Purification and Characterization of *Streptococcus sobrinus* Dextranase Produced in Recombinant *Escherichia coli* and Sequence Analysis of the Dextranase Gene

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The plasmid (pYA902) with the dextranase (*dex*) gene of *Streptococcus sobrinus* UAB66 (serotype g) produces a C-terminal truncated dextranase enzyme (Dex) with a multicomplex mass form which ranges from 80 to 130 kDa. The *Escherichia coli*-produced enzyme was purified and characterized, and antibodies were raised in rabbits. Purified dextranase has a native-form molecular mass of 160 to 260 kDa and specific activity of 4,000 U/mg of protein. Potential immunological cross-reactivity between dextranase and the SpaA protein specified by various recombinant clones was studied by using various antisera and Western blot (immunoblot) analysis. No cross-reactivity was observed. Optimal pH (5.3) and temperature (39°C) and the isoelectric points (3.56, 3.6, and 3.7) were determined and found to be similar to those for dextranase purified from *S. sobrinus*. The *dex* DNA restriction map was determined, and several subclones were obtained. The nucleotide sequence of the *dex* gene was determined by using subclones pYA993 and pYA3009 and UAB66 chromosomal DNA. The open reading frame for *dex* was 4,011 bp, ending with a stop codon TAA. A ribosome-binding site and putative promoter preceding the start codon were identified. The deduced amino acid sequence of Dex revealed the presence of a signal peptide of 30 amino acids. The cleavage site for the signal sequence was determined by N-terminal amino acid sequence analysis for Dex produced in *E. coli* χ 2831(pYA902). The C terminus consists of a serine- and threonine-rich region followed by the peptide LPKTGD, 3 charged amino acids, 19 amino acids with a strongly hydrophobic character, and a charged pentapeptide tail, which are proposed to correspond to the cell wall-spanning region, the LPXTGX consensus sequence, and the membrane-anchoring domains of surface-associated proteins of gram-positive cocci.

Understanding the biological role of extracellular polysaccharides produced by oral streptococci (*Streptococcus mutans*, *S. sanguis*, *S. sobrinus*, *S. cricetus*, and *S. rattus*), herein designated mutans streptococci, in contributing to dental caries has significantly increased in the past several years (13, 22, 34). It has been suggested (10, 31) that these extracellular polysaccharides are mainly composed of two classes, namely, α -1,6-linked water-soluble glucan (dextran) and α -1,3-linked water-insoluble glucan (mutan). It has been shown that on the tooth surface, pellicle-associated dextrans and glucans are involved in the first step in dental caries, a sucrose-independent attachment of *S. mutans* to the tooth surface (27). Also, there is considerable evidence for the importance of proteins on the surface of *S. mutans*, such as surface protein antigen A (SpaA) (39), being involved in this sucrose-independent phase. The second step is sucrose dependent and involves adherence of bacteria in a much firmer bonding to the tooth surface and also the aggregation reaction between adjacent bacterial cells in different chains of streptococci. Both adherence and aggregation in the sucrose-dependent phase involve the synthesis of water-soluble and water-insoluble glucans under the control of cell-associated glucosyltransferase enzymes (34, 43, 78). Several lines of evidence also suggest the involvement of dextranases in the virulence of oral streptococci by playing some role in adherence and aggregation (32). These observations include the following: (i) dextranase (α -1,6-glucan-6-glucanohydrolase; EC 3.2.1.11) can partially degrade water-soluble glucan (7, 31); (ii) dextranase can inhibit the production of water-

insoluble glucan (20, 33, 44, 63, 77); and (iii) dextranase can inhibit the adherence of oral streptococci (33, 63, 77). These findings implicate the involvement of dextranase activity in affecting the composition of dental plaques. Genetic analysis of dextranase-defective mutants (Dex⁻) (15, 16, 53, 54, 72, 73) for adherence, aggregation, and cariogenicity in rats has implicated dextranase as a determinant of *S. mutans* virulence since production of anomalous glucans in the sucrose-dependent stage correlated with reduced cariogenicity (14, 54, 71). Also, addition of the water-soluble glucan produced from some of these mutants to drinking water inhibited both plaque formation and cariogenicity by wild-type *S. mutans* in rats (71).

The precise roles that dextranase might play in facilitating normal glucan synthesis and in adherence on the tooth surface are as yet not clearly understood. Since some SpaA-deficient mutants lacked dextranase activity, it was postulated that the *spaA* and *dex* genes are coordinately controlled (14). However, it was shown that the *spaA* and *dex* genes are not homologous by DNA hybridization (14). The *S. sobrinus dex* gene has been cloned into the cosmid vector pHC79 (4), and dextranase from *S. sobrinus* has been purified and partially characterized (4).

To understand the role(s) of dextranase in dental caries induced by members of the *S. mutans* group of bacteria and the relationship between the SpaA and dextranase proteins more clearly, we describe in this report the purification and characterization of *S. sobrinus* dextranase and subcloning and subsequent sequencing of the entire *dex* gene.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. All bacterial strains and plasmids used are listed in Table 1. The *Escherichia coli*

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype	Reference(s)
<i>E. coli</i> K-12 χ 2831	F ⁻ <i>lacY1 glnV44 galK2 galT22</i> λ (c1857 b2 red β 3) <i>recA56</i> Δ <i>thyA57 metB1 hsdR2</i>	42
<i>S. sobrinus</i> UAB66	Serotype g, Str ^r Spc ^r , derivative of 6715	53
Plasmids		
pYA902	<i>amp tet dex</i> ; pHc79 with <i>Sau</i> 3AI partially digested <i>S. sobrinus</i> dextranase gene cloned into the <i>Bam</i> HI site	4
pYA3009	<i>cat dex</i> ; pACYC184 with <i>Sau</i> 3AI partially digested <i>S. sobrinus</i> dextranase gene cloned into the <i>Bam</i> HI site	70, 70a
pYA770	<i>cat spaA</i>	30
pYA181	<i>amp spaA</i>	30
pYA292	<i>asd lacZ</i> (α)	25
pUC8	<i>amp lacZ</i>	75

strains were grown at 30°C with aeration in L broth (47) with thymidine at 40 μ g/ml for all strains with a *thyA* mutation. Antibiotics were present at the following amounts per milliliter: 12.5 and 25 μ g of tetracycline $\cdot$ HCl for L broth and L agar, respectively; 100 μ g of ampicillin $\cdot$ Na and 30 μ g of chloramphenicol for L broth and L agar for *E. coli*. *S. sobrinus* 6715 strain UAB66 was cultured in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 37°C from a stock maintained in 50% glycerol at -40°C. An extracellular fraction was obtained from UAB66 grown in modified FMC medium (53) supplemented with 1% glucose.

