Metabolism of polysaccharides by the *Streptococcus mutans* *dexB* gene product

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The *Streptococcus mutans* *dexB* gene, a member of the multiple sugar metabolism (*msm*) operon, encodes an intracellular glucan 1,6- α -glucosidase which releases glucose from the non-reducing terminus of α -1,6-linked isomaltosaccharides and dextran. Comparison of primary amino acid sequences showed strong homology to *Bacillus* oligo-1,6-glucosidases and, like these enzymes, DexB was able to release free glucose from the α -1,4,6-branch point in panose. This suggested a role for DexB in the metabolism of either starch or intracellular polysaccharide, which contain such branch points. However, purified intracellular polysaccharide from the wild-type *S. mutans* strain LT11 and a mutant deficient in *dexB* revealed no substantial differences in the extent of branching as demonstrated by iodine staining spectra and the degree of polymerization. Furthermore, thin layer chromatography of radiolabelled intracellular polysaccharide digested with *S. mutans* wild-type and mutant cell extracts showed no differences in the products obtained. The involvement of DexB in dietary starch metabolism was investigated using α -limit dextrans produced from the action of α -amylase on starch. These induced the *msm* operon, including *dexB*, and the DexB enzyme was able to act on the α -limit dextrans to give further fermentable substrates. The transport system encoded by the *msm* operon can also transport α -limit dextrin. DexB may therefore be important in the metabolism of extracellular starch.

Introduction

The ability of oral micro-organisms such as *S. mutans* to utilize intracellular and extracellular polysaccharides has an important role in the formation of dental plaque and in the processes which may lead to dental caries.

S. mutans can utilize sucrose to produce extracellular glucans of which the main types are dextran (a predominantly α -1,6-linked polymer) and mutan (predominantly α -1,3-linked). These contribute to interbacterial aggregation and adhesion to the tooth surface and so may be determinants of cariogenicity (Hamada & Slade, 1980). *S. mutans* can also accumulate a glycogen-like intracellular storage polysaccharide (IPS) containing mainly α -1,4-linked glucose units (van Houte *et al.*, 1970). Stored IPS is believed to be of significance, in the absence of fermentable dietary carbohydrates, in production of acid which can lead to enamel demineralization and contributes to *S. mutans* cariogenicity *in vivo*

(Freedman & Coykendall, 1975; Tanzer *et al.*, 1976; Harris *et al.*, 1992).

Starch is a major constituent of the human diet and may influence the numbers and metabolic activity of plaque streptococci (Beighton & Hayday, 1984), possibly by altering bacterial adhesion, or it may be fermented to yield acidogenic substrates. Plaque exhibits starch-degrading activity which is probably due to a combination of bound salivary α -amylase (Douglas, 1983) and bacterial enzymes capable of hydrolysing starch. *S. mutans* shows some starch-degrading activity although the enzymes involved have not been characterized (Glor *et al.*, 1988).

S. mutans has been shown to produce an intracellular enzyme activity classified as dextran glucosidase (EC 3.2.1.70), encoded by the *dexB* gene (Russell & Ferretti, 1990). *dexB* is a member of the multiple sugar metabolism (*msm*) operon which contains four genes with homology to the periplasmic binding protein-dependent transport systems of Gram-negative bacteria (Russell *et al.*, 1992). The sugar-binding protein encoded by *msmE* is a lipoprotein, which presumably serves to anchor the binding protein at the cell surface (Sutcliffe *et al.*, 1993). The transport system has been shown to be

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Abbreviation: IPS, intracellular storage polysaccharide.

responsible for the uptake of melibiose, raffinose and isomaltosaccharides (Russell *et al.*, 1992; Tao *et al.*, 1993).

