Expression of a Gene for Glucan-binding Protein from *Streptococcus mutans* in *Escherichia coli*

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The structural gene for a glucan-binding protein (GBP) of *Streptococcus mutans* has been inserted into a bacteriophage λ vector and expressed in *Escherichia coli* K12. Lysates of *E. coli* infected with the recombinant phage contain an antigenic protein of the same size as *S. mutans* GBP. The GBP synthesized in *E. coli* can be affinity-purified on immobilized glucan and antiserum raised against it has been shown to precipitate fructosyltransferase activity from *S. mutans*.

INTRODUCTION

Streptococcus mutans is believed to be important in the initiation of dental caries (Hamada & Slade, 1980) but investigation of certain aspects of its physiology and antigenic composition has been hampered by the lack of a system of genetic analysis. Recently, however, several authors have described the transfer of *S. mutans* genes to other organisms, using techniques of *in vitro* DNA manipulation (Holt *et al.*, 1982; Jagusztyn-Krynicka *et al.*, 1982; Macrina *et al.*, 1980; Robeson *et al.*, 1983). A genetic approach should be of particular value in studies of glucosyltransferase (GTF) and fructosyltransferase (FTF), extracellular enzymes of *S. mutans* which synthesize polymers from sucrose, since their products are involved in the formation and metabolism of dental plaque. Both GTF and FTF exist in multiple forms, the relationship of which is unclear (Russell, 1979*a*). In addition to GTF and FTF, *S. mutans* produces glucan-binding proteins which have been demonstrated to contribute to its ability to adhere to surfaces (Russell, 1979*b*; Douglas & Russell, 1982; Russell *et al.*, 1983*a*).

This paper describes the first use of bacteriophage λ for the cloning of *S. mutans* genes and provides additional evidence for the recent observation (Russell *et al.*, 1983*a*) that one of the *S. mutans* proteins which has the ability to bind to glucans is related to FTF. This glucan-binding protein has molecular weight of 74000 and is referred to here as GBP.

METHODS

Construction of gene bank. Details of the DNA manipulation techniques used have been described in detail elsewhere (Kehoe *et al.*, 1983). Briefly, chromosomal DNA from *S. mutans* strain Ingbritt was partially digested with restriction endonuclease *Sau*3A to give fragments in the 5–20 kbp range. These fragments were then cloned into the *Bam*HI sites of the replacement bacteriophage vector λ L47.1 (Loenen & Brammar, 1980) and packaged into phage particles *in vitro*. This phage population was then plated on an *E. coli* strain lysogenic for phage P2, in order to select recombinant phage (since only recombinants of λ L47.1 in which the *red* and *gam* genes have been replaced by a cloned insert can grow on P2 lysogens). Plaques formed on the P2 lysogen were pooled to form a 'gene bank' stock.

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Abbreviations: FTF, fructosyltransferase; GTF, glucosyltransferase; GBP, glucan-binding protein.

Screening of gene bank. The gene bank was plated on *E. coli* C600 to give approximately 500 plaques per plate. A disc of nitrocellulose with an embossed grid (Schleicher & Schüll BA 85/23) was laid on top of the agar overlay and left in contact at 4 °C for at least 30 min. The nitrocellulose disc was then placed in 0.05 M-Tris/HCl (pH 7.5) containing 0.15 M-NaCl (Tris/saline buffer, TSB) and 5% (w/v) haemoglobin at 37 °C for 1 h. It was then shaken gently at room temperature for 2 h in TSB containing 5% (w/v) haemoglobin and rabbit antiserum raised against *S. mutans* antigens, diluted 1/100 (v/v). The nitrocellulose disc was washed by shaking for 10 min in TSB, 10 min in TSB + 0.05% Nonidet P40 and 10 min in TSB (Burnette, 1981) before shaking for 90 min at room temperature in TSB + 5% (w/v) haemoglobin containing swine anti-rabbit serum conjugated to peroxidase, diluted 1/200 (Dako P217, Mercia Brocades, Weybridge, UK). The washing procedure was repeated as before and the nitrocellulose disc placed in 0.05 M-Tris/HCl (pH 7.5) containing 0.06% (w/v) 4-chloro-1-naphthol (Sigma) and 0.01% (v/v) H₂O₂ (Hawkes *et al.*, 1982). In some experiments, instead of peroxidase-conjugated second antibody, rabbit antibody was located by means of sheep antiserum to rabbit immunoglobulins (Sigma) labelled with ¹²⁵I by means of Iodobeads (Pierce & Warriner Ltd, Chester, UK). Washing was as before, and the dried nitrocellulose disc was exposed to Kodak X-Omat AR film between two DuPont Lightning Plus intensifying screens at -20 °C (Swanstrom & Shank, 1978). All phage plaques have a faint image due to binding of natural antibodies to *E. coli* present in the rabbit serum, but those in which *S. mutans* antigens were expressed were readily detectable by their greater intensity of labelling. Such positive plaques were purified by streaking and single plaques picked and stored over chloroform. Background labelling due to antibodies to *E. coli* was much less when the peroxidase conjugate was used, compared to radiolabelled conjugate.

