Nucleotide sequence of the *Streptococcus mutans* *gtfD* gene encoding the glucosyltransferase-S enzyme

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The nucleotide sequence of the *Streptococcus mutans* GS-5 *gtfD* gene coding for the glucosyltransferase which synthesizes water-soluble glucan (GTF-S) has been determined. The complete gene contains 4293 base pairs and the unprocessed protein is composed of 1430 amino acids with a molecular mass of 159 814 Da. The amino terminus of the unprocessed protein resembles the signal sequences of other extracellular proteins secreted by *S. mutans* and that of the GTF-I secreted by *Streptococcus downei*. In addition, the GTF-S protein exhibits high amino acid similarity with the strain GS-5 enzymes responsible for insoluble glucan synthesis (GTF-I, GTF-SI) previously isolated and sequenced in this laboratory. These results indicate that all three *gtf* genes evolved from a common ancestral gene.

Introduction

Human dental caries is one of the most prevalent bacterial infections occurring in man (Gibbons & van Houte, 1975). Although several different oral bacteria may contribute to caries formation, a variety of approaches have strongly implicated *Streptococcus mutans* as the principal etiological agent in this disease (Loesche, 1986). One of the important pathogenic properties of these organisms appears to be their ability to colonize tooth surfaces abundantly in the presence of dietary sucrose. This activity is dependent upon the production of extracellular glucosyltransferases (GTF; EC 2.4.1.5) which synthesize water-insoluble adherent glucans from sucrose. An examination of different strains of mutans streptococci has revealed two different classes of GTF: GTF-I, which synthesizes primarily water-insoluble glucans and GTF-S, which synthesizes predominantly water-soluble glucans (Kuramitsu & Wondrack, 1983; Shimamura *et al.*, 1983).

The genes which express the different GTF activities

have recently been isolated (Gilpin *et al.*, 1985; Aoki *et al.*, 1986; Hanada & Kuramitsu, 1988; Hanada & Kuramitsu, 1989). Our laboratory has demonstrated that for one strain of *S. mutans*, GS-5, three distinct *gtf* genes are present on the chromosome (Aoki *et al.*, 1986; Hanada & Kuramitsu, 1988, 1989). Two of these, the tandemly-arranged *gtfB* and *gtfC* genes, code for the GTF-I and GTF-SI activities, respectively, which synthesize predominantly water-insoluble glucans. In addition, the *gtfD* gene expresses the GTF-S activity of strain GS-5.

Recent nucleotide sequencing of the *gtfB* and *gtfC* genes (Shiroza *et al.*, 1987; Ueda *et al.*, 1988) has revealed extensive homology between the two gene products. In addition, both enzymes are homologous to the GTF-I protein from another species of mutans streptococci, *Streptococcus downei* (previously named *Streptococcus sobrinus*) strain MFe28 (Russell *et al.*, 1988). It was, therefore, of interest to sequence the *gtfD* gene in order to initiate an examination of the molecular basis for the diverse synthetic activities of the GTF. The present communication describes the nucleotide sequence of the strain GS-5 *gtfD* gene and an analysis of the deduced amino acid sequence relative to the structures of the previously analysed GTF.

Methods

DNA manipulations. Plasmid pNH5 containing the intact *gtfD* gene has been recently described (Hanada & Kuramitsu, 1989). DNA

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isolation, endonuclease restriction digestion, ligation, and transformation of *Escherichia coli* were carried out as previously described (Aoki *et al.*, 1986).

Nucleotide sequencing. Overlapping DNA fragments encompassing the entire *gtfD* gene (Fig. 1) were isolated from pNH5 in either M13mp18 or M13mp19 (Bethesda Research Laboratories) with the exception of fragment 10B, which was isolated in pBlueScript SK (Stratagene). A series of deleted bacteriophage clones was then prepared as described by Henikoff (1984). Single-stranded DNA fragments were isolated and sequenced using the dideoxy chain-termination procedure (Sanger *et al.*, 1977) as previously described (Shiroza *et al.*, 1987). Both strands of the 5.74 kb *Xba*I–*Kpn*I DNA fragment containing the intact *gtfD* gene were completely sequenced.

