be important components in key regulatory systems that control a wide variety of cellular responses in various species of eukaryotes and prokaryotes [8,9]. Since G proteins control proliferation and differentiation of eukaryotic cells, they should play pivotal roles in the development of malignant and infectious diseases [10]. G protein of Escherichia coli was first reported as Era in the bacterial world. It had considerable similarity to yeast RAS proteins, and therefore it was regarded as a superfamily member of G protein [11]. Since then, Era has been extensively characterized by several research groups. For example, disruption of the era gene by insertional inactivation resulted in inhibition of cell growth and viability [12,13]. However, little is known concerning G pro-

0378-1097/97/$17.00 © 1997 Federation of European Microbiological Societies. Published by Elsevier Science B.V.
P11 50378-1097(97)00426-6
tein in streptococcal species [14]. We report here a gene (sgg) encoding GTP-binding protein of *S. gordonii*, and the characterization of recombinant G protein (rSgg) encoded by sgg.

2. Materials and methods

2.1. Bacterial strains, vectors, media, and chemicals

*S. gordonii* Challis was grown in brain heart infusion broth (Disco). Other strains of streptococcal species were arbitrarily selected from the culture collection of our Department. *E. coli* JM109 was cultured in Luria-Bertani medium. Plasmid pGEM-T (Promega), pUC19, and pMAL-c2 (New England Biolabs) were used for gene cloning, sequencing, and recombinant protein expression. [γ-32P]dCTP, -ATP, -CTP, -GTP, and -UTP were obtained from ICN Biomedicals Inc. Restriction endonucleases were purchased from New England Biolabs and Takara. Other chemicals were obtained from Wako Pure Chemicals and Sigma Chemical Co.

2.2. DNA manipulations

Isolation of chromosomal DNA of streptococcal species, plasmid DNA preparation, transformation, electrophoresis, and Southern blot analysis were performed as described previously [15–17]. DNA fragments were purified from agarose gel with Qiaex II Gel Extraction Kit (Qiagen). Ligation was performed with the DNA Ligation Kit Ver. 2 (Takara). The PCR mixture (40 μl) was added to each sample of AmpliTaq DNA polymerase (1.25 U per 50 μl, PE Applied Biosystems), upstream and downstream primers (0.2 μM each), MgCl2 (2 mM), and 1×PCR buffer II as recommended by the manufacturer. As a probe to obtain G protein ORF (sgg) from *S. gordonii*, upstream primer A and downstream primer B were synthesized according to the nucleotide sequence of the sgg gene (GenBank accession number L03428) from *S. mutans*. The nucleotide sequences of the oligonucleotides A and B were 5'-CTTGTCAATT-CAGGTTTGGC-3' and 5'-CGCGCCAGTTTTT- TGTACC-3', respectively. The reaction was carried out in a thermal cycler (Model 9600, PE Applied Biosystems) under optimal condition for 35 cycles. The PCR products were analyzed by 1% agarose gel electrophoresis.

2.3. Nucleotide sequence analysis

The plasmid harboring a target insert was purified with QIAwells 8 plus (Qiagen), followed by amplification with M13 primers (PE Applied Biosystems) and ABI Prism Cycle Sequencing Kit (PE Applied Biosystems). The resultant PCR products were sequenced by a DNA sequencer (Model 373, PE Applied Biosystems). Nucleotide and deduced amino acid sequences were analyzed with the GeneWorks program of IntelliGenetics, Inc.

2.4. Cloning and expression of the sgg gene

Chromosomal *S. gordonii* DNA was digested with *Hind*III and then ligated with the *Hind*III cassette of LA PCR in vitro Cloning Kit (Takara). The PCR product containing the sgg gene was amplified according to the manufacturer's instruction. A 2.3 kb DNA fragment was cloned into pGEM-T, resulting in plasmid pSK128. Approximately 900 bp of the Sgg ORF with EcoRI and PstI sites was generated by PCR amplification with primer C (5'-GAATT-CATGACTTTTAAATCAGGCT-3') and primer D (5'-CTGCAGTCATACTCCTTATTATAG-3') as shown in Fig. 1. The PCR product was digested with *EcoRI* and *PstI*, and then ligated into expression vector pMAL-c2. In the resultant plasmid pSK131, the sgg gene showed the same orientation with *lacZ* (Fig. 1). Recombinant Sgg was expressed as an MBP-Sgg fusion protein under the control of the Ptac promoter.

