Secretory IgA adsorption and oral streptococcal adhesion to human enamel and artificial solid substrata with various surface free energies

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Abstract—In this paper, secretory IgA adsorption from a single component sIgA solution and from
human whole saliva onto human enamel and artificial solid substrata with various surface free energies
was studied as a function of time. ELISA indicated that screening or displacement of adsorbed sIgA by
other salivary proteins occurred only on low surface free energy substrata, not on high surface free energy
substrata such as enamel. In addition, the adhesion of three oral streptococcal strains (*Streptococcus
mitis* BMS, *S. sanguis* 12, and *S. mutans* NS), also having widely different surface free energies, to sIgA-
coated surfaces was studied. The adhesion of all three streptococcal strains was significantly reduced in
the presence of a sIgA coating.

However, ranking the adhesion data with respect to the various substrata revealed a similar order to
that in the case of uncoated substrata, indicating that substratum properties were at least partly trans-
ferred by the adsorbed protein film to the interface with adhering micro-organisms. For *S. sanguis* 12 and
S. mitis BMS, adhesion decreased proportionally with the amounts of sIgA detected by ELISA, but for
S. mutans NS such relations with the amounts of sIgA detected on the protein-coated substrata were not
found. Thus, for *S. mutans* NS a specific antibody effect seems to exist in addition to a non-specific
protein effect like that observed for *S. sanguis* 12 and *S. mitis* BMS.

Key words: sIgA adsorption; oral streptococcal adhesion; surface free energies.

INTRODUCTION

The first event when foreign surfaces are exposed to a biological environment is the
adsorption of proteins at the solid–liquid interface. In a later stage, bacteria and
cells can adhere to this adsorbed protein layer which may lead to the formation of,
for example, thrombi or dental plaque. The adsorption of proteins on foreign sur-
faces depends on the properties of the solid surface (e.g. zeta potential or surface
free energy) and on the composition of the protein solution as, for example, serum,
plasma, or saliva. Preferential adsorption of competing proteins from plasma onto
the walls of artificial blood vessels is of well-known crucial importance for the
formation of thrombi. [1–3].

Like plasma, saliva is a complex body fluid consisting of over 60 protein com-
ponents, amongst which albumin, secretory immunoglobulin A (sIgA), a-amylase,

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and mucins are the main proteins identified. [4]. Selective adsorption of competing proteins from saliva onto enamel plays a key role in the formation of dental plaque, which can initiate dental caries and periodontal diseases, particularly since clean enamel is covered within minutes by a proteinaceous layer called the 'pellicle' [5]. However, the formation of a matured pellicle takes at least 2 h [6]. Albumin, phosphoproteins, lysozyme, sIgA, and mucins have been identified as the main salivary pellicle components [7]. The relative abundance of these proteins in the pellicle changes in time, therewith contributing to the ripening of the pellicle [6, 8, 9].

Since the adsorbed protein layer governs to a large extent the adhesion of oral bacteria to dental enamel [10], time-dependent changes in the pellicle composition will also affect the interactions between surface components of oral micro-organisms and macromolecules comprising the pellicle.

Studies of Kilian *et al.* [11] have shown that pellicle-bound sIgA can interfere with the adhesion of oral streptococci by two separate mechanisms, which are related to the protein effect and the inherent specific antibody effect, respectively. An sIgA coating of hydroxyapatite contributed positively to the adherence of two *Streptococcus sanguis* strains and negatively to the adherence of *S. mitior* and *S. mutans* as compared with the adherence to uncoated hydroxyapatite. For *S. mutans* this could be attributed mainly to a non-specific protein effect, while for *S. sanguis* and *S. mitior* this could be attributed to both a non-specific protein effect and an inherent specific antibody effect.

Recently we have demonstrated that streptococcal adhesion to an 'early' pellicle (formed after 5 min of saliva contact) differed from that to a 'matured' pellicle (formed after 2 h of saliva contact) [10]. Furthermore, it is of interest to note that streptococcal adhesion to uncoated substrata of various surface free energies can be described by a thermodynamical model [10, 12, 13]. Streptococcal adhesion to pellicle-coated substrata was far lower than that to uncoated substrata but nevertheless followed the same tendencies as those for the uncoated substrata [10].

The aim of this study was to determine the amount of sIgA at the outer pellicle surface during pellicle maturation and its effect on oral streptococcal adhesion. Therefore, sIgA was adsorbed from a single protein (sIgA) solution and from human whole saliva onto human enamel and artificial solid substrata with various surface free energies. The amount of adsorbed sIgA was subsequently quantified by an enzyme linked immunoassay (ELISA). In addition, the adhesion of three oral streptococcal strains to surfaces precoated with sIgA was investigated and compared with the adhesion to surfaces precoated with a salivary pellicle.

MATERIALS AND METHODS

### Saliva and salivary secretory IgA

Human whole saliva from ten healthy volunteers from both sexes was collected into ice-chilled cups after stimulation of the salivary flow by chewing Parafilm®. After pooling and clarifying by centrifugation at 10 000 g for 20 min at 4°C, 0.2 M phenylmethylsulphonyl fluoride (PMSF), a protease inhibitor, was added to a final concentration of 1 mM in order to prevent enzymatic protein breakdown. Subsequently the solution was recentrifuged, dialysed overnight at 4°C against distilled water, and lyophilized for storage.