Chemicals. Cyanogen bromide-activated Sepharose 4B and blue dextran 2000 (BD) were purchased from Pharmacia (Uppsala, Sweden). Prestained molecular weight markers for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), goat anti-rabbit immunoglobulin G-alkaline phosphatase conjugate, casein enzymatic hydrolysate, tetracycline, ampicillin, Nonidet P-40, Tween 80, Triton X-100, bovine hemoglobin, barbital buffer, phenylmethylsulfonyl fluoride, EDTA, and molecular mass standards for gel filtration were purchased from Sigma (St. Louis, Mo.). The biconchoinic acid protein assay reagent was purchased from Pierce (Rockford, Ill.). Acrylamide, bisacrylamide, and SDS were purchased from Bio-Rad (Richmond, Calif.). All other chemicals were of reagent grade. Radioactive isotopes for sequencing were purchased from New England Nuclear (Boston, Mass.).

Assays. Dextranase was assayed by the Somogyi assay (68, 69) modified by Nelson (56) for determination of glucose. The assay consisted of incubation of protein fractions in 50 mM sodium acetate (pH 5.5) with dextran T-2000 at 37°C under conditions of substrate excess. Reactions were stopped by boiling and were assayed spectrophotometrically. Dextranase activity was also assayed after electrophoresis of protein fractions in BD-SDS-PAGE followed by renaturation for enzyme activity (5) or in BD-native PAGE. Enzyme activities were visualized as bands of clearing on a blue background (5). The assays for β -galactosidase and alkaline phosphatase were done as described by Craven et al. (12) and Garen and Levinthal (26), respectively. Kinetic constants were determined by Lineweaver-Burk analyses of hydrolysis rates of a soluble dextran (18). Protein concentration was determined by the biconchoinic acid protein assay with bovine serum albumin as the standard.

Antisera. Antiserum to a purified dextranase protein encoded by pYA902 was raised in New Zealand White rabbits by multiple intradermal injections of 100 μ g of protein in 1 ml of Freund's complete adjuvant (Difco). Injections were given at 4-week intervals. Rabbit antisera against SpaA protein purified from *S. sobrinus* UAB66 (30) and antisera against the recombinant *E. coli* SpaA protein encoded by pYA726 were previously described (39). These antisera were preadsorbed with an extract of host *E. coli* or passed through a column of cyanogen bromide-Sepharose 4B coupled with host cell extract. Goat anti-rabbit immunoglobulin G serum conjugated with alkaline phosphatase was used as a secondary antibody. All antisera were used at a dilution of 1:2,000 or 1:3,000 and stored at -70°C.

Localization of dextranase protein in recombinant *E. coli*. Recombinant *E. coli* cells (carrying pYA902) were grown in a low-phosphate medium E (8) with 1 mM isopropylthiogalactopyranoside (IPTG) overnight at 30°C. The periplasmic fraction was obtained by cold osmotic shock (37), and the cytoplasmic fraction was obtained by disrupting the shocked cells by a Braun disruptor with one-third volume of glass beads (B. Braun Melsungen AG, Melsungen, Germany) at maximum speed for 3 min with intermittent CO₂ cooling. The resulting supernatant fluids were stored at 0 or -20°C for further assays.

Preparation of protein fractions. Protein fractions were prepared from 10 liters of recombinant *E. coli* cells grown with aeration in L broth at 30°C to late logarithmic phase (overnight). Periplasmic protein fractions of *E. coli* cells were prepared by the cold osmotic shock procedure (37). After periplasmic fractions were obtained, phenylmethylsulfonyl fluoride was added to 2 mM. These protein fractions were stored at 0 or -20°C prior to being used.

Purification of dextranase. Purification of dextranase from recombinant *E. coli* involved preparation of periplasmic fractions by cold osmotic shock (37) followed by (i) hemoglobin-Sepharose 4B column chromatography, (ii) anion-exchange fast protein liquid chromatography (FPLC) (Mono Q; Pharmacia), and (iii) gel filtration FPLC (Superose 6; Pharmacia). All chromatographic procedures were performed at room temperature.

(i) **Hemoglobin-Sepharose 4B column chromatography.** The affinity column was made by a modification of the method of Chua and Bushuk (11). The coupling procedure used was specified by the vendor (Pharmacia). Periplasmic fractions were loaded onto a hemoglobin-Sepharose 4B column and eluted with 50 mM sodium acetate buffer (pH 5.5). The eluent was dialyzed against 10,000 volumes of 50 mM sodium acetate buffer (pH 5.5) or 25 mM L-histidine $\cdot$ HCl buffer (pH 5.5).

(ii) **Anion-exchange chromatography.** The dialyzed protein from the step described above was applied at 0.5 ml/min to an FPLC HR5/5 Mono Q anion-exchange column equilibrated in 25 mM L-histidine $\cdot$ HCl (pH 5.5). Superloop 10 (10 ml) or Superloop 50 (50 ml) was used to load the proteins. The column was washed with 2 ml of the L-histidine $\cdot$ HCl buffer and eluted at a flow rate of 0.5 ml/min with a 25 ml of a 0 to 0.8 M NaCl gradient in 25 mM L-histidine $\cdot$ HCl (pH 5.5). Elution of protein was monitored at 280 nm. Volumes of 0.5 ml were collected and assayed for dextranase activity. Fractions with high dextranase activity were pooled from several chromatographic runs, dialyzed against 10,000 volumes of 10 mM sodium acetate buffer (pH 5.5) and then 100 volumes of deionized H₂O, and concentrated by lyophilization.

(iii) **Gel filtration chromatography.** The lyophilized proteins were suspended in 50 mM sodium acetate buffer (pH 5.5) containing 150 mM NaCl and eluted at 0.5 ml/min to an FPLC HR10/30 Superose 6 gel filtration column equilibrated with the

TABLE 2. Localization of dextranase enzyme activity in *E. coli* χ 2831(pYA902)

Enzyme	Total enzyme activity (U)		Relative enzyme activity	
	Periplasmic fractions ^a	Shocked cells ^b	Periplasmic fractions ^a	Shocked cells ^b
Dextranase	176.9	33.3	0.84	0.16
β -Galactosidase ^c	0.1	16.9	0.01	0.99
Alkaline phosphatase ^d	49.0	0.3	0.99	0.01

^a Enzyme released by cold osmotic shock.^b Cellular protein fractions of shocked cells produced in a Braun disruptor.^c Cytoplasmic protein marker.^d Periplasmic protein marker.

same buffer. The column was eluted at 0.5 ml/min with 50 mM sodium acetate buffer (pH 5.5) containing 150 mM NaCl, and the elution profile was monitored at 280 nm. Fractions of 0.25 ml were collected and assayed for dextranase activity. Fractions containing activities were pooled, dialyzed against deionized water, and concentrated by lyophilization. This purified protein was kept frozen at -70°C for further experiments.

