Oral Microbiology Immunology 2002: 17: 193–196
Printed in Denmark. All rights reserved

Oral Microbiology and Immunology
ISSN 0902-0055

Short communication

An essential amino acid residue for catalytic activity of the dextranase of *Streptococcus mutans*

T. Igarashi, H. Morisaki, A. Yamamoto, N. Goto
Department of Oral Microbiology, Showa University School of Dentistry, Shinagawa-ku, Tokyo, Japan

Igarashi T, Morisaki H, Yamamoto A, Goto N. An essential amino acid residue for catalytic activity of the dextranase of Streptococcus mutans.
Oral Microbiol Immunol 2002: 17: 193–196. © Blackwell Munksgaard, 2002.

Dextranase (Dex) is an enzyme that hydrolyzes glucan, a polymer of glucose synthesized from sucrose by glucosyltransferases (GTFs). By comparing amino acid sequences of Dexs and GTFs, we found that the Dex enzymes of *Streptococcus mutans, Streptococcus sobrinus, Streptococcus downei* and *Streptococcus salivarius* had similar amino acid sequences to those of the catalytic sites of GTFs of mutans streptococci. We therefore examined the amino acid essential in Dex catalysis by molecular genetic approaches in this study. Site-directed mutagenesis was used to convert the Asp-385 of the Dex molecule of *S. mutans* Ingbritt to Glu, Asn, Thr or Val. Replacement of Asp-385 with any of the amino acids resulted in complete disappearance of Dex activity. However, replacement of other Asp residues did not affect the enzyme activity. The inactive enzymes still retained dextran-binding ability. These results suggest that Asp-385 of the Dex of *S. mutans* Ingbritt was essential for enzyme activity and the catalytic and substrate-binding sites were located at different sites within the Dex molecule.

Key words: active site; catalytic activity; dextranase; *Streptococcus mutans*

T. Igarashi, Department of Oral Microbiology, Showa University School of Dentistry, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan
Accepted for publication September 25, 2001

Mutans streptococci have been strongly implicated in the etiology of human dental caries (6, 13). These organisms mainly accumulate on teeth by producing extracellular glucans from dietary sucrose catalyzed by glucosyltransferases (6, 13).

The mutans streptococci are predominant producers of the enzyme dextranase (Dex) (19, 20) which hydrolyzes α-1,6 linkages in the glucans synthesized by glucosyltransferases (6, 7, 21). In particular, the Dexs of *Streptococcus mutans* and *Streptococcus sobrinus* have been well characterized by biological and genetic studies (2, 3, 7–9, 21), and it has been proposed that Dex plays at least two roles in the ecology of dental plaque: supplying nutrients to the plaque bacteria by hydrolyzing the glucans as potential storage polysaccharides (4, 7, 16) and modifying the molecular structure of the glucans to make it firmer and more adhesive in nature (3, 6, 12, 18, 20). These studies therefore suggest that Dex is an important virulence factor of mutans streptococci.

In previous studies, we characterized the *dex* gene and the Dex enzyme of *S. mutans* (7–9). Genetic analysis showed that the open reading frame (2550 bp) of the *S. mutans dex* encoded a Dex protein consisting of 850 amino acids with a molecular mass of 94.5 kDa (9). Mature Dex enzymes produced by recombinant *Escherichia coli* showed multiple active forms: two major (133 and 104 kDa) and two minor (108 and 88 kDa) bands (8), and the higher molecular mass forms of the mature enzymes were bigger than the deduced molecular mass (94.5 kDa). Although the variation in the molecular mass forms of the mature enzymes was explained by proteolytic degradation (7), the discrepancy of the different molecular masses between the mature and deduced Dex is still unexplained. We have also shown that the Dex molecule consists of two variable domains and a con-

