Antiviral Activities in Human Saliva

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ABSTRACT

In this review, the authors survey the large number of antibacterial and antiviral proteins present in human saliva. Of interest, most of these antibacterial proteins display antiviral activity, typically against specific viral pathogens. The review focuses on one protein that interacts with both bacteria and viruses—gp340, originally referred to as *salivary agglutinin*. In the oral cavity, soluble gp340 binds to and aggregates a variety of bacteria, and this is thought to increase bacterial clearance from the mouth. However, when bound to the tooth surface, gp340 promotes bacterial adherence. In the oral cavity, most gp340 is found soluble in saliva and can function as a specific inhibitor of infectivity of HIV-1 and influenza A. In contrast, in the female reproductive track, most gp340 is bound to the cell surface, where it can promote HIV-1 infection.

Like many other mucosal surfaces, the oral cavity is exposed to a variety of infectious agents and toxic compounds. To provide a variety of biological functions (communication, nutrient intake, respiratory activity, etc), it needs to be an open system, yet this requires an equally diverse group of activities/biomolecules to insure its continued viability. Thus, copious salivary flow is important for mastication, taste, the maintenance of hard tissue structure, and the prevention of a range of toxic and infectious assaults. In this article, we focus on one aspect of the role of saliva—namely, protection from infection, specifically via the innate immune system. This system is an important first-line defense against bacterial and viral infection. In addition, mucosal surfaces are typically coated by a protective layer of mucins. Although salivary flow plays a major role in the continuous cleansing of the oral cavity (Dawes, 2008), we focus here on proteins in the oral cavity that demonstrate both antibacterial and antiviral activity.

To obtain an overview of antimicrobial proteins present in saliva, we carried out a PubMed search of the literature (http://www.ncbi.nlm.nih.gov/pubmed/). The Table lists the major proteins found in saliva and documented to demonstrate antibacterial activity. Though not exhaustive, this list contains proteins shown to be either bacteriostatic or bacteriocidal against multiple bacterial species. Of interest, most of these proteins have been reported to be antiviral for at least one virus. As noted on the table, the only entities reported having antibacterial but not antiviral activity are the histatins and calprotectin.

Table. Salivary Antibacterial and Antiviral Activities

Salivary Constituent	Antibacterial	Antiviral
Cathelcidin (LL-37)	✓	✓
Lactoferrin	✓	✓
Lysozyme	✓	✓
Mucins	✓	✓
Peroxidase	✓	✓
Salivary agglutinin (gp340, DMBT1)	✓	✓
sIgA	✓	✓
SLPI	✓	✓
α,β Defensins	✓	✓
Calprotectin (calgranulin)	✓	
Histatins	✓	

Despite the range of antiviral activities found in saliva, many reports still identify viruses in saliva, often infectious—including HSV, HIV, VZV, EBV, HPV, hepatitis A, hepatitis C, Ebola, Norwalk virus, HHV 6 and 8, measles, rabies, adenoviruses, and prions. Although infectious virus in the presence of potent antiviral activity appears counterintuitive, there are potential explanations (as suggested in the Discussion section).

Our studies have focused on a salivary protein initially identified as possessing antibacterial activity but subsequently found to have potent inhibitory activity against HIV-1. Significantly, this activity is manifest against only HIV-1 and influenza A (Wu *et al*., 2003; White *et al*., 2005a), with little or no activity against HSV, adenovirus, HIV-2, or SIV. This protein was referred to as *salivary agglutinin* and is now more commonly called gp340 or DMBT-1 (Wu *et al*., 2004). Figure 1 outlines the timeline of these discoveries. From 1986 to 1988, a series of publications—first by Fultz (1986) and then Fox *et al*. (1988, 1989)—reported that incubation of HIV-1 in human or chimpanzee saliva resulted in a loss of infectivity. Many groups subsequently began to report a variety of salivary proteins demonstrating anti-HIV activity (Archibald and Cole, 1990; Bergey *et al*., 1993a, 1993b; Malamud *et al*., 1993; McNeely *et al*., 1995). In 1997, we

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Key Words

HIV, AIDS, viral, antiviral, innate immune system.

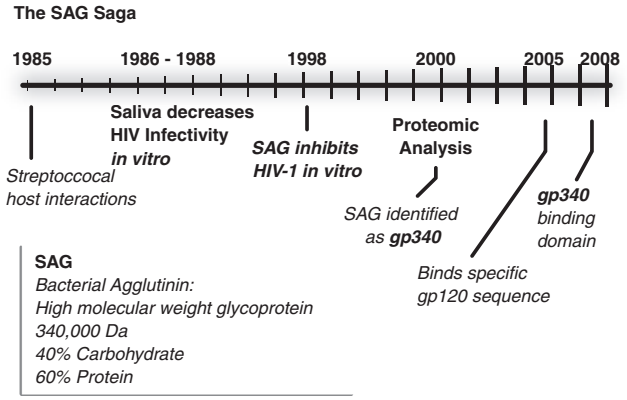

Fig. 1. Timeline of salivary agglutinin (SAG; *i.e.*, gp340) antibacterial and anti-HIV-1 activity.

