Volume 249, number 2, 383–388 FEB 07233 June 1989

Homology between surface protein antigen genes of *Streptococcus sobrinus* and *Streptococcus mutans*

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Received 7 April 1989

The structural gene (*pag* gene) for a 210 kDa protein antigen of *Streptococcus sobrinus* serotype *g* was cloned and compared with that (*pac* gene) of a 190 kDa protein antigen of *Streptococcus mutans* serotype *c*. Immunodiffusion analysis revealed that the product of the *pag* gene immunologically cross-reacted with that of the *pac* gene. Southern blot and nucleotide sequence analyses revealed that a significant homology existed between the middle regions of the two structural genes.

Protein antigen; DNA homology; (*Streptococcus sobrinus, Streptococcus mutans*)

1. INTRODUCTION

The *Streptococcus mutans* group has been strongly implicated as causative organisms of dental caries [1,2]. The *S. mutans* group is divided into seven genospecies [3]. Among the *S. mutans* group, *Streptococcus mutans* and *Streptococcus sobrinus* are frequently isolated from human dental plaque [2].

Wall-associated high-molecular mass protein antigens of the *S. mutans* group have recently been the focus of intense research. *S. mutans* produces a wall-associated protein antigen of M_r 190000 which has been designated antigen B [4], I/II [5], IF [6], P1 [7] and PAc [8]. This protein antigen has been successfully used as a vaccine to protect monkeys against dental caries [9,10]. Local passive immunization with monoclonal antibodies against the protein antigen prevents the colonization of *S. mutans* on the tooth surface and the development of dental caries in monkeys [11].

On the other hand, *S. sobrinus* produces a wall-associated protein antigen of M_r 210000 named SpaA [12] or PAg [13], which shows serological cross-reactivity with the protein antigen of *S. mutans*. Previous comparison of amino acid compositions of the purified protein antigens showed similarities between *S. sobrinus* PAg [13] and *S. mutans* P1 [7]. These findings suggest that the protein antigens may have the same function. In addition, the determination of antigenically cross-reactive and homologous amino acid sequences in the protein antigens may lead to the development of synthetic peptide vaccines directed against both *S. sobrinus* and *S. mutans*. It is, therefore, interesting to compare the two antigens at the gene level. In this study, we have cloned the structural gene for the protein antigen of serotype *g S. sobrinus*, and compared it with the gene for the protein antigen of serotype *c S. mutans* cloned by Okahashi et al. [8,14]. Here, we refer to the 190 kDa protein antigen of serotype *c S. mutans* as PAc [8] and the 210 kDa protein antigen of

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

serotype *g* *S. sobrinus* as PAg (protein antigen serotype g).

2. MATERIALS AND METHODS

2.1. *Bacterial strains, plasmids, antigens and antibodies*

Streptococcus sobrinus MT3791 (serotype *g*) *and S. mutans* MT8148 (*c*) were used in this study. Recombinant plasmid pPC41 containing the structural gene for the PAc (*pac* gene) was constructed by Okahashi et al. [8,14]. Rabbit anti-PAc serum and anti-PAg serum were prepared as described previously [8,13]. Sonic extracts of recombinant *E. coli* cells were prepared as described by Okahashi et al. [8].

2.2. *Cloning of the gene coding for PAg*

Chromosomal DNA of *S. sobrinus* MT3791 was prepared as described by Okahashi et al. [8]. This DNA was partially digested with *Sau*3A (Toyobo Co., Osaka, Japan) and ligated with T4 DNA ligase (Toyobo) to *Bam*HI-digested calf intestinal alkaline phosphatase (Boehringer Mannheim GmbH, Mannheim, FRG)-treated plasmid vector pUC19 [15]. *Escherichia coli* JM109 [15] was then transformed and plated on LB agar containing ampicillin (50 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal; 40 μg/ml) (Boehringer Mannheim).

