SUMMARY

The outer layer of our teeth is covered with a layer of enamel that varies in thickness from about 1-3 mm. Dental enamel consist of the mineral hydroxyapatite (~96 wt%), an organic matrix (~1 wt%, mainly proteins and lipids) and water.

Sometimes the enamel is locally covered with plaque, containing oral bacteria, proteins, polysaccharides and other components. Due to acids, originating from temporarily metabolic activities of oral bacteria after food consumption, the enamel can be demineralized locally. In vivo, this process is called: enamel caries.

The mineral distribution pattern of carious enamel as function of depth (perpendicular to the outer enamel surface) may be described as either a surface softening or a subsurface lesion. A surface softening is characterized by a low mineral content of the surface enamel that increase continuously inwards, while a subsurface lesion is characterized by a relatively intact surface layer covering the lesion body low in mineral content.

From clinical studies it is obvious that demineralized enamel can be repaired naturally (remineralized) due to the interaction with saliva.

This thesis deals with protein-mineral interactions in carious enamel. A short introduction is given in Chapter I.

The aim of this investigation was:
- to determine protein changes in dental enamel during demineralization.
- to obtain information in the protein-mineral interactions in demineralized enamel.

In Chapter II a literature survey on enamel proteins is given; a limited attention is given to the enamel mineral.
The protein changes in enamel during maturation, the amino acid compositions and some properties of proteins in embryonic and mature enamel are described. This information shows that human and bovine enamel proteins are similar. Furthermore, the results of other investigations dealing with proteins and enamel demineralization are surveyed.

Chapter III describes the analysis of enamel proteins released into a demineralizing solution during lesion formation. Demineralization was done with a system based on the principle of flow-dialysis. This flow-dialysis method combines the need for a large demineralizing volume to enamel surface ratio for mineral dissolution and a small ratio for easy handling in protein isolation. The results show that the protein release during lesion formation was not proportional with the mineral loss.

In Chapter IV the protein changes of enamel during lesion formation and surface softening are compared. The lost proteinaceous matter consists mainly of peptides (mol. wt < 1800). Protein loss during demineralization is roughly 50-60% of the proteins soluble under relatively mild acidic conditions (pH 4.5) and is about 20-25% of total proteins of sound enamel. Amino acid composition of lost material showed more acidic amino acids in case of surface softening as compared with lesion formation.

The following model is proposed for protein release during demineralization: acid from the external solution causes an initial mineral dissolution resulting in an increased porosity of the enamel. Acid soluble proteins are dissolved during or after mineral dissolution, diffuse outwards and are partially adsorbed on newly created sites of partially dissolved crystallites. In contrast with surface softening, extra new adsorption sites are available in the surface layer during lesion formation and may cause the differences in both amino acid composition and mol. wt distribution.

Demineralization with protein (BSA) present in the demineralizing solution is described in Chapter V. The mineral loss was strongly decreased by the presence of BSA in solution, while albumin penetration into the enamel was demonstrated. This protein penetration into enamel during demineralization was compared with protein penetration into porous enamel demineralized with protein free acidic solutions. Albumin treatment after demineralization at pH 4.7 showed quantitatively comparable uptake with albumin uptake during demineralization while treatment lowered protein uptake. Albumin treatment; there was no influence.

In Chapter VI the interaction of enamel with mineral has been analyzed (Chapter IV) was washed with 75 mM, while complete uptake of phosphate, fluoride albumin was completely (treatment) or partially (pretreatment with rough calculation indicated but mainly bound to the mineral. A considerable amount of albumin removed from the surface with 75 mM, while complete uptake of phosphate, fluoride albumin was completely (treatment) or partially (pretreatment with rough calculation indicated but mainly bound to the mineral. A considerable amount of albumin removed from the surface after albumin treatment after demineralization at pH 4.7 showed quantitatively comparable uptake with albumin uptake during demineralization while treatment lowered protein uptake. Albumin treatment; there was no influence.

Extrapolating the albumin interactions suggest a large role in remineralization processes, as proteins for the hydroxyapatite formation because of the presence of minerals.
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ation; there was no influence of fluoride on the albumin uptake.

In Chapter VI the interactions of albumin penetrated into the porous enamel with mineral have been investigated. The albumin containing enamel (Chapter IV) was washed with a non-complexing buffer solution (HEPES, pH 6.8) and subsequently the enamel was treated with increasing concentrations of phosphate, fluoride, chloride or calcium. It was found that the albumin was completely (treatment with low albumin concentration, Chapter IV) or partially (pretreatment with concentration >200 µg.ml⁻¹) washed out. A rough calculation indicated that this albumin was not in the enamel liquid, but mainly bound to the mineral.

A considerable amount of the non-washable albumin released by treatment with 75 mM, while complete removal occurred with 150-200 mM fluoride or phosphate solutions. Treatment with chloride or calcium did not remove significant amounts of albumin.

These results on albumin removal permit the following description of the protein mineral interactions: albumin removal upon washing indicates a weak interaction and might be caused by the loss of the native form of albumin upon binding to the partially dissolved crystallites.

Albumin removal with the lower fluoride or phosphate concentrations indicates interactions via Ca-bridges between albumin and mineral, whereas the removal at high fluoride or phosphate concentrations are due to the interaction of protein carboxyl groups with surface bound calcium of the crystallites.

Extrapolating the albumin-mineral interactions to salivary protein-mineral interactions suggest a large influence of salivary proteins on enamel de- and remineralization processes, because of the high affinity of several salivary proteins for the hydroxyapatite. This may even be more stronger than expected because of the presence of several salivary phosphoproteins.