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Analysis of a dextran-binding domain of the dextranase of *Streptococcus mutans*

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Aims: To examine the dextran-binding domain of the dextranase (Dex) of *Streptococcus mutans*.

Methods and Results: Deletion mutants of the Dex gene of *Strep. mutans* were prepared by polymerase chain reaction and expressed in *Escherichia coli* cells. Binding of the truncated Dexs to dextran was measured with a Sephadex G-150 gel. Although the Dexs which lacked the N-terminal variable region lost enzyme activity, they still retained dextran-binding ability. In addition, further deletion into the conserved region from the N-terminal did not influence the dextran-binding ability. However, the Dex which carried a deletion in the C-terminus still possessed both enzyme activity and dextran-binding ability. Further deletion into the conserved region from the C-terminal resulted in complete disappearance of both enzyme and dextran-binding activities.

Conclusions: Deletion analysis of the Dex gene of *Strep. mutans* showed that the C-terminal side (about 120 amino acid residues) of the conserved region of the Dex was essential for dextran-binding ability.

Significance and Impact of the Study: The dextran-binding domain was present in a different area from the catalytic site in the conserved region of the Dex molecule. The amino acid sequence of the dextran-binding domain of the Dex differed from those of glucan-binding regions of other glucan-binding proteins reported.

INTRODUCTION

Streptococcus mutans and *Strep. sobrinus* are frequently isolated from human dental plaque and have been implicated as primary causes of dental caries (Loesche 1986; Hamada and Slade 1980). In particular, the ability of extracellular glucan synthesis from dietary sucrose by these bacteria has been shown to contribute significantly to plaque formation and to the subsequent development of dental caries. In addition, *Strep. mutans* and *Strep. sobrinus* are predominant producers of the enzyme dextranase (Dex) which cleaves α-1,6-linkages of glucans (Walker *et al.* 1981; Igarashi *et al.* 1992; Wanda and Curtis 1994). The Dex is thought to be responsible both for the control of the amount and content of extracellular glucans and for the metabolic utilization of extracellular glucans (Schachtele *et al.* 1975; Germaine *et al.* 1977; Walker *et al.* 1981; Felgenhauer and Trautner 1983; Colby *et al.* 1995). From these results, the Dex has been thought to be an important determinant of virulence.

In previous genetic analyses, the authors have shown that the Dex molecule consists of two variable regions (VRs) and a conserved region (CR) and that the CR is related to the enzyme activity (Igarashi *et al.* 1995; Igarashi *et al.* 2002). Further analysis, utilizing site-directed mutagenesis, identified an Asp residue (Asp-385) essential for the Dex activity of *Strep. mutans* (Igarashi *et al.* 2002). This residue exists in a domain of the CR which is highly homologous to the active site domains of streptococcal glucosyltransferases (Gtfs). Although Dex activity was abolished in the mutant with the Asp-385 residue substitution, this mutant could still effectively bind to glucan (Igarashi *et al.* 2002). This suggested the existence of two functional domains, catalytic and glucan-binding sites, in the CR of the Dex molecule.

In this study, the glucan-binding domain of the Dex of *Strep. mutans* was examined using deletion analysis.

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MATERIALS AND METHODS

Bacterial strains

Streptococcus mutans Ingbritt was grown in Todd Hewitt broth (Igarashi *et al*. 1995). *Escherichia coli* JM109 was routinely used as a plasmid host and grown in Luria–Bertani broth supplemented with ampicillin (50 μg ml^{-1}).

DNA manipulations

Genomic DNA of *Strep. mutans* Ingbritt was purified by ultracentrifugation in a CsCl-ethidium bromide density gradient as described previously (Igarashi *et al*. 1995). Plasmid DNA was extracted by a Wizard miniprep purification kit (Promega, Madison, WI, USA) as described previously (Igarashi *et al*. 1995).

Preparation of deletion mutants

Deletion of the *Strep. mutans dex* was carried out by the long and accurate polymerase chain reaction (PCR) with LA *Taq* DNA polymerase (Takara Shuzo, Kyoto, Japan) as described previously (Igarashi *et al*. 2001). Primer sets used in this study are listed in Table 1. The genomic DNA of *Strep. mutans* Ingbritt was utilized as a PCR template. Shuttle PCR was used as a condition of amplification as recommended by the supplier (Takara Shuzo). Briefly, the reaction mixture (50 μl) was denatured at 94 °C for 1 min followed by 28 cycles of 20 s at 98 °C for denaturation and 2 min at 68 °C for annealing and extension. The amplified DNA fragments were subcloned into a pT7Blue T-vector (Novagen, Madison, WI, USA) and the truncated Dexs expressed in *E. coli* JM109.

