Nucleotide sequence of the dextran glucosidase (dexB) gene of Streptococcus mutans

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Nucleotide sequencing of DNA in the region of the Streptococcus mutans chromosome adjacent to the previously characterized gtfA gene revealed the presence of two long open reading frames. One of these corresponds to the dexB gene and has been shown to encode an intracellular exodextranase (dextran glucosidase) which has short isomaltosaccharides as preferred substrates. Comparison with other published sequences showed that the dexB gene product shares regions of similarity with enzymes, from a variety of sources, which attack other glucose polymers.

Introduction

Polymers produced from sucrose by extracellular enzymes of oral streptococci are believed to be important in the formation and metabolism of dental plaque. In particular the dextrans, which consist predominantly of α-1,6-linked glucose units, are involved in interbacterial aggregation events and bacteria-to-surface binding (Hamada & Slade, 1980), but are also available to the plaque microflora as a nutrient source (Parker & Creamer, 1971). A substantial proportion of plaque streptococci are capable of degrading dextran (Staat & Schachtele, 1974; Dewar & Walker, 1975) and hence dextrans can be regarded as a form of storage polysaccharide for organisms which synthesize the polymer or as an exploitable carbon source for other organisms.

Two classes of dextranase have been detected in bacteria – endodextranase (EC 3.2.1.11, dextranase) which cleaves within the glucan chain to release smaller isomaltosaccharides, commonly 3 to 5 glucose units long, and exodextranase (EC 3.2.1.70, glucan 1,6-α-glucosidase; also referred to as dextran glucosidase) which acts at the reducing terminus of dextran molecules to release free glucose units one by one (Walker, 1978). Dewar & Walker (1975) found that Streptococcus mutans appeared to have both these activities; the endodextranase activity was subsequently shown to be found in culture filtrates, whereas the dextran glucosidase was predominantly cell-associated (Walker et al., 1981). A dextranase from S. mutans was also reported by Burne et al. (1986), who found dextranase activity, encoded by a gene designated dexB, expressed from a fragment of S. mutans genome cloned into Escherichia coli. The cloned fragment also carried the gtfA gene, which we too have cloned, sequenced and shown to specify sucrose phosphorylase (Russell et al., 1985; Ferretti et al., 1988; Russell et al., 1988). In this report we describe further nucleotide sequence data for this region of the S. mutans chromosome and characterize the dexB product as a dextran glucosidase which is located intracellularly.

Methods

Bacteria, vectors and growth media. S. mutans strain Ingbritt was grown in Brain Heart Infusion broth (Oxoid) or the medium described by Terleckyj et al. (1976) with 0.5% (w/v) casein hydrolysate instead of the individual amino acids. Conditions for growth and handling of E. coli strain JM109, plasmids pUC18 and pUC19 and the single-stranded bacteriophage vectors M13mp18 and M13mp19 have been described by Yanisch-Perron et al. (1985).

Nucleotide sequencing. From a recombinant of bacteriophage λL47.1, previously shown to carry an insert of chromosomal DNA from S. mutans strain Ingbritt including the gtfA gene (Russell et al., 1985), a series of HindIII fragments were isolated. The 2·3 kb and 1·4 kb fragments were unidirectionally digested with BstXI and subcloned into bacteriophage M13 vectors mp18 and mp19 as previously described (Ferretti et al., 1986) for sequencing by the dideoxy chain-termination method (Sanger et al., 1977). All sequences were confirmed

Abbreviation: ORF, open reading frame.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number M30944.

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Fig. 1. Partial restriction map of the cloned segment of S. mutans Ingbrtit chromosome showing the position and orientation of gtfA, dexB and the ORF between them.

from at least two overlapping clones, and the entire sequence was determined on both strands. The region around the HindIII site was also confirmed using an EcoRI subclone. The sequence information was analysed by the James M. Pustell DNA/protein sequencing Biological Research Foundation database was done using the program (International Biotechnologies Inc.) and the Staden-Flus

Subcloning of the dexB gene. Inspection of the distribution of restriction sites and coding regions within the nucleotide sequence indicated that the entire open reading frame (ORF) suspected of carrying dexB was contained in a 2.44 kb RsaI fragment. Such a fragment was therefore isolated from the original recombinant phage and cloned into the HincII sites of plasmid vectors pUC18 and pUC19 to generate recombinant plasmids pSF104 and pSF105.

