The effect of ionic surfactants on salivary proteins adsorbed on silica surfaces

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The adsorption onto silicon oxide surfaces from water and 0.1 M acetate buffer containing 10% parotid saliva at 25°C and 35°C and at pH 6 was monitored in situ using ellipsometry. The silicon oxide surface was used as a model for dental enamel. The adsorption kinetics and the reversibility on rinsing were determined, and the desorbable fraction was found not to change after either 30 or 120 min of adsorption. Addition of sodium dodecyl sulfate after 30 or 120 min of saliva adsorption caused strong desorption. Rinsing 30 min after surfactant addition caused some redeposition if saliva was present, whereas continued desorption occurred in the absence of saliva. Cetyltrimethylammonium bromide caused either an increase or a slight decrease in the amount adsorbed when added after 30 min and 120 min, respectively. For both times, rinsing caused desorption, left the same amount adsorbed, and was not affected by the presence or absence of saliva in solution. No major effect from temperature and ionic strength was found. □ *Acquired pellicle; dental plaque; ellipsometry; toothpaste*

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The two major dental disorders, caries and periodontal disease, are caused by bacterial accumulations on tooth surfaces. The bacteria attach to a film consisting of organic matter, the acquired pellicle, which originates from adsorption of salivary protein material onto solid surfaces in the oral cavity. Under clinical conditions oral microorganisms may also be surrounded by a pellicle of the same type as or similar to that on teeth. The first adhesive events would then be controlled by the nature of the films on both the tooth surface and the microorganisms (for reviews, see, for example, Refs. 1–4). One way to prevent bacterial adhesion to tooth surfaces could thus be to alter the adhesive properties of the pellicle by, for example, addition of surfactants, either during its formation or by desorption of the film.

In the field of protein adsorption much interest has been focused on interactions between adsorbed proteins and detergents (5), and it is known that detergents may displace (or 'elute') proteins from interfaces under certain conditions (6). The purpose of the present study was therefore to investigate the influence of two surfactants, sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB), on salivary pellicles. SDS is present in the major part of all toothpastes and has been shown to affect the deposition of fluoride on enamel (7, 8). In addition, SDS has been reported to bind to hydroxyapatite and enamel (9). CTAB has been shown to be bactericidal against dental plaque-forming microorganisms (10). Further, pure samples of these surfactants can be prepared, and their association behavior is well known. The critical micellar concentrations (CMC) are 8.3×10^{-3} and 9.2×10^{-4} M at 25°C (11), and the Krafft points are 15.5°C (12) and 25.8°C (13) for SDS and CTAB, respectively. The surfactant concentration, 0.5% (w/v), chosen for this study corresponds to 17×10^{-3} M and 14×10^{-3} M for SDS and CTAB, respectively, which is well above the CMC for both compounds.

From in situ ellipsometry measurements of adsorption from saliva onto tooth enamel

and silicon oxide surfaces (14) it was concluded that the adsorption behavior was similar at the two kinds of surfaces and hence that the silicon oxide surface served as an appropriate model for the tooth enamel. Further, owing to the higher optical contrast (difference in refractive indices) between the surface and the film and the lower surface roughness, the accuracy in the determination of the amount adsorbed will be substantially increased for silicon oxide compared with enamel. Owing to these methodologic advantages, silicon surfaces covered with thermally grown silicon oxide was chosen for this study. Silicon oxide is known to have an isoelectric pH of about 2 (15) and is consequently negatively charged under the experimental conditions. Both silicon oxide and hydroxyapatite, the mineral component of tooth enamel, are known to be hydrophilic (16).

Materials and methods

Materials

The saliva consisted of unstimulated parotid saliva, which was collected from one person on one occasion with the use of Lashley cups (17). The saliva was thereafter stored at −20°C until used.

CTAB (AnalaR 10391, Lot 4550770 G) and SDS (specially purified for biochemical work 44215, lot 9449900E) were obtained from BDH Chemicals Ltd., England. The samples were further purified by recrystallization four times from aqueous solution. The detergents were dissolved (at a concentration of 80 g/dm^3 and 20 g/dm^3 for SDS and CTAB, respectively) in water at 30°C. The solutions were then slowly cooled until crystallization occurred. The crystals were filtered off and finally freeze-dried. Stock solutions of the surfactants, with a concentration of 10 times the final concentration in the cuvette, were used within 1 day of preparation.