Optical density

Optical density
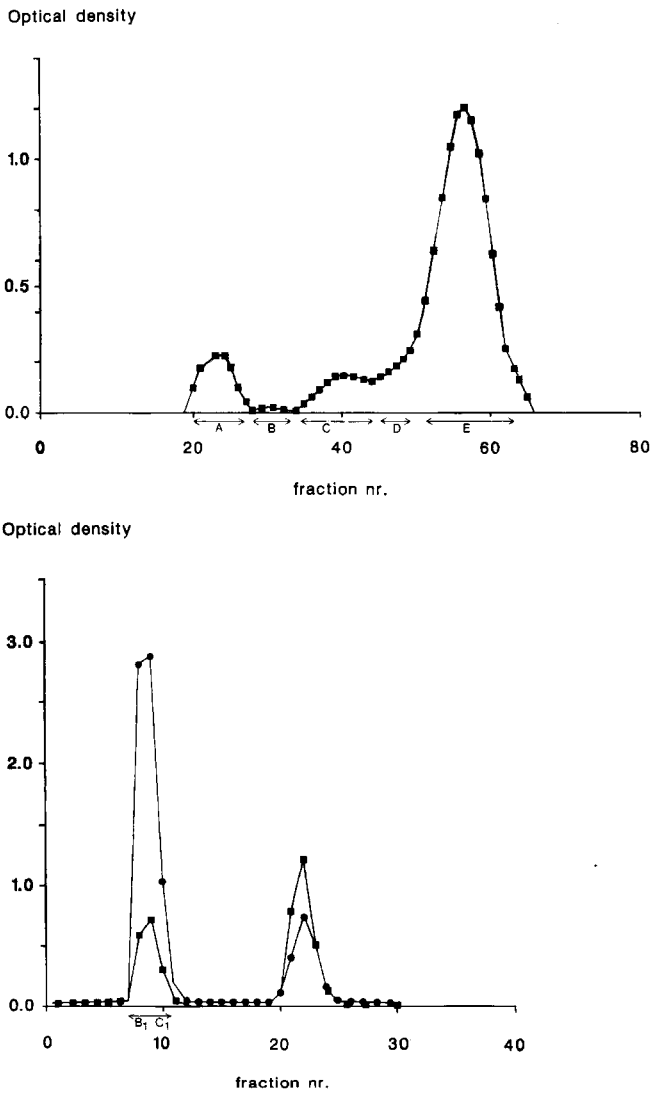
Figure 1. Top: Elution pattern of lyophilized human whole saliva from a Sephadex CL-4B (2.6 × 70 cm) column. The sIgA-containing fractions B and C were pooled as indicated by the solid bars. Bottom: Elution patterns of lyophilized fractions B and C from a Sephadex G-75 (1.5 × 70 cm) column. The sIgA-containing fractions B_1 (■) and C_1 (●) were pooled as indicated by the solid bars.

Part of the lyophilized material was employed for the isolation of sIgA. There-fore, lyophilized saliva (0.5 g) was dissolved in 10 ml of 0.05 M sodium phosphate buffer (pH 6.8) containing 50 mM NaCl and 10 mM EDTA. After centrifugation at 10 000 g for 20 min at 4°C, the supernatant was applied to a 2.6 × 70 cm Sepharose CL-4B column (Pharmacia, Uppsala, Sweden), pre-equilibrated with the same phosphate buffer and eluted at a flow rate of 20 ml h^{-1} at 4°C. The absorbances of the 5 ml fractions were monitored at 280 nm.

The IgA-containing fractions B and C (see Fig. 1) were dialysed overnight at 4°C against distilled water and lyophilized. For further purification pooled
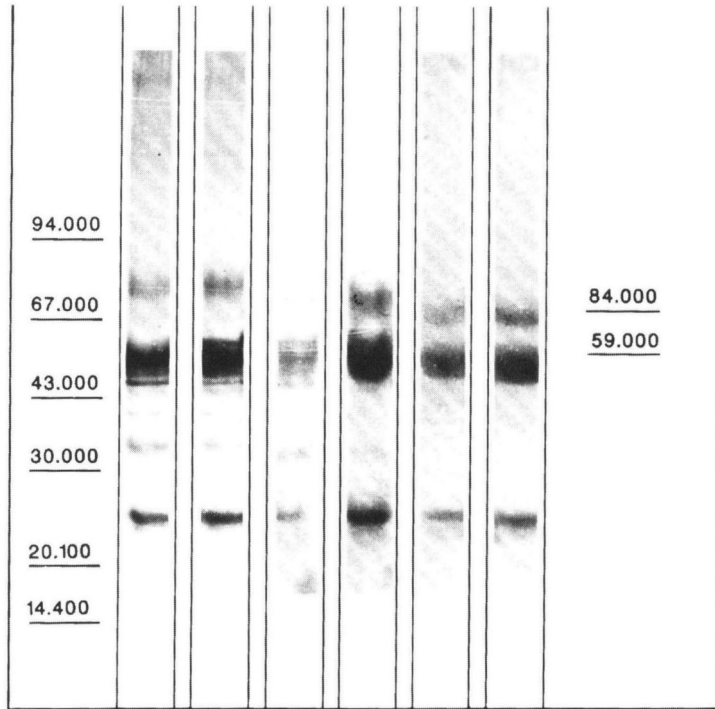
Figure 2. SDS-polyacrylamide gel electrophoresis. Lane A: molecular weight reference sample; lane B: fraction B; lane C: fraction C; lane D: fraction B_1; lane E; fraction C_1 (see also Fig. 1); lane F; rechromatographed salivary IgA (fractions B_1 and C_1); lane G: reference colostrum IgA; and lane H; Western immunoblotting pattern of salivary IgA, showing two bands with molecular weights of 84 000 and 59 000, which correspond to the a + light chain and the a chain, respectively. The band at 25 500, which was not seen during immunoblotting, represents the light chain of IgA.

lyophilized fractions B or C (0.5 g) were dissolved in 10 ml h^{-1} at 4°C. The fractions containing IgA (B_1 and C_1, see Fig. 1) were pooled, dialysed against distilled water at 4°C, and lyophilized. The isolated sIgA was checked for purity by comparison with reference colostrum secretory IgA (Cappel, Cochranville, USA) in SDS-PAGE and in Western immunoblotting experiments (see Fig. 2), with a-chain specific anti-IgA (TAGO Inc., Burlington, CA, USA).