Analysis of dextranase. Cell-free extracts or culture filtrates of S. mutans or recombinant E. coli were incubated for periods varying from 0.5 to 16 h with commercially obtained dextran (M, 72000, Sigma) and analysed by paper chromatography as described by Dewar & Walker (1975) or by thin-layer chromatography on silica gel (Binder et al., 1983).

Dextran glucosidase was quantified by the procedure of Walker & Pulkownik (1973) modified for microtitation trays. Enzyme preparations were added to substrates dissolved in 0.05 M-citrate buffer (pH 6.0) and incubated for 30 min at 37 °C. The reaction was stopped by the addition of an equal volume of 1 M-Tris/HCl (pH 7.0) and glucose oxidase reagent (Sigma) was added. After 30 min the A450 was read in a Flow Titretek plate reader.

Gel electrophoresis. SDS-PAGE was done using the buffer system of Laemmli (1970). For the detection of endodextranase activity, 2% (w/v) Blue Dextran (Sigma) was incorporated into the separating gel before polymerization. Following electrophoresis, the gel was washed for 1 h in several changes of 0.05 M-Tris/HCl (pH 7-5) then incubated overnight in 0.05 M-citrate (pH 6.0) containing 1% (v/v) Triton X-100 to remove residual SDS (Barrett & Curtiss, 1986; Russell, 1979).

Results

Analysis of dexB gene and its product

A partial restriction endonuclease map of the region containing gtfA and dexB is shown in Fig. 1. The positions of the HindIII and EcoRI sites are in close agreement with those presented by Burne et al. (1986) and Pucci & Macrina (1986), respectively. Nucleotide sequencing revealed the presence of three long ORFs, the deduced sizes of the polypeptides encoded by them corresponding to those specified by gtfA (55665), dexB (62103) and an ORF which lies between them (41964). These sizes also agree well with the sizes found in mini-cell experiments by Burne et al. (1986) and Pucci & Macrina (1986).

The nucleotide sequence of the dexB gene and its immediate flanking regions (from the base immediately after the termination codon of the upstream ORF to 102 bp downstream of the dexB termination codon) is shown in Fig. 2. No sequences corresponding to the –10 and –35 regions of known promoters are detectable upstream of the initiation codon, though there is a possible ribosome-binding site (AGGTGG) located 6 bp upstream. Just after the termination codon the sequence indicates the existence of an inverted repeat capable of forming a hairpin loop.

Analysis of the dexB ORF reveals that it codes for a 536 amino acid protein with a predominantly hydrophilic character and a predicted M, of 62103. There is no evidence for the existence of a signal peptide resembling those found in S. mutans products which are known to be secreted (von Heijne & Abrahamse, 1989). Furthermore, the size of the dexB gene product detected by SDS-PAGE in recombinant E. coli was 62000 (Fig. 3), close to that predicted from the sequence data. It therefore appears that the dexB gene product does not undergo post-translational modification and can be concluded to have an intracellular location.

Characterization of dextranase activity encoded by dexB

Although the DexB enzyme attacks dextran, with glucose being the sole low-M, product detectable by either paper or thin-layer chromatography (data not shown), the rate at which it releases glucose from dextran...
S. mutans dextranase

Fig. 3. SDS-PAGE of M, standards (lane 1) and recombinant E. coli carrying plasmids pSF104, in which dexB is orientated so as to be under control of the lac operon (lanes 2 and 3), or pSF105, where dexB is in the opposite orientation (lanes 4 and 5). The cultures used for lanes 3 and 5 were grown in the presence of 1 mM-IPTG.

Previous observations of an invertase-like activity encoded by the cloned dexB region (Burne et al., 1986).