reported that the effect of saliva on HIV inhibition was directly on the virus and specific for HIV-1 (Malamud *et al*., 1997), and in 1998 we demonstrated that salivary agglutinin could inhibit HIV infectivity by binding to gp120 on the surface of the virus (Nagashunmugam *et al*., 1998). Subsequent cloning of gp340 (Holmskov *et al*., 1999) and proteomic analysis by Prakobphol *et al*. (2000) identified salivary agglutinin as a previously cloned protein, DMBT-1 or gp340.

GP340 OVERVIEW

To briefly summarize the published data on gp340, we reviewed the literature in the field. Salivary agglutinin, gp340, and the tumor suppressor DMBT1 (deleted in malignant brain tumors) are encoded by the *dmbt1* gene. Proteins in the scavenger receptor cysteine-rich (SRCR) superfamily are either secreted or membrane-bound, demonstrating a variety of biologic functions (Resnick *et al*., 1994; Kang and Reid, 2003; Sarrias *et al*., 2004; Ligtenberg *et al*., 2007a, 2007b). Members of this family have at least one conserved domain, with either 6 or 8 cysteines forming characteristic disulfide bonds (S-S). The sequences of SRCR domains consist of about 110 amino acids that are conserved in evolution from sponges to humans. In sum, gp340 contains 14 SRCR domains, with the first 13 being highly conserved and the 14th somewhat less so (Holmskov *et al*., 1999). In general, the biological functions of the SRCR family of proteins consist of either tumor suppressor activity or innate immune defense properties. Salivary gp340 is able to aggregate a range of oral bacteria (Golub *et al*., 1985) and demonstrate antiviral activity against HIV-1 (Wu *et al*., 2003) as well as influenza A (White *et al*., 2005a, 2005b).

IDENTIFICATION OF A BIOACTIVE PEPTIDE WITHIN N-TERMINAL SRCR

In a series of elegant studies, a research team in Amsterdam, Netherlands, identified a bioactive peptide within a consensus SRCR sequence that binds to streptococcal receptors and mediates bacterial aggregation (Bikker *et al*., 2002). Using alanine scanning, they were able to identify a 5–amino acid motif required within this sequence for antibacterial activity (Bikker *et al*., 2004).

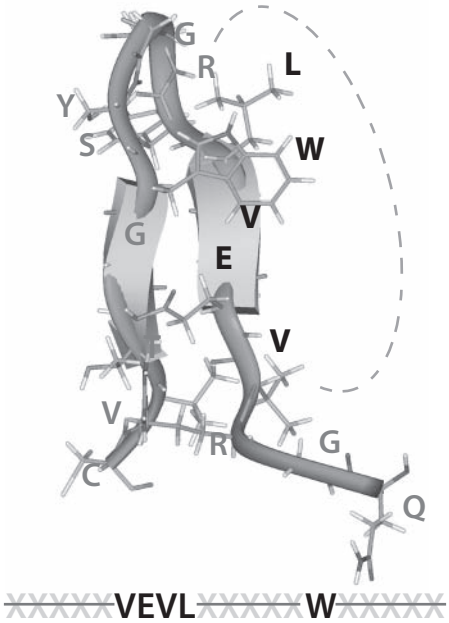

Fig. 2. Tertiary structure model for the active bacterial-binding motif in a scavenger receptor cysteine-rich (SRCR) domain that is proposed as being responsible for anti-HIV activity.

MOLECULAR MODELING STUDIES

Our group examined the reported antibacterial sequence in SRCR, and we used an available crystal structure for a single domain from the Mac-2 binding proteins to create a model for the active domain identified by Bikker *et al*. (2004) (see Fig. 2). Of interest, all the key amino acids identified by Bikker *et al*. occur on one face of the structure in this predicted model.

Because we had previously identified the sequence in HIV-1 gp120 involved in binding gp340 (Wu *et al*., 2004), we carried out a best-fit model of the binding between SRCR and HIV-1 env, using a docking module in the Discovery Studio software suite (Accelyris RDOCK, V 2.0; Accelrys, San Diego, California). Figure 3 presents the predicted interaction of gp340 SRCR with HIV gp120. Although it is not certain that the same peptide sequence of the SRCR binds both a bacterial and a viral target, the molecular modeling studies provide a hypothesis-generating approach that allows one to carry out targeted mutations. These studies are currently ongoing.

PARALLEL PATTERNS?