Gel electrophoresis and Western blot (immunoblot) analysis. Cells lysed in dissociation buffer were subjected to SDS-PAGE (45) by loading approximately 7 μg of protein per well, and cells disrupted mechanically in phosphate-buffered saline were subjected to native PAGE. Extracellular fractions from *S. sobrinus* UAB66 were obtained by a method described previously (4), the only change being precipitation with 60% ammonium sulfate. Prestained molecular weight markers were used as molecular mass standards for SDS-PAGE. The Western blot analysis procedure was as described previously (30).

Isoelectrofocusing. Isoelectric focusing was performed at 4°C by the procedure of Guilian et al. (29) with an SE600 vertical slab gel unit (Hoefer Scientific, San Francisco, Calif.). Electrofocusing was carried out for 2 h at 25 W on a 6.6% polyacrylamide gel containing 7% (vol/vol) Pharmalytes (pH 2.5 to 5.0; Pharmacia) with 20 mM NaOH as a catholyte and 20 mM acetic acid as an anolyte.

Plasmid analysis, restriction mapping, agarose gel electrophoresis, and transformation. Plasmid DNA from *E. coli* strains were isolated by a modification (67) of the Birnboim minilysate technique (6) followed by centrifugation in CsCl-ethidium bromide gradients (62). Chromosomal DNA from *S. sobrinus* UAB66 was isolated by a method reported by Hudson and Curtiss (40). Digestion with restriction endonucleases was carried out as recommended by the suppliers. Fill-in reactions using the Klenow fragment of DNA polymerase I were as described previously (62). DNA analysis was performed by horizontal agarose gel (0.8% [wt/vol]) electrophoresis in TAE buffer (40 mM Tris-acetate buffer containing 1 mM EDTA [pH 8.0]). DNA fragments were electroeluted from 0.8% agarose

gels by using Gene Clean kits (Bio 101, Inc., La Jolla, Calif.). *E. coli* strains were transformed by the method of Dagert and Ehrlich (17), and transformation mixtures were plated on appropriate selective plates after 60 min of incubation to allow phenotypic expression of drug resistance traits.

Nucleotide sequence analysis. The DNA sequence of the *dex* gene was determined by the bidirectional chain termination procedure (55), using modified T7 DNA polymerase supplied with the Sequenase version 2.0 kit (United States Biochemical, Cleveland, Ohio). Double-stranded templates of *E. coli* recombinants pYA993 and pYA3009, generated by Tn5seqI mutagenesis (55), were directly used for sequencing. The primers for the SP6 and T7 promoters (55) were synthesized by a DNA synthesizer. Also, UAB66 chromosomal DNA was sequenced by the Circumvent thermal cycle dideoxy DNA sequencing kit with Vent (Exo⁻) DNA polymerase (New England Biolabs, Beverly, Mass.), using PCR in a Perkin-Elmer Cetus model 480 thermal cycler. We used thermal cycles of 95°C for 3 min and then 30 cycles of 95°C for 1 min, 55 to 60°C (depending on T_m values of primers) for 1 min, and 72°C for 2 min. The primers (24-mer) were purchased from Cruachem, Inc. (Sterling, Va.).

Nucleotide sequence accession number. The nucleotide sequence for *dex* (Fig. 8) has been submitted to GenBank and assigned accession number M96978.

RESULTS

Cellular localization of *S. sobrinus* dextranase expressed in *E. coli* χ 2831 containing pYA902. The cellular localization of the dextranase enzyme in recombinant *E. coli* was determined by measuring the activity of dextranase released by cold osmotic shock (37) compared with the amount remaining in the shocked cells (Table 2). β -Galactosidase (cytoplasmic marker) and alkaline phosphatase (periplasmic marker) were assayed to assess the degree of contamination of one fraction with the other. Dextranase protein was present in both the periplasmic and shocked-cell fractions. However, 84% of the total dextranase protein was released from the periplasm by cold osmotic shock. Therefore, we concluded that this enzyme is periplasmic when specified by the *S. sobrinus* gene and expressed in *E. coli*.

Purification of *S. sobrinus* dextranase synthesized in recombinant *E. coli*. The stepwise purification scheme and recovery results are given in Table 3. After dialysis and lyophilization, the purified dextranase protein had a specific activity of greater than 4,000 U (μmol of glucose $\text{h}^{-1} \text{mg}^{-1}$). Final recoveries of activity units and protein were 50 and 1.7% respectively (Table 3). The significant increase of dextranase activity at steps 3 and 4 could be due to separation of substances inhibiting dextranase activity from dextranase by eliminating proteases through the hemoglobin-Sepharose column and Mono Q column chromatography steps. The results of SDS-PAGE and BD-SDS-PAGE of the total cellular protein from *E. coli* containing

TABLE 3. Summary of the procedure for purification of dextranase enzyme from *E. coli* χ 2831(pYA902)

Step	Fraction	Total protein (mg)	Total activity (μmol of glucose h^{-1})	Sp act μmol of glucose $\text{h}^{-1} \text{mg}$ of protein ⁻¹	Recovery of activity (%)
1	Periplasmic fraction from 10-liter culture	174	27,318	157	100.0
2	Concentration (lyophilized) ^a	89	17,355	195	63.5
3	Hemoglobin-Sepharose column	82	19,516	238	71.4
4	Mono Q (anion-exchange, FPLC)	15	31,740	2,116	>100.0
5	Superose 6 (gel filtration, FPLC)	4	14,224	3,556	52.1
6	Concentration (lyophilized) ^a	3	13,995	4,665	51.2

^a Lyophilized after dialysis.

FIG. 1. Protein fractions run in parallel on SDS-PAGE, stained with Coomassie blue (A), and BD-SDS-PAGE, renatured for activity (B). Lanes: 1, prestained molecular weight markers; 2, total cell extracts of *E. coli* χ 2831(pYA902), 80 μ g; 3, periplasmic fraction, 40 μ g; 4, Mono Q column-purified dextranase fraction, 15 μ g; 5, Superose 6 column-purified dextranase fraction, 5 μ g.

pYA902 are shown in Fig. 1, lanes 2. Each pair of lanes in Fig. 1 is composed of duplicate samples of the appropriate fraction stained with Coomassie blue (Fig. 1A) or assayed for dextranase activity (Fig. 1B) after in situ renaturation for dextranase activity (5). In Fig. 1, the smaller dextranase bands are not visible because of the shorter renaturation time. Getting clear bands on BD-SDS-PAGE is dependent on the amounts of sample loaded and length of the renaturation period.

Physical characteristics of dextranase. Proteins containing dextranase activity had masses of 160 to 260 kDa when fractionated in the Superose 6 gel filtration column (Fig. 2).