Control of expression of dexB in E. coli

A 2.44 kb RsaI fragment was subcloned into plasmid vectors pUC18 and pUC19 to produce recombinant plasmids pSF104 and pSF105. In E. coli carrying pSF104 a novel band of M, 62000 could be detected after SDS-PAGE (Fig. 3), although no such band was apparent in strains carrying pSF105. The addition of IPTG during overnight incubation to induce transcription from the lacZ promoter increased the amount of the 62000 band, and increased the level of dextran glucosidase detectable in strains carrying pSF104. These results indicated that the dexB gene is not being transcribed from its own promoter; when placed in appropriate orientation behind the lac promoter, however, as in pSF104, functional DexB protein is produced, the rate of transcription being further increased by induction of the lac promoter by IPTG. The low level of expression in strains carrying pSF104 even in the absence of inducer occurs because the host E. coli does not produce sufficient lac repressor to repress all the lac promoters present on the multicopy pUC plasmids.

Extracellular dextranase of S. mutans

Walker et al. (1981) described the occurrence of dextranase in culture filtrates of S. mutans. The ability of this enzyme to degrade high-M, Blue Dextran allows zones of activity to be detected by SDS-PAGE (Fig. 5). Two bands of activity from S. mutans cultures were detected, the M, values being 120000 and 105000.

is only 7% of the rate at which it releases the glucose from its shorter homologues, isomaltotriose being the preferred substrate (Fig. 4). The enzyme was unable to release glucose from maltose, maltotriose, maltotetraose, maltopentaose, starch, pullulan, inulin, levan or mutan. It did, however, split p-nitrophenyl α-D-glucopyranoside and also showed weak activity against sucrose after prolonged incubation. The effect on sucrose may explain...
During storage of samples, there was a gradual break-
activity encoded by Dextran was found in recombinant
with an apparent
Comparison of the deduced amino acid sequence of
corresponding to the highly conserved regions of
revealed the occurrence of regions of local similarity
bank and of enzymes known to attack glucan polymers
Comparison with other glucosidases

**Fig. 6. Comparison of the amino acid sequences of homologous regions in enzymes with varying specificities for cleaving glucans.**

**Table 6**

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For DEXB, S. mutans dextran glucosidase; AMY, α-amylase of Bacillus stearothermophilus and AMY consensus, a consensus sequence from 11 different amylases (Nakajima et al., 1986); IAM, isoamylase of Pseudomonas spp., (Amemura et al., 1989; Tognoni et al., 1989); PUL, pullulanase of Klebsiella pneumoniae (Katsuragi et al., 1987); NPL, neopullulanase of B. stearothermophilus (Kuriki et al., 1989); CGT, cyclodextrin glycosyltransferase of K. pneumoniae (Binder et al., 1986). Numbers indicate the position of amino acids within each sequence. Asterisks indicate amino acids identical to those in DEXB.

**Discussion**

The results presented in this paper confirm and extend the report of Burne et al. (1986) in showing that a dextranase gene, dexB, is closely linked to gtfA on the S. mutans chromosome. Burne et al. (1986) have previously established the physical properties and pH optimum of the dextranase and our further characterization of the primary sequence of the enzyme, its substrate range and reaction products show that it is an intracellular dextran glucosidase (exodextranase or glucan 1,6-α-glucosidase, EC 3.2.1.70), which releases glucose from dextran and isomaltosaccharides. The enzyme encoded by dexB thus corresponds to that first demonstrated in S. mutans Ingbritt by Dewar & Walker (1975) and resembles that characterized in Streptococcus mitis (Linder & Sund, 1981; Walker & Builder, 1967; Walker & Pulkownik, 1973). It is entirely distinct from the dextranase found in culture filtrates. The latter can most readily be assayed by its ability to release dye from Blue Dextran due to the fact that it cleaves the dextran into small diffusible fragments (isomaltosaccharides). This extracellular dextranase in S. mutans migrates on SDS-PAGE as a pair of bands with M_r values of 120000 and 105000 and this organism thus resembles Streptococcus sobrinus (Barrett et al., 1986, 1987) and Streptococcus downei (R. R. B. Russell, unpublished) which both have dextranase activity of M_r 190000 and active breakdown products as small as 70000. The intracellular dextran glucosidase and the extracellular dextranase also differ in