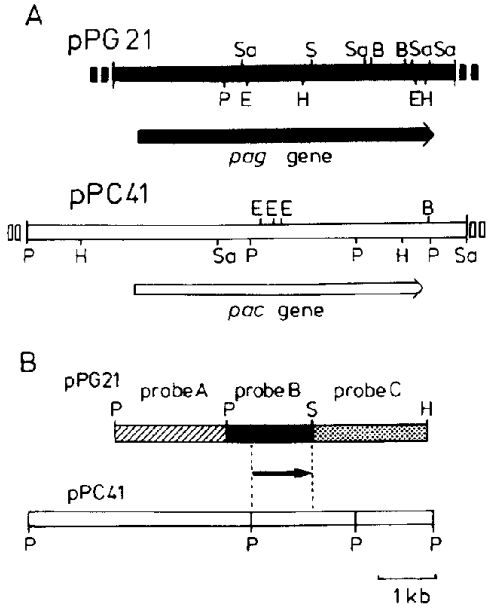

Fig.1. (A) Restriction endonuclease map of pPG21. The *S. sobrinus* DNA insert is indicated by the black bar. The broken bar indicates the plasmid vector. The map of pPC41 containing the *pac* gene was reported by Okahashi et al. [8]. (B) Probes A, B and C were used for the Southern hybridization in fig.3. The *Pst*I fragments of pPC41 used in the Southern hybridization in fig.3 are indicated by open bars (below). The arrow indicates the region for which the nucleotide sequences of the two genes are presented in fig.4. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sal*I; Sa, *Sac*I.

Colony immunoblot was performed for screening clones reactive with anti-PAg serum [8]. Protein in the *E. coli* sonic extracts was analyzed by SDS-polyacrylamide gel electrophoresis, Western immunoblotting and immunodiffusion, as reported in [8].

2.3. *Southern hybridization*

The DNA fragment (pPG21) containing the intact *pag* gene and the DNA fragment containing the *pac* gene [8] were digested with *Pst*I-*Sal*I and *Pst*I, respectively. Three probes covering the 5'-terminal region (2.0 kb *Pst*I-*Pst*I fragment; probe A, see fig.1), middle region (1.4 kb *Pst*I-*Sal*I fragment; probe B) and 3'-terminal region (2.1 kb *Sal*I-*Hin*dIII fragment; probe C) of the *pag* gene were radiolabelled by nick-translation [16] using ^{32}P-labelled deoxycytidine triphosphate (Amersham, Buckinghamshire, England). Hybridization on nitrocellulose membranes was performed according to the procedure of Southern [17] with 50% formamide at 42°C (allowing up to 15% base mismatch [17]) or 20% formamide at 42°C (allowing up to 35% base mismatch [14]).

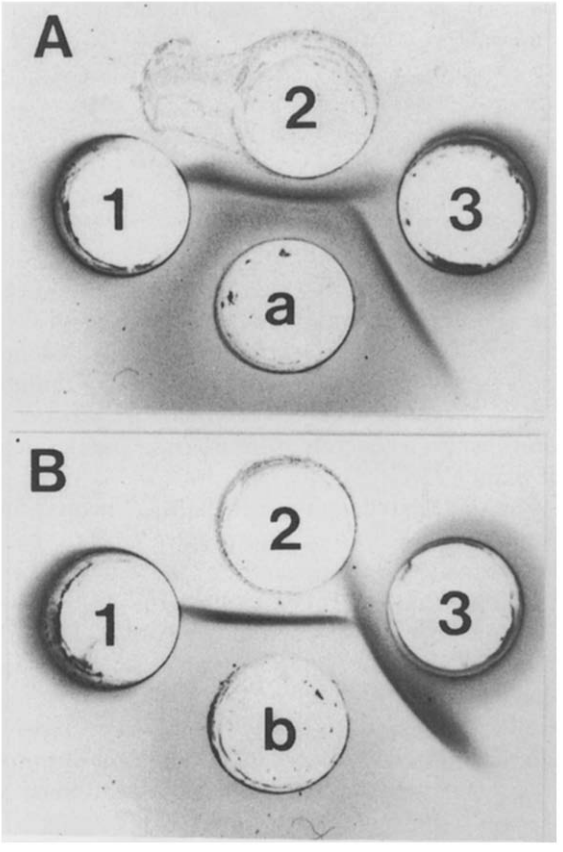

Fig.2. Immunodiffusion of recombinant PAg and PAc against anti-PAg serum (A) and anti-PAc serum (B). Wells: 1, the cell extract of *E. coli* JM109 (pUC19); 2, the cell extract of *E. coli* JM109 (pPG21); 3, the cell extract of *E. coli* JM109 (pPC41); a, rabbit anti-PAg serum; b, anti-PAc serum.

2.4. *Nucleotide sequence*

DNA fragments were subcloned into pUC118 or pUC119 (Takara Shuzo, Kyoto, Japan). Single-stranded DNAs were prepared as described in the Takara manual (Takara Shuzo). Nucleotide sequences were determined by the dideoxy chain termination method as described by Mizusawa et al. [18] with a 7-Deaza sequencing kit (Takara Shuzo). The nucleotide sequence of the *pac* gene was reported by us [14].