However, dextranase proteins with molecular masses of 80 to 130 kDa were enzymatically active on BD-SDS-PAGE (3–5). These results indicate that native dextranase probably exists in dimeric form (Fig. 2). The pH optimum for activity of purified dextranase from recombinant *E. coli* was determined to be 5.3 (Fig. 3A), essentially identical to the pH optimum of 5.2 to 5.4 for dextranase purified from *S. sobrinus* (4). The K_m value was determined to be 2.6×10^{-2} g of dextran T-2000 per ml (Fig. 3B). The optimum temperature was 39°C (Fig. 3C); however, dextranase purified from recombinant *E. coli* was less stable at higher temperatures (40 to 44°C) than dextranase from *S. sobrinus*, which has a optimum temperature of 36°C and was stable between 40 and 44°C (4). The purified dextranase has three different isoelectric points of 3.56, 3.6, and 3.7 (Fig. 3D). We expected that this purified dextranase existed as more than one molecular weight form due to proteolytic breakdown (4, 14), since we pooled fractions with molecular masses of 160 to 260 kDa on the gel filtration column. We thus expected that some correlation might exist between isoelectric points and molecular masses of dextranase protein. However, we detected no correlation (data not shown). Although the bands at pI 3.56 and 3.6 (Fig. 3D) appears to be overlapping, they are distinct in the original gels.

Immunological relationship between dextranase and SpaA protein by Western blot analyses. Rabbit antisera prepared against the purified SpaA protein from *S. sobrinus* reacted against SpaA protein specified by pYA181, *S. sobrinus* UAB66 extracellular fractions, and SpaA protein specified by pYA770 (Fig. 4A, lanes 1, 4, and 5), as did antisera raised against denatured SpaA protein purified from recombinant *E. coli* (pYA726) (39) (Fig. 4B, lanes 1, 4, and 5). Rabbit antisera prepared against the purified dextranase protein from recombinant *E. coli* (pYA902) reacted only with dextranase protein specified by pYA902 and contained in *S. sobrinus* extracellular fractions (Fig. 4C, lanes 2 and 4). Figure 4D is a BD-SDS-

FIG. 2. Elution profile on a Superose 6 gel filtration column. Molecular masses of dextranases were determined on a Superose 6 column, using various molecular mass markers: BD, 2,000 kDa ($\square$); horse spleen apoferritin, 443 kDa ($\blacktriangle$); β -amylase, 200 kDa ($\bullet$); yeast alcohol dehydrogenase, 150 kDa ($\blacksquare$); bovine serum albumin, 66 kDa ($\times$); and horse heart cytochrome c, 12.4 kDa ($\triangle$).

FIG. 3. Physical characteristics of purified dextranase from *E. coli* χ 2831(pYA902). Effect of pH (A), substrate concentration ($K_m = 2.6 \times 10^{-2}$ g of dextran per ml) (B), and temperature (C) on enzymatic activity. (D) pIs are 3.56, 3.6, and 3.7, determined by native PAGE. Lanes: A, pI markers (2.80 to 6.55); B, 3 μ g of purified dextranase.

FIG. 4. Immunoblot analyses of *S. sobrinus* antigens and *E. coli* recombinant antigens (dextranase specified by pYA902 and SpA specified by pYA770 and pYA181) on SDS-PAGE, using rabbit polyclonal antibodies against *S. sobrinus* SpA protein (A), *E. coli* SpA protein (B), and *E. coli* dextranase protein (C). (D) Renatured BD-SDS-PAGE for dextranase activity. Lanes: 1, *E. coli* containing *spaA* clone pYA181; 2, *E. coli* containing *dex* clone pYA902; 3, *E. coli* χ 2831 lacking plasmid (negative control); 4, extracellular fraction of *S. sobrinus*; 5, *E. coli* containing *spaA* clone pYA770. Samples containing 7 μ g of protein from total cell extracts were loaded on each lane.

FIG. 5. Partial circular restriction map of plasmid pYA902 and diagram of subsequent subcloning of *dex* from pYA993. Abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hind*III; P, *Pvu*II; S, *Sau*3AI; Ps, *Pst*I; Sm, *Sma*I; MCS, multiple cloning sites. The site designated B/S* or Sm/P* is no longer cleaved by *Bam*HI and *Sau*3AI or by *Sma*I and *Pvu*II, respectively. Positions numbered at *Pvu*II sites (P¹ to P⁷) correlate with those in Fig. 7 and 8.

PAGE analysis showing dextranase bands after renaturation which match the dextranase bands on the Western blot analysis. Similar results were observed after separation of proteins on nondenaturing gels followed by Western blot analysis (data not shown). Dextranases from *S. sobrinus* UAB66 have higher molecular masses than those from *E. coli* containing pYA902 (Fig. 4D). This means that pYA902 does not contain the entire *dex* sequence or has a possible mutation in the *Pvu*II-3 (P³)-to-*Pvu*II-4 (P⁴) fragment (Fig. 5) that terminates expression of dextranase.

Physical map of the *S. sobrinus dex* gene in recombinant *E. coli* recombinants pYA902. A partial physical map was obtained by restriction endonuclease analysis of the 12.2-kb insert from *S. sobrinus* (Fig. 5). Of the restriction enzymes used, *Sma*I, *Xho*I, *Nco*I, *Bgl*II, *Kpn*I, *Sph*I, *Xba*I, and *Sst*I had no cleavage sites. The total plasmid DNA size was 25 kb and

FIG. 6. Expression in *E. coli* of enzymatically active dextranase proteins encoded by pYA902 and its subclones as revealed by BD-SDS-PAGE. Lanes: 1, prestained molecular weight markers; 2, pYA902; 3, pYA991; 4, pYA992; 5, pYA993; 6, pYA3010; 7, pYA3011. Each lane was loaded with 40 μ g of total cell extract. The host strain for these plasmids was *E. coli* χ 2831.

contained a 12.2-kb *Sau*3AI fragment from *S. sobrinus* inserted into one of the *Bam*HI sites in two tandem pHC79 vectors.

Subcloning of the dextranase gene from *E. coli* recombinant pYA902. Plasmid DNA (pYA902) was partially digested with *Pvu*II, ligated, and transformed into χ 2831 to yield dextranase-producing subclones with a single pHC79 vector and smaller inserts, pYA991, pYA992, and pYA993 (Fig. 5). The 2.6-kb P²-to-P³ fragment encodes a major portion of the *dex* gene of UAB66 and causes synthesis in *E. coli* of enzymatically active dextranase. The 2.6-kb *Pvu*II fragment from pYA993 was inserted into the *Sma*I sites of the *Asd*⁺ vector pYA292 (25) and pUC8 (75) to generate pYA3010 and pYA3011, respectively (Fig. 5). The dextranases produced in *E. coli* by a series of subclones are shown by BD-SDS-PAGE after in situ renaturation (Fig. 6). Dextranases produced in pYA3010 and pYA3011 are localized mainly in the cytoplasmic region (data not shown). Also, the very faint higher-molecular-mass bands in Fig. 6, lanes 2, 3, and 5, could represent cytoplasmic forms with signal sequences intact.