Sequence analysis. Nucleic acid and protein analyses were carried out using the Pustell sequence analysis programs (International Biotechnologies).

Results

Nucleotide sequence of the *gtfD* gene

The sequencing strategy depicted (Fig. 1) was used to determine the complete nucleotide sequence of the 5.74 kb *S. mutans* GS-5 DNA fragment containing the *gtfD* gene. Only one of the two DNA strands contained a long open reading frame (ORF) capable of coding for the 155 kDa GTF-S enzyme (Hanada & Kuramitsu, 1989). The sequence detected contained 4293 bp and codes for a protein containing 1430 amino acids with an unprocessed molecular mass of 159814 Da (Fig. 2).

Seven base pairs preceding the putative initiation codon ATG (position 435; Fig. 2) was the sequence GGAGG (nucleotides 423–427), which is homologous to the ribosome-binding site (Shine & Dalgarno, 1974). In addition, the sequence TATAAT (positions 403–408), which is identical to the *E. coli* –10 promoter consensus sequence (Hawley & McClure, 1983), was identified. However, no sequence homologous to the –35 consensus sequence could be identified in this region. It will be necessary to analyse mRNA transcripts from this region to define the precise transcription start site. As with many Gram-positive bacterial genes previously sequenced (Hollingshead *et al.*, 1986; Shiroza & Kuramitsu, 1988; Shiroza *et al.*, 1987), the region upstream from the ribosome-binding site of the *gtfD* gene (180 bp) is AT-rich (73% AT compared to 63% for the entire *S. mutans* chromosome; Hamada & Slade, 1980). In addition, an inverted repeat sequence characteristic of rho-independent termination sequences was found 200 bp downstream from the *gtfD* stop codon (results not shown). In addition, an analysis of codon usage for the *gtfD* gene revealed generally similar utilisation to that of the previously sequenced *gtfB* and *gtfC* genes (Shiroza *et al.*, 1987; Ueda *et al.*, 1988).

Fig. 1. Strategy for sequencing the *gtfD* gene. The top line depicts the *S. mutans* strain GS-5 5.74 kb insert from plasmid pNH5 containing the *gtfD* gene. The thick line indicates the position of the gene, and the arrowhead indicates the direction of gene transcription. The overlapping fragments encompassing the gene, used for sequencing, are indicated below the insert with the directions of sequencing depicted. Bg, *Bgl*II; H, *Hind*III; K, *Kpn*I; P, *Pst*I; S, *Sal*I; X, *Xba*I.

Amino acid sequence of the GTF-S enzyme

The deduced amino acid sequence of the *gtfD* gene product indicated that the GTF-S protein contained an amino-terminal sequence typical of Gram-positive extracellular proteins (Hollingshead *et al.*, 1986; von Heijne & Abrahamson, 1989). This signal sequence region consisted of a strongly basic fourteen-amino-acid region followed by a relatively hydrophobic sequence (amino acids 15–29). The cleavage site for the signal peptidase has not yet been determined for the GTF-S protein. However, cleavage of the 159.8 kDa unprocessed protein between amino acids 30–40 would result in a protein with a molecular mass (155 kDa) approximating that of the enzyme recently purified from *E. coli* clones containing the *gtfD* gene (Hanada & Kuramitsu, 1989).

Like the GTF-I and GTF-SI proteins (Shiroza *et al.*, 1987; Ueda *et al.*, 1988), the GTF-S protein is highly hydrophilic (data not shown) with an estimated pI of 6.29. Furthermore, as with the GTF-I and GTF-SI enzymes of strain GS-5, no cysteine residues were identified in GTF-S.

