Characterization of Glucosyltransferase Expressed from a *Streptococcus sobrinus* Gene Cloned in *Escherichia coli*

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The gene encoding a glucosyltransferase which synthesized water-insoluble glucan, *gtfI*, previously cloned from *Streptococcus sobrinus* strain MFe28 (mutans serotype *h*) into a bacteriophage λ vector, was subcloned into the plasmid pBR322. The recombinant plasmid was stable in *Escherichia coli* and *gtfI* was efficiently expressed. The GTF-I expressed in *E. coli* was compared to the corresponding enzymes in *S. sobrinus* strains MFe28 (serotype *h*), B13 (serotype *d*) and 6715 (serotype *g*) and shown to resemble them closely in molecular mass and isoelectric point. The insoluble glucan produced by GTF-I from recombinant *E. coli* consisted of 1,3- α -D-glycosyl residues (~90%). An internal fragment of the *gtfI* gene was used as a probe in hybridization experiments to demonstrate the presence of homologous sequences in chromosomal DNA of other streptococci of the mutans group.

INTRODUCTION

The glucosyltransferases (GTFs) of the mutans group of oral streptococci are extracellular enzymes responsible for the synthesis of complex mixtures of water-soluble and insoluble glucans. These glucans are believed to be important in the formation and metabolism of dental plaque (Hamada & Slade, 1980). It was first shown by Guggenheim & Newbrun (1969) that a single strain could produce a number of electrophoretically distinct GTFs. Ciardi *et al.* (1976, 1977) showed that some of these enzymes synthesized soluble, and others insoluble, polymers but progress towards understanding the reason for the multiplicity of enzymes has been considerably hampered by technical problems, including the fact that GTFs exist in high-molecular-mass aggregates and have a strong tendency to bind to separation matrices commonly used in column chromatography (Germaine *et al.*, 1977; Russell, 1979; Figures & Edwards, 1981; Ono *et al.*, 1984). The latter fact has, however, proved useful in purification procedures involving affinity chromatography (McCabe & Smith, 1977; Russell, 1979; McCabe, 1985). At least some of the heterogeneity of GTFs is a consequence of proteolytic degradation (Asem *et al.*, 1986; Mukasa, 1986; Russell *et al.*, 1986), but many questions remain concerning the number of distinct GTFs and their individual functions.

Most progress has been made with strains that originally constituted *Streptococcus mutans* serotypes *d* and *g*, which are now classified as *Streptococcus sobrinus* (Coykendall, 1983; Coykendall & Gustafson, 1986). The purification from strains of these serotypes of GTFs capable of synthesizing soluble glucans (GTF-S) or insoluble glucans (GTF-I) has been described by a number of workers (Ciardi *et al.*, 1977; Germaine *et al.*, 1977; Hare *et al.*, 1978; Figures & Edwards, 1981; Fukushima *et al.*, 1981; Fukui *et al.*, 1982; Koga *et al.*, 1983; Robyt & Martin, 1983; Furuta *et al.*, 1985; McCabe, 1985; Namiki *et al.*, 1985; Mukasa, 1986). The glucan synthesized by GTF-S (dextran) consists mainly of 1,6- α -linked glucose units while that

Abbreviations: GTF, glucosyltransferase; PAS, periodic acid-Schiff.

synthesized by GTF-I (mutan) is rich in 1,3- α -linkages. Recently, the existence of another GTF, which makes a soluble glucan but is distinguished by the fact that it requires no dextran primer in order to function, has been reported (Tsumori *et al.*, 1983; McCabe, 1985). We refer to this as GTF-S2 and to the primer-dependent enzyme as GTF-S1 (Mukasa, 1986). These three different GTFs can be distinguished by isoelectric focusing (Tsumori *et al.*, 1983) and are immunologically distinct (Shimamura *et al.*, 1983; Mukasa, 1986).