Nucleotide sequencing. Double-stranded templates were generated by Tn5seqI mutagenesis (Fig. 7) from plasmids pYA993 and pYA3009 (Table 1). Plasmid pYA3009 was obtained from *S. sobrinus* UAB108 containing 2.5 kb of the *dex* gene that includes its control region and expresses 100-kDa enzymatically active dextranase (data not shown). The DNA sequence of 2,755 nucleotides that encodes the N-terminal portion of *dex* and its control region was generated by the bidirectional chain termination method (55). Sequence from 2,756 bp to the C-terminal end of *dex* was determined by UAB66 chromosomal DNA sequencing. To sequence the 1.7-kb C-terminal region in both strands, 17 24-mer primers (synthesized by Cruachem, Inc.) were used. The DNA sequence was determined in both strands and is shown in Fig. 8. The *dex* gene was identified as either an open reading frame of 4,011 nucleotides preceded by a putative ribosome-binding site (AGGA), 10 bases upstream from the putative GTG start codon beginning at position 121 and extending to the termination codon TAA at position 4132, or an open reading frame of 3,999 nucleotides beginning from the ATG start codon at position 133 and ending at position 4132. We would like to speculate that the GTG start codon at position 121 is more

FIG. 7. Map of Tn5seqI transposon mutagenesis of plasmids pYA993 and pYA3009. Dextranase activity was detected on BD-SDS-PAGE after renaturation. Abbreviations: B/S, BamHI-Sau3AI; EI, EcoRI; EV, EcoRV; H, HindIII; PS, PstI; S, Sau3AI; PV, PvuII.

likely to be the start codon, since ATG is situated at 23 bases downstream from the putative ribosome-binding site. A putative promoter region preceded *dex* with a probable -35 TTGTGT at positions 25 to 30 separated by 16 base pairs from a proposed -10 region TAAAAT at positions 47 to 52. Downstream of the termination codon TAA, a potential stem-loop structure is located at positions 4140 to 4173 (Fig. 8), with a free energy of about -12.3 kcal (ca. -51.5 kJ), plus a string of 5 T's, suggesting the existence of a transcription terminator.

Amino acid composition. The amino acid sequence deduced from the nucleotide sequence revealed that dextranase specified by the *S. sobrinus* insert in pYA902 is an acidic, hydrophilic protein of either 1,333 or 1,337 amino acids. Like other extracellular proteins from streptococci (2, 23, 28, 38, 66, 74), there were no cysteine residues detected in the deduced amino acid sequence of the *dex* gene product. The N-terminal portion of the protein corresponds to a signal peptide of either 26 or 30 amino acids. We believe that the latter length is correct because the VNNR N-terminal sequence is much more in accord with the expected basic N termini than the MLSF sequence if the ATG at bp 133 specifies the N termini. The typical basic N terminus of the preferred signal is followed by a hydrophobic central region and a polar C terminus (Fig. 8). Cleavage of the signal peptide should be after amino acid 30, in accordance with the -3 , -1 rule of von Heijne (76). The molecular weight of the unprocessed protein was 143,265; that of the processed mature protein was 140,088. To investigate whether the *dex* gene product was indeed cleaved at this site, the enzyme expressed in *E. coli* χ 2831(pYA902) was purified, transferred onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore Corp., Bedford, Mass.), and subjected to N-terminal amino acid analysis (49, 50). The first six amino acids were identified as Asp-Glu-Leu-Ala-Asn-Gln (DELANQ); this sequence is identical and unique to that of the deduced amino acid sequence directly following the pos-

tulated cleavage site (Fig. 8). Also, this signal peptide of 30 amino acids contains regions similar to those found in most secretory signal sequences (57).

A common motif for the C termini of gram-positive coccal surface proteins (GPC-SP) has been identified and extensively discussed (24, 64). The C-terminal portion of Dex has a number of features in common with GPC-SP, even though Dex has been found mainly in *S. sobrinus* supernatant culture. Located 27 residues from the end of Dex is the hexapeptide LPKTGD. In GPC-SP, the highly conserved peptide LPXTGX is found approximately 30 residues from the C termini of these proteins (24, 64). Following the Dex LPKTGD peptide is a charged tripeptide (HKT) and then 19 amino acids with a highly hydrophobic character. The protein terminates with a pentapeptide (RHEKK) carrying a net positive charge. This region is structurally similar to GPC-SP membrane-anchoring domains. Located immediately on the N-terminal side of the LPKTGD sequence is a Ser- and Thr-rich region (40% of 35 residues) which may correspond to the cell wall-spanning region of GPC-SP.

DISCUSSION

Extracellular dextranase from *S. sobrinus* UAB66 had been previously purified and partially characterized, and the *dex* gene had been cloned by Barrett et al. (4). The present study extended this work by purifying dextranase expressed in *E. coli* containing the truncated *dex* gene of *S. sobrinus* (pYA902) and characterizing its protein and antigenic properties. Also included was a study of an *E. coli* recombinant clone yielding the sequence of the 2,755-bp N-terminal region of the *dex* gene and its control region and UAB66 chromosomal sequencing yielding the sequence of the 1,445-bp C-terminal region.

The dextranase protein has been purified from *E. coli* χ 2831(pYA902) to near homogeneity by anion-exchange and gel filtration chromatography using the FPLC system. This