Sequence similarities of the GTF enzymes from *S. mutans*

Recent results from this laboratory (Ueda *et al.*, 1988) have indicated that the GTF-I and GTF-SI enzymes of *S. mutans* strain GS-5 share a high degree of identity for both the nucleotide and amino acid sequences. It was therefore of interest to compare the sequence of the GTF-S protein with these two proteins. An amino acid comparison (Fig. 3) revealed very strong similarity between the GTF-S and GTF-I proteins. This was especially true in the central regions of both proteins. Interestingly, the products of the *gtfB* and *gtfC* genes

Fig. 2 (continued overleaf). Nucleotide sequence of the 4.9 kb *Xba*I-*Aha*III fragment containing the intact *gltD* gene, and the deduced amino acid sequence of the GTF-S enzyme. The Shine-Dalgarno (SD) sequence is underlined (positions 423-427). The arrows indicate the positions of the three direct repeating units A, B, and C.

2410 2420 2430 2440 2450 2460
 SAT ATG TAC AGT GAT GAC GGT CAA TAT ATG GCG ACT AAA TCC CCT TAT TAT GAT GCT ATT
 Asp Met Tyr Ser Asp Asp Gly Gln Tyr Met Ala Thr Lys Ser Pro Tyr Tyr Asp Ala Ile
 2470 2480 2490 2500 2510 2520
 SAT ACT TTA TTA AAG GCA CGT ATT AAA TAT GCC GCC GGT GGT CAA GAC ATG AAG ATC ACC
 Asp Thr Leu Leu Lys Ala Arg Ile Lys Tyr Ala Ala Gly Gly Gln Asp Met Lys Ile Thr
 2530 2540 2550 2560 2570 2580
 TAT GTT GAA GGT GAT AAA AGT CAT ATG GAT TGG GAT TAT ACA GGC GTT TTG ACT TCT GTT
 Tyr Val Glu Gly Asp Lys Ser His Met Asp Trp Asp Tyr Thr Gly Val Leu Thr Ser Val
 2590 2600 2610 2620 2630 2640
 CGT TAT GGT ACA GGA GCT AAT GAA GCT ACA GAT CAA GGC AGT GAA GCA ACT AAA ACA CAA
 Arg Tyr Gly Thr Gly Ala Asn Glu Ala Thr Asp Gln Gly Ser Glu Ala Thr Lys Thr Gln
 2650 2660 2670 2680 2690 2700
 GGA ATG GCT GTC ATT ACC AGC AAT AAC CCT AGC CTT AAA TTG AAT CAA AAT GAT AAA GTA
 Gly Met Ala Val Ile Thr Ser Asn Asn Pro Ser Leu Lys Leu Asn Gln Asn Asp Lys Val
 2710 2720 2730 2740 2750 2760
 ATT GTC AAT ATG GGG GCT GCG CAT AAA AAT CAA GAG TAC GGT CCG CTC CTC TTA ACA ACT
 Ile Val Asn Met Gly Ala Ala His Lys Asn Gln Glu Tyr Arg Pro Leu Leu Leu Thr Thr
 2770 2780 2790 2800 2810 2820
 AAA GAT GGT TTG ACA AGC TAC ACT TCT GAT GCC GCT GCT AAA TCC CTT TAT GCG AAA AGC
 Lys Asp Gly Leu Thr Ser Tyr Thr Ser Asp Ala Ala Ala Lys Ser Leu Tyr Arg Lys Thr
 2830 2840 2850 2860 2870 2880
 AAT GAT AAA GGA GAA TTA GTC TTT GAT GCT AGT GAC ATT CAA GGT TAC CTG AAT CCG CAA
 Asn Asp Lys Gly Glu Leu Val Phe Asp Ala Ser Asp Ile Gln Gly Tyr Leu Asn Pro Gln