Comparison of the deduced amino acid sequence of
 DexB with sequences of other proteins in the NBRF data bank and of enzymes known to attack glucan polymers revealed the occurrence of regions of local similarity corresponding to the highly conserved regions of α-amylases (Nakajima et al., 1986). These regions of similarity are also found in other enzymes which attack the α-1,6 inter-chain linkages in starch and in the polymer pullulan, which has alternating α-1,4 and α-1,6 linkages: pullulanase from Klebsiella pneumoniae, isoamylase from Pseudomonas aeruginosa and neopullulanase from Bacillus stearothermophilus (Amemura et al., 1988; Katsuragi et al., 1987; Kuriki et al., 1989; Tognoni et al., 1989) as shown in Fig. 6. In addition to these regions common to all the enzymes, the dexB enzyme and isoamylase have a common region towards their C-terminal ends. Finally, a significant amount of overall sequence similarity, though with good match in only one of the conserved regions, was found between DexB and the cyclodextrin glycosyltransferase of K. pneumoniae, an enzyme which degrades starch to cyclodextrins (Binder et al., 1986).
that only the latter can be purified by affinity chromatography on immobilized dextran (unpublished observations).

The dextran glucosidase of *S. mitis*, which appears to be the direct counterpart of the *S. mutans* enzyme described in this paper, degrades macromolecular dextrins by removing terminal glucose residues along α-1,6-linked stretches until stopped by a branch point (Walker & Puilkownik, 1974). Although it has been suggested that such activity may be of physiological importance in generating free glucose from dextrins in plaque (Sund et al., 1989), the fact that the enzyme is located intracellularly both in *S. mitis* (Linder et al., 1983) and in *S. mutans* (this paper and Walker et al., 1981) make this doubtful. Indeed it may be inappropriate to refer to the *dexB* enzyme as a dextranase, since it is improbable that high-Mₘ dextrans will be taken up and become available as substrates within the *S. mutans* cell, and there is no evidence to suggest that dextrans are synthesized inside *S. mutans*. It is therefore attractive to advance the hypothesis that the extracellular and intracellular enzymes act in series, with dextrans first being cleaved into short isomaltosaccharides of a size which can be transported across the cell membrane. Dewar & Walker (1975) showed that the major products of the extracellular enzyme were isomaltotetraose and isomaltopentaose, which should be readily transported. Further experiments are needed to clarify the interrelationship of the two dextranasas in a catabolic pathway and the possibility that they are co-ordinately regulated. However, as pointed out by Walker et al. (1981), regulation of dextranase activity in *S. mutans* appears to be extremely complex so such studies should be helped by cloning of the gene for the extracellular dextranase, allowing measurement of mRNA levels.

The linkage of *dexB* to *gtfA* is interesting, and lends support to the notion that genes encoding functions associated with sugar metabolism are clustered on the chromosome of *S. mutans*. The data presented in this paper indicate that *dexB* is not transcribed from its own promoter and so its expression is likely to be coordinate with that of the protein encoded by the ORF immediately upstream. The nature of this protein is currently under investigation in our laboratories. Burne et al. (1986) concluded from their experiments that *dexB* did have its own promoter. Whether the discrepancy is attributable to the fact that they cloned the gene from a different strain of *S. mutans* (strain GS-5) or because they subcloned a larger fragment than the RsaI–RsaI piece used by us remains to be discovered.

Glucosidases are extremely diverse in their specificity for different substrates (Kelly & Fogarty, 1983) but primary sequence analysis has revealed the existence of conserved regions between amylases, α-glucosidases and transglucanosylases which are believed to be involved in substrate binding and active sites. In the α-amylases in particular, four highly homologous regions are conserved across a wide range of prokaryotic and eukaryotic species (MacKay et al., 1985; Nakajima et al., 1986; Svensson, 1988). Although the α-amylases and cycloextrin glycosyltransferase are specific for the α-1,4 linkages found in starch, similarity in the conserved regions is also shared by enzymes which attack α-1,6 linkages in glucose polymers such as pullulan (with alternating α-1,4 and α-1,6 linkages) and dextran. Despite their very different actions, there is a strikingly strong similarity between the *dexB* enzyme and the amylases in overall size, the location within the molecules of conserved regions, and the highly conserved amino acids. Accumulating information on the sequences of more enzymes will contribute to a mechanistic understanding of the basis of their specificity.

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References