-35 -10 Sau3AI²

GTCGGTGTTCCTCAAGTATTAGGGTGTGTTTCATAGATTTTCAGTAAATACACTTATGATTTTGGAGCACCTTAACAGGTCGCTCGATCATAAAAA 100

121
↓

SD PvuII²

ATTAGAAGGAGAAAAAGGCCGTGAATAACAGAATGCTATCTTCCCTAGCATGTTATTTCTACTTGCTTTTGAATAGTGCTCTCAGTTTCAGCTGGCAC 200

V N N R M L S F P S M L F L L A F G I V L S V S A G T 27

signal peptide sequences

TACTCATGCAGATGAGCTTGCAAACCAACCGCCAAGGTAGCCGATGAGGCTTCCATTTGGTTAGTACATCACAAGCCGCTGTTGAGCAGACCCAGTCA 300

T H A D E L A N Q T A K V A D E A S I V V S T S Q A A V E Q T Q S 60

↑ cleavage site

CAAGAGAAGGAGATCAGCCCAGCAATGGAAGAGGACACGAGCAATTTGAGCCTGAAACCCCAATGCTCAGCAAGAATCACAAAGTCTGACTCAAGTACAG 400

Q E K E I S P A M E E D T S N L S L K P N A Q Q E S Q S P D S S T E 94

AACCTCAAGATCCCGCTGAACAAACACCGCCTGAAACGTCTGATGCTTCTGCACCGCAGCAACCTCGGCGGATTCTGTTGAGAAATACGCTCAAGATGC 500

L Q D P A E Q T P P E T S D A S A P A T T S A D S V E K Y A Q D A 127

AACCCAAACCAATCATCTACCAGCAACGGACCTGGGGTTATCCGAGCAACAAGTGCTCAGGTAACCGCAACTCGATCTGTTGTTTCTTCAATCTGGC 600

T Q N Q S S T S N G P G V I R A T S A Q V T A T R S V V S S Q S G 160

GATGCAATTGTTGACTTGTCAGCCGACAAGGCCAGCTATCGTCAGGGTGAAGATGTCAACCTATCTGTTGACTTCAAGAACACAACAGATAAAGAGCAAG 700

D A I V D L S A D K A S Y R Q G E D V N L S V D F K N T T D K E Q D 194

ACGTAACGTGTTTACGCTGATGTTTATTATATTGACAACAACTAGGGACCTACAAGTTCAGCAACATCTCAAGCCGGTGAAGGCTACAAGATGCAATC 800

V T V Y A D V Y Y I D N K L G T Y K F S K H L K A G E G Y K M Q S 227

CGGCGACTTGAAGATTCTGCTTCAATTTGAAAACAATCATGGCTACCTGCTTAAGGTTGCGGTGACGCTGACAATAATACTCTCAGCGAGGTC 900

G D L K I P A S Q F E N N H G Y L L K V R V R D A D N N T L S E V 260

AACAAGGCCATTGCCGTTGAAAGTGATTGGACCAATTCCTCCGTTACGGAATGTTGGGGGTTCTCAAGATACCAACAACAGCCTGCTGCTCAAGGATG 1000

N K A I A V E S D W T K F P R Y G I V G G S Q D T N N S L L S K D A 294

CTGACCGTTACCGTGCAGAAATTGAAAAGATGAAGAATATGAACATCAATTCATCTTCTATGATGTTTACAAGACTGCTACCAATCCCTTCCCAAG 1100

D R Y R A E I E K M K N M N I N S Y F F Y D V Y K T A T N P F P S 327

TGATGAAGCCACTTTCAAACAAGACTGGAACACTTGGAGTGGCTCAGAAATTGATACCCAAGCCGTCAAAGATATTGTCAACCAAGTTTCATGATGGTGGC 1200

D E A T F K Q D W N T W S G S E I D T Q A V K D I V N Q V H D G G 360

GCTGTGCTATGCTTTACAACATGATTTTGGCAGAAAATACCAATACCGGTGAAGCTCCTGTTTGGCAGAGACGGAATACGCTTATAATAGCGATGACC 1300

A V A M L Y N M I L A E N T N T G E A P V L P E T E Y A Y N S D D R 394

GCGGTTATGGTGTCTAAGGACAACCGATGCTTACACCGTGAAAATTCCAAAAGACGGGCAAGAAGAGGATGTGAAATCCAACGCTACTACAACCCAAAC 1400

G Y G A Q G Q P M S Y T V K I P K D G Q E E D V E I Q R Y Y N P T 427

TAGCAAACTCTGGCAAGATTACATTGCTGACAAGATGGGTCAAGCCATGAAGAATGGTGGCTTTGATGGTTGGCAGGGTGATACTATCGGAGATAACGAA 1500

S K L W Q D Y I A D K M G Q A M K N G G F D G W Q G D T I G D N E 460

EcoRI

GTTTACAGCTATGCTGATAAGGATAGCAATGACCCCTCTAAGAAATTTTGGCTGACCGAAGGTTATGCGGAATTCCTTCGTGCCATTAAGGAAAAATTAC 1600

V Y S Y A D K D S N D P S K K F W L T E G Y A E F L R A I K E K L P 494

CCAATACTACTTAACCGTAAACGATGTTAACGGTGAACAAATCTATCGTCTCAAAGACGGTAATCAAGATGTTATCTACAATGAAATCTGGCCTTTCCG 1700

N Y Y L T V N D V N G E Q I Y R L K D G N Q D V I Y N E I W P F G 527

ACCGGCTCTGCCCTCGGAGATGGCCGAGTCAAACCGAATACCGTGACCTCAAGGCCCGTGTGACGAAGGTTCCGCAAGGTGACTGAAAATCTCTATT 1800

P A L P S E M A A V K P N T V T S R P V L T K V R Q G D W K I S I 560

GTCGGTGCCTATATGGAAGGTAGCGAAAATGGCGGTTCCAAGGCTGATGCTGAAGCAGGTAAATCCCTGCAAACAGATGCGGTTCTCTTGACCTCTGCCT 1900

V G A Y M E G S E N G G S K A D A E A G K S L Q T D A V L L T S A S 594

CAATCGCGCTGCGGTTGTTTACCATATGTCCCTCGCAGCTCTTGCTAACCAGCAAGACGAAACGGACGGAGGCCAAGGAATTGGCGTTCTCCAACTGC 2000

I A A A G G Y H M S L A A L A N Q Q D E T D G G Q G I G V L Q T A 627

HindIII

TTATTACCAACTCAAGCTTGAAGACCTCAAGCGAATTAACCCGCAAAAATAATGATTATCAACAGTTCATCACCGCTTATGAAAACGTTTTGCGTGAT 2100

Y Y P T Q S L K T S S E L T R K N N D Y Q Q F I T A Y E N V L R D 660

HindIII

GGTGTGAAATGATGATGCCAAGTAAATACCTTTGACTCAAATGGTCAAAAGCTTTCGACCGACGCCAAAGGTATTACTGGCAATCAAGTTTGGACTT 2200

G V E N D D A Q V N T F D S N G Q K L S T D A K G I T G N Q V W T Y 694

FIG. 8. Nucleotide sequence of the *dex* gene of *S. sobrinus*. The 30-amino-acid signal sequence, putative -35, -10, and Shine-Dalgarno (SD) ribosome-binding sites, and the start codon (arrow at position 121) are underlined. The underlined arrowed sequence downstream of the termination codon (identified as * after the arrow at position 4131) represents a potential stem-loop structure. The N-terminal cleavage site is shown with an arrow. Also shown with an arrow is the starting position where the sequence was determined by chromosomal sequencing. Positions numbered at *PvuII* sites correlate with those in Fig. 5 and 7, and positions at *Sau3AI* sites correspond to those in Fig. 7. A restriction site marked as *PvuII** at position 3027 is not the same site as P⁴ in plasmids pYA902, pYA991, and pYA992.