3. RESULTS

3.1. *Cloning of the* pag *gene in* E. coli

Fragments of chromosomal DNA from *S. sobrinus* MT3791 partially digested with *Sau*3A (serotype *g*) were ligated into the *Bam*HI site of pUC19. Western blot analysis of sonic extracts of *E. coli* clones positive for antigen expression showed that one clone, pPG21 (5.8 kb insert), expressed a 210 kDa PAg. Immunodiffusion analysis revealed that a precipitin line formed between the sonic extract of *E. coli* (pPG21) and anti-PAg serum was fused with that produced between the PAg from *S. sobrinus* MT3791 and the antiserum (data not shown). Fig.1A shows the restriction map of pPG21. The map of pPG21 differed from that of pPC41 containing the *pac* gene (fig.1A). This 5.8 kb insert fragment (pPG21) appeared to contain a promoter that was functional in *E. coli*, since the clones containing the insert in both orientations with respect to the *lac* Z gene produced the PAg (not shown). Expression of the PAg in various deletion mutants indicated that the cloned gene was transcribed from the left side to the right side of the restriction map of pPG21 (fig.1A, arrow).

3.2. *Immunological homology between recombinant PAg and recombinant PAc*

In immunodiffusion testing, a precipitin line produced between recombinant PAg and anti-PAg serum formed a spur with that produced between recombinant PAc and anti-PAg serum (fig.2A). On the other hand, a precipitin line produced between recombinant PAc and anti-PAc serum also formed a spur with that produced between recombinant PAg and anti-PAc serum (fig.2B). These results indicate that PAg and PAc share a common antigenic determinant.

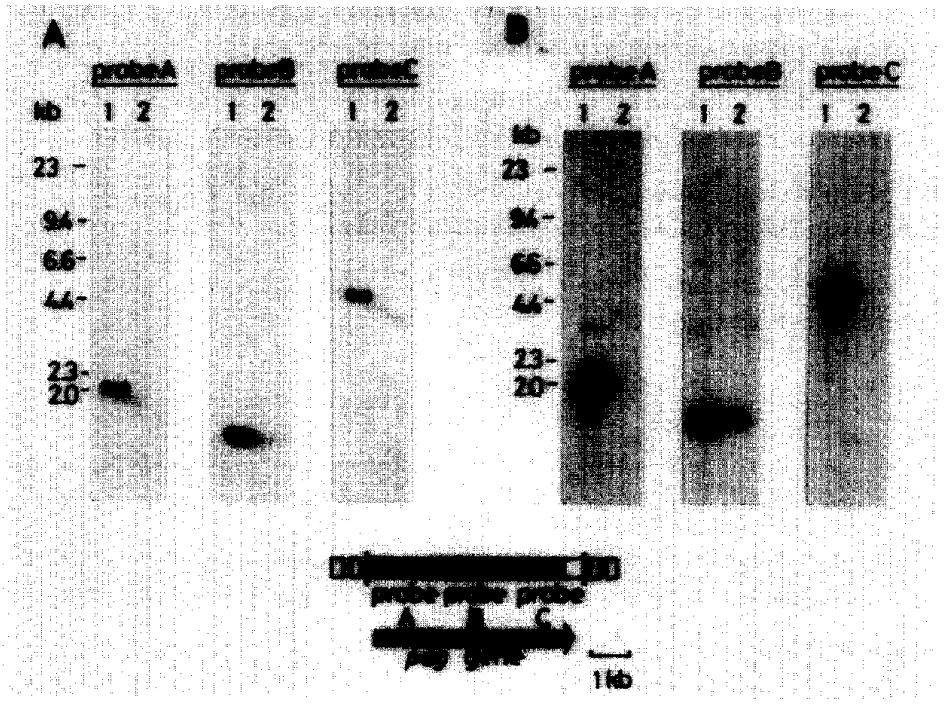

Fig.3. Southern hybridization analyses at 42°C in 50% formamide (A) and at 42°C in 20% formamide. The ^{32}P-labelled *Pst*I-*Pst*I fragment (probe A), *Pst*I-*Sal*I fragment (probe B) and *Sal*I-*Hin*dIII fragment (probe C) of pPG21 were used as probes. Lane 1, *Pst*I-*Sal*I digest of pPG21; lane 2, *Pst*I digest of pPC41.