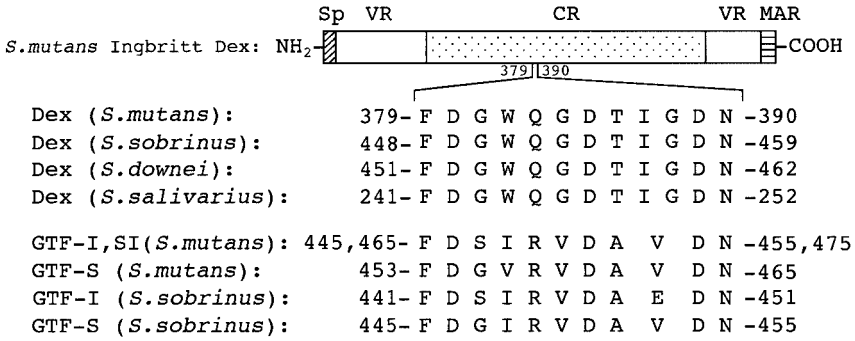

Fig. 1. Alignment of the amino acid sequences in the catalytic sites of dextranase and glucosyltransferase of mutans streptococci. Sp, signal peptide; VR, variable region; CR, conserved region; MAR, membrane-anchor region.

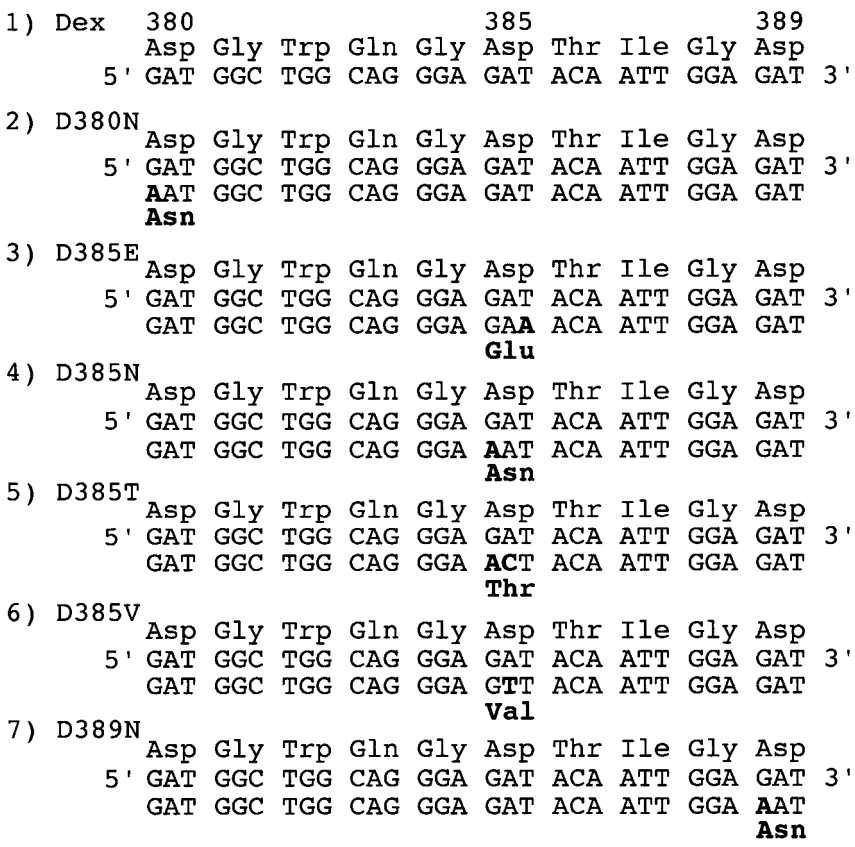

Fig. 2. Mutagenesis strategy to introduce a point mutation in Dex. The first line of each mutant shows the amino acid sequence of a wild-type enzyme; the second line is the nucleotide sequence encoding each amino acid. The third line shows the nucleotide sequence of the mutagenic PCR primer designed to replace a wild-type amino acid with the amino acid shown in the fourth line.

served domain (9, 10) and that the conserved domain (about 540 amino acid residues) was responsible for the enzyme activity (8, 9, 21). However, the catalytic and substrate-binding sites in the conserved domain of the Dex have not yet been determined. The primary structures of the Dexs from *S. mutans*, *S. sobrinus*, *Streptococcus downei* and *Streptococcus salivarius* have been reported (9, 10, 15, 21). Based on a comparison of these enzymes, we analyzed the catalytic site of the *S. mutans* Dex enzyme using site-directed mutagenesis and pinpointed the amino acid residue essential for catalytic activity on the molecular map of Dex.