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ACGGAAGGCGATACTTTAGAACCGTTCACTCCTTAACCTGATGGGCATTAACCTCTGACTGGAAGATGAAGATGGTTCGGCAGCCAAACAGAC 2300
G K K G D N F R T V Q L L N L M G I N S D W K N E D G S A A N K T 727

CCCAGATGAGCAACCAACCTGACGGTTAAGTATGCTCTGGGGGATGTCTCAATGGAAGATGCTCAACGGATGGCTAACCAACCTATGTGACCTTCCA 2400
P D E Q T N L T V K Y A L G D V S M E D A Q R M A N Q T Y V T S P 760

GATGACTGGTCTAAATCTAATCTGAAAAGTTTCTGCATCTGTAAAACTGACGAAATGGTAAACCTGTTTGGTTATTATGTGCCTAAATTGACTC 2500
D D W S K S N L Q K V S A S V K T D E N G K P V L V I N V P K L T L 794

TTTGGGACGTGGTCTACATCTCCAATGCCAACCAAGAATCTGCTCCTGAAGCTGATCAAGCTCAAAACCTGACGCTCAATCTAGCGATGATAAAGTTGC 2600
W D V V Y I S N A N Q E S A P E A D Q A Q T P A A Q S S D D K V A 827

TGAAAACGAACCAAGTCAACCGAGCAGAGACGCAAGAGCAAACTTCAGAACGAGCTCAAGACCAAGCGGACCGCGAGCAAGGACAAGCTATT 2700
E N E T S Q P A A E D A K E Q T S E P A Q D Q A A P A E Q G Q A I 860

AACCAAGCAGAATCTCCAGCTACTGAACCTGAAGCTGAGGTGATCCAGCGACAGCTGAACCTGCTAAGGTAGATGCACCTGAAGCAACCAAGCGCTG 2800
N Q A E S P A T E P E A E V T P A T A E P A K V D A P E A N Q A D 894
⇒ Determined by chromosomal sequencing

ATCAAGCGTAGCGCTGAGCCTGCAAGTCAAGAGCAAGCGGCTAGCCAAAGTCAGCTGAAGCGAACCACCAAGCTTCAATGAACTCCAGCAAC 2900
Q A V S P E P A S Q E Q A A S Q S Q P E A N Q T P A S N E T P A 927

TCAAGGAAATTCAGAACCACTGAATTAATGAACCAACAGCTCAACGCAACCAAGTTCCTAAGTTAGTCCAGCCAACTTCACTTACAGTTACAGTAGCT 3000
Q N E T S Q P E L N E P T A Q T Q P S S Q V S P A N T S V T P V A 960

GAACAACCGACTAACCAAGGTCAAGCTGATAAGGCAGACCAAGCTCCGACAAATAGCACTTCTACTCTGAGTCAACTAGTTCAGTTGAGCCAGCAG 3100
E Q P T N Q G Q A A D K A D Q A P T N S T S T P E S T S P V E P A A 994

CAACAGCAGCTCTTCAGATCTCTATAGTTACTGCCGCAATCTCTCTGTTCAACCGGACAGAGACCGGACCGCTTCCAGACAGCAAGGAGA 3200
T D Q S S D T P I V T A G N L S V Q P A E T E T P T V P D K Q G D 1027

CTCTAAGGTAATCAATCATCTACCGAGACCCAGTTGCTGACCAAGTCCAGCAGTAGCTGAACAACCAAGCAACAGCAACCAAGCGCTAAACCT 3300
C K A N Q S S T E T P V A D Q V P A V A E Q P Q A T E P N Q A K P 1060

TCTGTGCAAGCGACCGCACCAGAGCGCTAAGCCTGATTAACCTCAAGCAGCAGACCAAGCAATCCAGGCAAGAGGAGCAGATGATCCAGAAGTTG 3400
S V D K A A A P E A L S L I Q L K Q Q T P A I Q A K E A D D P E V D 1094

ATGAGACTAAGTCAGAGGTCACTCCAGATTCCGGAACAGATAAAGCTCCAGAAGCTGGTCAGGTAGACTCAGACAAGGCTCCAACTGTTAAGCCATCAAC 3500
E T K S E V T P D S G T D K A P E A G Q V D S D K A P T V K P S T 1127

ACCAGAAAATATGACAACTAGCCTAATATGCAAAAGATGCAGATAAGAATAAGACTAATGAGGCTGATTCACCAAGGCTAATCAGGATTCACAAA 3600
P E N N D N Q P N N A N D A D K N K T N E A D S N K A N Q D S T K 1160

GGGTCTTCAGCGGATCAATCTGAAAATCAACGACTCCGGAGGATGGTCCAGACAATTCAGTCCAGAAGATCCTGAGACCAACCGCTCAGATCCAAATA 3700
G S S A D Q S G K S T T P E D G P D N S S P E D P E T K P S D P N T 1194

CTGATACACGATCAGGAACAGGTGAAGCAAGTCTACCAAGTGTCTTAATCAACAGTAGATGATCCTAAACGAGCAGACTGATACTCCAGCGAA 3800
D T S D Q E Q V K P S L P V V P N Q T V D D P K T D D T D T P A N 1227

TACAGACTCAGCTAAGTCTAAAAAGTAGCGATGCAGATAAGAACAAGTGGCACTGATTCAGAAGCGCGTCAAAAATCTTCAGAGTTCCCTAAGAA 3900
T D S A K S K K V A D A D K N K V A T D S E G R Q K S S E F P K E 1260

GCTACAGACTTGGAAAAAGTTGGTCAACGAGCTCTCCCAAGTAGCAGGAGTAAATCATCAGTTGCAACTAGCCAGAAAAGAAATCAGAGCCTGTTT 4000
A T D L E K V G Q P A S P Q V A G V K S S V A T S P E K K S E S 1294

CTAAAACAGCAGACTTCATCTTCAGACAAGTTGCTAAGACTGGGGATCACAAGACCGTGTCTCATTATTGTTCTTGGTCTTGTCTTGGTGTAT 4100
K T S T T S S S D K L P K T G D H K T V V L I I V L G L V F V G M 1327
4131
↓
GACAGGACTTTTGGCAAGGCATGAAAAAATAAGCTAGAGGAAGTGGTGGGCTCTGCCATCTTTCTTGTCTTGCAGTTGGTAATAGTTTTT 4200
T G L L A R H E K K * stem-loop structure 1377

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FIG. 8—Continued.

separation showed one major protein band on analytical PAGE (Fig. 1A, lane 5) which corresponded to a single band of activity (Fig. 1B, lane 5). This purification scheme for this dextranase protein has been a very simple, quick, high-yielding, and reproducible procedure. The K_m s, pH optima, temperature optima, and isoelectric points for the dextranase synthesized in *E. coli* are similar to those previously reported for *S. sobrinus* dextranases (4, 20, 32, 58), with a difference in K_m (decreased 100-fold) compared with that for *S. sobrinus* dextranases (4). The molecular weight of the purified dextranase from *E. coli* 2831(pYA902) was estimated to be 130,000 after SDS-PAGE analysis. The fact that this value did not agree with the one obtained by gel filtration (about 260,000; Fig. 2) indicated that native dextranase might exist as an aggregated dimeric form. Dextranase expressed in *E. coli* 2831 was mainly transported into the periplasmic space, and this fact was confirmed by the nucleotide sequence analysis specifying a signal sequence in the N-terminal region.