The precise function of DexB is unclear: it acts at the non-reducing terminus of α -1,6-linked chains such as dextran to release free glucose units (Dewar & Walker, 1975; Walker, 1978), although the preferred substrates are short isomaltosaccharides of three to five glucose units rather than macromolecular dextran (Russell & Ferretti, 1990). It has therefore been suggested that it may act in series with extracellular endodextranase, dextran first being cleaved outside the cell to form smaller isomaltosaccharides which can be transported across the cell membrane and then further cleaved by the intracellular dextran glucosidase to yield free glucose (Dewar & Walker, 1975; Russell & Ferretti, 1990). Such a scheme would allow *S. mutans* to utilize plaque dextrans (produced by itself or by other plaque bacteria) as a form of extracellular storage polymer (Parker & Creamer, 1971; Hotz *et al.*, 1972; Johnson, 1990). The inclusion within a single operon of genes concerned with transport and subsequent metabolism of isomaltosaccharides would be consistent with this two-stage utilization of dextran.

The *msm* operon also contains genes for α -galactosidase (*aga*) (Aduse-Opoku *et al.*, 1991*a, b*) and sucrose phosphorylase (*gtfA*), the latter enzyme generating glucose-1-phosphate from intracellular sucrose (Russell *et al.*, 1988). Since glucose-1-phosphate is a precursor in IPS synthesis (Birkhed & Tanzer, 1979), an alternative function for *dexB* may be in IPS metabolism and this would provide an alternative explanation for these enzymes being located in the same operon. That the deduced amino acid sequence of the *dexB* enzyme has regions of homology to the conserved regions found in amylase and other starch-degrading enzymes (Russell & Ferretti, 1990) provides further support for a role of DexB in IPS metabolism.

The work described here was undertaken to clarify the possible functions of the *dexB* gene product in the utilization of extracellular dextran or starch and in the synthesis and breakdown of IPS.

Methods

Bacterial strains. *S. mutans* strains were grown in Brain Heart Infusion broth (Oxoid), Todd Hewitt broth (Oxoid) or the semi-defined medium of Terleckyj *et al.* (1975) modified by replacement of individual amino acids with 0.5% (w/v) casein hydrolysate (Russell, 1979). Carbohydrates, at 0.5% (w/v) unless otherwise stated, and erythromycin (10 μ g ml⁻¹) were added as required. Mutants 517 and 522 were constructed previously by cloning of internal *Bgl*II–*Hind*III fragments of the *msmE* and the *dexB* genes, respectively, into the erythromycin resistance plasmid pVA891 and transformation of the resultant constructs into *S. mutans* strain LT11. Homologous recombination of

the entire plasmid into the chromosome resulted in insertional inactivation of *msmE* and *dexB* (Russell *et al.*, 1992).

Construction of recombinant plasmid pSF104, carrying the *dexB* gene, has also been described previously (Russell & Ferretti, 1990). *Escherichia coli* strain JM109(pSF104) was grown in LB broth (Maniatis *et al.*, 1982) with 100 μ g ampicillin ml⁻¹ and 1 mM-IPTG.

Sequence analysis. DNA and protein sequences were manipulated using IBI-Pustell and PC-Genie software packages and the databases searched with the FASTA program of Wilbur & Lipman (1982). Multiple alignments of amino acid sequences were performed using the CLUSTAL program of Higgins & Sharp (1988).

Fermentation assays. *S. mutans* strain LT11 and the mutant 522 were tested for their ability to ferment a variety of sugars. These were obtained from Sigma except for gentiobiose (Cambridge Research Laboratories) and dextran T10 (Pharmacia) and were of 95–98% purity. Sugar solutions were sterilized by autoclaving (115 °C, 10 min). Cells from a 5 ml sample of an overnight culture of each strain, grown in Todd Hewitt broth with erythromycin where required for the mutant, were harvested by centrifugation, washed twice with sterile saline and then inoculated into Phenol Red Broth (Merck; 15 g l⁻¹) with appropriate carbohydrates at 1% (w/v) and incubated anaerobically at 37 °C for 24 h. A positive fermentation result was recorded if the pH indicator changed to a distinct yellow colour. To determine whether fermentation of dextran T10, wheat starch or amylopectin resulted in acid production, Todd Hewitt broth-grown overnight cultures of *S. mutans* LT11 and mutant 522 were inoculated into 200 μ l Todd Hewitt broth in a microtitre well to an OD₆₂₀ of 0.2 and substrate was added to 0.5% (w/v). This was incubated anaerobically overnight at 37 °C. The pH of the resulting suspension was measured using a micro-electrode.