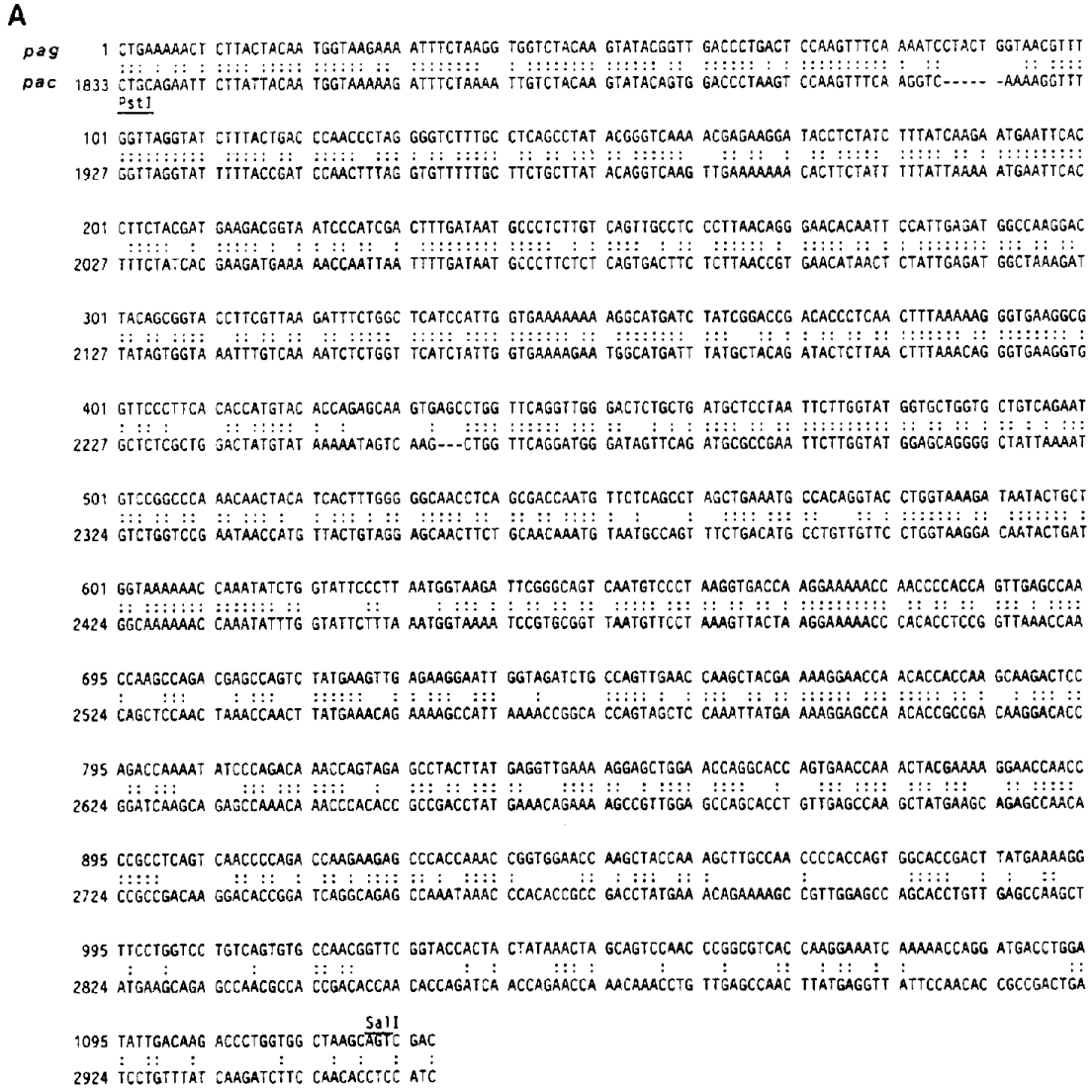
A
pag
pac
PstI
SalI
B
PAg
PAc

Fig.4. (A) Nucleotide sequence of the middle region of the *pag* in comparison with that of the *pac* gene. Nucleotides that are identical with those of the *pac* gene are indicated by a colon. The numbers on the left of the *pac* sequence correspond to the base number starting from the initiation codon of the open reading frame of the *pac* [14]. The location of these two sequences is presented in fig.1B. (B) Amino acid sequence of the middle region of PAg protein deduced from the nucleotide sequence presented in A. Amino acids that are identical with those of PAc protein are indicated by a colon. The number on the left of the PAc amino acid sequence corresponds to the amino acid number starting at the first methionine of the PAc protein [14]. Gaps in the sequences are indicated by a dash.