Table 1 Primer sets used in this study

Dex mutants	Primers (nos)
Wild type	1 and 2
mut-1	1 and 3
mut-2	1 and 4
mut-3	3 and 5
mut-4	3 and 6
mut-5	3 and 7

1, 5′–CCCGGGATGGAACAGTCAAATAGGCAAACGGC–3′.
2, 5′–CTCGAGTATGAATGGCTATCAGTCGGACACCT–3′.
3, 5′–ATATCTCGAGTCACCTAGCAGGATGTTGGGGC–3′.
4, 5′–CTCGAGTCAAGCATCTTGCGATAGCTGCCG–3′.
5, 5′–AATTCCCGGGGCAGTCTATCAGGCGGGAGA–3′.
6, 5′–CCCGGGTTAGAGCAGGGCAATCGGGC–3′.
7, 5′–AATTCCCGGGTGGTGGAGCCATTCGCAGGT–3′.
Dex, Dextranase.

Detection of truncated dextranase enzymes

Crude enzymes of the truncated Dexs were prepared as follows. Recombinant *E. coli* cells were sonicated in phosphate-buffered saline containing 1% Triton-X100 and centrifuged. The supernatant fluid was collected and utilized as crude enzymes of the Dex and truncated Dexs. Dextranase activity was detected by sodium dodecyl sulphate-polyacrylamide gel electrophoresis containing blue dextran 2000 (Pharmacia Fine Chemicals, Uppsala, Sweden) as described previously (Igarashi *et al*. 1992). Binding of the truncated Dexs to dextran was assessed with a Sephadex G-150 gel (Pharmacia Fine Chemicals, Uppsala, Sweden) by modification of a method described previously (Abo *et al*. 1991; Igarashi *et al*. 2002). The proteins bound to the resin were detected by Western blotting with an anti-Dex antibody.

RESULTS

The deletion mutants were prepared using the genomic DNA of *Strep. mutans* Ingbritt as a template for PCR. The truncated Dexs were expressed in *E. coli* cells (Fig. 1). Western blot and active staining analyses of these truncated Dexs showed that, although all of the mutants produced Dex proteins of smaller sizes (Fig. 2a), none of the truncated Dexs, except that from mut-1, retained dextran-hydrolysing activity (Fig. 2b, lanes 2–6). In contrast, the dextran-binding ability of these truncated Dexs was examined with Sephadex G-150 gel. Any Dexs which truncated the N-terminal region, such as mut-3, mut-4 and mut-5, still possessed dextan-binding ability although they lost enzyme activity (Fig. 3, lanes 7–9). A non-specific band, derived from *E. coli*, with almost the same molecular size as the truncated Dex from mut-3, was observed in all lanes in Fig. 3. Although the size of the truncated Dex from mut-3 was, in fact, slightly smaller than that of the non-specific band, these two bands in lane 7 were observed as one band which had a higher intensity than the non-specific bands in lanes 6 and 8–10 of Fig. 3. However, mut-2 resulted in the loss of both catalytic and dextran-binding activities (Fig. 2b and Fig. 3, lanes 1 and 5) although mut-1 retained enzyme activity (Fig. 2b). Hence, these results suggest that the C-terminal domain (amino acids 615–732) in the CR was responsible for the dextran-binding activity of Dex. The amino acid sequence of the dextran-binding domain of *Strep. mutans* was compared with the corresponding sequences of other streptococcal Dexs (Fig. 4). Although the amino acid sequence in this domain was widely homologous among these Dexs, the direct repeating units which are commonly present in the glucan-binding region of Gtfs, glucan-binding protein (Gbp) and the Dex inhibitor (Dei) were not observed in the sequence of the dextran-binding region of the streptococcal Dexs.

