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

Glucansucrases from *Streptococcus mutans* (GTF-SI) catalyze an essential factor in the pathogenesis of dental caries. Resolution of the GTF-SI structure confirmed that the domain order of glucansucrase-SI was circularly permuted compared with that of the well-known α-amylase, which catalyses the breakdown of starch into sugars. Based on the structure of GTF-SI and a comparison of the amino acids of other glucansucrases, it was revealed that the position of Asp593 in glucansucrase-SI is the most critical point for the orientation of the acceptor sugar, and this influences the transglycosyl reaction specificity of GTF-SI to produce insoluble glucan with α(1-3) glycosidic linkages or soluble glucan with α(1-6) linkage. This structural information should be useful for designing new inhibitors to prevent the formation of biofilms.

Sweetness is an important taste quality linked to food intake in humans, but it is also inextricably linked to the risk of dental caries. Sucrose, the most common form of sugar, is the most highly consumed sweetener but it 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 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 enamel on the surface of teeth. Acids produced by the bacteria in the biofilm as a result of fermentation of dietary carbohydrates such as fructose and glucose, including sucrose, demineralize 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.

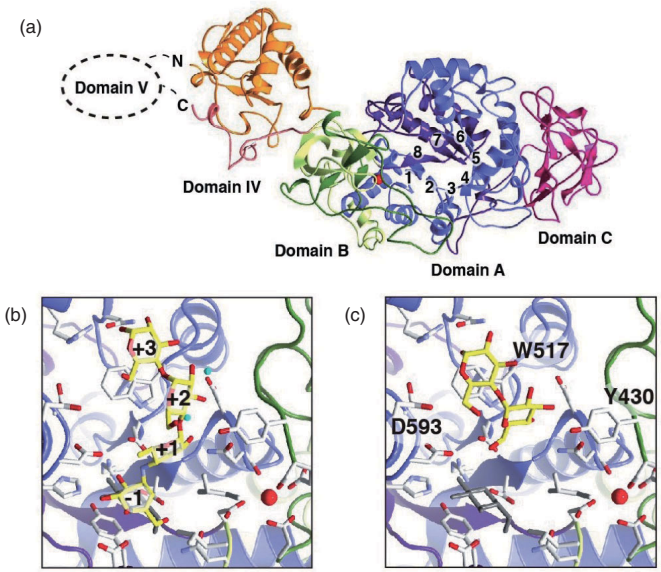

Figure 1
Structure of GTF-SI. (a) Ribbon diagram of GTF-SI. Domain IV, A and B are composed of chains IV1 (orange) and IV2 (pink), chains A1 (blue) and A2 (purple) and chains B1 (green) and B2 (yellow green), respectively. (b) Substrate-binding site with acarbose. Acarbose is a pseudotetrasaccharide and a strong inhibitor for glucansucrases. The numbers indicate the subsite numbering. (c) Substrate-binding site with maltose. Maltose is a transglycosyl acceptor for glucansucrases, but not a donor. The glycosyl intermediate in the structure of another amylase family member is shown in gray.

Glucansucrases are members of the 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 the oral cavity, 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 AR-NE3A and 5A beamlines to identify the 3D structure of GTF-SI, which plays a key role in tooth decay caused by sugar (Fig. 1(a)) [1]. 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. 1(a)). The 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 and previous studies of glucansucrases and sugar-cutting enzymes indicate that these enzymes share a similar reaction mechanism via glycosyl-enzyme intermediate in catalytic sites.

Meanwhile, GTF-SI also possesses unique structural features. The domain order of GTF-SI was circularly permuted compared with that of sugar-cutting enzymes. As a result, domains A, B and IV of GTF-SI are each comprised of two separate polypeptide chains (Fig. 1(a)). Several structural features were also revealed in second sucrose binding site, namely, subsite +1 and +2 (Fig. 1(b) and 1(c)). Trp517 provides the platform for glycosyl-acceptor binding, whereas residues such as Tyr430, Asn481, and Ser589 comprising subsite +1 are conserved in glucansucrases but not in sugar-cutting enzymes. Among these residues, the position of Asp593 in GTF-SI is critical for glucansucrases that make insoluble and sticky glucan with α(1-3) glycosidic linkages. This is because Asp593 is mutated to Thr in GTF-S, which is known to synthesize 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.

REFERENCE

[1] K. Ito, S. Ito, T. Shimamura, T. Kobayashi, K. Abe, A.D. Cameron and S. Iwata, *J. Mol. Biol.* **408** (2011) 177.

BEAMLINES

AR-NE3A and 5A

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