Both lyophilized saliva and lyophilized isolated sIgA were dissolved in a potassium phosphate buffer (2 mM potassium phosphate, 50 mM potassium chloride, and 1 mM calcium chloride, pH 6.8 and surface tension 72.5 mJ m^{-2}) to a concentration of 2.0 or 0.2 mg ml^{-1} for lyophilized whole saliva and 0.2 mg ml^{-1} for sIgA for adsorption studies.

Preparation of enamel and artificial solid substrata

Human enamel slabs (3 × 3 × 0.4 mm) were cut under running tap-water from freshly extracted human incisors and molars. The labial surfaces were ground plan-parallel under water with abrasive paper (Siawat, P1200) and polished with a slurry

of Al_2O_3 powder (Buchler Ltd., Evanston, IL, USA) in distilled water to a stylus roughness $Ra \leqslant 0.1 \mu m$.

Previous studies have shown the surface free energy γ_s of similarly prepared human enamel to be $88 \, mJ \, m^{-2}$ [14]. Before embedding in a polymethylmethacrylate sample holder ($10 \times 15 \times 10 \, mm$), the polished enamel slabs were cleaned in distilled water in an ultrasonic bath for 15 min operating at a frequency of 50 Hz.

The following three artificial solid substrata with widely different γ_s values were also used: fluoroethylenepropylene co-polymer (FEP Teflon; Fluorplast, Raamsdonkveer, The Netherlands), $\gamma_s = 20 \, ml \, m^{-2}$; cellulose acetate (CA; Hawe Néos Dental, Gentilino, Switzerland), $\gamma_s = 50 \, mJ \, m^{-2}$; and glass (microscopic slides, Menzel, F.R.G.) $\gamma_s = 109 \, mJ \, m^{-2}$. Because the artificial solid substrata did not pose the same dimensional limitations as the enamel specimens, rectangles of $10 \times 15 \times 1.0 \, mm$ could be used for the experiments without embedding in a sample holder. The artificial solid substrata were extensively cleaned before the adhesion experiments by rinsing with a 1% sodium dodecyl sulphate solution for 24 h, followed by rinsing with 0.1 M HCl for 1 h and finally by rinsing three times for 1 min with distilled water.

Bacterial strains

Three strains of oral streptococci with known and widely different surface free energies γ_b, were selected for this study and included *Streptococcus mutans* NS ($\gamma_b = 117 \, mJ \, m^{-2}$), *S. sanguis* 12 ($\gamma_b = 107 \, mJ \, m^{-2}$), and *S. mitis* BMS ($\gamma_b = 38 \, mJ \, m^{-2}$) [15]. All strains were originally isolated from the human oral cavity and stored at $-20°C$ in Todd Hewitt broth (Oxoid, Basingstoke, UK) containing 7% (v/v) dimethyl sulphoxide (DMSO). The bacteria were grown from the frozen state in Todd Hewitt broth overnight at $37°C$. This culture was used to inoculate a second culture of Todd Hewitt broth containing 0.5% (w/v) glucose for 16 h at $27°C$ and then harvested by centrifugation. For the adhesion studies the bacteria were washed three times in potassium phosphate buffer and finally suspended in buffer.

Protein adsorption and streptococcal adhesion

Protein adsorption and bacterial adhesion experiments were both carried out in a previously described flow cell system [12] in order to minimize the passages of a given substratum through liquid–air interfaces, which can give rise to severe artefacts both in protein adsorption and in bacterial adhesion. The cleaned slabs of human enamel, FEP Teflon, cellulose acetate, and glass were vertically positioned in the flow cells and air was purged from the system by flowing with buffer. Then the slabs were exposed for various time intervals to a continuous pulse-free flow of salivary proteins at a moderate shear rate of $21 \, s^{-1}$.

For experiments in which the amount of sIgA adsorbed was to be quantitated, the slabs were removed after rinsing with buffer in the flow cell system also at a shear rate of $21 \, s^{-1}$ (2 min) and carefully blotted dry.

The amount of adsorbed sIgA at the outer surface was quantitated with an ELISA technique described later in this paper. The curves representing the amount of sIgA

adsorbed from a single protein sIgA solution at time t, $\Gamma(t)$, versus the flow time, t, were fitted with an iterative least-squares method yielding $j_{0,p}$ and τ_p according to

$$\Gamma(t) = j_{0,p}\, \tau_p\, (1\text{-exp}\, (-t/\tau_p)), \tag{1a}$$

where $\Gamma(t)$ is the amount of adsorbed sIgA at time t (in ng cm^{-2}), $j_{0,p}$ is the initial adsorption rate (in ng min^{-1} cm^{-2}), τ_p is the relaxation time (in min), and t is the flow time (in min).