The GTFs of strains of *S. sobrinus* serotype *h*, which is closely related to serotypes *d* and *g*, have not been investigated in detail, although they were reported to have similar molecular masses and isoelectric points to serotype *d* and *g* enzymes and to cross-react antigenically with the latter (Beighton *et al.*, 1981; Gilpin *et al.*, 1985). Serotype *h* strains were, however, remarkable in that virtually all the glucan they produced was insoluble and bound to the bacteria in sucrose-containing media (Beighton *et al.*, 1981).

In order to investigate the GTFs of *S. sobrinus* serotype *h* strains and aid in the characterization of the different forms of GTFs, we have introduced a genetic approach and have described the cloning of genes for a GTF-S and a GTF-I in a bacteriophage λ vector (Gilpin *et al.*, 1985). This paper describes further studies on the cloned gene for GTF-I and compares the enzyme specified by it with GTF of the *S. sobrinus* strain from which it was derived.

METHODS

Strains and culture conditions. The strains used in this study were as follows: *Streptococcus cricetus* strain AHT (mutans serotype *a*); *Streptococcus rattus* strain FAI (serotype *b*); *S. mutans* strains Ingbritt (serotype *c*), C40 (serotype *e*) and 151 (serotype *f*); and *S. sobrinus* strains K1 (serotype *g*), 6715 (serotype *g*) and MFe28 (serotype *h*). All were from existing laboratory stocks apart from *S. mutans* strain C40, which was recently isolated from plaque of a child in Hampshire (D. Beighton, personal communication). For biochemical studies, mutans bacteria were grown in either the chemically defined medium of Terleckyj *et al.* (1975) or the same medium with 0.5% (w/v) casein hydrolysate (Oxoid) substituted for the mixture of individual amino acids. Cultures of *E. coli* strains DH1 (Davis *et al.*, 1980) or DS410 (Dougan & Kehoe, 1984) carrying plasmids were grown in either LB broth (Davis *et al.*, 1980) or Brain Heart Infusion broth (Oxoid) containing the appropriate antibiotics: tetracycline (15 $\mu\text{g ml}^{-1}$), ampicillin (100 $\mu\text{g ml}^{-1}$) or both antibiotics.

Isolation of streptococcal DNA. The bacteria were grown in Todd-Hewitt broth (Oxoid) supplemented with DL-threonine (20 mM) and DNA was prepared as described by Monsen *et al.* (1983). The mutanolysin M1 enzyme (Yokogawa *et al.*, 1975) used to lyse the bacteria was a gift from M. Yokogawa, Dainippon Pharmaceutical Company Japan.

Isolation of plasmid DNA. Plasmid DNA was extracted using the Triton X-100 procedure (Davis *et al.*, 1980) and purified by ultracentrifugation in caesium chloride gradients containing ethidium bromide (Maniatis *et al.*, 1982).

Isolation of bacteriophage DNA. Bulk phage lysates were prepared by propagating bacteriophage on *E. coli* strain C600 (Davis *et al.*, 1980) grown in liquid M9 minimal salts medium containing the following supplements: casein hydrolysate (10 g l⁻¹), glucose (4 g l⁻¹), maltose (4 g l⁻¹) and thiamin (10 $\mu\text{g l}^{-1}$). DNA was isolated from the phage as described by Maniatis *et al.* (1982).

DNA manipulations. Restriction endonuclease digestions and ligation reactions were performed according to the supplier's guidelines (Boehringer). Transformation of *E. coli* recipients with plasmid DNA was done according to the method of Cohen *et al.* (1973).

Subcloning gene *gtfI* on to plasmid pBR322. The construction of recombinant bacteriophage λ MFeA carrying gene *gtfI* from *S. sobrinus* strain MFe28 has been described (Gilpin *et al.*, 1985). DNA purified from bacteriophage λ MFeA was digested to completion with *Hind*III and ligated with pBR322 DNA cleaved with *Hind*III, using T4 DNA ligase. The ligation mixture was used to transform *E. coli* strain DH1. Transformants were selected on LB agar plates containing ampicillin (100 $\mu\text{g ml}^{-1}$). Transformants containing inserts were detected as tetracycline-sensitive (Tc^s) by replica-plating on tetracycline-containing medium. Each Tc^s transformant was streaked on sucrose indicator medium containing agar (10 g l⁻¹), sucrose (10 g l⁻¹), tryptone (5 g l⁻¹), sodium chloride (5 g l⁻¹) and bromothymol blue (200 mg l⁻¹), adjusted to pH 7.0. Transformants capable of utilizing sucrose were detected by the appearance of yellow-orange colonies.