Hydrolysis of dextran and starch. The ability of *S. mutans* LT11 and mutant 522 to hydrolyse high molecular mass dextran was determined by culturing on blue dextran agar plates (Donkersloot & Harr, 1979). Following incubation, a clear zone around the area of growth indicated dextranase activity. The ability of *S. mutans* LT11 and mutant 522 to degrade starch was determined by culturing on starch agar plates (Glor *et al.*, 1988).

Preparation of cell extracts. Cell-free extracts were prepared by sonication of cells of recombinant *E. coli* JM109(pSF104) followed by centrifugation (10000 g, 10 min) to remove cell debris. Extracts of *S. mutans* harvested from 1 litre cultures of *S. mutans* LT11 and mutant 522 were prepared by homogenization with a Braun MSE homogenizer with 0.17 mm Ballotini beads (Braun). Crude cell extracts were recovered by filtration under vacuum (Whatman GF/D filters) and centrifuged at 50000 g to remove cell debris.

Analysis of DexB activity. To determine substrate specificity of the DexB enzyme, cell-free extracts of recombinant *E. coli* JM109(pSF104) with dextran glucosidase activity were incubated for periods varying from 0.5 to 24 h with the substrates. α -Glucosidase activity was determined using the procedure of Walker & Pulkownik (1973), modified for microtitre plates (Russell & Ferretti, 1990). Enzyme preparations were added to substrates dissolved in 0.1 M-sodium 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 using a Flow Titertek plate reader.

Production of α -limit dextrans. Human salivary α -amylase Type IX-A (100 U) (Sigma) was used to digest 250 mg amylopectin (48 h, 37 °C). α -Limit dextrans were separated from glucose and maltose by gel filtration on a Sephadex G-25 column (60 $\times$ 1.5 cm) and identified by thin layer chromatography (TLC) as described below.

Production of radiolabelled α -limit dextrans. Radiolabelled α -limit dextrans were produced by the digestion of 17 μ Ci [U-¹⁴C]starch

(NEN) with 15 U human salivary amylase Type IX-A (sigma). The major product of this digestion was demonstrated by TLC and autoradiography to be maltose, as expected. The principal α -limit dextrin fraction was recovered by preparative TLC.

Radiolabelled α -limit dextrans were incubated (24 h, 37 °C) with crude cell extracts prepared from *S. mutans* LT11 and mutant 522 grown on raffinose. Products of the digestions were analysed by TLC.

TLC. TLC was used to analyse products from the action of DexB enzyme on commercially obtained substrates, on purified [14 C]glucose-labelled IPS and on α -limit dextrans prepared from labelled starch and amylopectin. Products from the action of α -amylase on labelled IPS, labelled starch and amylopectin were also analysed by TLC. Digestion products were separated on Silica gel 60 TLC (Merck) plates by two ascents in 5:2:4:4 (by vol.) isopropanol–nitromethane–acetonitrile–water solvent, as described by Binder *et al.* (1983) or by three ascents in 2:3:5 (by vol.) nitromethane–water–propanol (Robyt & White, 1987). The sugars were detected by spraying with 20% (v/v) H_2SO_4 in methanol and heating to 120 °C for 10 min and by autoradiography for the products obtained from the digestion of radiolabelled substrates by DexB and α -amylase.

Determination of IPS content. IPS of stationary phase cultures of LT11 and mutant 522, grown in Todd Hewitt broth with 5% (w/v) glucose, was determined by alkaline hydrolysis followed by the iodine complex method of DiPersio *et al.* (1974). Samples of whole cells were collected by centrifugation and washed twice in saline. The cells were then resuspended in 0.5 ml 5.3 M-KOH to a final OD_{620} of 1.0 and boiled for 90 min. After cooling, 0.5 ml 5.3 M-HCl, 1.0 ml 1 M- K_2HPO_4 and 0.5 ml 0.2% (w/v) iodine in 2% (w/v) KI solution were added and the absorbance of the resulting polysaccharide–iodine complex (E_{PS-I}) was determined by measurement of OD_{540} (van Houte & Jansen, 1968).