According to Eqn. (1a), the amount of sIgA adsorbed at the stationary state (Γ_s) can be calculated from

$$\Gamma_s = j_{0,p}\, \tau_p \tag{2a}$$

For experiments in which streptococcal adhesion to protein-coated substrata was to be studied, the flow cell system was switched from the protein solution to a bacterial suspension. The protein-coated slabs were exposed to a bacterial suspension without liquid–air passages of the samples for various time intervals ($1 < t < 60$ min), again at the same moderate shear rate of 21 s^{-1}. Rinsing (1 min) and fixation [22 min with 2% (v/v) glutardialdehyde in potassium phosphate buffer] were also performed in the flow cell system at identical shear rates to these during adsorption and adhesion. Then the slabs were removed, air-dried, and stained with 5% crystal violet. The number of attached bacteria was determined with a light microscope by counting ten squares of 1000 μm^2 each in the central part of the substratum.

Analogous to Eqn. (1a), the curves representing the number of adhering bacteria versus the flow time were fitted to

$$n_b(t) = j_{0,b}\, \tau_b\, (1\text{-exp}\, (-t/\tau_b)), \tag{1b}$$

where $n_b(t)$ is the number of adhering bacteria at time t (in cm^{-2}), $j_{0,b}$ is the initial deposition rate (in min^{-1} cm^{-2}), τ_b is the relaxation time (in min), and t is the flow time (in min).

Similar to Eqn. (2a), the number of bacteria adhering in a stationary state to a given substratum was calculated from

$$n_s = j_{0,b}\, \tau_b. \tag{2b}$$

Quantification of adsorbed sIgA (ELISA)

The immunological reaction between horse-radish peroxidase (HRP) conjugated anti-IgA and IgA was used for the detection and quantification of adsorbed sIgA. The quantification was performed by an enzymatic reaction of the HRP of conjugated anti-IgA with hydrogen peroxide in a buffered *ortho*-diphenyldiamine (OPD) solution. The subsequent colour change of the OPD in solution is proportional to the amount of adsorbed sIgA [16].

Calibration curves were constructed by adsorbing known amounts of sIgA (0, 20, 50, 100, 200, 300, 400, 500, 750, and 1000 ng) from 1.5 ml of potassium phosphate buffer to disposable polystyrene cuvettes (Lab Center, Breda, The Netherlands). After incubations for 16 h at room temperature, the supernatants were removed and the cuvettes were washed extensively with potassium phosphate buffer. Then the cuvettes were incubated for 45 min at 37°C with 1.5 ml of 1:1000 diluted HRP conjugated anti-IgA (TAGO Inc., Burlington, CA, USA) in buffer (0.01 M Tris–HCl,
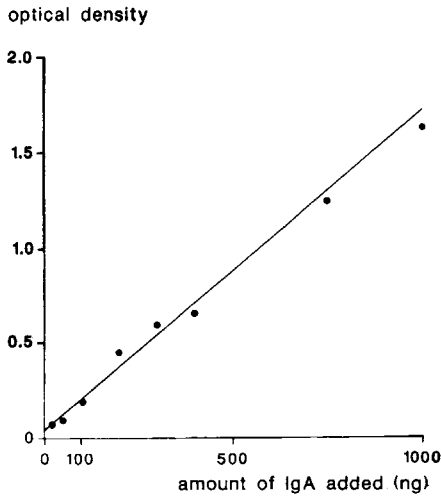
optical density

Figure 3. Linear regression line for optical densities vs. the amounts of sIgA added and adsorbed to cuvettes (coefficient of correlation $r = 0.995$).

0.2 M NaCl, 0.05% Tween, 1% BSA, pH 8.0). Thereafter the cuvettes were washed three times with rinsing buffer (0.01 M Tris–HCl, 0.15 M NaCl, 0.05% Tween, pH 8.0). Finally, 1.5 ml of phosphate-buffered OPD solution (Sigma Chemical) containing 0.05 M sodium phosphate, 0.02% OPD, and 0.15% H_2O_2 was added and after 30 min incubation at room temperature in the dark the reaction was stopped with 0.1 ml at 0.05 M H_2SO_4. The optical densities (OD) of 0.3 ml of the solutions were measured at 492 nm using a multiscan EIA reader (Flow Reader Amstelstadt, Amsterdam, The Netherlands) with a precision of 0.5%, yielding the calibration curve shown in Fig. 3. This curve could be fitted by linear regression according to
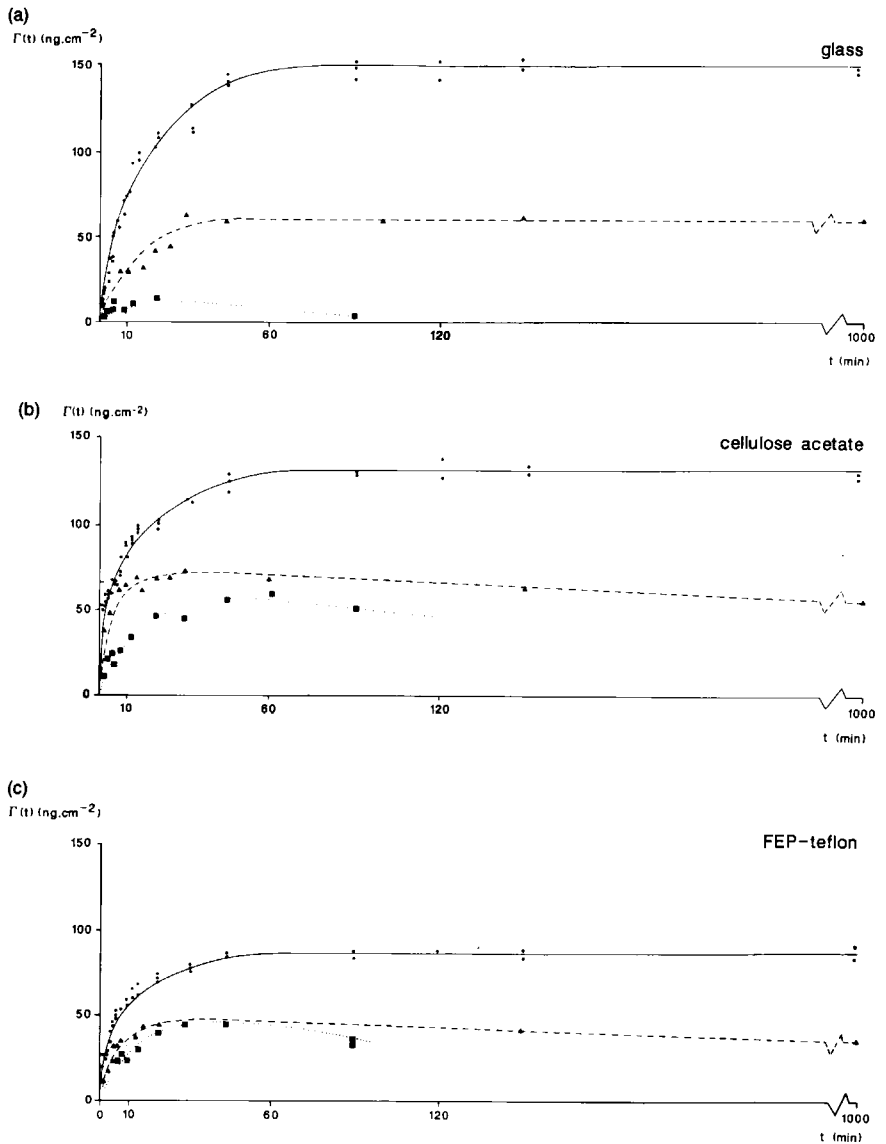
$$OD = 1.67 \times 10^{-3} \times \text{amount of sIgA added (ng)} + 0.040 \qquad (3)$$
$$\text{(linear correlation coefficient } t = 0.995).$$