E. coli JM109 cells were routinely used as a plasmid host and grown in Luria-Bertani broth supplemented with ampicillin (50 μg/ml). Plasmid pSDU18 was utilized as the source of the *S. mutans* Ingbritt *dex* gene encoding dextranase (8). The *dex* gene was subcloned into a pT7Blue T-vector (Novagen, Madison, WI), which was used for Dex expression, site-directed mutagenesis and nucleotide sequence analysis.

Plasmid extraction was carried out with a Wizard miniprep purification kit (Promega, Madison, WI) as described previously (9). Site-directed mutagenesis of DNA fragments was performed with a Quik change site-directed mutagenesis kit (Stratagene, La Jolla, CA) as recommended by the supplier.

Detection of Dex activity following SDS-PAGE containing blue dextran was performed as reported previously (7, 8). Binding of the mutated Dexs to dextran was measured on a Sephadex G-150 gel by modification of a procedure described previously (1). The proteins bound to the resin (dextran, Pharmacia Fine Chemicals, Uppsala, Sweden) were detected by Western blotting with an anti-Dex antibody.

The nucleotide sequence was determined with the ABI prime cycle sequencing kit and a Model 373S automated DNA sequencer (Applied Biosystems, Foster City, CA) as described previously in order to confirm the site-directed mutagenesis (9).

Although previous deletion analyses suggested that the conserved domain in Dex is responsible for enzyme activity (8, 9, 21), details of active and substrate-binding sites in the conserved domain are still uncertain. In order to determine the active site of Dex, the homology of the amino acid residues in the conserved domains of the *S. mutans*, *S. sobrinus*, *S. downei* and *S. salivarius* Dexs was analyzed. Homologous residues were widespread in the conserved region (9, 10). Comparing the amino acid sequences of glucosyltransferases, we noticed a similarity between a limited portion of the conserved region of the Dexs and the catalytic site of the glucosyltransferases (Fig. 1). As enzymes that use glucans as a substrate have an Asp residue as an essential amino acid in their active site (5, 11, 14, 17), the Asp residues (Asp-380, -385 and -389 of the *S. mutans* Dex in Fig. 1) were targeted for the functional analysis of the *S. mutans* Dex enzyme.