Experiments were performed in pure water at pH 6 and in 0.1 M sodium acetate buffer also at pH 6. In the experiments performed in pure water solutions the pH was adjusted with 1 M sodium hydroxide or hydrochloric acid. Acetate buffer, 0.1 M, pH 6, was prepared by dissolving sodium acetate in water. The pH was then adjusted to 6.0 by addition of acetic acid.

Silicon wafers (p-doped, Boron; resistivity, 0.01–0.02 ohm cm) were obtained from Okmetic Ltd., Finland. The wafers were cut into slides with a width of 12.5 mm. These slides were washed in a mixture of NH_4OH, H_2O_2 and H_2O (1:1:5) (v/v/v) for 5 min at 80°C, then in HCl, H_2O_2, and H_2O (1:1:5) (v/v/v) for 5 min at 80°C. They were then treated with hydrofluoric acid and finally rinsed in water. The slides were then transferred to an oven, where they were oxidized at 920°C for 27 min in a dry oxygen atmosphere. The slides were then exposed to room atmosphere and temperature to cool down. The thickness of the oxide layer thus obtained ranged from 300 to 350 Å. The oxidized slides were cleaned again in a mixture of NH_4OH, H_2O_2, and H_2O (1:1:5) (v/v/v) at 80°C for 5 min, followed by HCl, H_2O_2, and H_2O (1:1:5) (v/v/v) for 5 min at 80°C, and finally they were thoroughly rinsed in water and then kept in absolute ethanol until used.

All chemicals were of analytical grade, and the water used was distilled, passed through an ion-exchanger and activated charcoal, and finally doubly distilled in a glass still. Glassware was cleaned in a 1:1 (v/v) mixture of concentrated sulfuric and nitric acid and thoroughly rinsed in water.

Methods

The adsorption/desorption was monitored in situ using an automated Rudolph Thin-Film ellipsometer, type 43603-200E, equipped with a thermostated cuvette. The experimental setup has previously been described in detail (24). The principles of the method are described elsewhere (18). From the ellipsometric angles, Δ and Ψ, it is possible to calculate the thickness and the refractive index of an adsorbed layer on a surface (19). As shown by Cuypers et al. (20), the adsorbed amount can be calculated from these values with higher accuracy than either the refractive index or the thickness, especially at low surface coverage. The adsorbed

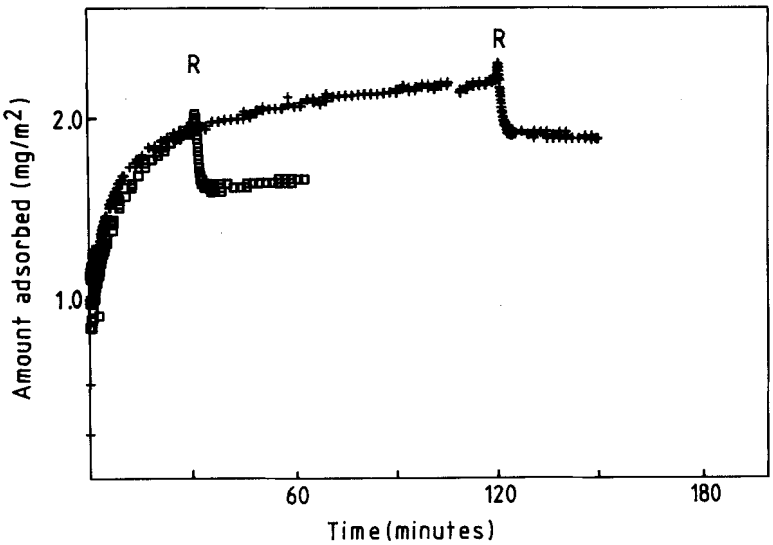

Fig. 1. Amounts of material adsorbed versus time on silica surfaces from a 10% solution of saliva in water at pH 6. Two separate experiments are shown, in which rinsing (R) was performed after 30 (□) and 120 min (+) of adsorption, respectively.

amounts were calculated in accordance with Cuypers et al. (20). These calculations require knowledge of the partial specific volume (v) and the ratio of the molar mass and the molar refractivity (M/A) of the adsorbed compounds. Since the composition of the adsorbed layer is unknown in these cases, the following typical values were chosen for these factors: v = 0.75 ml/g and M/A = 4.1 g/ml. It should be noted that these values do not influence dramatically the calculated amounts adsorbed and that the values chosen are in the range expected for both proteins and lipids (20). In the calculation of the refractive index and thickness of the adsorbed film, the silicon surface and the oxide layer were treated as one unit with an 'effective' refractive index.