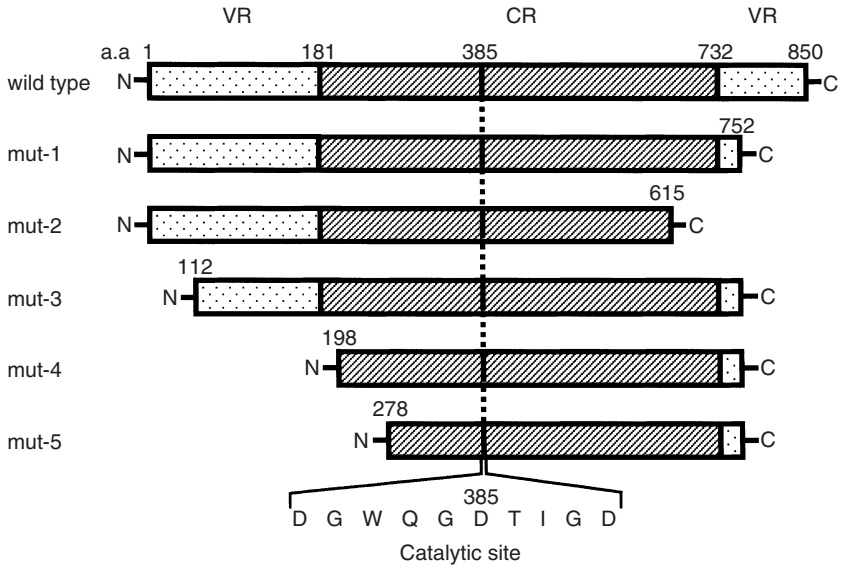

Fig. 1 Schema of the dextranase mutants. CR, Conserved region; VR, variable region

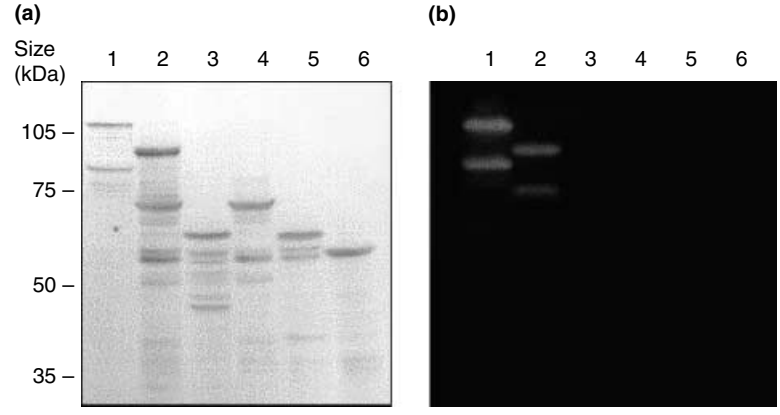

Fig. 2 Expression of truncated dextranase (Dex) by the mutants. The crude enzyme was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. (a) Western blot analysis with an anti-Dex antibody. (b) Active staining in the gel containing blue dextran. Lanes: 1, wild type; 2, mut-1; 3, mut-2; 4, mut-3; 5, mut-4; 6, mut-5

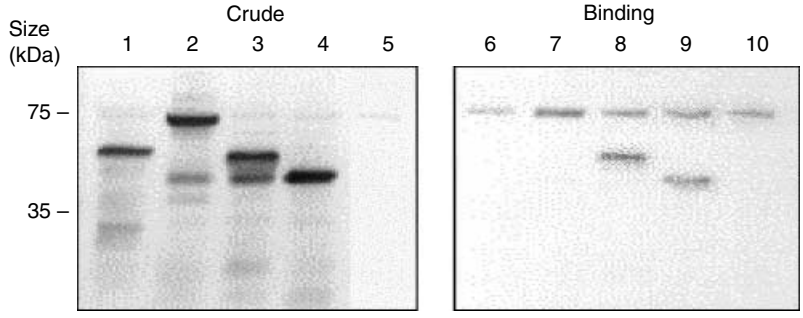

Fig. 3 Binding of truncated dextranases (Dexs) to dextran. The truncated Dexs bound to Sephadex G-150 were detected by Western blot with an anti-Dex antibody. Lanes: 1–4, truncated Dexs in crude enzyme before binding to the resin; 6–9, truncated Dexs bound to the resin; 1 and 6, mut-2; 2 and 7, mut-3; 3 and 8, mut-4; 4 and 9, mut-5; 5 and 10, *Escherichia coli* containing pT7Blue T-vector (negative control)