Site-directed mutagenesis was em-

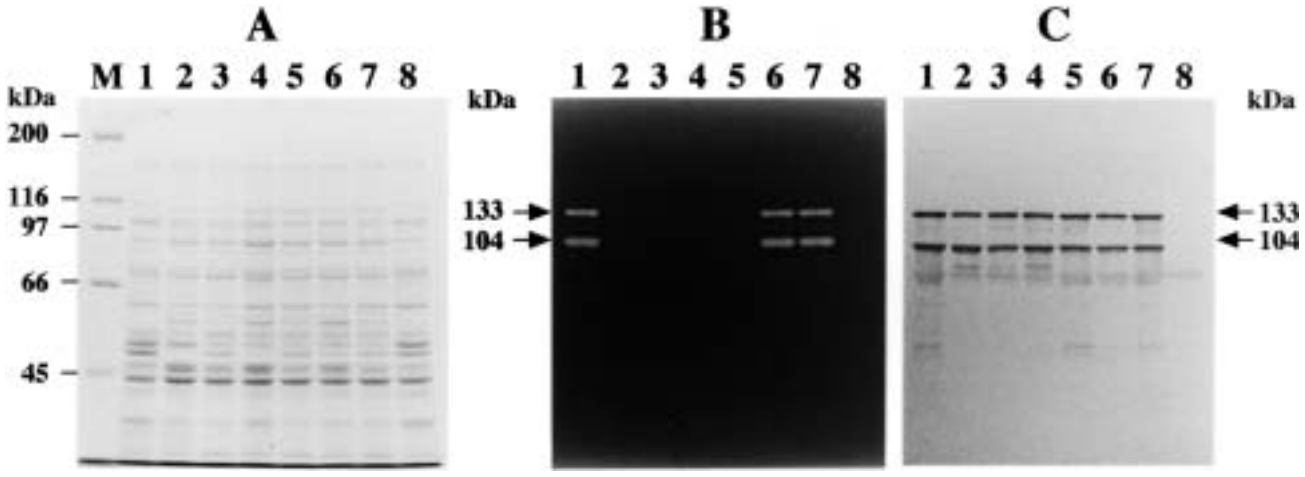

Fig. 3. Expression of Dex activity by the mutants. The *E. coli* cell lysates (50 μg/lane) were analyzed by SDS-PAGE. (A) Protein staining with Coomassie blue. (B) Active staining for dextran hydrolyzing activity. (C) Western blot analysis of SDS-polyacrylamide gel with anti-Dex antibody. The *E. coli* cells contained: lane 1, parental Dex from pT7 Blue; 2, mutant D385N; 3, mutant D385E; 4, mutant D385T; 5, mutant D385V; 6, mutant D380N; 7, mutant D389N; 8, pT7Blue T vector (negative control). Arrows indicate the major Dex enzymes (133 and 104 kDa) detected by active staining or Western blot.

ployed to convert Asp-385 to an Asn, Glu, Thr or Val residue (Fig. 2). Following mutagenesis, the generated mutations were confirmed by nucleotide sequencing. The replacement of Asp-385 produced proteins which failed to display enzyme activity, implying that Asp-385 was an essential amino acid residue for the enzyme activity (Fig. 3B, lanes 2–5). Western blot analysis with the anti-Dex antibody revealed that the mutants replacing Asp-385 with Asn, Glu, Thr or Val expressed their gene products (Fig. 3C, lanes 2–5). In contrast, replacement of Asp-380 with Asn or Asp-389 with Asn brought no apparent change in the enzyme activity (Fig. 3B, lanes 6 and 7). These results indicate that Asp-385 plays a decisive role in Dex activity. Homology analysis of the deduced amino acid sequences of the Dexs of the streptococcal species revealed that an analogous Asp residue is present in all conserved regions of the four enzymes (Fig. 1).

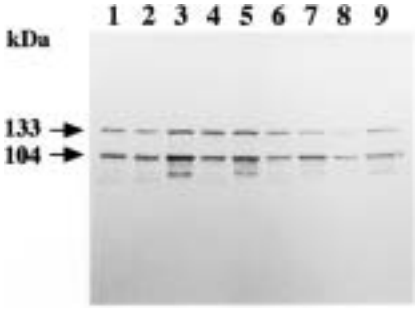

Fig. 4. Binding of the Dex mutants to dextran. The Dex mutants D385N, D385E, D385T, and D385V bound to Sephadex G-150 were detected by Western blot with an anti-Dex antibody. Lanes 1, 2, 4, 6 and 8 indicate the Dex in crude extract (40 μg) before binding to the resin. Lanes 3, 5, 7 and 9 indicate the Dex bound to the resin. Crude extract (90 μg) was mixed with Sephadex G-150 suspension and the Dex bound to the resin was loaded into each lane indicated. Lanes: 1, parental Dex; 2 and 3, D385N; 4 and 5, D385E; 6 and 7, D385T; 8 and 9, D385V. Arrows indicate the major Dex proteins (133 and 104 kDa) detected with the anti-Dex antibody.