 2890 2900 2910 2920 2930 2940
 GTA TCA GGT TAT TTA GCC GTT TGG GTT CCA GTA GGA GCT AGT GAT AAT CAA GAT GTT GGT
 Val Ser Gly Tyr Leu Ala Val Trp Val Pro Val Gly Ala Ser Asp Asn Gln Asp Val Arg
 2950 2960 2970 2980 2990 3000
 GTA GCA GCA AGC AAT AAG GCA AAT GCT ACT GGT CAA GTC TAC BAA TCA TCA AGT GCT CTT
 Val Ala Ala Ser Asn Lys Ala Asn Ala Thr Gly Gln Val Tyr Glu Ser Ser Ser Ala Leu
 3010 3020 3030 3040 3050 3060
 GAT TCT CAA TTG ATT TAC GAA GGT TTC TCA AAC TTC CAA GAT TTT GTA AGC AAA GAT TCA
 Asp Ser Gln Leu Ile Tyr Glu Gly Phe Ser Asn Phe Gln Asp Phe Val Thr Lys Asp Ser
 3070 3080 3090 3100 3110 3120
 GAC TAT ACT AAT AAG AAG ATT GCT CAA AAT GTC CAA CTC TTA CAA TCT TGG GGT GTC ACT
 Asp Tyr Thr Asn Lys Lys Ile Ala Gln Asn Val Gln Leu Phe Lys Ser Trp Gly Val Thr
 3130 3140 3150 3160 3170 3180
 TCC TTT GAA ATG GCA CCG CAA TAT GTC TCT TCT GAA GAT GGT TCT TTT CTA GAC TCT ATT
 Ser Phe Glu Met Ala Pro Gln Tyr Val Ser Ser Glu Asp Gly Ser Phe Leu Asp Ser Ile
 3190 3200 3210 3220 3230 3240
 ATT CAA AAT GGT TAT GCC TTT GAG GAT GGT TAT GAT CTT GCT ATG AGT AAG AAT AAC AAA
 Ile Gln Asn Gly Tyr Ala Phe Glu Asp Arg Tyr Asp Leu Ala Met Ser Lys Asn Asn Lys
 3250 3260 3270 3280 3290 3300
 TAC GGT TCT CAG CAA GAC ATG ATT AAT GCA GTT AAA GCT CTG CAT AAA AGC GGT ATT CAG
 Tyr Gly Ser Gln Gln Asp Met Ile Asn Ala Val Lys Ala Leu His Lys Ser Gly Ile Gln
 3310 3320 3330 3340 3350 3360
 GTT ATT GCG GAT TGG GTA CCA GAT CAA ATC TAT AAT CTT CCG GGC AAA GAA GTC GTA AGC
 Val Ile Ala Asp Trp Val Pro Asp Gln Ile Tyr Asn Leu Pro Gly Lys Glu Val Val Thr
 3370 3380 3390 3400 3410 3420
 GCT ACA GGT GTC AAC GAT TAT GGT GAG TAT GCG AAA GAC TCT GAA ATC AAA AAT ACA CTC
 Ala Thr Arg Val Asn Asp Tyr Gly Glu Tyr Arg Lys Asp Ser Glu Ile Lys Asn Thr Leu
 3430 3440 3450 3460 3470 3480
 TAT GCT GCC AAC ACT AAG AGT AAT GGT AAG GAT TAT CAA GCG AAG TAT GGC GGT GCT TTC
 Tyr Ala Ala Asn Thr Lys Ser Asn Gly Lys Asp Tyr Gln Ala Lys Tyr Gly Gly Ala Phe
 3490 3500 3510 3520 3530 3540
 CTT AGT GAA CTC GCT GCT AAG TAC CCT AGT ATC TTT AAC GCG AGC CAA ATT TCA AAT GGT
 Leu Ser Glu Leu Ala Ala Lys Tyr Pro Ser Ile Phe Asn Arg Thr Gln Ile Ser Asn Gly
 3550 3560 3570 3580 3590 3600
 AAG AAG ATT GAT CCA AGC GAA AAA ATC ACA GCA TGG AAA GCA AAA TAC TTC AAT GGG ACA
 Lys Lys Ile Asp Pro Ser Glu Lys Ile Thr Ala Trp Lys Ala Lys Tyr Phe Asn Gly Thr
 3610 3620 3630 3640 3650 3660
 AAT ATT CTA GGC GGT GGT GTT GGT TAT GTT CTT AAA GAT AAT GCT AGT GAT AAA TAC TTT
 Asn Ile Leu Gly Arg Gly Val Gly Tyr Val Leu Lys Asp Asn Ala Ser Asp Lys Tyr Phe