DISCUSSION

Homology analysis of deduced amino acid sequences among Dexs of streptococcal species showed that the CRs were highly homologous and with lengths similar to those of other species (Igarashi *et al*. 1995; Igarashi *et al*. 2002). In addition, the CRs contained an active site for enzyme activity (Igarashi *et al*. 2002). In contrast, the VRs were less homologous and the lengths were varied. These observations led to the conclusion that the CRs are functionally important but the VRs are not. The present study allowed further extension of the hypothesis: the C-terminal VR has no relationship with

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Strep. mutans       616 LGINGDQVWTYAKKGNDFRTIQLLNLMGITSDWKNEDGYENNKTPDEQTNLLVTYPLTG 674
Strep. sobrinus     684 KGITGNQVWTYGKKGDNFRTVQLLNLMGINSDWKNEDGSAANKTPDEQTNLTVKYALGD 742
Strep. downei       686 KGINGHQVWTYGKKGDNFRTVQLLNLMGINSDWKNEDGSAANKTPDEQTNLTVRYALGD 744
Strep. salivarius   481 LGVTGDQVWTFAKSGKGFSTVQMINMMGINAGWHNEEGYADNKTPDAQENLTVRLSLAG 539
                             *  * ****  * *  * * *  * ***   * ** *   ***** * ** *   *

Strep. mutans       675 VSMAEADRIAKQVYLTSPDDWLQSSMISLTTQVKTNENGDPVLYIQVPRLTLWDMIYI 732
Strep. sobrinus     743 VSMEDAQRMANQTYVTSPDDWSKSNLQKVSASVKTDENGKPVLVINVPKLTLWDVVYI 800
Strep. downei       745 ISMEDARRMAKQTYVTSPDDWSKSNLQKVSASVETDENGKPVLIINVPKLTLWDVVYI 802
Strep. salivarius   540 KTAQEAAKIADQVYVTSPDDWATSSMKKAQASLETDENGQPVLVISVPKLTLWNMLYI 597
                               *   * * * ******  *          * *** *** * ** ****   **
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Fig. 4 Homology of the DNA sequences of the C-terminal domain in the conserved region of the streptococcal dextranases (Dexs). Deduced amino acid sequences of the dextran-binding domain of the *Streptococcus mutans* Dex (accession no. D49430) and the corresponding regions of the Dexs of *Strep. sobrinus* (accession no. M96978), *Strep. downei* (accession no. AB052705) and *Strep. salivarius* (accession no. D29644) were compared. The number indicates the position of the amino acid residue of each Dex. Asterisks show identical amino acids

either catalytic or glucan-binding activities because mut-1 showed catalytic activity (Fig. 2). As seen in the case of mut-3, however, the deletion in the N-terminal VR resulted in loss of enzyme activity even though dextran-binding activity was retained. It is postulated that the deletion in the N-terminal VR might cause conformational change of the Dex molecule to abolish enzyme activity. As indicated by mut-4 and mut-5 in Fig. 1, further deletion to the N-terminal side of the CR also did not affect dextran-binding ability (Fig. 3, lanes 8 and 9). Involvement of the C-terminal side in the CR with dextran binding was demonstrated by mut-2 (Fig. 3, lanes 1 and 6). Mut-2 resulted in complete loss of both enzyme activity and dextran-binding ability. Comparing mut-1 and mut-2 (Fig. 1), it is suggested that the C-terminal domain (117 amino acid residues) in the CR of the Dex molecule, corresponding to amino acid position 616–732 in the *Strep. mutans* Dex (Igarashi *et al*. 1995), was necessary for dextran-binding ability. As glucan-binding proteins, such as Gtfs, Gbp and Dei, possess A and C repeats in glucan-binding sites (Ferretti *et al*. 1987; Mooser and Wong 1988; Russell *et al*. 1988; Banas *et al*. 1990; Sun *et al*. 1994), it was expected that the Dex, one of the glucan-binding proteins, would also have similar sequences. However, the expected sequences have not been detected in the Dex, implying that the binding mode of Dex to dextran is different from that of Gtfs, Gbp and Dei. Further analysis is required to clarify the dextran-binding mode of Dex.

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