Bacterial adhesion onto apatite minerals
electrokinetic aspects

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Abstract

The role of the electrokinetic properties of Streptococcus sanguis and Actinomyces naeslundii in determining their
adhesion to apatite minerals is examined. It is shown that bacterial adhesion onto a mineral surface takes place even
when both the surfaces are negatively charged, and that the adhered layer is resistant to washing. Also, the mineral
fines after exposure to bacteria exhibit zeta potential values which are in between those of the mineral and the bacteria,
but be closer to that of the mineral. In the absence of salivary proteins, the inorganic species present in saliva alter the
magnitude of the surface charge but do not affect the bacterial adhesion process. Dissolved apatite mineral species in
supernatant solutions are also shown to decrease the magnitude of the zeta potential of the bacteria. The observed
adsorption of negatively charged bacteria onto negatively charged minerals suggests that electrostatic interactions are
not the primary factors determining adhesion.

Keywords: Actinomyces naeslundii; apatite minerals; oral bacteria; Streptococcus sanguis; zeta potential

Introduction

Electrostatic and hydrophobic interactions play an important role in several adhesion processes in
the oral cavity [1,2]. The main constituent of teeth, hydroxyapatite mineral, is a substrate for adhesion
by oral bacteria. The majority of the latter, including the first colonizers on the enamel surface, are
most likely to be negatively charged under physiological pH conditions [3]. Their initial interaction
with the saliva-coated surface of the teeth is believed to be influenced by electrokinetic effects
[1,2], although hydrophobic interactions between bacteria and tooth pellicle are also known to play
an important role [4]. Even in the presence of a salivary pellicle, electrostatic interactions between
the bacteria and the mineral can become important. In addition to proteins, saliva contains many inorganic
species, including Ca²⁺, Mg²⁺, K⁺ and various phosphate species, which are expected to have a significant influence on the electrostatic
interactions. Information on the extent of the latter can be obtained from electrokinetic studies, which
involve measurement of the particle zeta potential in aqueous solutions containing the desired chemical
species.

In this work, the effect of the bacterial conditioning of apatite minerals with the oral bacteria Streptococcus sanguis (G9B) and Actinomyces naes-
lundii (PK29) in a synthetic saliva, referred to as adherence buffer (containing various inorganic
species in saliva) solution, on the zeta potential of the minerals is described, and the role of electroki-
netics on the adhesion of bacteria to minerals is discussed.
Materials and methods

Minerals

Tooth apatite minerals used in this study are hydroxyapatite (Ca$_5$(PO$_4$_$_2$)OH) and fluorapatite (Ca$_{10}$(PO$_4$_$_6$)F$_2$). Brushite (CaHPO$_4$.2H$_2$O) is also included because it is present during the early stages of tartar formation on teeth. Both hydroxyapatite and brushite used in the zeta potential measurements consist of fine particles less than 5 μm in size, obtained from BDH Inc. Coarse non-porous fluorapatite particles (600 μm), also used for the streaming potential measurements, are of interest because this tooth surface mineral is formed by the frequent use of dentifrice and drinking water containing fluoride [5]. Its non-porous surface is desirable for the bacterial adsorption/desorption studies.

Bacterial strains and culture conditions

Oral bacterial strains Streptococcus sanguis (G9B) and Actinomyces naeslundii (PK29) (obtained from the culture collection at Unilever Research, US, Inc.) were chosen, since both species are early colonizers of teeth and are found in most dental plaque [6]. All the measurements were carried out using cultures freshly grown as mentioned elsewhere [7] in tryptone–yeast extract (2.5% tryptone, 1.5% yeast extract, 0.5% K$_2$HPO$_4$, 0.1% MgSO$_4$ plus 1% fructose) (TYE) broth.

Medium

The medium used for these experiments was a buffered KCl solution, often referred to as synthetic saliva [8], containing the inorganic ionic species present in oral saliva (5.0·10$^{-2}$ M KCl, 10$^{-5}$ M CaCl$_2$, 10$^{-3}$ M K$_2$PO$_4$ and 10$^{-4}$ M MgCl$_2$). In order to understand the influence of these species on the adhesion process, measurements were also carried out in solutions of single components or in triply distilled water (TDW). Hydrochloric acid and sodium hydroxide were used for adjusting the system to the desired pH.

Methods

Zeta potential measurements

Information on the surface charge of bacteria and minerals (except fluorapatite) was obtained by measuring the zeta potential using a Model D-Zeta meter system at a solids' concentration of 0.01%. For conditioning with bacteria, about 7.9·10$^7$ cells ml$^{-1}$ of S. sanguis and about 1.2·10$^7$ cells ml$^{-1}$ of A. naeslundii were used. Cell concentrations were measured using a UV–visible spectrophotometer at 540 nm. Minerals were first equilibrated in solutions adjusted to the desired pH for 30 min and for bacterial adhesion studies, an additional 30 min of conditioning was carried out by stirring the suspension containing both the bacteria and the mineral.

