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 λ L47.1, 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 *Hind*III fragments were isolated. The 2.3 kb and 1.4 kb fragments were unidirectionally digested with *Bal*31 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.

Fig. 1. Partial restriction map of the cloned segment of *S. mutans* Ingbritt 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 *Hind*III site was also confirmed using an *Eco*RI subclone. The sequence information was analysed by the James M. Pustell DNA/protein sequencing program (International Biotechnologies Inc.) and the Staden-Plus programs (Amersham International). Comparison with protein sequences held in the Protein Information Resource of the National Biological Research Foundation database was done using the FASTP program of Wilbur & Lipman (1982).

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 *Rsa*I fragment. Such a fragment was therefore isolated from the original recombinant phage and cloned into the *Hinc*II 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_r 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 microtitration 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 A_{450} was read in a Flow Titretrek 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 *Hind*III and *Eco*RI 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_r 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_r product detectable by either paper or thin-layer chromatography (data not shown), the rate at which it releases glucose from dextran

Fig. 2. (on facing page; continued overleaf). Nucleotide sequence of the *dexB* gene of *S. mutans* Ingbritt and the deduced amino acid sequence of its product. The sequence shown begins with the base immediately following the termination codon of the upstream ORF. Nucleotides are numbered underneath the sequence and amino acid residues on the right. The putative ribosome-binding site is boxed and an inverted repeat region indicated by arrows.

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AAAACGGCTTAAATTGTTTTAGATTTTCTCAGTCTCTTTATTAGAACTAAAGAACTAAC
  10      20      30      40      50      60

ATGTCATAATAAATTAAGGTGGTGATAAGATGCAAAAACATTGGTGGCACAAGGCAACT 10
  70      80      90     100     110     120

V Y Q I Y P K S F M D T N G D G I G D L 30
GTTTATCAAATTTATCCAAAATCTTTATGGATACAAATGGTGATGGAATTGGTGATCTC
 130     140     150     160     170     180

K G I T S K L D Y L Q K L G V M A I W L 50
AAAGGTATTACGAGTAAATTGGATTATTTGCAAAAGTTAGGGGTTATGGCTATTTGGCTA
 190     200     210     220     230     240

S P V Y D S P M D D N G Y D I A N Y E A 70
TCTCCAGTTTATGATAGCCCATGGATGACAATGGCTATGACATTGCGAATCATGAAGCA
 250     260     270     280     290     300

I A D I F G N M A D M D N L L T Q A K M 90
ATTGCGGATATTTTGGCAATATGGCTGATATGGATAATTGCTGACGCAGGCAAAAATG
 310     320     330     340     350     360

R D I K I I M D L V V N H T S D E H T W 110
CGCGACATAAAAAATCATTATGGATCTAGTGGTTAATCATACTCAGATGAACATACTTGG
 370     380     390     400     410     420

F I E A R E H P D S S E R D Y Y I W C D 130
TTTATTGAAGCACGTGAGCATCCAGACAGTTCTGAACGCGATTATTATATTTGGTGTGAC
 430     440     450     460     470     480

Q P N D L E S I F G G S A W Q Y D D K S 150
CAGCCAAATGATTTGGAATCTATTTTCGGTGGTCTGCTTGGCAGTATGATGATAAGTCC
 490     500     510     520     530     540

D Q Y Y L H F F S K K Q P D L N W E N A 170
GATCAATATTTTGCATTTTTTAGTAAGAAGCAGCCAGATCTAAACTGGGAAAACGCA
 550     560     570     580     590     600

N L R Q K I Y D M M N F W I D K G I G G 190
AACTTACGTCAGAAGATTTATGATATGATGAATTTCTGGATTGATAAAGGTATTGGCGGC
 610     620     630     640     650     660

F R M D V I D M I G K I P A Q H I V S N 210
TTTCGGATGGACGTCATTGATATGATTGGGAAAATTCCTGCTCAGCATATTGTCAGTAAC
 670     680     690     700     710     720

G P K L H A Y L K E M N A A S F G Q H D 230
GGACCAAAATTCATGCTTATCTTAAGGAGATGAATGCCGCTAGTTTTGGTCAACATGAT
 730     740     750     760     770     780

L L T V G E T W G A T P E I A K Q Y S N 250
CTGCTGACTGTGGGGGAACTTGGGGAGCAACGCCTGAGATTGCGAAGCAATATTCAAAT
 790     800     810     820     830     840

P V N H E L S M I F Q F E H I G L Q H K 270
CCAGTCAATCACGAACTCTCTATGATTTTTCAATTTGAACATATTGGTCTTCAGCATAAA
 850     860     870     880     890     900

P E A P K W D Y V K E L N V P A L K T I 290
CCAGAAGCTCCTAAATGGGATTATGTGAAGGAACCTAATGTTCTGCTTTAAAAACAATC
 910     920     930     940     950     960

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F N K W Q T E L E L G Q G W N S L F W N 310
TTTAATAAATGGCAGACTGAGTTGGAATTAGGACAGGGGTGGAATTCGTTATTTCTGGAAT
970          980          990          1000          1010          1020

