# Saliva - The Sentinel of the Oral Cavity

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**Abstract**

It is generally accepted that saliva is of paramount importance for the maintenance of oral health. This is based on the numerous studies reporting subjective and objective functional losses that occur in persons who lack the ability to produce adequate volumes of saliva. These include dry mouth feeling (xerostomia), difficulty with swallowing food and an increased susceptibility for opportunistic infections. The last issue points to an active protective role of saliva in maintaining oral health under normal conditions.

**Introduction**

Studies in the past have highlighted the importance of saliva in maintaining the integrity of oral tissues. The major functions of saliva are summarized in Table 1. These functions serve to protect the hard and soft oral tissues and can be attributed , in part to particular glycoproteins in saliva, including mucins, praline-rich glycoproteins, α-amylases, lactoferrin, salivary peroxidase, secretary IgA and kallikerin. It has been recognized that saliva contains many components that, in one way or another interact with microorganisms, in this way controlling the composition of the oral microflora. This review, primarily dealing with the defensive systems in saliva, will in particular focus on the more recently obtained data, aiming to (re)interpret earlier observations on the basis of our present knowledge. In this respect it is intended to give an update of the current knowledge and insights in the protective role of salivary components.

**Proposed Functions of Saliva**

1. Physico-mechanical flushing of the oral cavity
2. Tissue coating: lubrication & permeability barrier- (a) soft tissue: mucous coat concentrates protective molecules (b) soft tissue/prosthetic interface: thin film for retention and cushion (c) hard tissue: acquired enamel pellicle
3. Modulation of oral flora: (a) promotes selective clearance and adherence of microbial flora (b) anti-microbial actions (c) utilization as a metabolic substrate
4. Antacid and neutralization of deleterious materials: (a) buffering capacity (b) formation of base (c) complex formation with tannins
5. Regulation of calcium/phosphate equilibrium (a) intracellular: secretary granule maturation (b) extracellular : mineralization
6. Digestion : (a) taste acuity (b) neutralization of esophageal contents (c) dilution of gastric chyme (d) formation of a food bolus (e) initial breakdown of starches
7. Extracellular post-translational processing of salivary molecules.

**Blood group active glycoproteins: mucins and salivary agglutinin**

**Salivary mucins:** Salivary mucins occur as a single high molecular weight species containing blood group activity. Saliva contains two genetically distinct mucin types, designated MG1 and MG2 originating from the MUC5B and MUC7 gene respectively. MUC7 (MG2) has been found to exist in two glycoforms, MG2a and MG2b respectively. Salivary MUC5B (MG1), which displays blood group activity, exists in at least three different glycoforms, differing in sialic acid and sulphate content, depending on the glandular source. MUC5B functionally and structurally belongs to the classical mucins, which are the main constituents of the slime layers that cover the mucous epithelia throughout the body, e.g. in the gastrointestinal tract, the urogenitary tracts and the respiratory tracts. These function as barriers protecting the underlying epithelium against mechanical damage and preventing direct entrance of noxious agents including bacteria and viruses into the underlying vulnerable epithelium. In the oral cavity, MUC5B is present in the protein films (pellicles) covering the enamel and epithelial surfaces and in this quality protect against acidic attacks and modulate the microbial colonization of these surfaces.

**Agglutinin :** Salivary agglutinin is highly glycosylated protein, with a molecular mass of approximately 340 kDa that carries blood group active antigens. Except for the presence of blood group antigens, salivary agglutinin shares a number of features with MUC7: both are monomeric, heavily glycosylated proteins, with extremely sticky properties. As a consequence, under native conditions these proteins occur associated with a variety of salivary proteins, including S-IgA. Agglutinin, initially identified as protein responsible for the S. mutans aggregating properties of parotid saliva was later found to mediate also the binding between S. mutans and S. sanguis. This salivary glycoprotein is also detectable as a component of the salivary pellicle on the tooth surface.

**Cystatins-cysteine proteinase inhibitors :** In saliva at least nine different cystatin isoforms are secreted, including the neutral cystatin SN, three moderately anionic isoforms of cystatin SA, three or four isoforms of the more anionic cystatin S and cystatin C, a cationic cystatin. Since they have their proteinase inhibiting properties, cystatins have been suggested to play a role in controlling proteolytic activity, either from the host (released during inflammatory processes) or from microorganisms. However, there is experimental evidence suggesting that cystatin C, although a minor constituent of the total population of oral cystatins, contributes most to the cysteine proteinase inhibiting activity of saliva. Salivary cystatins are constitutively secreted in humans, but evidence has been produced that in particular cystatin C levels are increased following severe inflammation in periodontal diseases.

**Von Ebner glands protein (VEGh) and secretary leucocyte proteinase inhibitor (SLPI)**

**VEGh, as a cysteine proteinase inhibitor:** VEGh is a salivary protein secreted by the Von Ebner glands located around the circumvallate and foliate papillae of the tongue. Originally it has been assumed that VEGh was involved in the perception of bitter taste by binding lipophilic bitter compounds and transporting them to the taste buds. However, later it was demonstrated that VEGh can act as inhibitor of cysteine proteinases. Evidence has also been produced that VEGh can act as an oxidative-stress induced scavenger of peroxidation products.

