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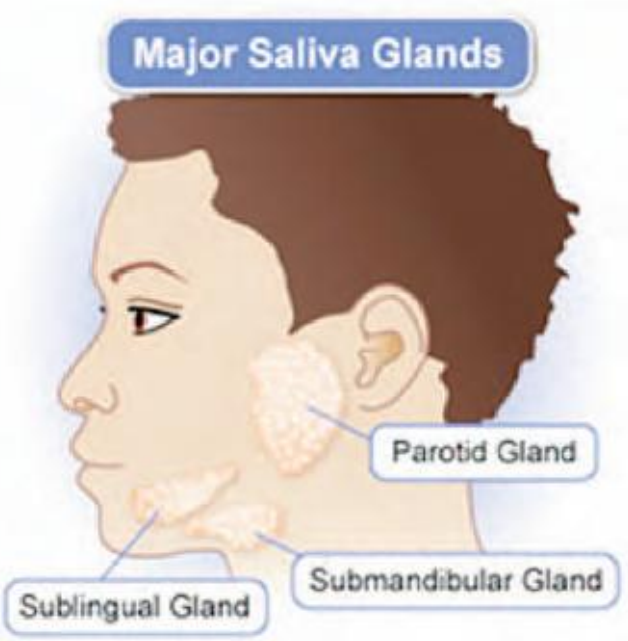
Biochemistry of Saliva & Teeth

Total Saliva

- Total (whole) saliva: the complex mixture of fluids from the salivary glands, the gingival fold, oral mucosa transudate, in addition to the mucous of the nasal cavity and pharynx, non-adherent oral bacterial, food remainders, desquamated epithelial & blood cells, as well as traces of medications or chemical products



Saliva – composition

- Salivary fluid is an exocrine secretion
  - ✓ Water (99%); the remainder 1% are:
  - ✓ Electrolytes: Na, K, Ca, Cl, Mg, HCO_3, PO_4
  - ✓ Proteins: enzymes, Igs, antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides
  - ✓ Also: glucose and nitrogenous products (urea & ammonia)
    - ❖ The components interact & are responsible for the various functions attributed to saliva
- Salivary Flow (SF) index:
  - ✓ Normal salivation (1-1.5L)
  - ✓ Very low (hyposalivation)
  - ✓ Low
  - ✓ *High or very high (hypersalivation)*

Saliva – Function

- **1. Taste**
- ✓ **The SF is hypotonic (plasma; low levels of glucose, Na, Cl, & urea)**
- ✓ **Gustin (salivary protein): necessary for the growth & maturation of taste buds**

- **2. Protection and Lubrication**
- ✓ **Mucosal covering to lubricate & protect against irritating agents**
- ✓ **Mucins: high carbohydrate content; responsible for lubrication, protection from dehydration, & maintenance of salivary viscosity**
- ✓ **Mastication, speech, & deglutition (swallowing) are aided by the lubricant effects of these proteins**

Saliva – Function

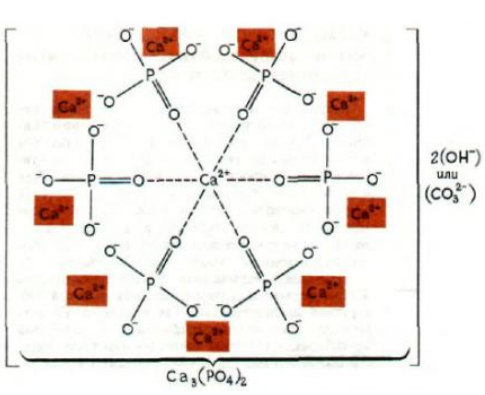
- 3. Dilution & Cleaning
  - ✓ Its' fluid consistency provides mechanical cleansing (non-adherent bacteria & cellular & food debris)
  - ✓ SF tends to eliminate excess carbohydrates, thus, limiting the availability of sugars to the biofilm microorganisms
  - ✓ SF reduction causes a drastic change in the level of oral cleaning

- 4. Tissue Repair
  - ✓ The bleeding time of oral tissues is shorter than other tissues although the resulting clot is less solid than normal
  - ✓ Experimental studies in mice have shown that the epidermal growth factor is the cause (produced by submandibular glands)