Streaming potential measurements

Streaming potential measurements were carried out using a cell described elsewhere [9]. About 21 g of the fluorapatite mineral was placed in the cell, along with 300 ml of solution adjusted to the desired pH. A packed bed of the mineral prepared as described previously [9] was equilibrated overnight inside the cell, and the pH was recorded. The conductivity of the solution was measured with a Thomas meter, Model 275. The streaming potential is related to the zeta potential in accordance with the Helmoltz relationship [10]:

$$E = \frac{D\zeta}{4\pi\mu \lambda}$$

where $E$ is the streaming potential, $P$ is the pressure difference (dyn cm$^{-1}$), $D$ is the dielectric constant, $\zeta$ is the zeta potential (mV), $\mu$ is the viscosity of the liquid (P) and $\lambda$ is the specific conductivity.

Results and discussion

The zeta potentials of hydroxyapatite, S. sanguis, and A. naeslundii, as well as of hydroxyapatite...
after conditioning with bacteria in triply distilled water, as a function of pH. are shown in Fig. 1. Apatite exhibits an isoelectric point (IEP) at pH 8 and an unusual minimum at pH 6.5. Reasons for this abnormal behavior are not clear at present. (Since this aspect is not the major focus of this study, the reasons for it have not been investigated. Preliminary studies, however, show that atmospheric CO₂ may have a marked effect on the zeta potential of apatite to the extent of even forming calcium carbonate on the surface under high pH conditions [11]. The presence of more than one IEP in this type of system is not uncommon). Repeat measurements of the zeta potential of both the bacteria showed some scatter, which is probably due to minor differences in their surface properties at different stages of growth. It can be seen that S. sanguis is positively charged below pH 3.2 and negatively charged above it and A. naeslundii is positively charged below pH 3.7. Also, for A. naeslundii, the zeta potential remains essentially constant at above pH 8.

Upon conditioning the hydroxyapatite with bacteria, significant heterocoagulation with the mineral was observed. The zeta potentials of hydroxyapatite–bacteria aggregates were found to be negative over the entire pH range studied, but always lower than those of pure bacteria, indicating that the cell concentration used was not sufficient to cover the entire mineral surface.

Analogous effects were obtained with the brushite mineral in the presence of S. sanguis and A. naeslundii in triply distilled water as a function of pH (Fig. 2). In contrast to hydroxyapatite, brushite exhibits a negative potential in triply distilled water over the entire pH range studied. Evidently, the adherence of bacteria to phosphate-type minerals occurs even when both surfaces bear the same sign of charge.

Zeta potentials of hydroxyapatite, S. sanguis, and A. naeslundii as well as hydroxyapatite–bacteria aggregates in the described buffer solution are given in Fig. 3. A comparison with Fig. 1 shows that this buffer causes an increase in the negative charge of the mineral over the entire pH range with no apparent IEP. Both kinds of bacteria remained negatively charged, and at pH > 6, the potential is higher in the buffer solution than in pure water. Obviously, the solutes in the buffer have a significant influence on the surface charge.

![Fig. 1. Zeta potential of hydroxyapatite, Streptococcus sanguis (Ss) and Actinomyces naeslundii (An) and of hydroxyapatite with and without conditioning with bacteria in triply distilled water.](image1)

![Fig. 2. Zeta potential of brushite after conditioning with and without Streptococcus sanguis (Ss) and Actinomyces naeslundii (An) in triply distilled water.](image2)
of hydroxyapatite and both bacteria, as discussed elsewhere [12–14].

The potential of the hydroxyapatite mineral became more negative when mixed with bacteria. For example, at pH 6.8, hydroxyapatite has a potential of about −17 mV and S. sanguis about −45 mV, while the mineral–bacteria aggregate has a potential of −27 mV. The results obtained suggest that electrostatic factors alone do not control the adhesion process. It is, however, important to recognize that the zeta potential only provides information on the potential at the shear plane and in the case of heterogeneous surfaces it may not be adequate to explain the behavior of the particles in a system. For example, it has been reported that two unlike particles bearing the same sign of zeta potential but differing in the magnitude of the charge can actually attract at short distances of separation [15]. Various factors considered for such behavior include surface roughness, hydrodynamic effect, non-uniform charge distribution, size distribution, uncertainty of zeta potential, and dissolution of material. Similarly, adsorption of anionic surfactants onto surfaces with net negative charge has been attributed to surface heterogene-