Subsequently, the binding ability of the mutants to the substrate, dextran, was examined and it was revealed that replacement of Asp-385 did not alter dextran-binding ability (Fig. 4). These results suggest that the Asp-385 residue was identified as an essential amino acid in the *S. mutans* Dex catalysis and a substrate-binding site was present at another location from the catalytic site in the conserved domain of the Dex molecule. Further analysis will be needed to clarify the substrate-binding site.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (no. 11671813) from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Abo H, Matsumura T, Kodama T, Ohta H, Fukui K, Kato K et al. Peptide sequences for sucrose splitting and glucan binding within *Streptococcus sobrinus* glucosyltransferase (water-insoluble glucan synthetase). J Bacteriol 1991: **173**: 989–996.
2. Barrett JF, Barrett TA, Curtiss R III. Purification and partial characterization of the multicomponent dextranase complex of *Streptococcus sobrinus* and cloning of the dextranase gene. Infect Immun 1987: **55**: 792–802.
3. Colby SM, Whiting GC, Tao L, Russell RRB. Insertional inactivation of the *Streptococcus mutans dexA* (dextranase) gene results in altered adherence and dextran catabolism. Microbiology 1995: **141**: 2929–2936.
4. Dewar MD, Walker GJ. Metabolism of the polysaccharides of human dental plaque. Caries Res 1975, 1993: **9**: 21–35.
5. Funae K, Shiraiwa M, Hashimoto K, Ichishima E, Kobayashi M. An active-site peptide containing the second essential carboxyl group of dextransucrase from *Leuconostoc mesenteroides* by chemical modifications. Biochemistry 1993: **32**: 13696–13702.
6. Hamada S, Slade H. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol Rev 1980: **44**: 331–384.
7. Igarashi T, Yamamoto A, Goto N. Characterization of the dextranase purified from *Streptococcus mutans*. Microbiol Immunol 1992: **36**: 969–976.
8. Igarashi T, Yamamoto A, Goto N. Characterization of the dextranase gene (*dex*) of *Streptococcus mutans* and its recombinant product in an *Escherichia coli* host. Microbiol Immunol 1995: **39**: 387–391.
9. Igarashi T, Yamamoto A, Goto N. Se-

quence analysis of the *Streptococcus mutans* Ingbritt *dexA* gene encoding extracellular dextranase. Microbiol Immunol 1995: **39**: 852–860.

10. Igarashi T, Yamamoto A, Goto N. Nucleotide sequence and molecular characterization of a dextranase gene from *Streptococcus downei*. Microbiol Immunol 2001: **44**: 341–348.
11. Kato C, Nakano Y, Lis M, Kuramitsu HK. Molecular genetic analysis of the catalytic site of *Streptococcus mutans* glucosyltransferases. Biochem Biophys Res Commun 1992: **189**: 1184–1188.
12. Larrimore S, Murchison H, Shiota T, Michalek SM, Curtiss R III. In vitro and in vivo complementation of *Streptococcus mutans* defective in adherence. Infect Immun 1983: **42**: 558–566.
13. Loesche WL. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 1986: **50**: 353–380.
14. Mooser G, Hefta SA, Paxton RJ, Shively JE, Lee TD. Isolation and sequence of an active-site peptide containing a catalytic aspartic acid from two *Streptococcus sobrinus* α-glucosyltransferases. J Biol Chem 1991: **266**: 8916–8922.
15. Ohnishi Y, Kubo S, Ono Y, Nozaki M, Gonda Y, Okano H et al. Cloning and sequencing of the gene coding for dextranase from *Streptococcus salivarius*. Gene 1995: **156**: 93–96.
16. Parker RB, Creamer HR. Contribution of plaque polysaccharides to growth of cariogenic microorganisms. Arch Oral Biol 1971: **16**: 855–862.
17. Russell RRB, Ferretti JJ. Nucleotide sequence of the dextran glucosidase (*dexB*) gene of *Streptococcus mutans*. J Gen Microbiol 1990: **136**: 803–810.
18. Schachtele CF, Staat RH, Harlander SK. Dextranases from oral bacteria: inhibition of water-insoluble glucan production and adherence to smooth surfaces by *Streptococcus mutans*. Infect Immun 1975: **2**: 309–317.
19. Staat RT, Schachtele CF. Evaluation of dextranase production by the cariogenic bacterium *Streptococcus mutans*. Infect Immun 1974: **9**: 467–469.
20. Walker GJ, Pulkownik A, Morrey-Jones JG. Metabolism of the polysaccharides of human dental plaque. Release of dextranase in batch cultures of *Streptococcus mutans*. J Gen Microbiol 1981: **127**: 201–208.
21. Wanda S-Y, Curtiss R III. Purification and characterization of *Streptococcus sobrinus* dextranase produced in recombinant *Escherichia coli* and sequence analysis of the dextranase gene. J Bacteriol 1994: **176**: 3839–3850.