Saliva – Function

- 5. Buffer Capacity
  - ✓ Buffers: neutralizes acids produced by acidogenic organisms, thus, preventing enamel demineralization
  - ✓ The bicarbonate & phosphate buffer systems (the two most important)
  - ✓ Negatively loaded residues on salivary proteins work as buffers
  - ✓ Sialin (salivary peptide): increases the biofilm pH after exposure to fermentable carbohydrates
  - ✓ Urea: causes a rapid increase in biofilm pH by releasing ammonia and CO_2 when hydrolyzed by bacterial ureases
    - Children with chronic renal insufficiency present with less caries than healthy children
    - Ammonia: is cytotoxic to gingival tissues initiating gingivitis

Saliva – Function



- **6. Integrity of Tooth Enamel (remineralization & demineralization)**
  - ✓ **Main factors for stability of hydroxyapatite: Ca, PO_4^{3-}, F^-, & pH**
  - ❖ **Ca, PO_4^{3-}: ionic exchanges towards/from the tooth (saturation)**
    - ○ **Post-eruptive maturation & Remineralization of a carious tooth before cavitation**
    - ○ **Conc. of salivary Ca varies with SF and is not affected by diet**
  - ❖ **Fluoride in saliva:**
    - ○ **Its concentration in total saliva is related to its consumption**
    - ○ **F^- reduces mineral loss when biofilm pH drops (↓ solubility of hydroxyapatite; more resistant to demineralization)**
    - ○ **Fluoride also reduces the production of acids in biofilm**

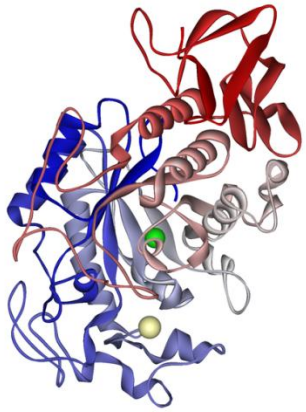
Saliva – Function

- **6. Integrity of Tooth Enamel (remineralization & demineralization)**
  - ✓ **Depending on pH, salivary Ca can be ionized or linked**
    - **Ionized: establishing equilibrium between the calcium phosphates of enamel and its adjacent liquid (saturation)**
    - **Non-ionized: can be linked to**
      - **Inorganic ions (phosphate, bicarbonate, fluoride)**
      - **Small organic ions (citrate)**
      - **Macromolecules (statherin, histidine-rich peptides, and proline-rich proteins)**
  - ✓ **A special case of the combination of calcium is its strong link with α-amylase, where it acts as a co-factor necessary for the enzyme function**

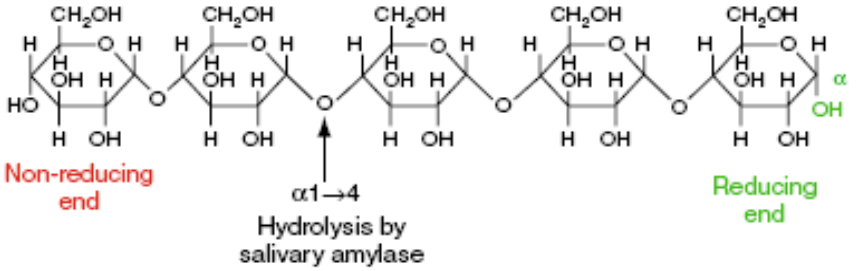
Saliva – Function

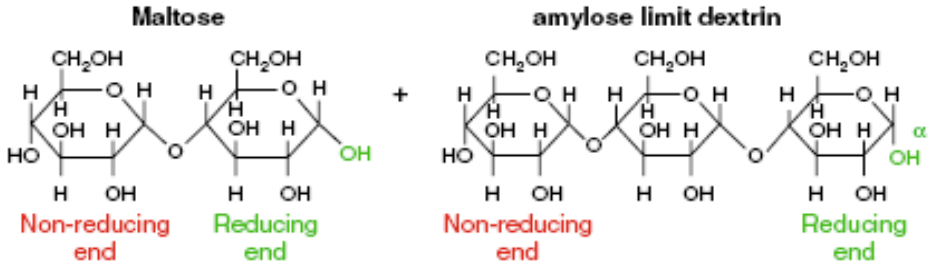
- **7. Digestion**
  - ✓ **Initial digestion of starch (maltose, maltotriose, & dextrins); favoring formation of food bolus**
  - ✓ **Mainly due to the digestive enzyme α-amylase (ptyalin); Parotid (80%)**
  - ✓ **It is considered a good indicator of properly functioning salivary glands? (40-50)% of total salivary protein produced**
  - ✓ **Lingual lipase: has a pH optimum ~4. So, it is not activated till entering an acidic environment**