IPS isolation and purification. *S. mutans* LT11 and mutant 522 were grown in 1 litre semi-defined medium with 2% (w/v) glucose for 24 h. IPS was extracted and purified by the method of alkaline extraction of DiPersio *et al.* (1974). Cells were harvested and washed twice with cold (4 °C) distilled H_2O . A solution of 30% (w/v) KOH was added to make a final volume of 40 ml and the suspension was boiled for 90 min. Extracts were centrifuged (14000 g, 20 min) and the pellet was discarded. IPS was purified by three precipitations with 95% ethanol (v/v) and then lyophilized. Purity of IPS was estimated from the amount of glucose liberated after acid hydrolysis (1 M- H_2SO_4) for 2 h at 100 °C, measured by glucose oxidase, as a percentage of the total carbohydrate, measured by the phenol–sulphuric method of Dubois *et al.* (1956) modified for microtitre plates by Fox & Robyt (1991). For the preparation of radiolabelled IPS, *S. mutans* LT11 was grown in the above medium containing 1% (w/v) glucose (Freedman & Coykendall, 1975) and 50 μ Ci [14 C]glucose (Amersham). Cells were then solubilized and the labelled IPS was ethanol-precipitated three times as described above.

Degree of polymerisation of IPS. The degree of polymerisation (d.p.) was calculated as

$$d.p. = \frac{\text{total carbohydrate } (\mu\text{g})}{\text{reducing sugar (as } \mu\text{g maltose)}} \times 2$$

(Jane & Robyt, 1984). Reducing value was determined using copper bicinchoninate (Fox & Robyt, 1991). Maltose was used as the standard in both analyses.

Spectral analysis of IPS. The visible spectrum of a solution of 0.01% (w/v) purified IPS in 0.02% I_2 in 0.2% KI (prepared fresh) was determined against an appropriate iodine/iodide blank (Archibald *et al.*, 1961) using a Phillips PU8630 spectrometer. The degree of cross-linking was estimated as described by Archibald *et al.* (1961).

Induction of *dexB*. DexB activity can be measured by the hydrolysis of *p*-nitrophenyl α -D-glucopyranoside (Russell & Ferretti, 1990). *S.*

mutans LT11 and mutant 522 were grown overnight in semi-defined medium with selected carbohydrates as sole carbon source. Cells were harvested by centrifugation and resuspended in 100 mM-sodium phosphate buffer, pH 6.0, to a standard OD_{620} . *p*-Nitrophenyl α -D-glucopyranoside was added (10 mM final concentration) to cell suspensions in a microtitre plate and the cells incubated at 37 °C for 30 min. Substrate hydrolysis by DexB was measured as the increase in A_{405} after correction for the OD_{620} of the assay suspension.

Sugar transport assay. The transport of [14 C]-labelled α -limit dextrans prepared from radiolabelled starch was assayed as described previously (Russell *et al.*, 1992). Briefly, *S. mutans* was grown on semi-defined medium with either glucose or raffinose as sole carbon source. Raffinose is an effective inducer of the *msm* operon (Russell *et al.*, 1992). The harvested cells were washed and resuspended in carbohydrate-free medium and radiolabelled α -limit dextrin (10^5 d.p.m. per assay) added. Cell aliquots were removed during incubation at 37 °C, recovered by filtration and washed extensively with PBS (about 25 ml). The radioactivity retained was determined by liquid scintillation counting.

Results

Homology of *DexB* with oligo-1,6-glucosidase

Comparison of the primary amino acid sequence of the *dexB* gene product with sequences available in the databases showed the greatest similarity to be to oligo-1,6-glucosidases from several species of *Bacillus*. When compared with the enzyme from *Bacillus cereus* ATCC 7064 (Watanabe *et al.*, 1990), 53% of the residues were found to be identical and 11% functionally similar, the two proteins aligning throughout their entire length. A slightly lower similarity was found with two other *Bacillus* enzymes from *Bacillus thermoglucosidasius* KP1006 (Watanabe *et al.*, 1991) and an alkalophilic *Bacillus* sp. designated strain F5 (Yamamoto & Horikoshi, 1990), though all three *Bacillus* enzymes were more closely related to each other than to the *S. mutans* enzyme. The sequence from *Bacillus* sp. strain F5 appears truncated in comparison to the others. A multiple alignment of the sequences (Fig. 1) shows the existence of several regions which are particularly well-conserved, including those regions identified previously as being conserved in all of the 'amylase' family (Russell & Ferretti, 1990).