Minicell analysis of the polypeptides encoded by pMLG10. Plasmids were introduced into the minicell-producing *E. coli* strain DS410 by transformation. Use of the minicell system and the methods employed were as described by Dougan & Kehoe (1984).

Southern-blot hybridization. A 3.3 kbp *Hpa*I fragment from within the cloned *gtfI* sequence was isolated from pMLG1 DNA using the procedure given by McDonnell *et al.* (1977). The *Hpa*I fragment was radiolabelled with

[α - 32 P]dATP using the nick translation method described by Feinberg & Vogelstein (1983) and used to probe for homologous sequences in *Hpa*I chromosomal DNA fragments from representative strains of each of the eight mutants serotypes, using the method of Southern (1975) as described by Maniatis *et al.* (1982).

Electrophoresis and isoelectric focusing. The conditions for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), detection of GTF activity on gels and detection of antigens by Western blotting were all as used previously (Russell, 1979; Russell *et al.*, 1985). Molecular mass standards (carbonic anhydrase, ovalbumin, bovine serum albumin, phosphorylase *b*, β -galactosidase and myosin) were from Sigma.

The procedure for isoelectric focusing of GTFs in the range pH 3–10 on thin layers of polyacrylamide and subsequent detection of GTF activity was as described by Tsumori *et al.* (1983). Calibration was done with the Broad pI kit supplied by Pharmacia. the pI of GTF-I purified from *E. coli* strain MAF10 was also determined using ultrathin layer polyacrylamide gels (Servalyt Precotes 3-10, Serva Feinbiochemical), using the manufacturer's instructions for electrophoresis and staining and a kit of standard proteins (pH 2.4–5.65; BDH). After focusing, standard marker proteins were stained with Coomassie brilliant blue. Glucosyltransferase activity was detected by overnight incubation of the gel at 37 °C in phosphate buffer containing sucrose (Tsumori *et al.*, 1983). Insoluble glucan was easily seen by dark-ground illumination, while soluble glucans were detected by staining with periodic acid-Schiff (PAS) reagent.

DNA fragments were analysed in 0.7 or 0.8% (w/v) agarose gels, stained with ethidium bromide (0.5 μ g ml⁻¹) for 30 min and photographed under UV light through a Kodak Wratten 23A orange filter (Maniatis *et al.*, 1982).

Purification of GTF-I. GTF-I was purified from lysates of *E. coli* strain C600 infected with recombinant bacteriophage λ MFeA and from cells of *E. coli* strain MAF10 (which carries plasmid pMLG10) by subjecting the bacteria to ultrasonic disruption and using a single-step affinity chromatography procedure: the bacterial lysate or extract was passed through a column containing Sepharose CL-6B (Pharmacia) and mutan. After washing with buffer, bound GTF-I was eluted with 6 M-guanidine.HCl (Russell *et al.*, 1985).

Preparation of antisera. Antiserum to GTF-I prepared as described above was raised in a New Zealand White rabbit by a schedule of three intramuscular injections, three weeks apart, each of 100 μ g GTF-I combined with aluminium hydroxide (Alu Gel S, Serva) as adjuvant. The rabbit was bled one week after the final injection. Antisera to GTF-S1, GTF-S2 and GTF-I, purified from *S. sobrinus* strain 6715 as described elsewhere (Mukasa, 1986), were raised as described by Mukasa & Slade (1973).