N H D L P R V L S I W G N T G K Y R E K 330
AACCATGACCTGCCTCGTGTGTTTTATCAATCTGGGGAAATACGGGCAAATATCGTGAGAAG
1030          1040          1050          1060          1070          1080

S A K A L A I L L H L M R G T P Y I Y Q 350
TCTGCTAAAGCACTGGCTATTCTTCTTCACCTTATGCGTGGGACACCTTATATTTATCAA
1090          1100          1110          1120          1130          1140

G E E I G M T N Y P F K D L N E L D D I 370
GGTGAAGAGATTGGGATGACCAATTATCCTTTTAAAGATTTAAATGAACTTGATGATATT
1150          1160          1170          1180          1190          1200

E S L N Y A K E A F T N G K S M E T I M 390
GAATCACTTAATTATGCTAAGGAAGCTTTTACAAATGGTAAGTCTATGGAAACTATCATG
1210          1220          1230          1240          1250          1260

D S I R M I G R D N A R T P M Q W D A S 410
GACAGTATTCGATGATTGGCCGTGATAATGCCAGAACACCTATGCAATGGGATGCTTCT
1270          1280          1290          1300          1310          1320

Q N A G F S T A D K T W L P V N P N Y K 430
CAAAATGCCGGATTTTCAACAGCGGATAAAACATGGCTGCCAGTTAATCCAAACTATAAA
1330          1340          1350          1360          1370          1380

D I N V Q A A L K N S N S I F Y T Y Q Q 450
GACATCAATGTTCAAGCAGCTCTGAAAAATTCCAATTCTATCTTTTACACCTATCAACAA
1390          1400          1410          1420          1430          1440

L I Q L R K E N D W L V D A D F E L L P 470
CTCATTGAGCTTCGAAAAGAAAATGATTGGCTAGTAGATGCCGATTTTGAATTGCTCCCT
1450          1460          1470          1480          1490          1500

T A D K V F A Y L R K V R E E R Y L I V 490
ACAGCGGACAAAGTATTTGCCTATTTACGAAAGGTAAGAGAAGAAAGGTATCTTATAGTG
1510          1520          1530          1540          1550          1560

V N V S D Q E E V L E I D V D K Q E T L 510
GTCAATGTTTCAGATCAGGAAGAAGTTCTAGAGATTGATGTTGACAAACAAGAACTCTC
1570          1580          1590          1600          1610          1620

I S N T N E S A A L A N H K L Q P W D A 530
ATTAGCAATACAAATGAAAGCGCTGCTCTTGCCAATCACAACTCCAGCCTTGGGATGCT
1630          1640          1650          1660          1670          1680

F C I K I N *
TTTTGTATTAAGATAAACTAGGATAGAATAAGAAAAACAACAGACGATACCACTTAAAAAG
1690          1700          1710          1720          1730          1740

TGACGGCCTGTTGTTTTTTTAAATGTTTGTATTAAGTAATAAAGCGATTCTGATTGACTAT
1750          1760          1770          1780          1790          1800

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Fig. 3. SDS-PAGE of M_r 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.

Fig. 4. The rates of release of glucose by DexB enzyme from isomaltose (IM), isomaltotriose (IM3), isomaltotetraose (IM4), isomaltopentaose (IM5) and dextran of M_r 10000 expressed relative to that obtained with IM3 as substrate. The source of the enzyme was a cell extract of recombinant *E. coli* expressing the *dexB* gene. No activity against any of the substrates was detectable in extracts of non-recombinant *E. coli*.

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

Fig. 5. SDS-PAGE of *S. mutans* cells on a gel containing Blue Dextran. The gel was washed and incubated to reveal dextranase activity before staining. Left-hand lane contains M_r standards.

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_r 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_r 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_r values being 120000 and 105000.

DEXB	98	DLVVNH	190	GFRMDVIDM	308	FWNNHD
AMY	98	*DVVLNH	227	***GFRIDAAKH	323	*FVEN***HHD
AMY consensus		*DAVINH		***GFRIDAAKH		*FVDN***HHD
IAM	291	*DVVYNH	370	***GFRFDLASV	502	*FIDV***HHD
PUL	600	*DVVYNH	671	***GFRFDLMGY	827	*YVSK***HHD
NPL	242	*DAVFNH	324	*GWRLDVANE	419	LLGSHD
CGT					358	*FMDN***HHD
DEXB	443	SIFYTYQQ-LIQLRK				
IAM	639	*SNFYTFQAQRLIAFRK				

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

During storage of samples, there was a gradual breakdown of the proteins to give an active band of dextranase with an apparent M_r of 70000. No activity against Blue Dextran was found in recombinant *E. coli* expressing the activity encoded by *dexB*.

Comparison with other glucosidases

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).

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

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 dextrans by removing terminal glucose residues along α -1,6-linked stretches until stopped by a branch point (Walker & Pulkownik, 1974). Although it has been suggested that such activity may be of physiological importance in generating free glucose from dextrans 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- M_r 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 dextranases 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 with our data 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

transglucanoylases 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 cyclodextrin 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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