Preparation of *E. coli* lysates. λ vectors carrying *S. mutans* genes were diluted and plated on *E. coli* C600 so as to yield confluent lysis after 16 h incubation of plates at 37 °C. Then 2 ml of 0.05 M-Tris/HCl (pH 7.5) was added to each plate, and the soft agar layer was scraped off, macerated and placed in an ultrasonic bath for 15 min. The lysates were then centrifuged at 47000 g for 3 h to deposit agar and approximately 99% of the phage particles, leaving the proteins in the supernatant.

Electrophoresis. The conditions for SDS-PAGE and rocket- and crossed-immunoelectrophoresis were as described previously (Russell, 1979*a, b*). Proteins were transferred to nitrocellulose after SDS-PAGE (Western blotting) as described by Burnette (1981), antigens being detected as described above for gene bank screening.

Affinity chromatography. Glucan-binding proteins were purified by passing the *E. coli* lysates or *S. mutans* culture supernatant down a chromatography column made by mixing a slurry of 2 ml Sepharose CL-6B (Pharmacia) with 200 mg of the insoluble polymer mutant (synthesized by *S. mutans* GTF: Russell *et al.*, 1983*a*). The column was eluted with 6 M-guanidine HCl and fractions of 1 ml were collected and dialysed against 0.05 M-Tris/HCl buffer (pH 7.5) before analysis by SDS-PAGE, immunoelectrophoresis, or assay for FTF activity (Russell *et al.*, 1983*a*).

RESULTS

The conditions chosen for cleaving the *S. mutans* chromosome with *Sau*3A should result in the entire gene bank being represented in approximately 1000 phage plaques. In our initial screening, approximately 1200 plaques were replica blotted to nitrocellulose and probed with a polyvalent antiserum to *S. mutans*, capable of detecting at least 12 antigens in crossed immunoelectrophoresis experiments. Seven plaques expressing *S. mutans* antigens were detected. These were purified by restreaking and lysates were prepared. Samples of the lysates were then examined for their antigen content by rocket immunoelectrophoresis using rabbit antisera specific for *S. mutans* wall-associated protein antigens A, B and C (Russell *et al.*, 1983*b*), GTF and GBP (Russell, 1979*a*; Russell *et al.*, 1983*a*). Four of the lysates gave precipitin peaks with the antiserum to GBP. The height of the rockets indicated that lysates contained 50 μ g GBP ml⁻¹, a concentration approximately 100 times that found in cultures of *S. mutans*. None of the other lysates reacted with the sera tested, indicating that they contain antigens to which we do not yet have specific antisera or in amounts insufficient to be detected by rocket immunoelectrophoresis. However, by further screening of the gene bank with antisera directed against specific antigens, we have been able to find recombinant plaques in which other antigens, including antigens A, B and GTF, are expressed (R. R. B. Russell & M. L. Gilpin, unpublished).