Characterization of activity encoded by *dexB*

Mutant 522, deficient in DexB, was compared to the wild-type LT11 for its ability to ferment a variety of sugars. The results shown in Table 1 demonstrate that LT11 is able to produce acid from a variety of sugars with an α -1,6 linkage which the mutant is unable to ferment, thus confirming the previous report by Russell & Ferretti (1990) that the DexB enzyme cleaves α -1,6 linkages. Both *S. mutans* strains LT11 and the mutant 522 demonstrated starch hydrolysis after growth on starch agar plates and hydrolysis of dextran following growth on blue dextran agar plates. *S. mutans* LT11 was

Table 1. Carbohydrates fermented by *S. mutans* LT11 and mutant 522 deficient in *DexB*

Overnight cultures of *S. mutans* were inoculated into Phenol Red Broth with carbohydrates at 1% (w/v) and incubated anaerobically for 24 h at 37 °C. +, pH indicator changed to yellow colour; ±, pH indicator changed to orange colour and acid production was confirmed by pH measurement; –, no colour change or drop in pH.

Substrate	Structure	Fermentation	
		LT11	522
Cellobiose	4- <i>O</i> -β-D-glucopyranosyl-D-glucopyranose	+	+
Gentiobiose	6- <i>O</i> -β-D-glucopyranosyl-D-glucopyranose	+	+
Maltose	4- <i>O</i> -β-D-glucopyranosyl-D-glucopyranose	+	+
Trehalose	1- <i>O</i> -α-D-glucopyranosyl-D-glucopyranose	+	+
Dextran T10	6- <i>O</i> -α-D-glucan	±	–
Isomaltose	6- <i>O</i> -α-D-glucopyranosyl-D-glucopyranose	+	–
Palatinose	6- <i>O</i> -α-D-glucopyranosyl-D-fructofuranose	+	–
Panose	α-D-glucosyl-(1 → 6)α-D-glucosyl-(1 → 4)-D-glucose	+	–
Melezitose	α-D-glucosyl-(1 → 3)-β-D-fructosyl-(2 → 1)α-D-glucoside	–	–
Turanose	3- <i>O</i> -α-glucopyranosyl-β-D-fructose	–	–

to glucose. Following growth on raffinose, the *dexB* mutant 522 had negligible substrate hydrolytic activity compared to *S. mutans* LT11.

Rate of glucose release from selected substrates by *DexB*

Isomaltose, isomaltotriose and panose were incubated with *DexB* enzyme over 30 min and rate of glucose release was assayed by glucose oxidase. Panose and isomaltotriose were hydrolysed to a similar extent, whilst the rate of release of glucose from isomaltose was reduced (Fig. 2).

Role of *DexB* in IPS metabolism

Quantification of the IPS produced by bacteria grown in excess glucose showed that the mutant 522 produced only 70% of the iodine-positive material produced by LT11. Iodine values ($E_{\text{PS-1}}$) were obtained of 0.28 ± 0.03 and 0.20 ± 0.02 ($n = 4$; $P > 0.01$) respectively. However, qualitative differences between the spectral characteristics of IPS purified from LT11 and 522 could not be detected (data not shown). Both spectra had the characteristics expected of bacterial glycogens (Archibald *et al.*, 1961; van Houte & Jansen, 1968) with absorbance maxima of 490–510 nm with a similar degree of cross-

Fig. 2. The rate of release of glucose, expressed as mmol-glucose released per mol substrate, from isomaltose (Δ), isomaltotriose (□) and panose (▽) by *DexB* from a recombinant *E. coli* strain expressing the *S. mutans* *dexB* gene. No activity was detectable against the substrates in extracts of non-recombinant *E. coli*. Results shown are from a representative experiment.