**SLPI :** Another example of salivary protein with more than one function is SLPI which is expressed in various secretary tissues, including the submandibular, sublingual, parotid and minor salivary glands. In addition to its proteinase inhibitory properties, SLPI has antimicrobial and antiviral properties.

**Tissue Inhibitors of Metallo-proteinases (TIMPS):** In parotid and submandibular secretions TIMP-1 has been identified, a member of the family of tissue inhibitors of matrix metalloproteinases (MMPs). Considering their potent inhibitory action against MMPs, including collagenase, gelatinase and stromelysin, TIMPs are thought to play an important role in turnover and remodeling of the extracellular matrix. In saliva the main type of collagenase derives from the host

leucocytes, suggesting a role of salivary TIMP-1 in control of inflammatory diseases. Such a function would also be suggested by the finding that TIMP levels in saliva of periodontitis patients, which have elevated collagenase activities, are decreased compared with those of healthy controls.

**Lactoferrin:** The presence of lactoferrin in secretions such as tears, milk and saliva is usually linked with its iron binding properties. There is general consensus that the long known bacterostatic effects of lactoferrin are the result of its iron depriving effects. One of the target microorganisms of lactoferrin is the peripathogenic bacterium A. actinomycetemcomitans. It has been reported that in periodontitis patients, carrying A. actinomycetemcomitans, a negative correlation exists between the number of subgingival A. actinomycetemcomitans and the lactoferrin concentration in saliva. It is now clear that lactoferrin is a multifunctional protein having bacteriostatic, bactericidic, fungicidal, antiviral and anti-inflammatory and immunomodulatory properties.

**Antimicrobial enzymes-lactoperoxidase, lysozyme and chitinase:** Salivary peroxidases catalyse the formation of bactericidic compounds, e.g. hypothiocynate, by peroxidation of thiocynate. Dentrifices and mouthrinses have been marketed which enhance the endogenous activity of salivary peroxidase, by supplementing $H_2O_2$-generating enzyme systems because of the antimicrobial effects of the lactoperoxidase system.

Lysozyme is ubiquitously present in body fluids, including saliva, tears, bronchial mucous and sweat. The well-known antimicrobial activity of lysozyme is generally linked with its lytic action on bacteria by catalyzing the hydrolysis of cell wall polysaccharides. In addition, non-enzymatic bactericidic activity has been documented for lysozyme as well, which has been attributed to activation of bacterial autolysins.

Chitinase which catalyses the hydrolytic cleavage of chitin is present in saliva, where it is derived from the parotid, submandibular, sublingual and palatine glands. As chitin is a constituent of the yeast cell walls, chitinase activity may play a role in the protection against colonization of oral epithelial cells by yeast. Interestingly, in saliva of periodontal patients, chitinase levels are significantly elevated compared with those in healthy controls.

**Antimicrobial peptides-histatins and defensins:** Besides killing of bacteria such as S. mutans and yeasts, histatin 5 has been implicated in a variety of processes, including pellicle formation, neutralization of potentially noxious substances, e.g. polyphenols, chelation of metal ions, inhibition of inflammatory cytokine induction, and inhibition of host and bacterial proteinases, including metalloproteinases and cysteine proteinases.

Recently, β-defensins have also been demonstrated in saliva. These antimicrobial peptides, which are induced in epithelial tissues upon inflammation, are also expressed in duct cells of salivary glands. These peptides are part of the innate immune system; they have broad spectrum antibacterial and antifungal activity and they have properties that may serve to link innate immunity with the acquired immune system.

**Calprotectin :** Calprotectin is a calcium and zinc-binding protein derived mainly from granulocytes, monocytes and macrophages. It appears to inhibit microbial growth through competition for zinc. The main sources of salivary calprotectin appear to be the gingival crevicular fluid and the oral surface epithelium. As the calprotectin concentration rises markedly in some inflammatory diseases, this protein has been thought to be a marker of inflammatory disease.

**Equilibrium in microbial ecology :** The oral cavity is colonized by a great number species of microorganisms, varying greatly in quantity. For example, S. oralis and S. sanguis are abundantly present on the surfaces of teeth, whereas S. mutans forms only a minority of supragingival plaque. That an equilibrium system in microbial ecology exists in the oral cavity can be illustrated by the fact that about half of the population carries Candida albicans without phenomena of candidiasis. In other words, in these cases candidiasis is an opportunistic disease emerging after lowering of the immune system. The equilibrium in the ecology of the microorganisms in the oral cavity will be maintained not only by the diet but also through the interactive inhibition between the bacterial species themselves. An example of the last mechanism is the discovery of the release of Lantibiotics by S. salivarius that can inhibit the growth of S. pyogenes.

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