We recently reported that, in contrast to the oral cavity, the female reproductive track is characterized by low levels of soluble gp340 (Cummins *et al*., 2006) but has high levels of gp340 on the surface of vaginal and cervical epithelial cells, as detected by immunohistochemistry and FACS analyses (Stoddard *et al*.,

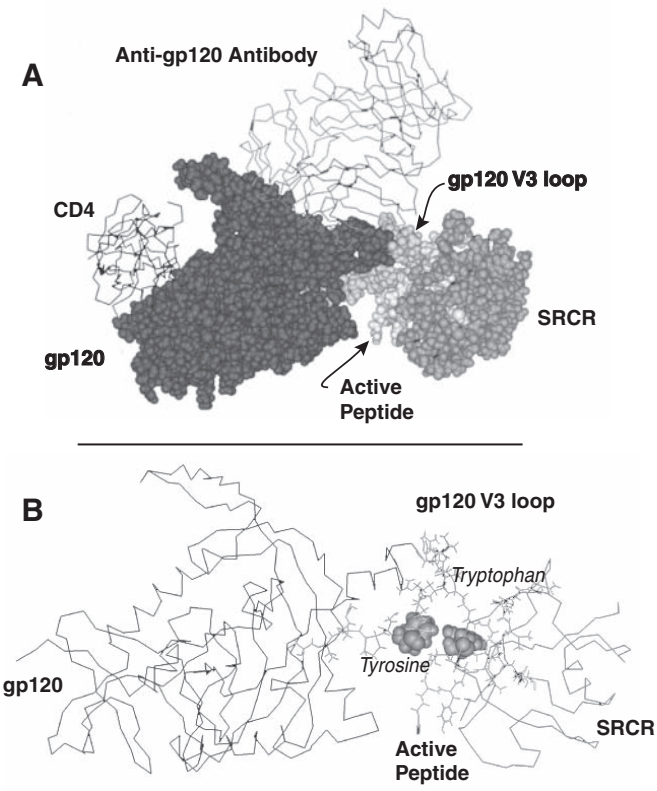

Fig. 3. Space filling (A) and wire frame (B) models showing the putative interactions of the active peptides on an scavenger receptor cysteine-rich (SRCR) domain with the V3 loop of gp120. The positions of tryptophan and tyrosine on the intersecting face are indicated.

2007; Cannon *et al*., 2008). When incubated with HIV-1, cells derived from the oral cavity do not bind virus; in contrast, when vaginal or cervical epithelial cells are incubated with HIV-1, they bind virus and are subsequently able to transfer the captured virions to CD4+ T cells (Stoddard *et al*., 2007). It is thus possible that in the mouth, soluble gp340 aggregates bacteria and inhibits HIV infection whereas bound gp340 increases bacterial adhesion (Rosan *et al*., 1982a, 1982b) and promotes oral disease by increasing bacterial adherence. Instead, in the female reproductive track, gp340 is primarily on the cell surface and is able to bind HIV; these virions can then remain infectious for > 4 days, thereby potentially promoting HIV infection.

DISCUSSION

In this brief overview, we describe a variety of antibacterial proteins present in saliva, most of which also demonstrate antiviral activity. Despite this range of antimicrobial proteins, the oral cavity remains a site for colonization by multiple bacterial species and numerous viruses. These findings suggest a complex interplay between microbial colonization and clearance in the oral cavity. The antimicrobial proteins may be in solution or bound to hard and soft tissues in the oral cavity. Thus, the levels of the antibacterial proteins and the site within the oral cavity where these proteins are found modulate the balance between bacterial adherence and clearance. If salivary agglutinin (gp340) is in its soluble form in saliva, then it acts primarily as a bacterial aggregator leading to clearance. In contrast, when salivary agglutinin is localized to the tooth surface, it serves to foster bacterial adhesion (Carlen *et al*., 1998). In addition, this same glycoprotein can be involved in biofilm formation (Demuth *et al*., 2001; Ahn *et al*., 2008) and thereby interact with other bacteria to promote either attachment to a surface or clearance from the oral cavity.

In attempting to understand the persistence of infectious viruses in the oral cavity despite the presence of potent antiviral activities, it is important to note that most of the antiviral proteins have relatively limited antiviral potency and a narrow range of activity. As such, gp340 interacts with both bacteria and only 2 viruses: HIV-1 and influenza A. Note that these are both enveloped RNA viruses with a susceptible glycoprotein target on their surfaces—gp120 and hemagglutinin, respectively—which suggests a common mechanism of action of gp340 with these 2 viruses. The concentration of specific salivary proteins differs among individuals such that some individuals have higher levels of specific antibacterial and/or antiviral proteins. These levels could be part of the explanation for the differential infectivity with bacteria such as *Streptococcus pyogenes* and *Streptococcus mutans* and viruses such as influenza A and HIV-1, which infect some individuals more efficiently than they do others. It also appears that the oral cavity is susceptible to colonization by a variety of microorganisms and that there is a diversity of antimicrobial activities with unique modes of action. In addition to "backup" in the case of resistance mutations, the existence of multiple active antimicrobial molecules with different modes of bioactivity ensures protection against diverse pathogens.

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