ities and the presence of positive patches on the surface [16]. In the case of bacteria, the surface is very complex with a polysaccharide envelope with hydroxyl, hydrophobic, carboxylic acid and phosphate moieties [17]. Adhesion under these conditions can occur for several reasons. For example, hydrogen bonding by hydroxyl groups of the polysaccharides with calcium phosphate sites and bridging by the dissolved calcium from the mineral can lead to adhesion. The fimbriae on the bacterial surface can change their configuration, depending upon the nature of the approaching surface, and can regulate its charge characteristics. In addition to such complexities at the bacterial surface, it is also important to recognize the heterogeneities at the calcium phosphate–water interface. Even though the net potential of the mineral surface is negative under the tested conditions, it has both positive calcium sites and negative phosphate sites on it. Binding of negative species can occur at the localized calcium sites. Furthermore, calcium phosphate minerals undergo dissolution and dissolved species can have a significant effect on the potential of the bacteria. The dissolved calcium can cause bridging between the anionic mineral sites and the anionic moieties on the bacterial surface. The effect of the dissolved species is examined in more detail in a later section. The main point to be noted is that the measured zeta potential is only a net effect and may not represent the local and dynamic potentials experienced by the approaching moieties and therefore the lack of direct correlation between the zeta potential and the adhesion cannot be taken as an indication of the absence of any electrostatic interactions.

Similar observations were made with brushite and bacteria in the buffer medium, with the potential values obtained in buffer solution being somewhat more negative than those in pure water (Fig. 4).

Some of the possible structures for the particle–bacteria aggregates are shown in Fig. 5. Depending on the extent of adhesion and the structure of the aggregates, the net potentials of the latter could be
bacteria or the bacterial concentration is not high enough to saturate the mineral surface.

The minerals used in this work are known to undergo dissolution in aqueous medium. The dissolved species can interact with the bacterial surface, and thus can influence the coaggregation process. Studies were therefore carried out to determine the influence of such solutes on the potential of the bacteria. The mineral dissolution also causes an increase in the pH: after conditioning for 30 min the pH of hydroxyapatite dispersion stabilizes at about 7.2 while that of brushite stabilizes around 8.3 (Fig. 6). The changes of the pH in the brushite dispersion are greater than in hydroxyapatite, indicating a higher solubility of the former. In order to study the influence of dissolved species from the minerals on the potential of the bacteria, a solution was prepared as follows: 0.1 g of the mineral was conditioned in 100 ml of distilled water for 24 h by adjusting the pH to the desired value during the initial 6–8 h. The zeta potentials for both *S. sanguis* and *A. naeslundi* in these prepared supernatant solutions of hydroxyapatite and brushite are given in Figs. 7 and 8 respectively, and show that the potential is influenced in both cases, the effect being more pronounced in supernatant solutions anywhere between that of the mineral and the bacteria, although the measured potentials are as a rule closer to that of the bare mineral, suggesting that either more mineral particles adhere to the

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**Fig. 4.** Zeta potential of brushite before and after conditioning with and without *Streptococcus sanguis* (Sa) and *Actinomyces naeslundi* (An) in adherence buffer solution.

**Fig. 5.** Model showing the types of bacterial adherence to fine particles of apatite minerals.

**Fig. 6.** Change in solution pH due to conditioning with minerals as a function of the initial pH.
dissolved species the zeta potential is markedly reduced, indicating that the electrostatic repulsion is reduced and, consequently, that the coaggregation is promoted by the dissolved species.

The zeta potentials of fluorapatite, estimated by measuring the streaming potential in different solutions, yield the IEP at pH 3.7 (Fig. 9). The corresponding curves for S. sanguis and A. naeslundii (also included) show that the zeta potential values of A. naeslundii are closer to that of fluorapatite; therefore only the effect of the interactions with S. sanguis were studied. The results in Fig. 10 indicate that at pH 9.0, the zeta potential becomes increasingly more negative as the cell concentration was increased up to $5 \times 10^6$ cells ml$^{-1}$ and then remains constant, suggesting the saturation of the mineral surface with bacteria. The mineral suspension was further washed three times with a supernatant solution of fluorapatite and the streaming potential was found to be about $-40$ mV (Table I) in contrast to $-30$ mV, indicating that the attached bacteria cannot be completely removed by washing.

A comparison of data in Figs 7 and 8 with those in Figs 1 and 3 clearly shows that even though the potential of bacteria in water and in buffer solutions is relatively high, in the presence of the

obtained with brushite than with hydroxyapatite, indicating, again, the higher solubility of the former.

Fig. 7. Zeta potentials of Streptococcus sanguis (Ss) and Actinomyces naeslundii (An) in the supernatant of hydroxyapatite.

Fig. 8. Zeta potentials of Streptococcus sanguis (Ss) and Actinomyces naeslundii (An) in the supernatant of brushite.

Fig. 9. Zeta potentials of fluorapatite (FAP), obtained from streaming potential measurements in a fluorapatite supernatant, and Streptococcus sanguis (Ss) and Actinomyces naeslundii (An) in triply distilled water.
takes place even when both the bacteria and the minerals are negatively charged. The possible factors in controlling such adhesion of similarly charged particles include surface heterogeneity of the bacteria with a polysaccharide envelope and hydroxyl, hydrophobic and ionic moieties. Also, the flexible fimbriae can possibly regulate the surface charge characteristics, depending on the environment. The dissolved species from the minerals also alter the surface charge of the bacteria in a direction which reduces repulsion and thus favor adhesion.

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References