The supernatants of the solutions used for the preparation of the calibration curve were found to be essentially free of sIgA, as checked by a conventional ELISA, indicating that all the sIgA added had adsorbed from the solution to the polystyrene cuvettes.

In order to quantify the unknown amounts of sIgA adsorbed to the various artificial solid substrata, the slabs with adsorbed sIgA were put in clean polystyrene cuvettes and treated as described above, starting with the incubation with 1.5 ml conjugated anti-IgA. Subsequently the optical densities could be transformed into surface concentrations using the calibration curve shown in Fig. 3.

Owing to the dimensional limitation of the available enamel samples, the above procedure, including calibration, was carried out in microtiter plates for the quantification of sIgA adsorbed onto enamel. Also for this reason, no adsorption experiments were done on enamel with diluted saliva solutions.

(a)
$\Gamma(t)$ (ng.cm^{-2})

glass

[Graph (a): y-axis marked 0, 50, 100, 150; x-axis marked 10, 60, 120, 1000 t (min)]

(b) $\Gamma(t)$ (ng.cm^{-2})

cellulose acetate

[Graph (b): y-axis marked 0, 50, 100, 150; x-axis marked 10, 60, 120, 1000 t (min)]

(c)
$\Gamma(t)$ (ng.cm^{-2})

FEP−teflon

[Graph (c): y-axis marked 0, 50, 100, 150; x-axis marked 0, 10, 60, 120, 1000 t (min)]

Figure 4. sIgA adsorption, Γ, from a sIgA solution and human whole saliva onto glass (a), cellulose acetate (b), and FEP Teflon (c) as a function of the adsorption time t. (—●—) sIgA solution with a concentration of 0.2 mg ml^{-1} lyophilized sIgA; (- -▲- -) human whole saliva with a protein concentration of 2 mg ml^{-1}; (··■··) human whole saliva with a protein concentration of 0.2 mg ml^{-1}.

RESULTS

Protein adsorption

Figure 4 shows the amounts of sIgA adsorbed onto the various artificial substrata from reconstituted human saliva with concentrations of 2 and 0.2 mg ml^{-1} lyophilized whole saliva and purified human salivary sIgA with a concentration of 0.2 mg ml^{-1} as a function of the adsorption time. The amount of sIgA adsorbed

Table 1.
Initial adsorption rates $j_{0,p}$ (in ng min^{-1} cm^{-2}), stationary state surface concentrations Γ_s (in ng cm^{-2}), and maximal surface concentration Γ_m (in ng cm^{-2}), of adsorbed sIgA from a single protein component (sIgA) solution, human whole saliva, and diluted saliva to various artificial solid substrata and human enamel[a]

Substratum	sIgA (0.2 mg ml^{-1})		Whole saliva (2.0 mg ml^{-1})			Diluted Saliva (0.2 mg ml^{-1})		
	$j_{0,p}$	Γ_s	$j_{0,p}$	Γ_s	Γ_m	$j_{0,p}$	Γ_s	Γ_m
FEP	8.3	90	9.4	$-$[b]	47	4.8	$-$[b]	47
CA	10.8	132	14.3	$-$[b]	73	4.2	$-$[b]	57
Glass	10	153	5	$-$[b]	65	1.3	$-$[b]	12
Enamel	$-$[c]	60	$-$[c]	$-$[b]	28	$-$[d]	$-$[d]	$-$[d]

[a]All experiments were done in triplicate, yielding an average intra-assay SD ($n = 3$) in $j_{0,p}$ and Γ_s and Γ_m of 15%.
[b]Owing to the existence of a maximum surface concentration, no stationary state surface concentration could be determined.
[c]Owing to the low surface concentrations, the initial adsorption rates were not calculated.
[d]The sensitivity of the ELISA technique did not allow quantification of the amount of sIgA on small dental enamel samples from diluted saliva.

from a single protein solution could be fitted to Eqn. (1a), yielding the initial adsorption rate $(j_{0,p})$ and the stationary state surface concentrations, which are summarized in Table 1. However, sIgA adsorption from reconstituted human whole saliva did not follow Eqn. (1a); after an initially linear increase, a maximum surface concentration was reached after approximately 1 h followed by a steady decrease in the surface concentration. These maximal surface concentrations Γ_m are also presented in Table 1.