Characterization of glucans. For analysis by gas-liquid chromatography (GLC), the insoluble polymer formed by incubation of a crude extract of *E. coli* strain MAF10 (harbouring plasmid pMLG10) with sucrose was washed twice with water, subjected to complete methylation and hydrolysed to the partially methylated glucose derivatives. The derivatives were further reduced, acetylated, and the resultant partially methylated glucitol acetates analysed by GLC (Shimamura *et al.*, 1982). The composition of the glucan was also analysed by 13 C nuclear magnetic resonance (NMR) spectrometry: glucan was washed with distilled water, dried and assayed at a concentration of 100 mg ml⁻¹ in D₂O containing 0.5 M-NaOD. Sample temperature was 21 °C. Natural-abundance 13 C NMR spectra were recorded at 67.8 MHz on a Jeol JNM-GX270 FT NMR spectrometer with spinning sample tubes of 5 mm outer diameter. The sample was pulsed with 45° radio-frequency pulses (3.6 μ s pulse width). A spectral width of 8000 Hz was used, and spectra accumulated at 16384 data points. Chemical shifts were measured digitally and are reported in parts per million downfield from internal methyl carbon of 3-(trimethylsilyl)propanesulphonic acid sodium salt. Assignment of peaks was based on the report by Colson *et al.* (1974).

RESULTS

Size of insert of S. sobrinus DNA in recombinant bacteriophage λ MFeA

Originally gene *gtfI* was cloned into the *Bam*HI sites of bacteriophage vector λ L47.1 on a *Sau*3A fragment of DNA from *S. sobrinus* strain MFe28. The recombinant bacteriophage was designated λ MFeA (Gilpin *et al.*, 1985).

DNA purified from bacteriophages λ MFeA and λ L47.1 was digested separately with *Eco*RI, *Hind*III and *Bam*HI, and analysed by agarose gel electrophoresis. It was found that only one of the two *Bam*HI sites of bacteriophage λ L47.1 had been regenerated in the recombinant λ MFeA. Digestion of λ MFeA DNA with *Hind*III gave six fragments of sizes 23, 8.2, 5.0, 2.6, 2.3 and 0.5 kbp (total 41.6 kbp). Digestion of λ MFeA DNA with *Eco*RI gave four fragments of sizes 21.7, 7.6, 7.2 and 5.1 kbp (total 41.6 kbp). Given that the size of the bacteriophage λ L47.1 genome with the *Bam*HI stuffer fragment removed is 34 kbp (Loenen & Brammar, 1980), the size of insert of *S. sobrinus* strain MFe28 DNA in the recombinant bacteriophage λ MFeA was calculated to be 7.6 kbp.

Fig. 1. Restriction map of recombinant plasmid pMLG1, which carries gene *gtfI* on a 5 kbp *Hind*III fragment subcloned from recombinant bacteriophage λMFeA into the *Hind*III site of vector plasmid pBR322. The double open lines represent plasmid pBR322 sequences, the solid bars represent *S. sobrinus* strain MFe28 DNA, and the hatched area represents bacteriophage λL47.1 DNA. Deletions introduced at either end of the insert produced plasmids pMLG1-A and pMLG1-B. pMLG1-A lacks a 1.55 kbp *Bgl*II–*Bam*HI fragment found in pMLG1, whereas pMLG1-B lacks a 1.33 kbp *Eco*RI–*Sac*I fragment. The arrow indicates the direction of transcription of gene *gtfI* (deduced from deletion analysis). The zig-zag line indicates the 3.3 kbp *Hpa*I fragment used as probe in Southern hybridization experiments. Restriction endonucleases: A, *Ava*I; B1, *Bam*HI; B2, *Bgl*II; C, *Cla*I; E1, *Eco*RI; E5, *Eco*RV; H1, *Hpa*I; H3, *Hind*III; N, *Nde*I; S, *Sac*I.

Sub-cloning gene *gtfI* on to plasmid pBR322

DNA from recombinant bacteriophage λMFeA was completely digested with *Hind*III and ligated with *Hind*III-cleaved pBR322 DNA. The ligation mixture was used to transform *E. coli* strain DH1 to ampicillin resistance (Amp^r). Of 440 Amp^r transformants tested, 21 (4.8%) were Tc^s, of which 11 gave a positive sucrose-utilization phenotype when streaked on sucrose-indicator agar medium. Confirmation that gene *gtfI* had been sub-cloned came from a Western blot experiment using antiserum raised to GTF-I isolated from a bacteriophage λMFeA lysate, and from the detection of bands of GTF activity in SDS-PAGE gels (not shown).