Immediately before the experiments the silicon slides were blown dry with nitrogen, and then plasma was cleaned in low-pressure air (0.2–0.3 torr) using a radio frequency glow discharge unit (Harrick PDC 3 X G, Harrick Scientific Corp., Ossining, N.Y., USA). The surfaces obtained were hydrophilic, obvious from their water wettability. The substrate was then immediately placed in the ellipsometer cuvette containing 4.5 ml of water or buffer. Stirring was performed with a magnetic stirrer at a rate of 325 rpm. When the ellipsometer angles were constant for the clean surface, 0.5 ml saliva was added, and the adsorption was monitored for 30 min or 120 min before rinsing or addition of surfactant took place. Rinsing was performed by a continuous flow of 100 ml of water or buffer through the cuvette. Addition of surfactant was performed by addition of 0.5 ml of stock solution to obtain a final concentration in the cuvette of 0.5% (w/v). Experiments were performed at 25°C unless otherwise stated.

Results

The experiments performed in water and and in 0.1 M sodium acetate buffer showed a deviation within 10% of the amounts adsorbed. The time course of the adsorption of salivary components onto silicon oxide surfaces is given in Fig. 1. The reversibility on rinsing after 30 min and 120 min of adsorption is illustrated. The experiments shown were performed in water.

The effect of SDS addition at different stages of the adsorption is shown in Fig. 2. Rinsing was performed 30 min after the

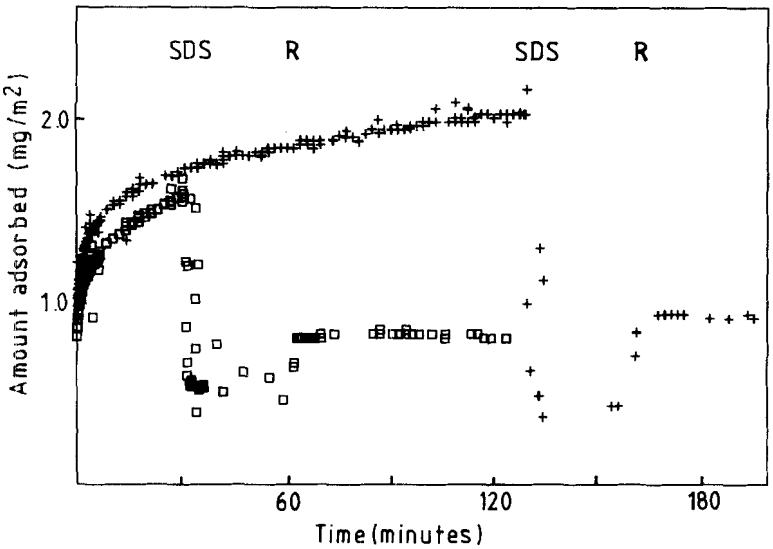

Fig. 2. Amounts of material adsorbed versus time on silica surfaces from a 10% solution of saliva in 0.1 M sodium acetate buffer at pH 6. Two experiments are shown, in which 0.5% sodium dodecyl sulfate (SDS) is added at 30 (□) and 120 min (+), respectively. Owing to the low amounts adsorbed and low refractive index of the adsorbed film, the calculation of the amount adsorbed could only be performed for a few data points in the time interval between addition of SDS and the rinse. R indicates rinse.