3670 3680 3690 3700 3710 3720
 GAA CTG AAA GGG AAT CAA ACC TAT CTG CCA AAA CAG ATG ACT AAC AAA GAA GCT TCG ACT
 Glu Leu Lys Gly Asn Gln Thr Tyr Leu Pro Lys Gln Met Thr Asn Lys Glu Ala Ser Thr
 3730 3740 3750 3760 3770 3780
 GGT TTT GTT AAT GAT GGC AAT GGG ATG ACT TCT TAT TCA ACT AGT GGT TAT CAA GCC AAG
 Gly Phe Val Asn Asp Gly Asn Gly Met Thr Phe Tyr Ser Thr Ser Gly Tyr Gln Ala Lys
 3790 3800 3810 3820 3830 3840
 AAC AGC TTT GTT CAA GAT GCC AAA GGA AAT TGG TAT TAC TTT GAT AAT AAT GGC CAT ATG
 Asn Ser Phe Val Gln Asp Ala Lys Gly Asn Trp Tyr Tyr Phe Asp Asn Gly His Met
 3850 3860 3870 3880 3890 3900
 GTT TAT GGC TTA CAG CAG CTA AAT GGC GAA GTG CAA TAC TTT TTA TCA AAT GGT GTT CAA
 Val Tyr Gly Leu Gln Gln Leu Asn Gly Glu Val Gln Tyr Phe Leu Ser Asn Gly Val Gln
 3910 3920 3930 3940 3950 3960
 TTG GGT GAA TCT TTC TTG GAA AAC GCT GAT GGC AGC AAG AAC TAT TTT GGT CAT CTA GGA
 Leu Arg Glu Ser Phe Leu Glu Asn Ala Asp Gly Ser Lys Asn Tyr Phe Gly His Leu Gly
 3970 3980 3990 4000 4010 4020
 AAT AGA TAT AGT AAT GGT TAT TAT TCA TTT GAT AAT GAT AGT AAG TGG GGT TAT TTT GAT
 Asn Arg Tyr Ser Asn Gly Tyr Tyr Ser Phe Asp Asn Asp Ser Lys Trp Arg Tyr Phe Asp
 4030 4040 4050 4060 4070 4080
 GCC AGT GGA GTC ATG GCT GTA GGT TTG AAA ACA ATT AAC GGC AAT AGC CAG TAC TTT GAT
 Ala Ser Gly Val Met Ala Val Gly Leu Lys Thr Ile Asn Gly Asn Thr Gln Tyr Phe Asp
 4090 4100 4110 4120 4130 4140
 CAA GAT GGT TAT CAA GTC AAA GGT GCT TGG ATA CAA GGC AGC GAT GGC AAA AAG GGT TAT
 Gln Asp Gly Tyr Gln Val Lys Gly Ala Tyr Ile Thr Gly Ser Asp Gly Lys Lys Arg Tyr
 4150 4160 4170 4180 4190 4200
 TTT GAT GAC GGA TCT GGA AAT ATG GCT GTT AAT GCT TTT GCA AAT GAT AAA AAC GGC GAT
 Phe Asp Asp Gly Ser Gly Asn Met Ala Val Asn Arg Phe Ala Asn Asp Lys Asn Gly Asp
 4210 4220 4230 4240 4250 4260
 TGG TAC TAT CTC AAT TCA GAT GGC ATT GCT TTG GTT GGT GTC CAA ACC ATT AAT GGT AAG
 Trp Tyr Tyr Leu Asn Ser Asp Gly Ile Ala Leu Val Gly Val Gln Thr Ile Asn Gly Lys