The antigen content of lysates of the GBP-recombinants was also examined by Western blotting. All four gave identical patterns, with a band of mol. wt 74000 identical in mobility to the GBP of *S. mutans* and an additional band, mol. wt 76500, also reacting with antisera to GBP (Fig. 1). It has previously been reported that GBP from *S. mutans* tends to break down to yield

Fig. 1. Western blot of (a) *S. mutans* culture supernatant and (b) lysate of *E. coli* infected with recombinant λ carrying the GBP gene. Antigens were located with antibody raised against pure *S. mutans* GBP.

Fig. 2. Fused-line immunoelectrophoresis in which concentrated *S. mutans* culture supernatant (Sm) and lysate of *E. coli* (Ec) infected with recombinant λ specifying GBP were electrophoresed into antiserum raised against either GBP purified from *S. mutans* (a, c) or against GBP produced in *E. coli* (b, d). (a) and (b) show the immunoprecipitates stained with Coomassie Blue; (c) and (d) show the location of FTF activity detected by autoradiography of the same slides after incubation in [^{14}C]sucrose.

multiple bands in the mol. wt range 50000–70000 (Russell *et al.*, 1983*a*) and the GBP in *E. coli* lysates (Fig. 1*b*) appears also to yield such minor bands.

Lysates of the GBP-recombinants were passed down affinity chromatography columns of immobilized mutan. After washing, the retained proteins were eluted and analysed by gel electrophoresis. This efficient, single-step purification procedure showed that both the 76500 and 74000 mol. wt bands bound to mutan and could be eluted by soluble dextran or by chaotropic agents such as guanidine.HCl.

To investigate the relationship between GBP and FTF further, antiserum was raised in rabbits by immunization with GBP produced in lysates of *E. coli* and purified by affinity chromatography. This antiserum precipitated antigen from both *S. mutans* and *E. coli* lysate, the single precipitation line showing identity of the two antigens (Fig. 2*b*). This identity was also revealed by reaction with antiserum raised against GBP from *S. mutans* (Fig. 2*a*). The precipitation lines formed by *S. mutans* GBP contained FTF activity whereas those formed by *E. coli* GBP did not (Figs 2*c*, *d*).

DISCUSSION

Cloning of *S. mutans* genes in *E. coli* has hitherto used either plasmid or cosmid vectors (Holt *et al.*, 1982; Jagusztyn-Krynicka *et al.*, 1982; Robeson *et al.*, 1983). The use of λ as a vector has a number of advantages, including the ability to clone large DNA fragments, the presence of the strong pL promoter and the fact that cloned products are released during lytic burst. The results presented above demonstrate that the expression of *S. mutans* antigens in recombinant plaques can readily be detected and, at least in the case of GBP, the responsible gene is transcribed by *E. coli* to yield a product that has the same molecular weight as, and is antigenically identical to, that made in *S. mutans*. The molecule of mol. wt 76500 most probably consists of GBP with an attached signal peptide which would normally be cleaved during export of GBP across the cytoplasmic membrane of *S. mutans* (Michaelis & Beckwith, 1982).

The cloned GBP gene product, produced in *E. coli*, retains the ability of *S. mutans* GBP to bind to dextran or mutan. It does not, however, retain the FTF activity found in immunoprecipitates of *S. mutans* GBP. Although in early experiments we believed we could detect FTF activity in *E. coli* lysates (Russell *et al.*, 1983*a*) we have not been able to reproduce those results. A possible explanation for lack of FTF activity is that the cloned GBP gene product, mol. wt 76500, is not processed in *E. coli* to give an active enzyme even though a fragment, mol. wt 74000, apparently the same size as the *S. mutans* GBP is generated. Despite the lack of enzyme activity in the cloned GBP gene product, the fact that antiserum raised against GBP purified from *E. coli* lysate precipitates FTF from *S. mutans* supports our previous evidence for the apparent identity of GBP to one of the forms of FTF of *S. mutans* (Russell *et al.*, 1983*a*).

Although we have failed to find expression of *S. mutans* FTF activity in *E. coli* lysates in the experiments described above, we have been able to detect activity of several other *S. mutans* enzymes in lysates made with other recombinants (R. R. B. Russell & M. L. Gilpin, unpublished). These findings, together with the results presented here, indicate that a cloning system based on bacteriophage λ provides a convenient and effective technique for the study of the properties of *S. mutans*.

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