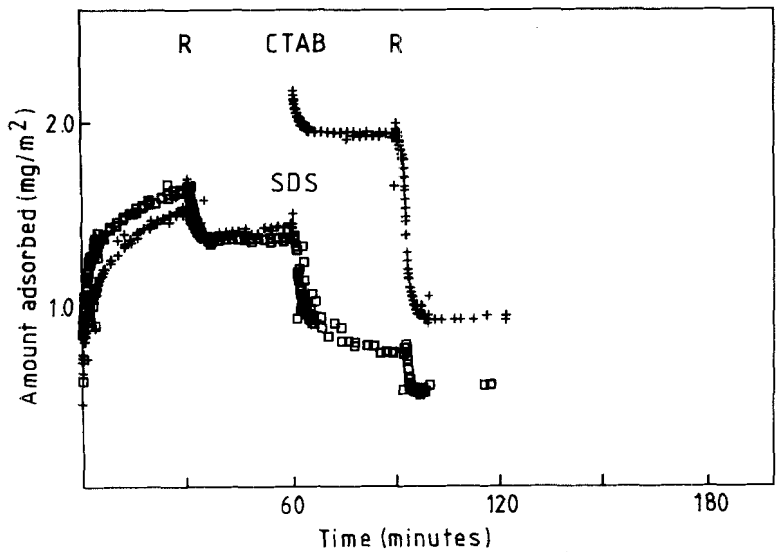

Fig. 3. Amounts adsorbed versus time on silica surfaces from a 10% solution of saliva in 0.1 M sodium acetate buffer at pH 6. After 30 min of adsorption the cuvette is rinsed (R), and after 60 min sodium dodecyl sulfate (SDS) (□) or cetyltrimethylammonium bromide (CTAB) (+) is added at a concentration of 0.5%. Rinsing (R) is then again performed at 90 min. After the final rinse following the SDS addition, the low amounts of adsorbed material in combination with a low refractive index of the adsorbed film enabled calculation of the amount adsorbed only for a few data points.

addition of SDS. The experiments shown were in 0.1 M acetate buffer. A strong desorption obviously took place on addition of SDS, and after the start of rinsing some redeposition at the surface seemed to occur. The behavior was equivalent after 30 min and 120 min of adsorption. Experiments featuring 30 min of adsorption at 35 °C in buffer showed similar behavior but with slightly lower values (≈10%) of the amount adsorbed. To investigate further the nature of the redeposition phenomenon, experiments were performed in which SDS was added after removing the salivary components from the solution by rinsing (Fig. 3). In this case a gradual decrease in the amount adsorbed was found on addition of SDS, and this decrease proceeded further on rinsing.

Addition of CTAB, followed by rinsing, produced an altogether different behavior (Fig. 4). After 30 min of adsorption the addition caused an increase in the amount adsorbed, a plateau was reached almost immediately, and then a quick desorption took place on rinsing, followed by a new plateau. In acetate buffer at 35 °C a similar behavior but approximately 10% lower amounts adsorbed was observed. After 120 min the effect was different. On addition the amount adsorbed was slightly reduced, and a plateau was attained, at about the same level as after addition at 30 min, and again rinsing caused the amount to decrease to a plateau in a similar manner as after 30 min. The experiments shown were performed in 0.1 M acetate buffer.

Finally, the effect of CTAB addition, with no saliva present in the solution, was investigated (Fig. 3). For this surfactant the behavior was similar to that when saliva was present (Fig. 4, 30-min curve).

Discussion

The adsorption of salivary proteins onto the hydrophilic negatively charged silicon oxide surface may be partly governed by electrostatic forces, such as interactions between positively charged residues of the proteins and the negatively charged surface. Since the major protein components can be assumed to bear a negative net charge under the con-

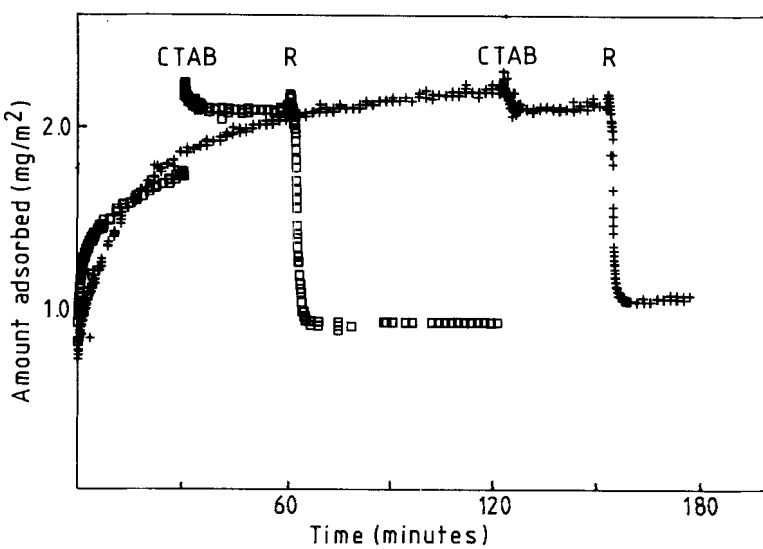

Fig. 4. Amounts of material adsorbed versus time on silica surfaces from a 10% solution of saliva in 0.1 M sodium acetate buffer at pH 6. Two experiments are shown, in which 0.5% cetyltrimethylammonium bromide (CTAB) is added at 30 (□) and 120 min (+), respectively. R indicates rinse.

ditions chosen (21, 22), other types of contributions, such as entropically favorable conformational changes on adsorption (23), may also be involved. In this connection it should be mentioned that the presence of divalent ions, such as Ca^{2+} in saliva, may affect the surface potential and thus the adsorption of both proteins and surfactants. Even though electrostatics may not be the only driving force for adsorption, we believe that the different nature of action of the cationic and anionic surfactants may be explained by considering the charges of the surface and the proteins in relation to the charge of the surfactant.