In contrast to the situation with artificial solid substrata, sIgA adsorption onto human enamel from both a purified sIgA solution and from reconstituted human whole saliva obeyed Eqn. (1a) and no apparent maximum coverage was observed, as can be seen in Fig. 5. The characteristics of sIgA adsorption on enamel are also summarized in Table 1.
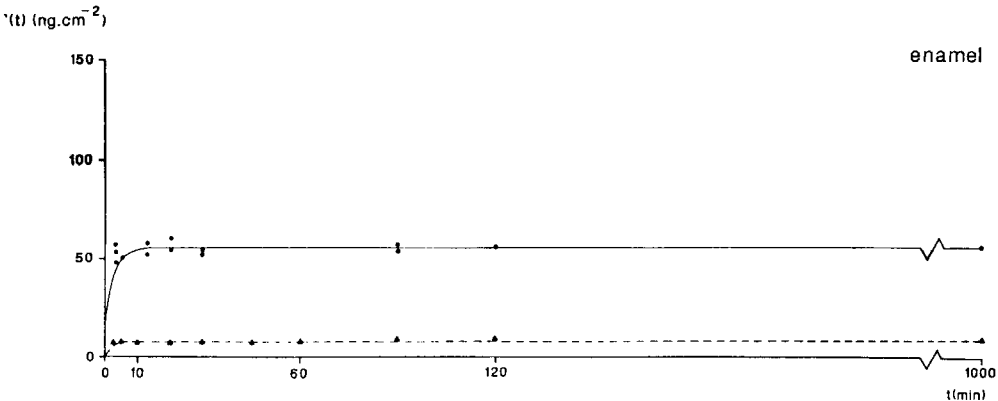
Figure 5. sIgA adsorption, Γ, from a sIgA solution and human whole saliva onto enamel as a function of the adsorption time t. (—●—) sIgA solution with a concentration of 0.2 mg ml^{-1} lyophilized sIgA; (- -▲- -) human whole saliva with a protein concentration of 2 mg ml^{-1}.

Table 2.
The numbers of oral streptococci ($\times$ 10^6 cm^{-2}) adhering in the stationary state, n_s, to various artificial solid substrata and enamel before and after exposure for 120 min to a purified sIgA (0.2 mg ml^{-1} solution and after exposure to human whole saliva (2.0 mg ml^{-1}) for 5 and 120 min[a].

Substratum/strain		Uncoated[b]	120 min sIgA	5 min saliva[c]	120 min saliva[c]
FEP/	*S. mitis* BMS	1.5	0.4	0.6	0.4
	S. sanguis 12	3.3	0.7	**1.1**	0.9
	S. mutans NS	3.6	0.5	**0.9**	1.5
CA/	*S. mitis* BMS	1.5	0.3	**0.5**	0.3
	S. sanguis 12	6.0	0.9	**2.2**	0.9
	S. mutans NS	5.7	0.5	**1.1**	1.6
Glass/	*S. mitis* BMS	1.0	0.2	**0.5**	0.2
	S. sanguis 12	7.3	0.6	**1.7**	0.9
	S. mutans NS	17.0	0.6	**1.4**	1.5
Enamel/	*S. mitis* BMS	0.7	0.4	**0.5**	0.6
	S. sanguis 12	4.1	0.8	**1.9**	0.7
	S. mutans NS	13.8	0.5	**1.3**	1.9

[a]The inter-assay coefficients of variation for the number of bacteria adhering at the stationary state varied for uncoated substrata from 10 to 15%, whereas they varied for the coated substrata from 20 to 50%.
[b]Data taken from ref. [12].
[c]Data taken from ref. [10].

Streptococcal adhesion

Table 2 summarizes the numbers of oral streptococci adhering in the stationary state, n_s, to various artificial solid substrata in the absence and presence of various protein coatings. From Table 2 it can be seen that precoating of the surfaces with purified sIgA from human whole saliva as well as with human whole saliva significantly reduces the adhesion of all strains.

DISCUSSION

The ELISA technique described in this paper enabled the quantification of adsorbed sIgA on artificial solid substrata as well as on human enamel surfaces. In addition, this technique could be used to study the competitive adsorption characteristics of sIgA from saliva. At this stage it must be emphasized that this technique cannot be used for the quantification of sIgA adsorbed in multilayers, because only the outer layer of adsorbed sIgA can bind HRP conjugated anti-IgA and thus be determined. Although this can be considered as a disadvantage, it is most relevant when studying protein adsorption in relation to bacterial and cellular adhesion, as these bioparticles interact primarily with the outermost adsorbed protein layer.