α-amylase



- Two isoforms: salivary & pancreatic
- Salivary amylase (ptyalin):
  - ✓ Breaks starch (insoluble) into maltose and dextrins (soluble)
  - ✓ Acts on linear α(1,4) glycosidic linkages
  - ✓ Inactivated in the stomach
- Optimum conditions for ptyalin:
  - ✓ Optimum pH (~6) & T (37°C)
  - ✓ Anions & activators:
    - Cl & Br: most effective
    - Iodide: less effective
    - SO_4 & PO_4: least effective





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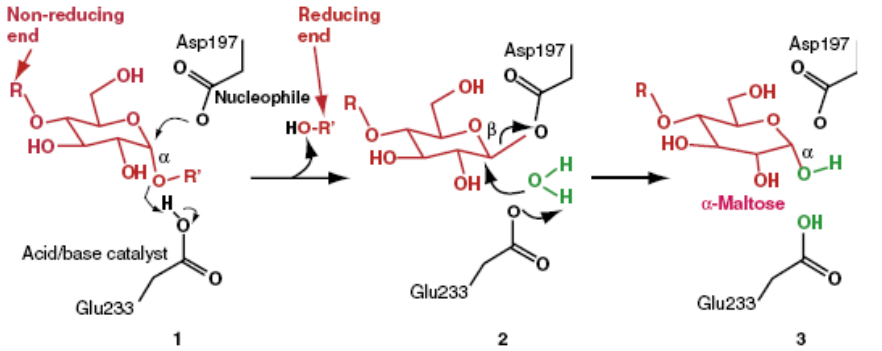


Saliva – Proteins

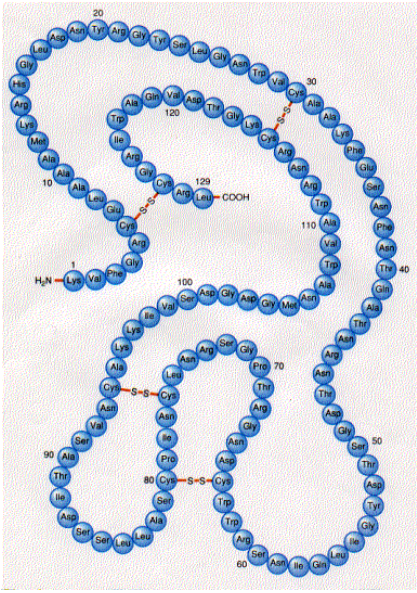
- **8. Antibacterial Properties & Participation in Film & Calculus Formation**
  - ✓ **Immunologic & non-immunologic proteins with antibacterial properties**
  - ✓ **Also, some proteins are necessary for inhibiting spontaneous precipitation of calcium & phosphate ions in the salivary glands & in their secretions**
  - ✓ **Secretory IgA: the largest immunologic component of saliva. It can neutralize viruses, bacterial, and enzyme toxins. It serves as an antibody for bacterial antigens and is able to aggregate bacteria, inhibiting their adherence to oral tissues**