The transformants (*E. coli* SK131) were grown to A600=0.5 and induced by adding IPTG to a final concentration of 0.3 mM. Following further incubation at 37°C for 2 h, the cells were harvested and lysed. The supernatant which contained fusion protein of maltose-binding protein (MBP) and rSgg was frozen at −20°C until use. rSgg was purified with the Protein Fusion and Purification System (New England Biolabs). Briefly, the cell lysate was applied onto an amylose resin column and then bound MBP-Sgg fusion protein was eluted with TNE buffer (20 mM Tris-HCl, pH 7.4; 200 mM NaCl; 1 mM EDTA) supplemented with 10 mM maltose. Then,
the fusion protein was cleaved with factor Xa in order to separate rSgg and MBP. rSgg was finally purified by DEAE-Sepharose column chromatography (Pharmacia).

2.5. Nucleotide binding assay

rSgg (500 pmol) was incubated with [α-32P]NTP (1 nmol, 1 Ci/mmol) by shaking at 37°C for 1 h as described previously [14,18] with minor modifications. Aliquots were spotted onto nylon membranes (1×1 cm, HyBond-N; Amersham), which were dried at room temperature. The membranes were washed 3 times and dried. The bound radioactivity was measured by a liquid scintillation counter (Aloka).

3. Results and discussion

To examine whether oral streptococcal species including S. gordonii, S. milleri, S. pyogenes, and S. salivarius possess G protein, we designed several pairs of primers for PCR amplification according to the nucleotide sequence of the sgg gene of S. mutans. PCR analysis using primers A and B showed that S. gordonii chromosomal DNA alone was amplified. Therefore, the PCR product was inserted into pGEM-T cloning vector and the resultant plasmid pMA1 was sequenced. The nucleotide sequence analysis revealed that 75% similarity existed between the insert from pMA1 and the sgg gene and that no ORF sequences were found in the PCR product. Thus, the LA PCR in vitro Cloning Kit was employed to obtain the complete ORF of the sgg gene of S. gordonii. The resultant 2.3 kb DNA fragment was amplified and cloned into pGEM-T (termed pSK128).

Sequence analysis revealed that pSK128 harbored a 897 bp ORF corresponding to the gene (sgg) encoding G protein and that the sgg ORF is able to encode a polypeptide of 299 amino acids with a calculated molecular mass of 33200 (Fig. 2). The nucleotide sequence upstream of the ATG showed a high similarity to the C-terminal region of the dkg

![Diagram](https://example.com/diagram.png)

Fig. 1. Scheme of construction of the expression vector for recombinant G protein (rSgg). The 900 bp of sgg gene was amplified by primers C and D and then digested with EcoRI and PstI. Subsequently, the fragment was cloned into pMAL-c2 that pre-digested with EcoRI and PstI. The recombinant protein is produced as a fusion form of MBP and rSgg by induction with IPTG.
The gene of *S. mutans* [19] (data not shown). The *dgk* gene has been located immediately upstream of the *sgg* gene [14,19]. Based on these findings, it is considered that a gene corresponding to the *dgk* gene should exist upstream of the *sgg* gene. The upstream region of the *sgg* gene remains to be investigated.

Comparison of the deduced amino acid sequence of Sgg with Sgp and Era revealed amino acid sequence identity/similarity of 93/97% and 44/62%, respectively (Fig. 3). Three boxed residues in N-terminal region (Fig. 3) are common to all GTP-binding proteins, i.e. *S. cerevisiae* RAS, human Ha-ras, and *E. coli* Era [10,11]. Sgg also possessed the same structural motifs that were highly conserved when compared with other G proteins. Southern blot analysis of several streptococcal species revealed that S. pyogenes, S. milleri, and S. salivarius chromosomal DNA hybridized to the *sgg* gene under medium stringency (data not shown). The results suggest that G protein in the genus *Streptococcus* appears conservative in spite of the difference of species.

