



Salivary proteins

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Functions of salivary secretions

- Physico-mechanical flushing of the oral cavity
- Tissue coating: hydration and formation of a permeability barrier
- Modulation of the oral flora
- Regulation of Ca/PO₄ equilibrium
- Anti-acid and neutralization of deleterious materials
- Digestion: formation of a food bolus and deglutition



Inorganic composition of saliva

- Ca
- PO₄
- K
- Na
- HCO₃
- Cl
- pH 6.8-7.3



Organic composition of saliva

- Small MW: urea, uric acid, lipids, amino acids
- Proteins: synthesized by glands or not synthesized but released

Acquired enamel pellicle precursor proteins

- AEP: organic film covering teeth that appears to protect the tooth surface
 - Formed by selective adsorption of salivary proteins on tooth enamel
- Determination of proteins content largely dependent on *in vitro* studies
 - *In vivo* samples- small
 - *In vitro* incubation of HAP beads or ground enamel in whole saliva
 - *In situ*: enamel slabs placed in the mouth
- Pellicle precursor proteins are those that exhibit high affinity to HAP

Identification of proteins in pellicle

- Salivary proteins
 - sIgA, acidic PRP, cystatin, amylase, MG1, salivary CA, lactoferrin, lysozyme, histatin 1, statherin
- Other non salivary proteins
 - albumin, GTF, complement
- *In vitro* studies show that PRPs are the most abundant followed by cystatins and lysozyme
- The exact composition *in vivo* is not known due to the difficulty in obtaining sufficient sample for analysis

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are needed to see this picture.

Acquired enamel pellicle precursor proteins

- Acidic proline-rich proteins
 - Encoded by a gene family (6 genes) and secreted mainly by parotid and SMG
 - 6 major proteins: PRP1-4, PIFs, and PIFf
 - Similar primary structure with asymmetric distribution of a.a.(polar toward N-terminal)
 - Functions
 - » Pellicle formation
 - » Inhibit crystal growth
 - » Inhibit spontaneous precipitation of CaPO_4 salts
 - » Modulation of bacterial colonization (cryptitopes)

Acquired enamel pellicle precursor proteins

- Statherin

- Small asymmetric molecules with high negative charges on the N-terminal
- Secreted by parotid and SMG
- Functions
 - » Pellicle formation
 - » Inhibition of spontaneous precipitation
 - » Inhibition of crystal growth

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Acquired enamel pellicle precursor proteins

- Histatins

- Histidine-rich protein family secreted by parotid and SMG
- 3 major histatins: Hst 1, 3, and 5
- Function
 - » Pellicle formation
 - » Antibacterial effect- prevent co-aggregation of *P. gingivalis* and *S. mutans*, inhibit bacterial collagenase and trypsin-like proteases, and induce histamine release from mast cells
 - » Antifungal effect- Hst 5 most potent, candidacidal effect apparently mediated by specific binding to a cell protein followed by internalization of Hst and interaction with intracellular targets
 - » Inhibits crystal growth (Hst 1 only)



Acquired enamel pellicle precursor proteins

- Cystatins
 - Cysteine-containing proteins secreted by SMG and SLG
 - 3 proteins identified, cystatin SA-1 best characterized- negatively charged terminals and positive charge on middle part
 - Function
 - » Pellicle formation
 - » Inhibition of crystal growth
 - » Inhibits cysteine proteinases (SA-1)

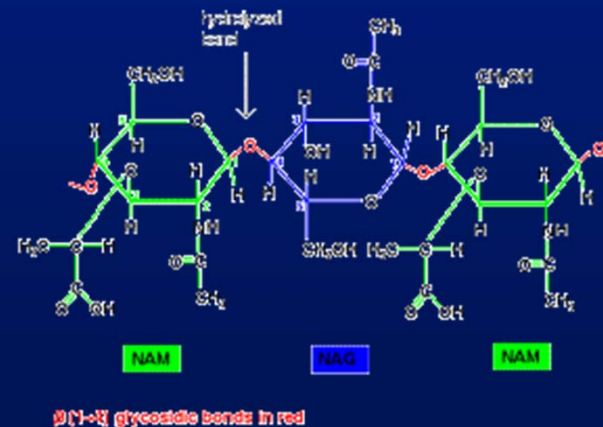
Antibacterial salivary proteins

- Lysozyme

- Secreted by parotid and SMG

- Function

- » Muramidase activity- hydrolyze $\beta(1-4)$ bond between N-acetylmuramic acid and N-acetyl glucosamine in the peptidoglycan layer of the bacterial cell wall. Gram⁻ bacteria resistant due to LPS layer.
 - » Strongly cationic protein can activate bacterial autolysins which can destroy the cell wall
 - » Effective against *S. mutans*