sIgA adsorption

The results presented in Table 1 suggest that sIgA adsorption is related to the surface free energy of the artificial solid substrata, as is illustrated graphically in Fig. 6. The data point for enamel in Fig. 6 shows that there is less sIgA adsorbed on enamel than
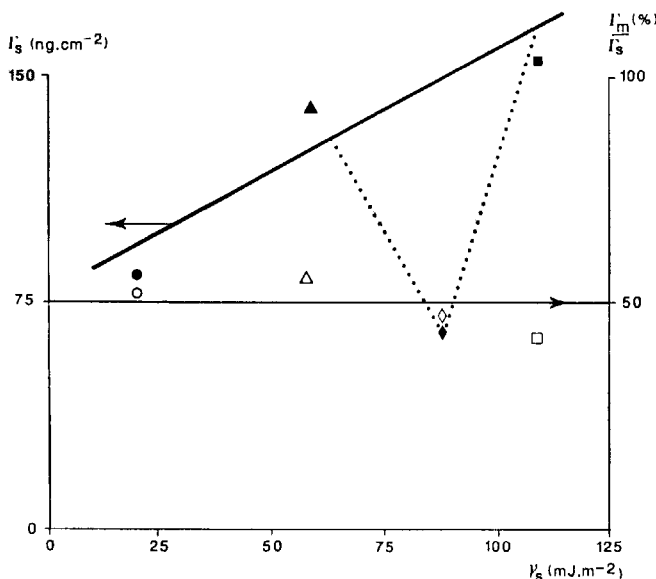
Figure 6. sIgA adsorption onto materials with different surface free energies. (—) Adsorbed at the stationary state, Γ_s, from a sIgA solution onto FEP Teflon (●), cellulose acetate (▲), glass (■), and enamel (♦) as a function of the surface free energy, γ_s. (····) The ratio $\Gamma_m/\Gamma_s \times 100\%$ for FEP Teflon (○), CA (△), glass (□), and enamel (◇) as a function of the surface free energy (γ_s).

expected on the basis of the findings for the artificial solid substrata. This can probably be explained by considering the complex structure of enamel, which consists of hydroxyapatite prisms interconnected by an organic matrix. Proteins leaching out of the organic matrix adsorb immediately on the prism surface, thereby leaving less surface available for sIgA [17]. It is also interesting to consider the ratio Γ_m/Γ_s as a function of the surface free energy, in which Γ_m is the maximum amount of sIgA adsorbed from saliva and Γ_s is the stationary state surface concentration of sIgA adsorbed from a purified sIgA.

This ratio, also shown in Fig. 6, has an almost constant value for both the artificial solid substrata and the enamel surface. This percentage (approximately 50%) is significantly higher than the percentage sIgA in saliva (approximately 10%), which indicates that all surfaces preferentially adsorb sIgA from saliva.

On the high surface free energy substrata for the concentrations of 2.0 and 0.2 mg ml^{-1} saliva, a clear concentration dependence is observed for selective sIgA adsorption from saliva; for the low surface free energy substratum FEP Teflon this concentration dependence is absent. Hence, it was concluded that sIgA adsorbs essentially irreversibly to FEP Teflon. However, a maximum in the concentration of sIgA at the outer pellicle surface during pellicle ripening was evident only for FEP Teflon. Therefore, the existence of this maximum in surface concentration is most likely due to screening of readily adsorbed sIgA by other proteins, rather than to displacement, which requires a more reversible binding.

A 'Vroman-like' effect has not yet been described in the literature for pellicle formation on an enamel surface [6, 8, 9, 18–21]. Although the results of this study do not present direct evidence for such a screening or displacement process for salivary protein adsorption on enamel, this phenomenon was observed on FEP Teflon.

Thus, a 'Vroman' effect may exist for pellicle formation on enamel too, but more research including other proteins is required to establish the existence of this phenomenon.

Thermodynamic analysis of streptococcal adhesion

The adhesion of bacteria to solid substrata involves specific interactions between complementary surface components [22] and physico-chemical surface characteristics such as charge, hydrophobicity [23–25], and surface free energies [26–29]. Using a thermodynamic approach in which electrical charge interactions are neglected, adhesion can be predicted on the basis of an interfacial free energy balance. According to Absolom et al. [27] and Busscher et al. [26], adhesion will be energetically favourable if

$$\Delta F_{adh} = \gamma_{sb} - \gamma_{sl} - \gamma_{bl} < 0, \tag{4}$$

where ΔF_{adh} is the interfacial free energy of adhesion, γ_{ab} is the solid–bacterium interfacial free energy, γ_{sl} is the solid–liquid interfacial free energy, and γ_{bl} is the bacterium–liquid interfacial free energy.

Adhesion will be energetically unfavourable if

$$\Delta F_{adh} > 0. \tag{5}$$

The interfacial free energies can be calculated from an approach based on separating surface free energies into a dispersion and a polar component denoting van der Waals interactions and the capacity to donate or accept hydrogen ions, respectively [30]. According to the geometric mean equation, the interfacial free energy between any two surfaces, 1 and 2, can be expressed using the dispersion and polar components by

$$\gamma_{12} = \gamma_1 + \gamma_2 - 2(\gamma_1^d \gamma_2^d)^{1/2} - 2(\gamma_1^p \gamma_2^p)^{1/2},$$

where γ_{12} is the interfacial free energy between surfaces 1 and 2; γ_1 and γ_2 are the surface free energies of surfaces 1 and 2 respectively; γ_1^p and γ_2^p denote the polar components of the surface free energies of surfaces 1 and 2, respectively; and γ_1^d and γ_2^d denote the dispersion components of the surface free energies of surfaces 1 and 2, respectively.

Recently we have demonstrated that the adhesion of a wide variety of oral streptococcal strains adhering at the stationary state to homogeneous artificial solid substrata as well as to an inhomogeneous enamel surface can be described by the following thermodynamic model:

$$n_s = a(\Delta F_{adh} - 23.1) + 1.2,$$

where n_s is the number of bacteria adhering at the stationary state to a solid substratum (in $10^6 \, cm^{-2}$); ΔF_{adh} is the interfacial free energy of adhesion (in $mJ \, m^{-2}$); and a is a strain-specific microbial factor (in $10^{10} \, mJ^{-1}$), which is associated with the absence or presence of fibrillar surface appendages and the capacity of the strain to excrete substances with a tendency to modify the substratum properties.

