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Crystal Structure of Glucansucrase from the Dental Caries Pathogen, *Streptococcus mutans*

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1 Introduction

Sweet is an important favorable taste quality linked to food intake in humans, but is inextricably linked to dental caries risks. Sucrose, the most common form of sugar, is the most highly consumed sweetner but also causes tooth decay. According to the World Oral Health Report 2003, dental caries is a major health problem in most industrialized countries, affecting 60-90% of school children and the vast majority of adults. If left untreated for a long period of time, dental caries it can result in pain and tooth loss, and can lead to additional infections and, in some cases, even death by sepsis. Caries formation is initiated when glucan, a sticky glucose polymer produced by *Streptococcus mutans*, forms a biofilm (dental plaque) on teeth, which then traps oral bacteria, food debris and salivary components. As they grow, the bacteria secrete acids which break down the tooth's enamel on the surface . Acids produced by the bacteria in the biofilm as a result of fermentation of dietary carbohydrates such as fructose and glucose, including sucrose, demineralizes the tooth surface, leading to dental caries. Therefore, high molecular-weight sticky glucan synthesized from sucrose by glucansucrases, extracellular enzymes from *S. mutans*, plays an essential role in the etiology and pathogenesis.

2 Experiment, Results and Discussion

Glucansucrases are members of glycoside hydrolase family 70, and catalyze the formation of glucan with various types of glucosidic linkages, α(1-3), α(1-4) or α(1-6) bonds, from sucrose via transglycosylation reactions. In oral cavities, glucan synthesis by *S. mutans* involves three extracellular enzymes, GTF-I, GTF-SI and GTF-S. GTF-I and GTF-SI synthesize mainly insoluble sticky glucan with α(1-3) glycosidic linkages. We have used the beam line NE3A of the Photon Factory to solve the 3D structure of GTF-SI that plays a key role in tooth decay cased by sugar (Fig 1a). We resolved the crystal structures of the GTF-SI in the free enzyme form and in complex with acarbose and maltose, respectively. The structure of GTF-SI comprised four separate domains: A, B, C and IV (Fig 1a). Overall catalytic domain (domains A, B and C) structure is similar to those of well-known sugar-cutting enzymes (such as α-amylases), and some of the catalytic amino acid residues are conserved. The results indicate that these enzymes share a similar reaction mechanism via glycosyl-enzyme intermediate in catalytic site.

Meanwhile, GTF-SI also possesses unique structural features. The domain order of GTF-SI was circularly permuted as compared to that of sugar-cutting enzymes. As a result, domains A, B and IV of GTF-SI are eachcomprised of two separate polypeptide chains (Fig 2a). A novel structural features were also revealed in second sucrose binding site, namely, subsite +1 and +2 (Fig 1b and 1c). Trp517 provide the platform for glycosyl-acceptor binding, and such as Tyr430, Asn481, and Ser589 residues comprising the subsite +1 are conserved in glucansucrases but not in sugar-cutting enzymes. Among these residues, the position of Asp593 in GTF-SI is most critical point for glucansucrases that make insoluble and sticky glucan with α(1-3) glycosidic linkages. And the reason for that is because Asp593 is mutated to Thr in GTF-S, which is known to synthesizes predominantly soluble glucan with α(1-6) glycosidic linkages. Acarbose contains a nonhydrolyzable nitrogen-linked bond that blocks the catalytic activity of various glycosyl hydrolases, including maltase-glucoamylase in the small intestine. This could underlie some of the side effects of this inhibitor, such as hypoglycemia. New inhibitors that specifically target subsites +1, +2 and +3 of glucansucrases can now be designed based on the structure of the GTF-SI-maltose complex reported herein.

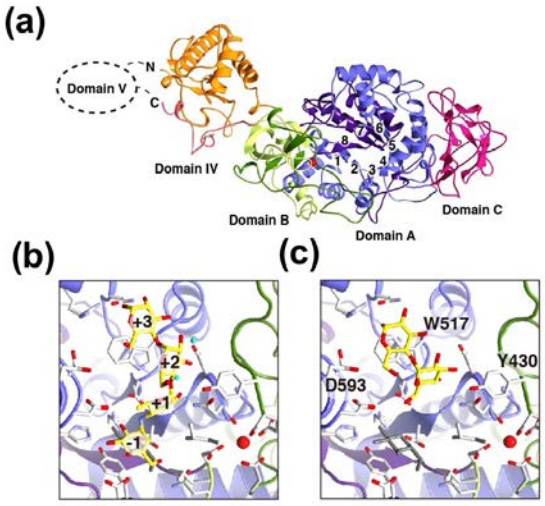

Figure 1: Structure of GTF-SI.

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