Plasmids in two of the Amp^r transformants have been characterized further. Plasmid pMLG1 has a 5 kbp *Hind*III insert in pBR322, whereas pMLG10 has a 10 kbp insert made up of two copies of the same 5 kbp insert as in pMLG1 linked in tandem. A restriction endonuclease site map of pMLG1 is shown in Fig. 1. Approximately 0.5 kbp at the right end of the insert is derived from the λ phage vector (M. L. Gilpin, unpublished data). Thus the insert of *S. sobrinus* strain MFe28 DNA in pMLG1 is 4.5 kbp. Given that the molecular mass of GTF-I is about 160 kDa (see below), and that 1 kbp of DNA can code for a protein of molecular mass 37 kDa, it was estimated that gene *gtfI* would occupy about 4.3 kbp of DNA. The insert of *S. sobrinus* strain MFe28 DNA in pMLG1 therefore consists almost entirely of gene *gtfI*.

Deletions extending into either end of the insert in pMLG1 were created by cleaving the DNA simultaneously with *Bgl*II and *Bam*HI, or *Eco*RI and *Sac*I (followed by 'polishing' the fragment ends with DNA polymerase I to create blunt ends), religating and transforming the deletion plasmids into *E. coli* strain DH1. Transformants bearing plasmids with the generated deletions were detected by their inability to produce acid on sucrose-indicator agar medium. Neither deletion plasmid pMLG1A, which lacks a 1.55 kbp *Bgl*II–*Bam*HI fragment found in pMLG1, nor deletion plasmid pMLG1B, lacking a 1.33 kbp *Eco*RI–*Sac*I fragment, produced an

Fig. 2

Fig. 3

Fig. 2. Southern blot hybridization of a 3.3 kbp *HpaI* DNA fragment from within gene *gtfI* (see Fig. 1) to: lane 1, 3.3 kbp *HpaI* DNA probe; lanes 2–9, *HpaI*-cleaved chromosomal DNA of representative strains of mutans streptococci: lane 2, *S. cricetus* strain AHT (serotype *a*); lane 3, *S. rattus* strain FA1 (serotype *b*); lane 4, *S. mutans* strain Ingbritt (serotype *c*); lane 5, *S. mutans* strain C40 (serotype *e*); lane 6, *S. mutans* strain 151 (serotype *f*); lane 7, *S. sobrinus* strain B13 (serotype *d*); lane 8, *S. sobrinus* strain K1 (serotype *g*); lane 9, *S. sobrinus* strain MFe28 (serotype *h*).

Fig. 3. Analysis of the polypeptides expressed by minicells of *E. coli* strain DS410 carrying plasmids pBR322 or pMLG10. The polypeptides were analysed by SDS-PAGE after incorporation of L-[³⁵S]methionine and stained with Coomassie brilliant blue (lanes 1 and 2) followed by autoradiography (lanes 3 and 4). Minicells harboured: lanes 1 and 3, pBR322 (control); lanes 2 and 4, pMLG10. GTF-I, Tc and Amp indicate migration of proteins encoded by the *gtfI*, *tet* and *amp* genes.

active GTF-I (Fig. 1). However, pMLG1B codes for an enzymically inactive protein of about 120 kDa which was detected by Western blotting using antiserum raised against GTF-I (data not shown). This suggests that the promoter for initiating transcription of gene *gtfI* lies within the 1.55 kbp *Bgl*II–*Bam*HI fragment of pMLG1. Moreover, some of the other recombinant pBR322 subclones studied carried the 5 kbp *Hind*III insert in reverse orientation (data not shown) and expressed *gtfI*. This strongly suggests that transcription of *gtfI* originates from the cloned DNA of *S. sobrinus* strain MFe28 and not from the vector plasmid pBR322.

