The adsorption of peptides and purified salivary proteins onto tooth enamel. A study on pellicle formation
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SUMMARY

The most common diseases that occur in the oral cavity are dental decay (caries) and inflammation of the soft tissues surrounding the teeth (periodontal disease). Both caries and periodontal disease are caused by bacterial metabolites in the plaque, an organic layer on the tooth surface, which consists of bacteria in a matrix composed of proteins and polysaccharides. The bacteria in the plaque digest sugar to form organic acids, which cause the dissolution of the tooth enamel (caries).

The dental plaque is attached to a bacteria-free protein layer present on the tooth enamel. This protein layer is called the acquired enamel pellicle or more simply the "pellicle". The pellicle forms on the tooth surface as a result of selective adsorption of salivary proteins, e.g. some proteins are adsorbed in larger quantities than others. The pellicle forms very rapidly. In consequence this layer determines the attachment of bacteria onto the tooth surface. Furthermore the pellicle has been shown to protect the enamel against acid attack.

The aim of this investigation was to:

a) isolate and characterize some of the salivary proteins found in the pellicle,

b) investigate which of the properties of salivary proteins are important in the adsorption process,

c) obtain information on the mechanism of protein adsorption onto tooth enamel.

Chapter 1 is a short introduction to this thesis. It can be seen that adsorbed protein layers form on any surface in contact with body fluids. A short description of the pellicle is included and a number of its functions are discussed.
Chapter 2 describes the results of other investigations relevant to this thesis. These are mainly concerned with tooth enamel, human saliva and protein adsorption onto solid surfaces, in particular onto enamel or onto pure hydroxyapatite (tooth enamel is composed of 98% hydroxyapatite by weight). The reasons for using intact enamel surfaces, submandibular saliva and synthetic homopolypeptides in this study are also discussed.

Chapter 3 describes the isolation and characterization of some of the proteins present in both human submandibular saliva and 'in vitro' pellicles. Four of these proteins were purified; three of them (I, II, III) had acidic properties (isoelectric point - 4.7), whereas the fourth (IV) had an isoelectric point of 6.9. The proteins II and III contained phosphate. The phosphoproteins were adsorbed onto hydroxyapatite in larger quantities than the non-phosphorylated proteins.

The adsorption of two positively and two negatively charged homopolypeptides onto tooth enamel is described in Chapter 4. The adsorption was studied by incubating bovine enamel slabs in a peptide solution for differing periods of time. The amount of peptide adsorbed was calculated from the differences in peptide concentration in solution before and after incubation. The two positively charged homopolypeptides poly-L-lysine (PL), poly-L-ornithine (PO) and the negatively charged poly-L-aspartic acid (PA) gave similar results. From the amounts adsorbed it has been concluded that these peptide molecules are adsorbed using only very few amino acid residues. Although no adsorption could be measured for the negatively charged poly-L-glutamic acid (PG), contact angle measurements showed that adsorption of PG did, in fact, occur.

Since the peptide molecules are highly charged under the experimental conditions, the amount adsorbed can not be explained unless a charge neutralization occurs in the adsorbed layer. The number of calcium and phosphate ions in solution after the adsorption process indicates that:

a) polypeptides which are adsorbed onto the surface release ions of the
same charge from the surface (e.g. calcium ions as a result of adsorption of positively charged PL and PO),
b) ions of opposite charge are bound to the surface (e.g. phosphate as a result of adsorption of PL and PO).

A model for the adsorbed layer has been proposed as a result of these measurements, in which the mutual repulsive charges of the adsorbed peptide molecules are neutralized by divalent ions of opposite charge which dissolve from the enamel surface. The affinity of the peptide molecules for divalent counter ions was confirmed by dialysis experiments. Circular dichroism measurements of peptides in solution suggest that divalent counter ions induce a more compact molecular shape. Molecular entwining in the adsorbed state may therefore be expected.

The difference in adsorption behaviour between poly-L-glutamic acid (PG) and poly-L-aspartic acid (PA) is tentatively explained by an adsorption model in which PG is adsorbed by an interaction of many amino acid side chains forming a flat monolayer, whereas PA is only adsorbed through some amino acid residues only.

A model has been proposed for the interaction between enamel and protein at the atomic level, based on these experiments and on data obtained from column chromatography on hydroxyapatite as described by others. In this model positively charged amino groups interact with negatively charged phosphate groups on the enamel surface. An exchange reaction has been proposed for the negatively charged carboxyl groups of the proteins in which the carboxyl groups replace a phosphate group in the enamel crystal surface.

The adsorption of the purified salivary proteins (chapter 3) is described in chapter 5. A remarkable difference was found between the amounts of phosphoproteins and non-phosphorylated proteins adsorbed. The phosphoproteins reached adsorption plateaus similar to the values found for the homopolypeptides (chapter 4). The amounts of polypeptides adsorbed could be explained by the effect of the neutralizing ions and the flexibility of the peptide chain. Since these native molecules are not as highly charged as the polypeptides, the effects of adsorption on the concentra-
tration of calcium and phosphate in solution could not be measured. These ions are not essential in the adsorption process since native proteins contain both positively and negatively charged groups. No repulsion between adsorbed molecules in the adsorbed layer therefore occurs.

Since no aggregation between protein molecules was observed in solution in the presence of calcium and phosphate ions, it can be concluded that the adsorption of the molecules in a multilayer is unlikely and that adsorption in a monolayer is more probable. This monolayer model for protein adsorption suggests a random coil conformation for the phosphoproteins, which allows the molecules to orientate at the surface in a very dense layer.

It is not only the amount adsorbed, but also the strength of adsorption which provides important information on the adsorption process. Column chromatography experiments using powdered enamel were carried out to measure the strength of adsorption. Both the type and the concentration of the buffers at which the proteins were released from the column were used as a quantitative measurement. These experiments revealed that phosphoproteins were bound more firmly to the enamel surface than non-phosphorylated proteins. This observation can be explained by ascribing the greatest importance in protein adsorption onto enamel to an exchange reaction in which phosphate groups on the protein molecule replace phosphate groups on the enamel surface.

Extrapolation of the data on adsorption strength, to the 'in vivo' pellicle situation suggests that the pellicle/enamel interface will be saturated with phosphoproteins as a function of time. Probably this saturation of the surface with phosphoproteins is essential to give the pellicle its protective function.