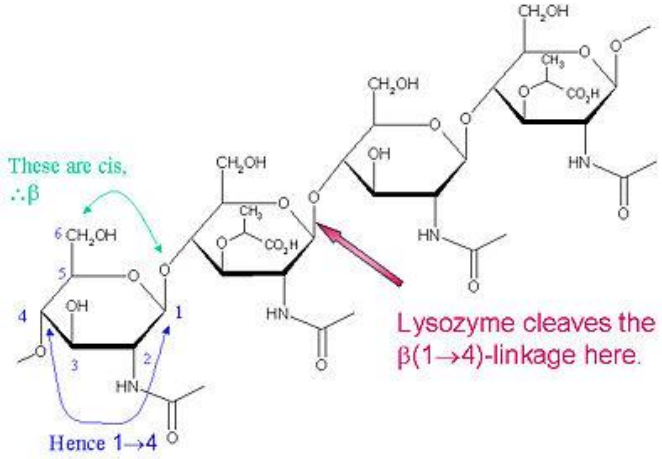
Saliva – Function

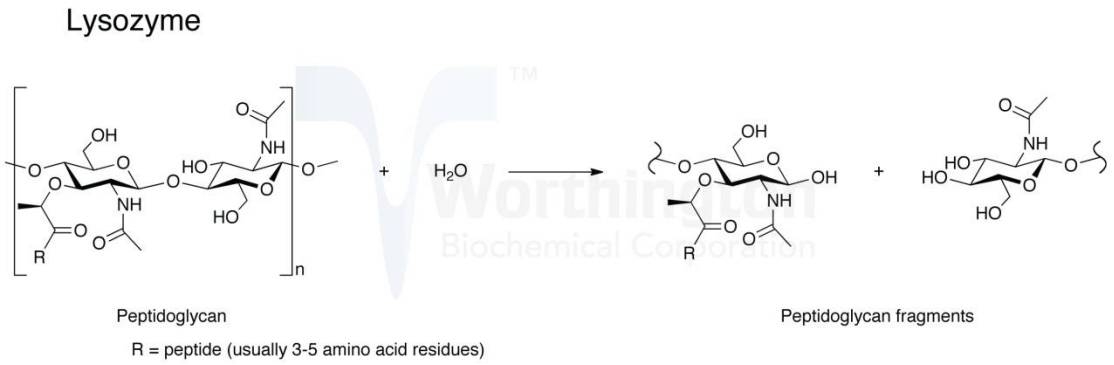
- **8. Antibacterial Properties and Participation in Film and Calculus Formation**
  - ✓ **Other immunologic components, such as IgG and IgM, occur in less quantity and originate from gingival fluid**
  - ✓ **Non-immunologic salivary protein components:**
    - **Enzymes: lysozyme, lactoferrin, and peroxidase**
    - **Mucin glycoproteins**
    - **Agglutinins**
    - **Histatins**
    - **Proline-rich proteins**
    - **Statherins**

Saliva – Enzymes - Lysozyme

- Lysozyme (4 S-S bonds) can hydrolyze the <u>cellular wall of some bacteria (β 1-4 linkage)</u>, and because it is <u>strongly cationic</u>, it can activate the bacterial "autolysines" which are able to destroy bacterial cell wall components
- <u>Gram-negative bacteria are more resistant</u> to this enzyme due to the protective function of their <u>external lipopolysaccharide layer</u>

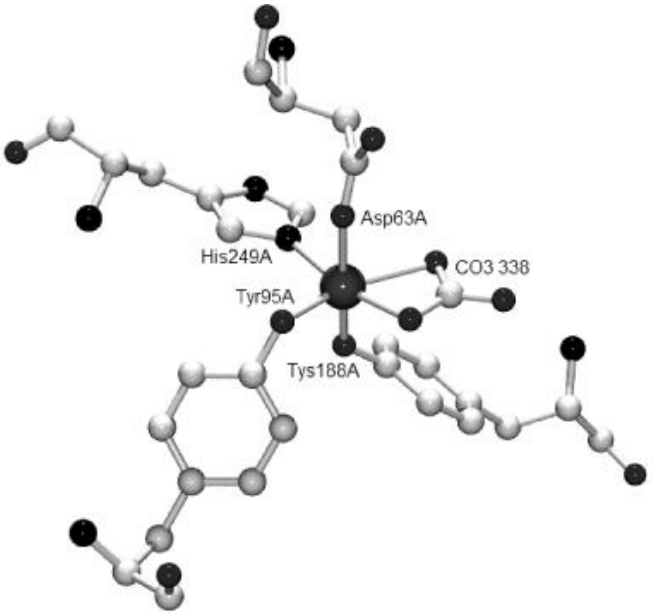






Saliva – Enzymes - Lactoferrin

- Lactoferrin scavenges free iron in the saliva causing bactericidal or bacteriostatic effects on various microorganisms requiring iron for their survival
- Lactoferrin also provides fungicidal, antiviral, anti-inflammatory, and immunomodulatory functions