SpaA (39) appears to be one of the proteins necessary for

sucrose-independent adherence (19, 59). The observation that the antiserum against SpaA protein, whether made by recombinant *E. coli* or by *S. sobrinus*, is also capable of causing immunoprecipitation of dextranase activity from *S. sobrinus* UAB66 (3, 14) implied that dextranase may also play a role in sucrose-independent adherence to the pellicle-coated tooth surface. These results led us to hypothesize that the SpaA and dextranase proteins were immunologically related and shared at least one antigenic determinant (3, 14). To further investigate the relationship between the SpaA and dextranase proteins, immunoblot analyses were performed with antisera made against dextranase expressed in *E. coli* and antisera made against SpaA from *S. sobrinus* and *E. coli* recombinants. However, the results indicated no immunological cross-relationship between the SpaA and dextranase proteins. The dextranase protein that we purified to raise antiserum turned out to be a truncated gene product missing the 1,377-bp C-terminal region from bp 2755 to 4131. This may imply that the common antigenic determinant is at the missing C-terminal

region. However, comparison of amino acid sequences deduced from nucleic acid sequences for the entire *dex* gene and *spaA* (30, 46) indicate little homologies of any significance between the two proteins.

Compared to the dextranase produced by *S. sobrinus* UAB66 as recovered in the extracellular fraction (175 kDa) (4), dextranase protein expressed in *E. coli* was smaller (130 kDa). Moreover, since dextranase as small as 80 kDa still has enzymatic activity (3, 14), only about half (2.2 kb) of the *dex* gene appears to be needed to specify enzyme activity. Subsequent subcloning from the original pYA902 clone revealed that the 2.6-kb *PvuII* fragment specified a 110,000-Da enzymatically active dextranase. This 2.6-kb *PvuII* fragment in pBR (pYA993), p15A (pYA3010), and pUC (pYA3011) vectors specify synthesis of dextranase-active proteins of molecular masses (130 to 100 kDa; Fig. 6). Since a DNA sequence of 4.8 kb would be needed to specify the 175-kDa dextranase found in *S. sobrinus* (4) by SDS-PAGE analysis, it is apparent from the nucleic acid sequence of pYA993 that a large portion of the C terminus of the *dex* gene was absent without effect on enzymatic activity, implying that this activity resides in the N-terminal region.

Analysis of the nucleotide sequence of the *dex* gene confirmed the restriction map of clones prepared by restriction endonuclease digestion. The nucleotide sequence immediately upstream of the putative initiation codon GTG of the *dex* gene contains sequences characteristic of a procaryotic ribosome-binding site (65) and promoter sequences (35, 36, 52). Nucleotide sequencing of the *dex* gene indicated that codon occurrence frequencies showed several characteristics in common with those of other known streptococcal genes (38, 48, 61) and significant differences from those in *E. coli* (1) except for phenylalanine and tyrosine codon uses (data not shown). Since we do not know the *S. sobrinus* 16S rRNA sequence, we can only hypothesize that a potential ribosome-binding site, AGGA, is located 10 bases upstream of the GTG start site. The nucleotide sequence upstream of this region is extremely AT rich and contains likely -10 and -35 promoter sequences. The 4,011 nucleotides (1,337 amino acids) of the *dex* gene would specify an enzyme with a molecular size of 143,265 Da.

Analysis of the deduced amino acid sequence at the N-terminal end of the dextranase protein suggests the presence of a signal sequence typical of most extracellular or periplasmic proteins (57). A putative signal peptidase cleavage site was detected after alanine residue 30, applying the rules of von Heijne (76), and was confirmed by N-terminal amino acid analysis. This yielded a processed protein of 140,088 Da. It is not clear why the deduced molecular mass of dextranase from the nucleotide sequence (143,265 Da) is smaller than the molecular mass of *S. sobrinus* dextranase (175,000 Da) determined from mobilities in SDS-PAGE analysis. It seems that the dextranase protein is one of a number of proteins that exhibit aberrant electrophoresis migration in SDS-gels. Analogous situations have been described and have been interpreted as due to the presence of either a high negative charge (9, 41) or a C-terminal proline-rich region adjacent to the hydrophobic region (38). In the case of dextranase, the anomaly may be the result of unusually high negative charge as well as a near-C-terminal proline-rich region. Also of note is that the putative cell wall-spanning domain of Dex contains 5 prolines and glycines within 35 amino acids (14%) and 11 prolines and glycines within 100 amino acids (11%) of the N-terminal side of the LPXTGX sequence, whereas it contains 14 serines and threonines (40%) and 27 serines and threonines (27%), respectively. In GPC-SP, this region is mostly composed of between 10 and 32% prolines and glycines (24). For

a few proteins, the concentration of prolines and glycines is significantly higher than that of threonines and serines, while in most others, this relationship is either reversed or nearly equal. This finding may be significant in that the Pro- and Gly-rich region has been postulated to augment the association of the GPC-SP with the cell wall by imparting a structure to the cell wall-spanning region that would allow the protein to intercalate within the peptidoglycan meshwork (24). The function of the threonines and serines is not immediately clear. The lower proline and glycine composition of this region in Dex compared with other GPC-SP may allow the enzyme to freely dissociate from the cell rather than be attached at the cell wall region.

Comparison with several other *S. sobrinus* and *S. mutans* cell surface protein amino acid sequences (DexB, SpaA, GtfB, GtfC, GtfI, and GtfS) (23, 28, 46, 60, 66, 74) by protein data base scanning showed few or no detectable homologies. Also, no tandem repeats as found in some other streptococcal glucan-binding proteins (2, 23, 51, 66, 74) or a region specific for cleaving glucans as found in other glucose polymer-attacking enzymes (60) occur in the C-terminal region. Few internal repeating polypeptide sequences (8 to 10 amino acids) exist at the proline-rich region (at amino acids 800 to 1227) adjacent to the C-terminal end, which is extremely AT rich with strings of A's. Also, nucleotide sequence homologies were detected in these internal repeating regions. We have attempted numerous times without success to clone the C-terminal region by using various vectors with cloned *dex* sequences as a probe. Since we have the entire sequence of the *dex* gene from *S. sobrinus* UAB66, it is still in our interest to clone the whole *dex* gene by PCR to study the entire gene product.

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