If we start by considering the reversibility on dilution (Fig. 1), we found that a fraction of the material was desorbable and that the size of this desorbable fraction did not change significantly with the residence time of the molecules at the surface. It should also be noted that this behavior was identical in the buffer.

In the case of SDS addition (Fig. 2) the effect was obviously an almost complete removal of the adsorbed layer. A possible explanation would be that the salivary components bind DS^- molecules, which prevent the protein from depositing by increasing the electrostatic repulsion between the surface and the protein and also between the protein molecules. A pure SDS solution does not cause any adsorption under the experimental conditions (31). This may be due to the absence of electrostatic attraction between the surfactant head-group and the surface (24). When the rinsing is performed, the concentration of saliva and of SDS will decrease. The adsorption of SDS at interfaces is known to decrease with concentration below the CMC (25). When the concentration of SDS drops, the amount of bound DS^- will decrease, and, consequently, the remaining protein in the solution will be destabilized and redeposit onto the surface. This means that if we rinse the system before SDS addition, we will expect no redeposition on the second rising, and instead further removal takes place at this point (Fig. 3).

For CTAB the effect is apparently dependent on the point of the adsorption process at which the addition is done (Fig. 4). A simple model explaining this behavior will be presented. Let us assume that we have two fractions of bound salivary proteins—one tightly bound fraction and a loosely bound one that can be displaced by CTA^+. Further, we assume that the tightly bound fraction remains constant over the time scale from 30 to 120 min. We know that CTA^+ adsorbs to the bare silicon surface under the experimental conditions and that this adsorption is reversible on dilution (31). Moreover, CTA^+ has been shown to adsorb reversibly to adsorbed protein (31). Thus, our observations will be explained if the increase in the amount adsorbed between 30 and 120 min mainly consists of loosely bound protein, which is elutable by CTA^+. The added surfactant will then bind to the surface or the firmly adsorbed protein, thereby replacing the loosely bound protein, and after the rinsing the surfactant will desorb, leaving only the tightly bound protein at the surface. According to this model, we would expect no difference in the behavior when protein is present or absent in solution, as can be seen when comparing Fig. 3 and Fig. 4 (30-min curve).

The various elutabilities of the adsorbed material obtained by rinsing, by CTAB, and by SDS are a strong indication of the presence of several fractions of adsorbed protein. This might indicate the presence of various molecules with different sizes, charges, and so forth at the interface. Similar effects are found also in single protein systems, however, and are attributed to multiple adsorption states of the molecules (5, 31).

This work initiated systematic studies of interactions between ionic surfactants and adsorbed protein. These studies involve the influence of the solid surface properties, and the charge of the surfactants and the net charge of the protein and models for the adsorption/displacement processes are presented (31).

When considering plaque formation in vivo, two main theories on bacterial colonization of tooth surfaces have been suggested. The so-called specific plaque hypothesis has been suggested to involve a selective bacterial colonization pattern, in

which the bacterial surfaces contain a highly developed recognition system that identifies and interacts with components of oral surfaces, such as the pellicle (for reviews, see, for example, Refs. 26, 27). The other theory, the unspecific plaque hypothesis, involves more general physicochemical aspects, and it was shown by Glantz (28) that the maximum weight of a dental plaque capable of adhering to a solid depends on the specific surface free energy of the solid. Further, Baier & Glantz (1) and Glantz (29) have presented a theory that the primary adhesion of oral microorganisms to solid surfaces is a process of low chemical specificity, and Simonsson & Glantz (30) have suggested that individual plaque formation can be partially related to the colloid–chemical properties of saliva and bacteria.

Judging from the results of the present study, both SDS and CTAB could have an effect on plaque formation irrespective of what theory explains plaque formation in vivo, either by blocking or removing specific receptors or by changing unspecific surface conditions necessary for bacterial adherence.

This preliminary study will be followed by systematic investigations in which the observed phenomena will be related to the surface properties of the substrate and the properties of the salivary samples such as concentration, secretions from the individual glands versus whole saliva, and so forth.

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