Fig. 3. Transport of radiolabelled α-limit dextrins by *S. mutans* strain LT11 grown in the presence of glucose (▽), LT11 grown on raffinose (Δ) and mutant 517 grown on glucose (□). Results shown are from a representative experiment.

linking calculable for each (18.8 for IPS from LT11 and 17.1 for that from 522). The degree of polymerization for LT11 IPS was 74 compared to 79 for IPS from mutant 522.

Products from the digestion of radiolabelled IPS with cell extracts from *S. mutans* LT11 and 522, and from recombinant *E. coli* expressing *dexB*, were analysed by TLC followed by autoradiography. In each case maltodextrins from four glucose units down to free glucose could be detected after 4–24 h digestion, independent of the presence or absence of *DexB* expression (data not shown).

Fig. 4. TLC showing action of *S. mutans* DexB on purified radiolabelled α -limit dextrans. Lanes: 1, α -limit dextrin; 2, α -limit dextrin following incubation with crude cell extract from LT11; 3, α -limit dextrin with mutant 522 extract. Standards are marked as follows: Glc, glucose; M2, maltose; M3–M6, maltodextrins with increasing numbers of glucose units; Pan, panose; IM3, isomaltotriose; IM4, isomaltotetraose.

Role of DexB in α -limit dextrin metabolism

As shown in Fig. 3, *S. mutans* LT11 clearly accumulated

Fig. 5. Induction of *S. mutans* dexB following growth of LT11 on chemically defined medium in the presence of selected carbohydrates: glucose ($\triangle$), maltose ($\blacktriangle$), raffinose ($\blacksquare$) and α -limit dextrin ($\square$). DexB activity was determined by monitoring the increase in absorbance resulting from hydrolysis of *p*-nitrophenyl- α -D-glucopyranoside. Results shown are from a representative experiment.

radiolabelled α -limit dextrin and this was increased following growth on raffinose, an inducer of the *msm* transport system. However, mutant 517, in which *msm* transport is non-functional due to insertional inactivation of *msmE*, failed to transport α -limit dextrin. Incubation of the radiolabelled α -limit dextrin preparation with a crude cell extract from *S. mutans* LT11 resulted in the release of radiolabelled maltodextrin material (Fig. 4, lane 2). This activity was not apparent after incubation with a comparable extract of mutant 522 (Fig. 4, lane 3).

S. mutans LT11 grew on α -limit dextrans at a rate similar to that observed on monosaccharides. The DexB activity following growth of LT11 on α -limit dextrans is shown in Fig. 5. Purified α -limit dextrans were clearly demonstrated to induce the production of DexB.

Discussion

The data presented here show that the DexB enzyme described previously as dextran glucosidase (EC 3.2.1.70) has a pattern of activity closely similar to other enzymes classified as oligo-1,6-glucosidases (EC 3.2.1.10) in having the ability to cleave α -1,6 linkages in a variety of contexts, and not solely in linear α -1,6-linked chains. The high degree of similarity between the DexB enzyme and *Bacillus* spp. oligo-1,6-glucosidases is striking and in marked contrast to the limited similarity found previously, in restricted regions of the sequence, to other starch-degrading enzymes (Russell & Ferretti, 1990). The similarity of the amino acid sequences is reflected in the predicted physical properties of the enzymes including hydrophobicity profiles and predicted location of α -helices and β -turns

(data not shown). Structure prediction and hydrophobic cluster analysis has also shown the *S. mutans* and *Bacillus* spp. enzymes to have similar domain structures, with a catalytic (β/α)₈-barrel and a smaller C-terminal domain (Jespersen *et al.*, 1991). The physiological function of the *Bacillus* enzymes does not appear to have been investigated, though there is interest in their commercial application in starch digestion.