Occurrence of DNA sequences homologous to gtfI among strains of mutans streptococci

An internal 3.3 kbp *HpaI* fragment of the *gtfI* sequence (Fig. 1) was selected to probe *HpaI*-digested chromosomal DNA from representatives of the different species of mutans streptococci. Sequences with homology with the probe were found in all the strains tested, but only *S. sobrinus* strain MFe28 (serotype *h*) and *S. cricetus* AHT (serotype *a*) carried fragments identical in size to the 3.3 kbp probe (Fig. 2). In all strains (most obviously in *S. cricetus* strain AHT) there existed more than one chromosomal fragment with a sequence capable of hybridizing with the probe. The possibility that the detection of such multiple hybridization fragments was due to incomplete digestion of the chromosomal DNA was excluded by a series of experiments in which digestion with *HpaI* was allowed to proceed for different times.

Fig. 4

Fig. 5

Fig. 4. Isoelectric focusing patterns of GTF activities in cultures of *S. sobrinus* strains B13 (serotype *d*, lanes 1 and 4), 6715 (serotype *g*, lanes 2 and 5) and MFe28 (serotype *h*, lanes 3 and 6). Insoluble glucan could be seen by dark-ground illumination (lanes 1–3) and soluble glucans after PAS staining (lanes 4–6). The arrows indicate the positions of standards from the isoelectric focusing calibration kit (Pharmacia).

Fig. 5. Immunodiffusion analysis of reactions between GTF-I purified from *E. coli* strain MAF10 (centre well) and antisera raised against GTF-S1 (well 1), GTF-S2 (well 2) and GTF-I (well 3) purified from *S. sobrinus* strain 6715. Well 4 contained homologous antiserum raised against GTF-I purified from *E. coli* strain MAF10.

Minicell analysis of the polypeptides encoded by pMLG10

The polypeptides encoded by plasmid pMLG10 were compared with those specified by plasmid pBR322 in a minicell system using *E. coli* strain DS410 as the host. The polypeptides were analysed by SDS-PAGE after incorporation of L-[³⁵S]methionine. Comparison of the Coomassie brilliant blue stained pattern of proteins in *E. coli* strain DS410 bearing either pBR322 or pMLG10 indicated a high level of expression of *gtfI* (Fig. 3). The major band of GTF-I had a molecular mass of approximately 160 kDa but multiple bands of lower molecular mass were also detectable in the autoradiograph (Fig. 3). It would appear that these are all degradation products of GTF-I, since antiserum to GTF-I reacted with bands of a multiplicity of sizes in Western blot experiments. In addition, several bands just below the one of 160 kDa had GTF activity on SDS-PAGE gels (not shown).

Isoelectric focusing of glucosyltransferases

Since the characterization of GTFs of *S. sobrinus* serotype *h* by isoelectric focusing has not previously been reported, culture supernatants of *S. sobrinus* strains B13 (serotype *d*), 6715 (serotype *g*) and MFe28 (serotype *h*) were subjected to isoelectric focusing on thin layers of polyacrylamide gel (Fig. 4). Although for each strain only a single band of GTF-I activity was visible in unstained gels, the two zones of GTF-S activity always gave a series of minor bands close to the major ones, visible after PAS staining, as previously reported (Tsumori *et al.*, 1983). The pI of major GTF bands resolved by isoelectric focusing varied by up to 0.2 pH units from experiment to experiment. The results summarized in Table 1 are the means of at least three determinations for each strain and show that the three strains of *S. sobrinus* (serotypes *d*, *g* and *h*)

Table 1. *Isoelectric points of glucosyltransferases*

Isoelectric points were determined by isoelectric focusing on horizontal polyacrylamide slab gels, followed by incubation in sucrose and PAS staining. Each value is the mean of at least three separate determinations.