 4270 4280 4290 4300 4310 4320
 ACT TAT TAC TTT GGC CAA GAT GGT AAG CAA ATC AAA GGT AAA ATT ATT ACA GAC AAT GGT
 Thr Tyr Tyr Phe Gly Gln Asp Gly Lys Gln Ile Lys Gly Lys Ile Ile Thr Asp Asn Gly
 4330 4340 4350 4360 4370 4380
 AAG CTG AAA TAT TTC CTT GCC AAT TCA GGA GAA TTA GCA GCG AAT ATC TTT GCA ACA GAT
 Lys Leu Lys Tyr Phe Leu Ala Asn Ser Gly Glu Leu Ala Arg Asn Ile Phe Ala Thr Asp
 4390 4400 4410 4420 4430 4440
 AGT CAA AAC AAT TGG TAT TAC TTT GGT TCA BAC GGT GTT GCG GTT ACA GGC AGT CAG ACA
 Ser Gln Asn Asn Trp Tyr Tyr Phe Gly Ser Asp Gly Val Ala Val Thr Gly Ser Gln Thr
 4450 4460 4470 4480 4490 4500
 ATT GCT GGT AAA AAG CTC TAT TTT GCA AGC GAC GGA AAA CAA GTC AAA GGC AGC TTT GTC
 Ile Ala Gly Lys Lys Leu Tyr Phe Ala Ser Asp Gly Lys Gln Val Lys Gly Ser Phe Val
 4510 4520 4530 4540 4550 4560
 ACT TAT AAT GGT AAA GTT CAT TAT TAT CAT GCT GAC TCA GGA GAA TTA CAA GTT AAC GCG
 Thr Tyr Asn Gly Lys Val His Tyr Tyr His Ala Asp Ser Gly Glu Leu Gln Val Asn Arg
 4570 4580 4590 4600 4610 4620
 TTT GAA GCA GAT AAG GAT GGT AAT TGG TAT TAT CTT GAT TCA AAT GGT GAA GCT CTG ACA
 Phe Glu Ala Asp Lys Asp Gly Asn Trp Tyr Tyr Leu Asp Ser Asn Gly Glu Ala Leu Thr
 4630 4640 4650 4660 4670 4680
 GGC AGC CAA GCG ATT AAC GAT CAG GGT GTC TTT ACB CBA GAA GGA AAA CAA GTT AAA
 Gly Ser Gln Arg Ile Asn Asp Gln Arg Val Phe Phe Thr Arg Glu Gly Lys Gln Val Lys
 4690 4700 4710 4720 4730 4740
 GGT GAT GTT GCT TAT GAT GAG CGA GGG CTT CTT GGT TAT TAT GAC TAA GAT AGT GGT AAC
 Gly Ala Gly Lys Tyr Asp Glu Arg Gly Leu Leu Arg Tyr Tyr Asp ---
 4750 4760 4770 4780 4790 4800
 ATG GTT TAC AAC AAA GTC GTC ACT TTA GCC AAT GGA AGA GCG ATT GGC ATT GAC GGT TGG
 4810 4820 4830 4840 4850 4860
 GGT ATC GCT AGA TAT TAT TAA CAC TGA CAT AGC TTA ACB TGA TCA ATA AAT TTG ATC GCG
 4870 4880 4890 4900 4910 4920
 TTT TCT TGT TTT AGA CCT TTT ATT CAC AGA AAA CCA TTC ATC TTG GAT AAT GTT GTC AAT