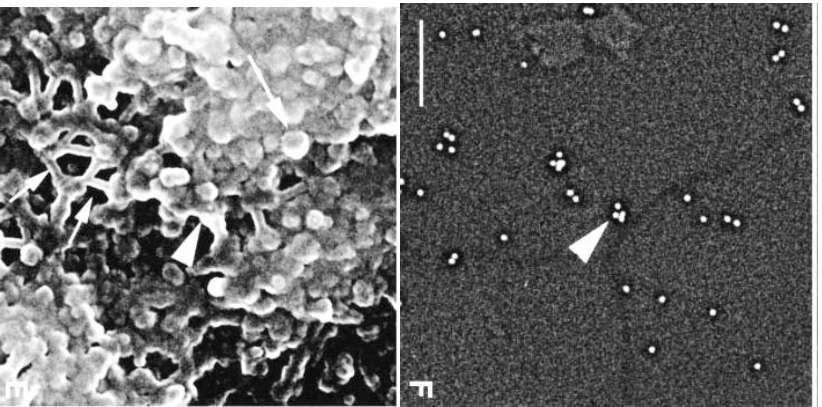
Saliva – Enzymes - Peroxidase

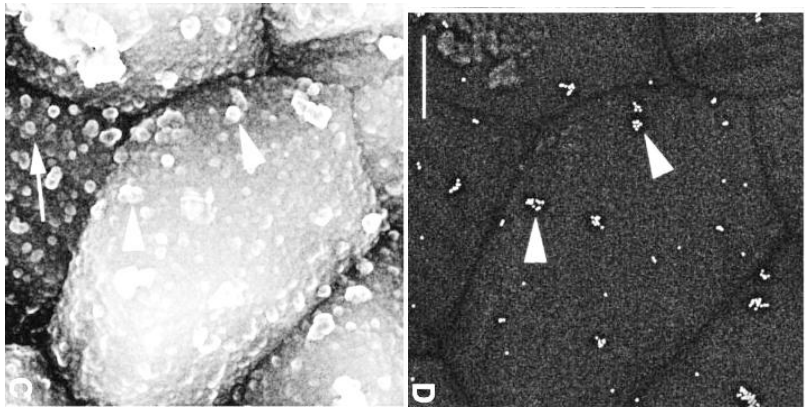
- **Peroxidase (sialoperoxidase) offers antimicrobial activity because it serves as a catalyst for the oxidation of the salivary thiocyanate ion by hydrogen peroxide into hypothiocyanate, a potent antibacterial substance**
- **As a result of its consumption, proteins and cells are protected from the toxic and oxidant effects of hydrogen peroxide**

$$H_2O_2 + \text{thiocyanate ion } (SCN^-) \xrightarrow{\text{Salivary Peroxidase}} \text{Hypothiocyanite ion } (OSCN^-/HOSCN) + H_2O$$

Saliva – Proteins – PRP_s & Statherins

- **Proline-Rich Proteins**
  - ✓ **Negatively charged Ca^{2+} binding phosphoproteins: Inhibit the spontaneous precipitation of calcium phosphate salts & the growth of hydroxyapatite crystals on the tooth surface; preventing the formation of salivary & dental calculus**
  - ✓ **Two phosphoserines - additional negative charges; favoring oral structure lubrication**
  - ✓ **Ca^{2+} balance, strongly prevents precipitation**





Saliva – Proteins – Histatins

- A family of His-rich peptides, largest is phosphoprotein, others not
  - ✓ Have antimicrobial activity against strains of bacteria & fungi
  - ✓ The bactericidal & fungicidal effects occur through union of positively loaded histatins with the membranes resulting in destruction of their architecture & altering their permeability
- Other functions:
  - ✓ Inhibition of histamine release by the mastocytes, suggesting a role in oral inflammation
  - ✓ Salivary agglutinin: a highly glycosylated protein frequently associated with secretory IgA, responsible for bacteria agglutination
  - ✓ Also Ca2+ balance