Antibacterial salivary proteins

- Lactoferrin
 - Iron-binding glycoprotein secreted by serous cells of most glands
 - Function
 - » Bacteriostatic effect by bacterial deprivation of essential Fe
 - » Bactericidal effect by direct binding of the iron-free form to the cell wall
 - » Active against *S. mutans*

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Antibacterial salivary proteins

- Salivary peroxidase
 - Secreted by acinar cells of parotid and SMG
 - Mechanism-oxidation of thiocyanate to hypothiocyanate
 - » $\text{SCN}^- + \text{H}_2\text{O}_2 \rightarrow \text{OSCN}^- + \text{H}_2\text{O}$
 - » OSCN⁻ affects some glycolysis enzymes, bact. metabolism, and growth inhibition
 - Antimicrobial effect *in vitro* on *S. mutans*, lactobacilli, yeast, some viruses, and some perio pathogens even after adsorption to HAP

Antibacterial salivary proteins

- Salivary sIgA
 - Predominant immunoglobulin class in oral secretions
 - Most important functional element of the oral immune defense system
 - Functions
 - » Aggregate oral microbes
 - » Prevent their adherence to mucosal surface receptors (binds to bacteria and coats mucosal surfaces)
 - » Neutralize viruses
 - Synthesis of IgA and J-chain in plasma cells with a secretory piece added in acinar cells which makes it more resistant to proteolysis
 - Levels of IgA detected since infancy, increases toward adulthood. Specific Ab levels, like to *S. mutans* GTF, are highest in adulthood



Antibacterial salivary proteins

- Agglutinins
- α -microglobulin
- Fibronectin

Other salivary proteins

- **Amylase**
 - Major protein produced by salivary glands, mostly by parotid and by SMG
 - Present in different isozymes (1-5) divided in two families depending on the degree of glycosylation
 - Cleaves < 1-4 bonds between glucose molecules in starch producing maltose and glucose (cariogenic potential)
 - Inactivated in stomach by acidic content

Other salivary proteins

- **Mucins**
 - Present in the mucous layer of all epithelial surfaces
 - High molecular weight glycoproteins consist of a protein core (approx. 15-20% of the molecule) and O-linked glycans (80% CHO) with abundant Ser and Thr
 - Encoded by 11 genes of which 2 are associated with salivary mucins (MUC5B and MUC7) but nonsalivary specific mucins are also expressed (MUC1 and 4)
 - Protein structure characterized by a central region of multiple tandem repeats rich in Ser and Thr
 - Classified in for groups
 - » Gel-forming mucins: MUC5B
 - » Small, soluble mucins: MUC7
 - » Membrane-bound mucins: MUC1 and 4
 - » Uncertain classification

Other salivary proteins

- Mucins

- Mostly secreted by SMG, SLG, and minor salivary glands
- Two distinct salivary mucins identified
 - » Mucin glycoprotein 1 (MG1)-product of MUC5B, gel-forming, multimers of very high molecular weight
 - Functions
 - Viscoelastic and rheologic properties of saliva facilitating mastication, speech, and swallowing (coating the bolus)
 - Binds and facilitate clearance of oral microbes- e.g. *P. gingivalis* and *C. albicans*
 - Covers tooth surface as a component of the AEP, this may promote bacterial binding to the tooth surface
 - » Mucin glycoprotein 2 (MG2)- product of MUC7, soluble form, monomeric
 - Functions
 - Promotes agglutination and clearance of bacterias
 - The products of MUC1 and 4 are membrane bound and help in the formation of the mucous protective layer on epithelial tissue

Other salivary proteins

- Mucins
 - The high degree of glycosylation and potential for hydration protect the tissue from desiccation and drying of tissues, from mechanical and chemical injury, microbial assault while lubricating tissues for mastication, speech and swallowing.

