

Poster Presentation

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Structure/Function studies on Glucan Binding Protein C of Streptococcus mutans

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Streptococcus mutans is a known etiological agent in dental caries. In the past several years, we have taken a concerted effort toward understanding the adhesion mechanisms adopted by the surface proteins *S. mutans*. The Glucan Binding Protein C (GBPC) is a LPXTG anchored surface protein on *S. mutans* that has been widely implicated to play a significant role in biofilm formation. GBPC displays limited homology to the V-region of Antigen I/II (Agl/II), another surface protein of *S. mutans* (1,2). We undertook to crystallize and resolve the structure of GBPC. Recombinant GbpC111-552 (residues encompassing 111-552) was cloned into a pET23d vector, and thereafter expressed in *E. coli* BL21(DE3). GbpC111-552 purified initially using affinity chromatography (his-tag), followed by anion exchange (MonoQ) and finally polished with size exclusion (Superdex 75). GbpC111-552 was crystallized using the vapor diffusion method by scanning various commercial crystallization kits on a 96 well plate format through the Art Robbins Gryphon robot. Large crystals were obtained from a refined droplet condition that contained 1 μ l of protein (43.3 mg/ml) mixed with 1 μ l of reservoir made of 100 mM Bis-Tris pH 6.5 and 25% (w/v) PEG3350. The crystal structure could not be resolved by molecular replacement. Therefore crystals were soaked in Sodium Iodide (NaI) and thereafter flash frozen with 9% glycerol as cryoprotectant. MAD data sets were collected and the crystal structure was resolved. We will present the crystal structure, and how based on its structural homology we discovered similar functionalities among the surface proteins of *S. mutans*. The structural and functional studies of these surface proteins we hope would allow the identification of the adherence motifs, which would aid in development of inhibitors. The development of such anti-adhesive inhibitors would aid in disease preventative therapies.

[1] Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, et al. (2010) Elongated fibrillar structure of a streptococcal adhesin assembled by the high-affinity association of alpha- and PP1I-helices. *Proc Natl Acad Sci U S A* 107: 5983-5988., [2] Larson MR, Rajashankar KR, Crowley PJ, Kelly C, Mitchell TJ, et al. (2011) Crystal structure of the C-terminal region of *Streptococcus mutans* antigen I/II and characterization of salivary agglutinin adherence domains. *J Biol Chem* 286: 21657-21666

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