Species	Strain (serotype)	pI values		
		GTF-S1	GTF-S2	GTF-I
<i>S. sobrinus</i>	B13 (<i>d</i>)	4.0	5.6	4.8
	6715 (<i>g</i>)	3.9	5.6	5.0
	MFe28 (<i>h</i>)	4.2	5.6	4.9
<i>E. coli</i>	MAF10*	—	—	4.9

* *E. coli* strain DH1 carrying recombinant plasmid pMLG10.

gave closely similar results. Recombinant *E. coli* strain MAF10 contained no detectable GTF-S activity, but had a GTF-I band with a pI the same as that of the GTF-I found in *S. sobrinus* strain MFe28 and close to those of the other *S. sobrinus* strains (Table 1).

Immunological relationship of GTF

We previously reported that the enzyme encoded by *gtfI* reacted with antiserum to GTF of *S. sobrinus* strain K1 (serotype *g*) (Gilpin *et al.*, 1985). Immunodiffusion analysis was used to investigate the reaction of the enzyme with antisera raised against GTF-S1, GTF-S2 and GTF-I from another *S. sobrinus* strain, 6715 (serotype *g*), purified by procedures described elsewhere (Shimamura *et al.*, 1985; Mukasa, 1986). No reaction was seen between GTF-I purified from *E. coli* strain MAF10 and antiserum to GTF-S1 or GTF-S2, but a strong precipitin line was observed between the antigen and antiserum raised against GTF-I. A line of identity was formed by the antisera raised against GTF-I purified from *E. coli* strain MAF10 and against GTF-I from *S. sobrinus* strain 6715 (Fig. 5).

By immunodiffusion, antigens related to the cloned GTF-I could be detected in culture supernatants only of mutants serotypes *a*, *d*, *g* and *h* (i.e. *S. cricetus* and *S. sobrinus*), but the more sensitive Western blotting technique revealed cross-reactive antigens in all eight serotypes *a-h* (data not shown). In every case multiple bands were detected by Western blotting, but it is not known whether this was due to the degradation of the highest molecular mass form of GTF-I or to antigenic cross-reactivity with other GTFs.

Analysis of glucan synthesized by E. coli strain MAF10

The insoluble glucan produced by incubation of crude extracts of *E. coli* strain MAF10 with sucrose was converted into partially methylated alditol acetates. The derivatives of 2,3,4,6-tetra-2,4,6-tri-, 2,3,4-tri- and 2,4-di-*O*-methyl D-glucitol were detected at 0.4, 88.4, 2.4 and 0.5 mol% respectively, with 8.2% unknown derivatives. The glucan thus consisted of 88% 1,3- α -D-glucosyl residues with 2.4% 1,6- α -glucosyl residues. NMR analysis of the glucan was in agreement with this composition as, based on peak areas (Fig. 6), the glucan was calculated to consist of 94.4% 1,3- α -D-glucan and 5.6% unknown components. The nature of the unidentified linkages is not, at present, clear.

DISCUSSION

We have previously reported that the GTFs of *S. sobrinus* serotype *h* strains closely resemble those of strains of serotypes *d* and *g* (Beighton *et al.*, 1981). Those findings were extended by the present work which showed that strain MFe28 (serotype *h*) contained three GTFs resolvable by isoelectric focusing and that these had isoelectric points close to those of enzymes GTF-S1, GTF-S2 and GTF-I previously described for serotype *d* and *g* strains (Shimamura *et al.*, 1983; Tsumori *et al.*, 1983; Mukasa, 1986). Despite these similarities between GTFs however, serotype *h* strains differ from those of serotypes *d* and *g* in a number of other respects, and

Fig. 6. ^{13}C NMR spectrum of water-insoluble glucan produced by an extract of recombinant *E. coli* strain MAF10, which carries *gtfI* on plasmid pMLG10. Based on peak areas, the glucan was calculated to consist of 94.4% 1,3- α -D-glucan and 5.6% unknown components.

DNA-DNA hybridization studies indicate that serotype *h* should constitute a distinct subspecies of *S. sobrinus* (R. A. Whiley, J. Hardie, R. R. B. Russell and D. Beighton, unpublished data). It should also be noted that the strains of serotypes *d* and *g* used in this study differed from each other and from strain MFe28 (serotype *h*) in the Southern blot experiment with the *gtfI* probe (Fig. 2).

