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A whole cell BIAcore assay to evaluate P1-mediated adherence of *Streptococcus mutans* to human salivary agglutinin and inhibition by specific antibodies

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Abstract

Researchers now recognize the utility of surface plasmon resonance technology to evaluate interactions of microbial pathogens with host components. The surface adhesin and candidate vaccine antigen P1 of *Streptococcus mutans*, the main causative agent of dental caries, interacts with a high molecular weight glycoprotein called salivary agglutinin, or gp340, in the salivary pellicle. We optimized a BIAcore assay to measure P1-mediated Ca^{2+} dependent binding of *S. mutans* whole cells to this physiological ligand immobilized on a Pioneer F1 sensor chip. Regeneration conditions allowed cells to be eluted from the sensor chip permitting multiple reuse of the agglutinin-coated surface. An isogenic P1-deficient *S. mutans* mutant did not bind to immobilized agglutinin demonstrating specificity of the detected interaction. Glutaraldehyde-fixation of bacterial cells showed the assay measured a whole cell–ligand interaction and was not an artifact of solubilized or leached proteins. Adherence inhibition assays demonstrated varying degrees of disruption of the *S. mutans*–agglutinin interaction by anti-P1 monoclonal antibodies recognizing different epitopes, whereas a polyclonal reagent demonstrated more complete inhibition. This report describes an improved method to assess salivary agglutinin-mediated adherence of *S. mutans* in vitro under physiological-like conditions and to evaluate the effectiveness of antibodies of differing specificities to inhibit binding.

Keywords: *Streptococcus mutans*; Adherence inhibition; SPR; BIAcore®; Whole cell

1. Introduction

Oral microorganisms colonize, survive and multiply in a dynamic host environment that is continually altered by dietary components as well as by the host immune system. *Streptococcus mutans* is the predominant etiologic agent of dental caries and results in billions of dollars in annual global healthcare costs (Hamada and Slade, 1980). This species and related members of the oral microflora have also been associated with bacteremias and endocarditis (Ayakawa et al., 1988; Chia et al., 2002; Gong et al., 1998; Harder et al., 1974). *S. mutans* possesses a number of virulence factors that allow it to colonize and eventually dominate its niche in the oral cavity. The wall-associated Mr~185,000 multi-domain protein known variously as P1, antigen I/II, or PAc is widely presumed to allow bacterial attachment to the acquired pellicle of teeth via calcium-dependent binding to the high molecular weight glycoprotein, salivary agglutinin (Demuth et al., 1988), now also known as gp340 (Demuth and Irvine, 2002). We previously constructed

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an isogenic mutant of *S. mutans* by eliminating the gene encoding P1, known as *spaP* or *pac* (Crowley et al., 1999). This enabled characterization of the cell surface-localized protein as an adhesive component of *S. mutans* and demonstrated its contribution to caries formation and invasion of dentinal tubules (Crowley et al., 1999; Love et al., 1997).

P1 has been studied as a candidate vaccine antigen, but not all anti-P1 antibodies are equally protective against dental caries or as effective at inhibiting the P1–agglutinin interaction (Brady et al., 1992; Kelly et al., 1995). It is therefore important to be able to measure P1-mediated adherence specifically to its physiological ligand and to be able to accurately assess disruption of *S. mutans* binding to immobilized agglutinin by antibodies of varying specificity. In the past, *S. mutans* adhesion to human salivary agglutinin and inhibition by specific antibodies has been studied by measuring the binding of ^{3}H-labelled bacteria to salivary agglutinin-coated hydroxyapatite beads (Brady et al., 1993, 1992). However, this assay requires use of a radiolabel and is costly in terms of the amounts of required reagents. More recently, investigators have begun to utilize biosensor technology to evaluate the interaction of different species of oral streptococci with human salivary components (Kawashima et al., 2003). In this report we describe an expansion of this approach to measure the specific interaction of cell surface localized P1 with immobilized agglutinin under physiological-like conditions and to discern differences in the relative abilities of anti-P1 antibodies to inhibit adherence.

Surface plasmon resonance (SPR) BIAcore® based technology has been widely used in drug design, antibody screening and epitope mapping and has provided functional and structural information in immunology and infectious disease research. The technique has most commonly measured the association and dissociation of two molecules on a sensor surface. One binding partner, the ligand, which can be nucleic acid, protein, carbohydrate, synthetic polymer, or lipid, is covalently attached to a sensor chip surface. The second binding partner, or analyte, in liquid phase is passed through a microfluidic system to come in contact with an immobilized ligand. If an analyte–ligand interaction occurs the mass on the sensor chip surface is altered and is detected as a change in refractive index. This change is monitored by an optical detection unit. BIAcore® is gaining popularity to gain insight into more complicated molecular interactions in infectious disease research including those involving whole bacterial cells (Berube et al., 1999; Holmes et al., 1997; Kawashima et al., 2003; Medina, 2001; Quinn and O'Kennedy, 2001; Quinn et al., 2000; Senpuku et al., 2001). Careful design and analysis of whole cell BAcore assays are essential to obtaining reliable data (Myszka, 1999; Rich and Myszka, 2001, 2002) and several factors should be considered when using whole cells in SPR analysis. Firstly, the interaction of large cells with their ligand is a complex interaction therefore simple binding constant equations cannot be applied and determination of K_Ds may be arbitrary. When determined, affinities should be measured by competition analysis (Nieba et al., 1996) and antibodies or ligand/fragments should be incubated with cells before SPR analysis. Secondly, repetition of experiments should be performed independently on surfaces made at different occasions, since repeated injection of bacterial cells onto the same surface may not account for all potential variability in experimental conditions. Thirdly, the significance of changes in resonance signals, especially small ones, should be carefully scrutinized in light of known biological properties. For example, it is questionable whether a delta RU of 10 in a cellular SPR assay is as meaningful as a small change in RU in protein based experiments (Kawashima et al., 2003). We optimized the whole cell assay described in this report with these considerations in mind.

It is possible to disrupt a host–pathogen interaction with molecules that bind to or compete with relevant regions of ligands or receptors. Data supporting a role for humoral immunity in protection against dental caries have been documented and P1 has shown promise as a therapeutic immunogen (reviewed in (Koga et al., 2002; Michalek et al., 2001)). Individual fragments of P1 have been studied and vary with regard to their ability to elicit protective responses or to interfere directly with *S. mutans* colonization (Kelly et al., 1999). In addition, a passively applied monoclonal antibody directed against antigen I/II has been reported to confer protection against *S. mutans* colonization in human clinical trials; however, this property is not shared by all anti-antigen I/II monoclonals (Ma et al., 1989). Adherence of *S. mutans* can be inhibited in vitro by addition of certain anti-P1 antibodies, fluid-phase P1 or defined P1 (antigen I/II) peptides (Brady et al., 1992; Kelly et al., 1999). Therefore, we also adapted our BIAcore SPR assay to evaluate the function of anti-P1 antibodies of varying specificities by measuring inhibition of *S. mutans* binding to salivary agglutinin immobilized on the chip surface.