From Fig. 7, representing the numbers of bacteria adhering at the stationary state to the different uncoated and sIgA-coated surfaces as a function of the interfacial
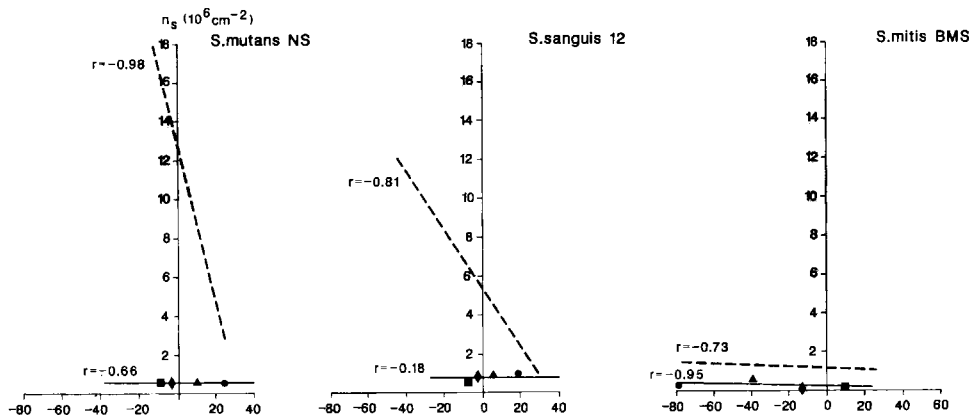
Figure 7. The numbers of bacteria adhering at the stationary state, n_s to glass (■), enamel (♦), CA (▲), and FEP Teflon (●) as a function of the interfacial free energy of adhesion, ΔF_{adh}, after 2 h exposure to a sIgA solution (0.2 mg ml^{-1}). The numbers of bacteria adhering at the stationary state to clean substrata are indicated by a dotted line. Data taken from regs [10, 12].

free energy of adhesion, it is clear that the sensitivity of *S. mutans* NS, *S. sanguis* 12, and *S. mitis* BMS to ΔF_{adh}, as represented by the '*a*' factor, is much lower for sIgA-coated substrata than for the uncoated substrata. However, the persistent tendency towards small negative slopes, albeit with admittedly low coefficients of correlation, suggests that the substratum properties are, in part, transferred to the bacterium–protein interface.

sIgA adsorption and oral streptococcal adhesion

sIgA is known to be a regular component of the acquired pellicle which can interfere with the adhesion of bacteria to solid surfaces [11]. We have determined the percentage reduction in adhesion for the three oral streptococcal strains, setting the adhesion to bare uncoated substrata to 100%. The percentage adhesion is plotted in Fig. 8 as a function of sIgA exposed at the outer pellicle surface. For *S. mitis* BMS and
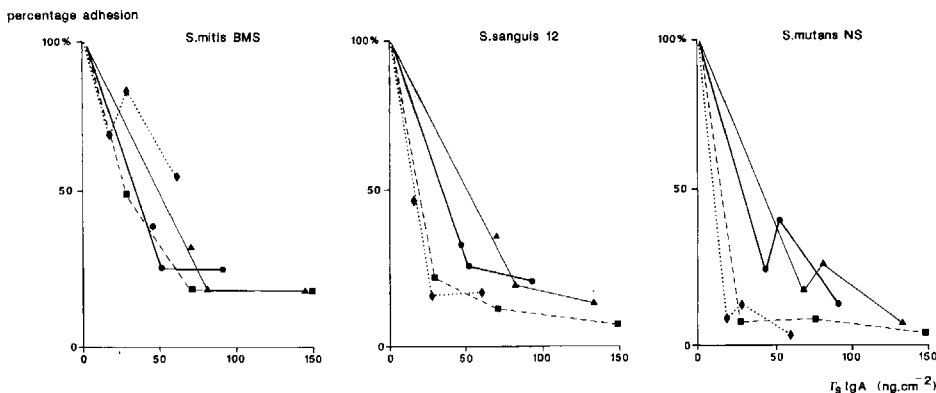
Figure 8. The percentage of adhesion of *S. mitis* BMS, *S. sanguis* 12, and *S. mutans* NS to FEP Teflon (●), cellulose acetate (▲), glass (■), and enamel (♦) (taking the adhesion to clean substrata as 100%) as a function of the amount of adsorbed sIgA at the outer pellicle surface adsorbed during 5 and 120 min.

particularly for *S. sanguis* 12, the percentage adhesion decreases gradually with increasing sIgA exposure, but irregularly for *S. mutans* NS. Speculatively, this could suggest that for *S. mitis* and *S. sanguis* this reduction is mainly due to a non-specific protein effect. In the case of *S. mutans*, a specific protein effect could be involved next to the non-specific protein effect, since for this strain the effect of sIgA seems to depend more on the substratum to which it is adsorbed. This can be due to the conformational state in which a protein is adsorbed and which is expected to change more on hydrophobic surfaces such as FEP Teflon than on hydrophilic materials [31], thus exposing different antigenic groups on various solid substrata. These results are partly in contradiction with the results of Kilian *et al.* [11], who concluded that the decreased adherence of *S. sanguis* was thought to be due to both a specific and a non-specific protein effect. However, the net effect of these specific and non-specific interactions may well be dependent on the titre of the specific antibodies, which can explain the differences between the results of Kilian *et al.* [11] and the results described in this paper.

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