AhaIII
 CTT TTT TAA A

Fig. 3. Amino acid similarity matrix between the GTF-I and GTF-S proteins. Window sequences of six amino acids are compared and each point represents greater than 50% comparative similarity. Dots represent relatively short similar sequences.

both contain relatively long (65 and 49 amino acids respectively) highly homologous direct repeating units at the carboxyl termini (Shiroza *et al.*, 1987; Ueda *et al.*, 1988). Likewise, three repeating units consisting of sixty-five amino acids were identified in the COOH-terminal region of the GTF-S protein (corresponding to base positions 4128–4319, 4320–4511 and 4512–4700; Fig. 2). In addition, the direct repeating units of the GTF-I protein exhibited significant identity with the GTF-S sequence (Fig. 4). Furthermore, the GTF-SI protein repeating unit was less similar to that of the GTF-S protein than to the GTF-I repeat sequences (data not shown). All three enzymes also share significant but lower similarity with the GTF-I protein produced by *S. downei* (Ferretti *et al.*, 1987).

Signal sequence regions of *S. mutans* extracellular proteins

The *gtfD* gene is the fourth *S. mutans* extracellular protein gene to be sequenced in this laboratory. Therefore, it was of interest to compare the signal sequence regions of these proteins, in order to identify amino acids which might be important in secretion. A comparison of the amino acids in these regions from the three GTF proteins and from the extracellular fructosyltransferase (FTF) secreted by strain GS-5 (Shiroza & Kuramitsu, 1988) revealed a number of similarities (Fig. 5). In addition to the initiator amino acid methionine, seven amino acid positions were identical within the first seventeen amino-terminal amino acids. This similarity between these regions of the GTF and FTF proteins

from *S. mutans* is in marked contrast to the lack of identity displayed between the processed forms of the two groups of enzymes (T. Shiroza & H. K. Kuramitsu, unpublished results). Comparison with the sequence of the GTF-I protein from another mutans streptococcus, *S. downei* strain MFe28 (Ferretti *et al.*, 1987), indicated that six amino acid positions were conserved for all five proteins. All of these putative signal sequences contained an acidic amino acid at position 2, Arg at position 6, Lys at positions 8, 11 and 13, Trp at position 16, and Val at position 17. As might be expected for similar enzymes from the same strain, all three *S. mutans* GTF shared a number of other conserved amino acid positions (sixteen of the first thirty amino acids). In addition, the three GTF enzymes from *S. mutans* strains GS-5 together with the GTF-I protein from *S. downei* exhibited thirteen identical amino acid positions in this region.

Discussion

The deduced amino acid sequence of the GTF-S enzyme expressed by the *gtfD* gene indicated that the protein is highly hydrophilic with an unprocessed molecular mass of approximately 159 kDa (Fig. 2). This size is similar to that determined for both the GTF-I (Shiroza *et al.*, 1987) and GTF-SI (Ueda *et al.*, 1988) proteins. Purification of the GTF-S enzyme from either *S. mutans* or *E. coli* indicated two forms, with molecular masses of 140 and 155 kDa (Hanada & Kuramitsu, 1989). This would suggest that cleavage with the signal peptidase within the first 30–40 amino acids is followed by proteolytic processing. However, the nature and location of these alterations remains to be determined for the GTF-S protein.

The predicted pI of 6.29 for the unprocessed GTF-S protein is somewhat more acidic than those for both the GTF-I and GTF-SI proteins (pI 6.87 and 9.4, respectively; Shiroza *et al.*, 1987; Ueda *et al.*, 1988). However, purification of the GTF-S enzyme from culture supernatant fluids of strain GS-5 produced a 140 kDa protein with a pI of approximately 7.5 (Kuramitsu & Wondrack, 1983). Therefore, it is likely that processing of the extracellular GTF-S protein in *S. mutans* is accompanied by modification of the primary translation product of the *gtfD* gene to a more basic protein.