The preliminary characterization of the GTF synthesized in *E. coli* infected with recombinant bacteriophage λ carrying the *gtfI* gene showed that its molecular mass matched that of an enzyme in *S. sobrinus* strain MFe28 (Morrissey *et al.*, 1985). The GTF expressed in *E. coli* also occurred in a form with a pI the same as that of the enzyme in *S. sobrinus* strain MFe28 (Table 1), but studies of both molecular mass and pI were complicated by the degradation of GTF during storage, regardless of the species by which it was synthesized. It is possible to reduce this degradation in streptococcal species by early harvesting of cultures, maintenance of low temperatures and addition of the protease inhibitor phenylmethylsulphonyl fluoride (Mukasa, 1986; Russell *et al.*, 1986), but we have not yet found a way of preventing degradation in *E. coli*. As a result, we are as yet unable to arrive at a precise molecular mass of GTF-I or indeed even to be fully confident that we have cloned the entire gene. This issue will, however, be resolved by DNA-sequencing studies currently in progress.

Analysis of the glucan produced from sucrose by the cloned GTF-I showed it to consist almost entirely of 1,3- α -D-glucosyl residues. The enzyme is thus confirmed to be comparable to that purified from strain 6715-15 by Fukui *et al.* (1982) and referred to by them as mutansynthetase (EC 2.4.1.?). The pI of 4.9 is close to that of GTF-I reported by others for strains of serotypes *d* and *g* (Tsumori *et al.*, 1983; Furuta *et al.*, 1985; Namiki *et al.*, 1985; McCabe 1985). There was also a close immunological relationship between GTF-I of serotypes *d*, *g* and *h*, detectable by immunodiffusion. The use of the more sensitive Western blotting revealed a wider conservation of antigenic determinants in all eight mutans serotypes *a-h*. The data from DNA hybridization experiments with a *gtfI* gene probe were fully consistent with this result since sequences homologous with an internal fragment of *gtfI* were found in chromosomal DNA from representatives of all serotypes. In some strains, there was also evidence for the existence of more than one sequence with homology, which suggests that multiple copies of *gtfI* genes are present or that there is some homology between the gene for GTF-I and genes for other GTFs.

The *gtfI* gene probe will be of value for the screening of recombinant gene banks constructed from other mutans streptococci for such *gtf* genes. This may be particularly useful for studies of *S. mutans* serotypes *c*, *e* and *f*, which are the most common isolates from humans but with which we have so far been unsuccessful in attempts to clone *gtfI*. There is, however, the possibility that the sequences of DNA which hybridize with the *gtfI* gene probe do not code for a GTF, but form part of some other gene with partial homology, for example a determinant coding for glucan-binding properties.

The restriction map of *gtfI* of *S. sobrinus* strain MFe28 will be of value for comparison with cloned genes for other GTFs. It bears no resemblance to that of a *gtfQ* gene cloned from *S. mutans* strain GS-5 (serotype *c*) studied by Kuramitsu & Aoki (1986). Moreover, our own experiments (unpublished data) indicate that the *gtfI* gene is quite distinct from the *gtfS* gene we have cloned from *S. sobrinus* MFe28 (serotype *h*) (Gilpin *et al.*, 1985). The nature of the product, independence of primer and pI of 5.5 (R. R. B. Russell, unpublished) show that the enzyme encoded by the latter gene corresponds to that referred to as GTF-S2 by Mukasa (1986) and as GTF-S4 by McCabe (1985). The isoelectric focusing results in this paper indicate that there also exists a third, primer-dependent GTF-S1 enzyme in MFe28 and we intend to rescreen our recombinant gene bank for this.

The availability of recombinant *E. coli* strains carrying the genes for individual GTFs will greatly facilitate purification of the enzymes for biochemical studies and also make it possible to investigate the genetic relatedness and evolution of the multiple forms of GTF found amongst the streptococci and related genera.

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