3.3. *Distribution of sequences homologous to the* pag *gene and the* pac *gene*

Three probes, 2.0 kb *Pst*I-*Pst*I fragment (5′-terminal region), 1.4 kb *Pst*I-*Sal*I fragment (middle region) and 2.1 kb *Sal*I-*Hin*dIII fragment (3′-terminal region) were prepared from the *pag* gene (fig.1B). Under the high stringent condition (50% formamide at 42°C), all the *pag* probes hybridized only to the fragments of the parent gene (fig.3A). On the other hand, under the low stringent condition (20% formamide at 42°C) the middle probe (probe B) within the *pag* gene hybridized to the 1.5 kb *Pst*I-*Pst*I fragment covering the middle region of the *pac* gene (fig.3B). These results indicate that there is a significant homology between the middle regions of the *pag* gene and the *pac* gene.

The nucleotide sequence of the middle region of the *pag* gene was determined and compared with that of the *pac* gene. Fig.4A shows the sequences of the two genes from the *Pst*I site of *pac* gene to the *Sac*I site of the *pag* gene (see fig.1B). The matching nucleotides in these sequences were 62%. The deduced amino acid sequence showed 66% homology between the two proteins (fig.4B). Within this homologous region, proline-rich tandem repeats were detected in the PAc protein. Although the PAg protein contained a similar proline-rich region, the sequence was not so regular as the PAc protein.

4. DISCUSSION

In the present study, the *pag* gene coding for a 210 kDa protein antigen (PAg) of *S. sobrinus* MT3791 (serotype *g*) has been cloned. The product of the *pag* gene immunologically cross-reacted with that of the *pac* gene cloned from *S. mutans* MT8148 (serotype *c*). Furthermore, Southern hybridization analysis under low stringency revealed that a low but significant homology existed between the middle regions of *S. sobrinus pag* gene and *S. mutans pac* gene (fig.3). Nucleotide sequence analysis revealed the existence of clusters of homologous sequence in the middle regions of the two genes.

These findings are of particular interest because of the involvement of these organisms in dental caries [1,2], and the evolutionary differences of the two species, i.e., *S. sobrinus* has a G + C content = 44–46% and *S. mutans* has a G + C content = 36–37% [1,2]. Although the biological significance of the homologous regions of the two antigens is not understood, the knowledge of the primary structures of these regions might be useful in designation and synthesis of peptides that act as anti-caries vaccines against both *S. sobrinus* and *S. mutans*.

Acknowledgements: This work was supported in part by Grant-in-Aid for Developmental Scientific Research (no.63870086) from the Ministry of Education and Culture of Japan.

REFERENCES

[1] Hamada, S. and Slade, H.D. (1980) Microbiol. Rev. 44, 331–384.
[2] Loesche, W.J. (1986) Microbiol. Rev. 50, 353–380.
[3] Whiley, R.A., Russell, R.R.B., Hardie, J.M. and Beighton, D. (1988) Int. J. System Bacteriol. 38, 25–29.
[4] Russell, R.R.B. (1980) J. Gen. Microbiol. 118, 383–388.
[5] Russell, M.W., Bergmeier, L.A., Zanders, E.D. and Lehner, T. (1980) Infect. Immun. 28, 486–493.
[6] Hughes, M., Machardy, S.M., Sheppard, A.J. and Woods, N.C. (1980) Infect. Immun. 27, 576–588.
[7] Forester, H., Hunter, N. and Knox, K.W. (1983) J. Gen. Microbiol. 129, 2279–2288.
[8] Okahashi, N., Sasakawa, C., Yoshikawa, M., Hamada, S. and Koga, T. (1989) Mol. Microbiol. 3, 221–228.
[9] Lehner, T., Russell, M.W., Caldwell, J. and Smith, R. (1981) Infect. Immun. 34, 407–415.
[10] Russell, R.R.B., Beighton, D. and Cohen, B. (1982) Br. Dent. J. 152, 81–84.
[11] Lehner, T., Caldwell, J. and Smith, R. (1985) Infect. Immun. 50, 796–799.
[12] Holt, R.G., Abiko, Y., Saito, S., Smorawinska, M., Hansen, J.B. and Curtiss, R., iii (1982) Infect. Immun. 38, 147–156.

[13] Okahashi, N., Koga, T. and Hamada, S. (1986) Microbiol. Immunol. 30, 35–47.
[14] Okahashi, N., Sasakawa, C., Yoshikawa, M., Hamada, S. and Koga, T. (1989) Mol. Microbiol., in press.
[15] Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Gene 33, 103–119.
[16] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[17] Southern, E.M. (1975) J. Mol. Biol. 98, 503–517.
[18] Mizusawa, S., Nishimura, S. and Seela, F. (1986) Nucleic Acids Res. 14, 1319–1324.