The action pattern of DexB suggests a number of possible functions that it could perform in cellular physiology. The first may be concerned with the utilization of extracellular dextran. This would involve a two-step process in which dextran is first degraded by extracellular dextranase to isomaltosaccharides, and these would then be further cleaved by intracellular DexB to give free glucose. Our results are consistent with such a role: the *msm* system transports isomaltotriose and isomaltotetraose across the cell membrane (Russell *et al.*, 1992; Tao *et al.*, 1993) and levels of DexB are elevated markedly by growth in isomaltose or isomaltotriose. However, despite the presence of a suitable transport system and an active enzyme (DexB) inside the cell for their utilization, growth on isomaltosaccharides is poor and we have been unable to demonstrate adequate growth on longer chain carbohydrates such as dextran T10, which is approximately 25 glucose units long. The explanation for this remains unknown but it should be noted that growth on semi-defined medium with melibiose (another *msm* substrate) is also poor. Growth may be limited by accumulation of free glucose or phosphorylated intermediates and experiments are in progress to define the metabolic fate of glucose derived from *msm* substrates. However, it is possible that conditions *in vitro* do not appropriately mimic the environment experienced by the organism during growth *in vivo* and that not all metabolic activities are expressed in the laboratory. A comparable situation occurs with utilization of extracellular starch, where *S. mutans* possesses enzymes for its degradation yet cannot be shown to produce acid from it.

The demonstration that the DexB enzyme is able to cleave the α -1,6 linkage in branched oligosaccharides such as panose to yield a maltosaccharide and free glucose suggests the possibility that DexB may remove α -1,6-linked glucose residues from predominantly α -1,4-linked polysaccharides. Growth on panose induces DexB and the rate of hydrolysis of panose is equivalent to that of isomaltotriose and greater than that of isomaltose (Fig. 2). A comparable enzyme in *S. mitis* (α -glucosidase; dextran glucosidase) has been demonstrated to act on molecules with a glucose joined through an α -1,6 bond to either a maltosaccharide or an isomaltosaccharide, and acts more readily on panose than on isomaltose (Walker & Builder, 1967). A second role for DexB in IPS

metabolism could therefore be proposed which would involve either 'trimming' of nascent α -1,6 branch points in IPS during its synthesis as a mechanism of modulating the extent of branching, or alternatively the enzyme may act during breakdown of IPS, either to cleave branch points or to release terminal glucose from α -limit dextrans. If DexB is involved in either of the IPS processes, then it would be expected to be induced during the appropriate stage in IPS metabolism. However, DexB levels do not increase during growth in excess glucose when IPS would be expected to accumulate. We have also been unable to detect any difference between IPS made by wild-type and the *dexB* mutant with regard to iodine-staining or degree of polymerization, despite the fact that the *dexB* mutant makes slightly less IPS than the wild-type. We are therefore unable to support a role for the DexB enzyme in the modification of nascent IPS.

Both mutant and wild-type cell extracts produce glucose and maltosaccharides of two to four glucose units from purified IPS and therefore it is unlikely that DexB has a role in utilization of the polysaccharide. It is not yet known what enzymes are involved in this degradation since we were unable to detect any α -amylase activity in extracts of *S. mutans*. Nevertheless, if branched oligosaccharides are a product of IPS breakdown in *S. mutans*, then DexB could have a role in facilitating the total degradation of IPS to glucose by removing α -1,6 glucose 'stubs'.

The third possible function suggested for the DexB enzyme is in the metabolism of α -limit dextrin products from the degradation of extracellular starch by human salivary α -amylase or plaque-derived amylase. Those produced from human salivary α -amylase acting on amylopectin have been determined to be of four to six glucose units with a maltotriose base chain and a side chain of one to three glucose units joined by α -1,6 linkage to the non-reducing terminus (Walker & Whelan, 1960). Our results show that the *msm* transport system is able to transport α -limit dextrans into the cell and the DexB enzyme is able to degrade these dextrans within the cell. Moreover, growth of *S. mutans* LT11 on purified α -limit dextrans induces *dexB*. The *msm* operon therefore contains genes for both the transport and subsequent breakdown of products from the hydrolysis of extracellular starch. The ability to degrade these carbohydrates could be an advantage for *S. mutans* when the supply of simple exogenous sugars is scarce.

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