Since the three GTF enzymes secreted by *S. mutans* synthesize glucans differing primarily in the proportions of α -1,6- and α -1,3-glucose linkages, it is not surprising that the proteins are similar to one another. The highest degree of similarity among the proteins exists within the central regions of the sequences (Fig. 3; Ueda *et al.*, 1988). A comparison of amino acids corresponding to the 2.0 kb central DNA fragments (*Hind*III fragment of the

(a) GTF-S

A GKKRYFDDGSGMNAVNRNFANDKNGDWYLLNSDGLALVGQTINGKTYFFGQDGKQIKGIITDN
 B GKLYFLANSSELARNIFATDSQNNWYFGSDGVAVTGSQTIAGKKLYFASDGKQVKSFTVTYN
 C GKVHYHADSSELQVNRFEADKNGDWYLLNSDGLALVGQTINGKTYFFGQDGKQIKGIITDN

(b) GTF-S and GTF-I

S-B GKLYFLANSSELARNIFATDSQNNWYFGSDGVAVTGSQTIAGKKLYFASDGKQVKSFTVTYN
 I-A GRISYYDNGSGDIQRNRFVRNAQGWYFDNNGYAVTGARTINGQLLYFRANGVQVKGFEVTDTR

Fig. 5. Comparison of the signal sequence regions of mutants streptococcal extracellular proteins. Identical amino acid positions for all five proteins are indicated by connecting vertical lines. (a) *S. mutans* GTF-S; (b) *S. mutans* GTF-I; (c) *S. mutans* GTF-SI; (d) *S. mutans* FTF; (e) *S. downei* GTF-I. The arrow depicts the signal cleavage site for the *S. downei* GTF-I as determined by amino acid sequencing (Ferretti *et al.*, 1987).

gtfD gene) revealed approximately 95% sequence identity at the amino acid level between the GTF-I and GTF-SI proteins. In this same region, 84% identity was determined between the GTF-I and GTF-S proteins. Such differences are consistent with the observations that both the GTF-I and GTF-SI enzymes synthesize primarily water-insoluble glucans (Aoki *et al.*, 1986; Hanada & Kuramitsu, 1989) while the GTF-S enzyme synthesizes water-soluble glucans (Hanada & Kuramitsu, 1989). Comparison of the three *S. mutans* GTF sequences also reveal regions of sequence dissimilarity (Fig. 3).

Recent results have indicated that the carboxyl termini of both the GTF-I and GTF-SI proteins from *S. mutans*, as well as the GTF-I from *S. downei*, contain relatively long homologous direct repeating units (Shiroza *et al.*, 1987; Ueda *et al.*, 1988; Ferretti *et al.*, 1987). The present results demonstrate that similar direct repeating units are present at the carboxyl terminus of the GTF-S protein. However, the similarity of the repeating units within the GTF-S protein (52% identity between units A and B; Fig. 4) was less than that observed between the homologous units in the GTF-I and GTF-SI proteins (95% and 75% respectively; Shiroza *et al.*, 1987; Ueda *et al.*, 1988).

A comparison of the GTF-S signal sequence with three

Fig. 4. Amino acid direct repeating units at the carboxyl termini of the GTF proteins. The amino acid direct repeating units for the *S. mutans* *gtfB* and *gtfD* gene products (GTF-I and GTF-S respectively) are depicted in single-letter code. Identical amino acid positions are depicted by stars. (a) The three direct repeating units of the GTF-S protein (see Fig. 2). (b) A comparison of the second GTF-S repeating unit (B) with the first repeating unit (A) of the GTF-I protein.

other *S. mutans* extracellular proteins revealed a number of identical amino acid positions (Fig. 5). Likewise, these four *S. mutans* signal sequence regions shared a number of amino acid positions with that of the GTF-I from *S. downei*. Comparison with the signal sequences of other streptococcal extracellular proteins, namely streptokinase (Malke *et al.*, 1985) and group A streptococcus M protein (Hollingshead *et al.*, 1986), did not reveal sequence similarity. Based on a limited comparison of the available sequences, the mutants streptococcal extracellular protein signal sequences appear to have conserved similar structural features. However, the role of individual amino acids in the secretion process remains to be elucidated.

Since the tandem *gtfB* and *gtfC* genes may be coordinately regulated (Ueda *et al.*, 1988) and this genetic locus is not present near the *gtfD* gene (D. Perry & H. K. Kuramitsu, unpublished results), it will be of interest to examine the regulation of expression of this latter gene.

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