Salivary protein adsorption and oral streptococcal adhesion

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SUMMARY

The importance of protein adsorption and subsequent bacterial and cellular adhesion to different solid substrata in various applications of medical and environmental interest is pointed out in chapter 1. Salivary protein adsorption to the enamel surfaces of teeth is followed by bacterial adhesion initiating dental plaque formation, the primary cause for caries and periodontal diseases. An understanding in the mechanisms by which both the underlying substrata and the adsorbed protein layers contribute to bioparticle adhesion may help in the development of new preventive measures on reducing bacterial adhesion in dentistry. This requires a detailed knowledge of the influence of the adsorbed proteins as well as of the underlying substrata on bacterial adhesion.
The aims of this investigation were:

1. to investigate whether oral streptococcal adhesion to artificial solid substrata and human enamel can be described by a thermodynamic model under conditions of moderate shear as encountered in the human oral cavity.
2. to determine the influence of salivary protein adsorption on oral streptococcal adhesion.
3. to elucidate some of the mechanisms by which adsorbed protein layers transfer substratum properties to the interface with adhering microorganisms.

Chapter 2 presents a literature survey on interactions between proteins and solid surfaces, bacteria and solid surfaces and between bacteria and proteins readily adsorbed to solid surfaces. Special attention is given to salivary protein adsorption (pellicle formation) and the two most important models by which bacterial adhesion to solid substrata is described, namely the physico-chemical model and the specific-receptor model. In chapter 3 the flow cell system is described, in which both bacterial adhesion and protein adsorption experiments were done. In addition, an overview is presented of the major experimental techniques employed in this study.
In chapter 4 a relation between interfacial free energy of adhesion (ΔF_{adh})-dependent and non-interfacial free energy of adhesion (non-ΔF_{adh}) dependent adhesion of oral streptococci to solid substrata is described and discussed. It was concluded that one strain-specific factor influences both ΔF_{adh}-dependent and non-ΔF_{adh}-dependent adhesion. The numerical value of this factor, which may be related to the presence of surface appendages, together with a surface energetic analysis predicts the number of streptococci that will adhere to a given artificial non-biological substratum such as FEP-teflon, glass or cellulose-acetate. In chapter 5 it is shown that the strain-specific factor "a" was not only associated with fibrillar appendages but also with the capacity of the adhering cells to excrete substances with a tendency to adsorb to the surface and to change therewith the surface characteristics. Recalculation of data provided by other authors confirmed the existence of a strain-specific factor in a general approach of bacterial adhesion to solid substrata. Tentatively, this strain specific factor must be considered as a parameter because of its possible dependence on temperature, pH, ionic strength and shear rate. In chapter 6 the adhesion of the oral streptococcal strains *Streptococcus mutans* NS, *Streptococcus sanguis* 12 and *Streptococcus mitis* BMS to uncoated and albumin coated artificial solid surfaces (FEP-teflon, glass and cellulose acetate) is described. In the absence of an albumin coating a linear

relation was found between the bacteria adhering at the stationary state and the interfacial free energy of adhesion. Although in the presence of a BSA coating the number of adhering bacteria greatly decreased, linear relations between the number of bacteria adhering at the stationary state and the interfacial free energy of adhesion remained, albeit with lower coefficients of correlation than for uncoated substrata. It was concluded that the bare uncoated substrata still influenced bacterial adhesion in spite of a marked influence of a BSA coating. Chapter 7 describes the effects of pellicle formation on streptococcal adhesion on human enamel and artificial solid substrata with various surface free energies. Streptococcal adhesion to artificial solid substrata exposed to saliva was low and the differences among uncoated materials were markedly reduced, already after only 5 minutes exposure to saliva. In addition, it was found that oral streptococcal adhesion to bare enamel, a heterogeneous, high surface free energy material, could be fitted as well by the thermodynamic model, previously described for adhesion to artificial substrata. Chapter 8 presents the effects of high molecular weight salivary mucin adsorption on oral streptococcal adhesion. With all other protein coatings applied, it appeared possible to use a thermodynamic model based on the bare substratum characteristics, however, this was found to be impossible after mucin adsorption. It was hypothesized that mucins reveal hidden binding sites upon adsorption (cryptitopes), invalidating the thermodynamic model. Speculatively, it was stated that the degree of cryptitope relevation, may depend on substratum surface free energies. In chapter 9 secretory IgA adsorption and oral streptococcal adhesion to human enamel and artificial solid substrata with various surface free energies are described. Screening or displacement of adsorbed sIgA by other salivary proteins is obvious for low surface free energy substrata but not for high surface free energy substrata such as enamel. sIgA adsorption did not invalidate the use of a thermodynamic model for oral streptococcal adhesion. For *S. sanguis* 12 and *S. mitis* BMS the reduction in adhesion to protein coated substrata with respect to uncoated substrata was said to be due mainly to an aspecific protein effect, while for *S. mutans* NS also a more specific antibody affect was observed in addition to the non-specific protein effect. Chapter 10 deals with the possibility of extrapolating the results of this thesis to the *in vivo* situation. It is emphasized that *in vivo* plaque formation on tooth surfaces during nine days shows a much more pronounced influence of substratum surface free energies than expected on basis of this *in vitro* study on bacterial adhesion. It is argued that *in vivo* shear forces fluctuate very strongly and that therefore retention become very important. A set of experiments is proposed through which *in vitro* results similar to *in vivo* results might be obtained.

In conclusion these investigations show that streptococcal adhesion:

- can be adequately described by a thermodynamic model;
- is mainly governed by surface adsorbed proteins;
- remains to be partly influenced by the substratum properties of the underlying surfaces.