In order to characterize Sgg, we constructed an expression vector, pSK131, which was constituted with plasmid pMAL-c2 and the *sgg* ORF amplified by primers C and D. SDS-PAGE showed that ~75 kDa of the MBP-Sgg fusion protein was abundantly expressed in *E. coli* SK131 when induced with IPTG (Fig. 4). The supernatant from the ultrasonic cell lysis was clear and no significant fusion protein was present as inclusion bodies. The MBP-Sgg fusion protein was purified by amylose resin affinity chromatography, followed by digestion with factor Xa which can cleave between MBP and Sgg (Fig. 4). Subsequently, Sgg (33 kDa) was separated from MBP (42 kDa) and factor Xa (42 kDa) by DEAE-Sepharose ion-exchange chromatography.
Fig. 4. Expression and purification of recombinant Sgg in E. coli carrying pSK131. Lane 1, marker; 2, E. coli lysate induced with IPTG; 3, E. coli lysate induced without IPTG; 4, MBP-Sgg fusion protein isolated by amylase resin column chromatography; 5, MBP and Sgg cleaved with factor Xa; 6, Sgg (33 kDa) purified by DEAE-Sepharose ion-exchange column chromatography; 7, marker.

The filter binding assay was employed to determine the ability of rSgg to bind nucleoside triphosphate (Table 1). It was found that rSgg specifically bound to GTP but not to ATP, CTP, and UTP. Furthermore, the binding of [32P]GTP to Sgg was investigated by adding excess amounts of unlabeled GTP, ATP, CTP, or UTP. It was shown that ATP, CTP, and UTP did not compete with [32P]GTP (Table 2). These results indicate again that Sgg possesses characteristics of GTP-binding proteins.

S. gordonii can adhere to substances found in the oral environment, i.e. mucin, fibronectin, and secretory immunoglobulin A [20,21]. In addition, the organism strongly coaggregates with several oral bacterial species, i.e. Actinomyces, Porphyromonas, and Fusobacterium [21,22]. Interaction of S. gordonii with other molecules which are involved in bacterial adherence may induce G protein-mediated signal transduction. It may be presumed that G protein could play an important role as second messenger in the bacterial world. However, much remains to be elucidated concerning the function of bacterial G proteins.

Eukaryotic and prokaryotic GTP-binding proteins are frequently reported to possess GTPase activities [10–12,23]. It has also been reported that GTP-binding protein with GTPase activity was autophosphorylated [24,25]. Therefore, Sgg is supposed to exhibit an intrinsic GTPase activity and autophosphorylation reaction. In this regard, putative GTP-binding and GTPase activating domains have been proposed within G protein molecules [26–28]. Furthermore, it was reported that disruption of the era gene encoding G protein from E. coli resulted in inhibition of cell growth and viability, indicating that era is essential for growth of E. coli at 43°C [13,29].

**Table 1**

<table>
<thead>
<tr>
<th>Nucleotide binding by recombinant Sgg</th>
<th>[α-32P]NTP</th>
<th>cpm bound/100 µl reaction*</th>
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<tbody>
<tr>
<td>GTP</td>
<td>50 349 ± 3 867</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>1 286 ± 254</td>
<td></td>
</tr>
<tr>
<td>CTP</td>
<td>364 ± 172</td>
<td></td>
</tr>
<tr>
<td>UTP</td>
<td>572 ± 121</td>
<td></td>
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</tbody>
</table>

*The reaction mixture (100 µl) contained 500 pmol of protein and 1 nmol of NTP (1 Ci/µmol). The reagent blank value containing bovine serum albumin (500 pmol) was subtracted from all counts. Data are the mean ± S.D. from at least three experiments.

**Table 2**

<table>
<thead>
<tr>
<th>Competing nucleotide</th>
<th>cpm bound/100 µl reaction*</th>
</tr>
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<tbody>
<tr>
<td>GTP</td>
<td>50 349 ± 3 867</td>
</tr>
<tr>
<td>ATP</td>
<td>3 768 ± 1 435</td>
</tr>
<tr>
<td>CTP</td>
<td>42 544 ± 6 731</td>
</tr>
<tr>
<td>UTP</td>
<td>48 190 ± 2 443</td>
</tr>
</tbody>
</table>

*The reaction mixture (100 µl) contained 500 pmol of Sgg, 10 nmol of cold competing nucleotide and 1 nmol of GTP (1 Ci/µmol). The reagent blank value containing bovine serum albumin (500 pmol) was subtracted from all counts. Data are the mean ± S.D. from at least three experiments.

**Acknowledgments**

The upstream and downstream nucleotide sequences including the sgp ORF gene were kindly provided by Dr. H.K. Kuramitsu (State University of New York, Buffalo, NY) for comparison with our data. This work was supported by grants from the Japan Society for the Promotion of Science and the Ministry of Health and Welfare.
References