

Influence of Divalent Cations and pH on Adsorption of a Bacterial Polysaccharide Adhesin

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Hyphomonas MHS-3 (MHS-3) elaborates a diffuse capsular material, primarily composed of polysaccharide, which has been implicated to serve as the holdfast of this prosthecate marine bacterium. A purified polysaccharide (fr2ps) from this capsular material exhibits a relatively large affinity for (Ge), or more precisely for the Ge oxide surface film. In its natural habitat MHS-3 attaches to marine sediments. This suggests that molecular properties of fr2ps have evolved to render it adhesive toward mineral oxides. In order to characterize these molecular interactions, the effect of divalent cations and pH on the adsorption of fr2ps to Ge has been measured using attenuated total internal reflection Fourier transform infrared (ATR/FT-IR) spectroscopy. The effect of adsorption of fr2ps on the Ge oxide film has been investigated using X-ray photoelectron spectroscopy (XPS). The results indicate that divalent cations participate in binding of fr2ps to Ge oxide and that atomic size of the cation is important. Evidence for significant participation of hydrogen bonding to the oxide surface is lacking. © 1998 Academic Press

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INTRODUCTION

Attachment of microorganisms to inert surfaces has been investigated in numerous publications [for reviews see (1–3)]. While proteins are known to mediate adhesion of microbes to biological surfaces in specialized ecosystems, presumably through stereospecific interactions (4–6), the adhesins and the molecular mechanisms involved in attachment of microbes to inert surfaces have not been as well characterized; however, there is evidence that specialized proteins (7, 8), polysaccharides (9, 10), and glycoproteins (11) have evolved to serve this purpose. In general, adsorption of globular proteins to inert surfaces is thought to be influenced by a large contribution from internal rearrangements (conformational changes) (12–14). Polysaccharides, as well as proteins lacking substantial secondary and tertiary structure, are more “deformable” (15) and therefore may provide a versatile set of interactions which

are directly related to functional group chemistry (16), and can be catered to serve specific types of adhesive functions to inert surfaces. In support of this assertion there is evidence that polysaccharides may have properties which are exploited by cells for either attachment to (17), or detachment from (18), inert surfaces.

The Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloidal stability predicts that biological cells, whose envelopes typically have a net negative charge, cannot approach closer than the secondary minimum, about 10–20 nm, from a typical (negatively charged) surface (19). Originating from this prediction is the hypothesis that cells utilize appendages to breach this electrostatic barrier, in order to induce irreversible attachment (20). In some cases proteinaceous fimbriae have been shown to provide this bridge (21, 22). Extracellular polysaccharides are invariably a major component of the polymeric matrix of most mature biofilms (23). Cells that elaborate an extracellular capsular polysaccharide while still in suspension could presumably utilize this as an appendage to initiate irreversible attachment.

In a number of previous studies, evidence has been presented indicating that the marine bacterium, *Hyphomonas* MHS-3 (MHS-3), utilizes a diffuse extracellular capsular material to bind to inert surfaces (24–26). A polysaccharide component of this capsular material (fr2ps) has been isolated and partially characterized. Studies of its adsorption behavior indicated fr2ps bound relatively strongly to an oxide surface (germanium) when ranked against an acidic polysaccharide (alginate), globular blood proteins, and a disordered protein which is a primary component of a natural marine epoxy resin. Adsorption behavior of fr2ps to various substrata was consistent with attachment preference of whole cells. In its normal habitat, MHS-3 is likely to encounter mineral surfaces and attach to them as part of its prosthecate lifecycle. Adsorption of organic molecules to mineral surfaces is thought to occur primarily through hydrogen bonding to oxide surfaces (27). It seems reasonable that divalent cations may also serve to link oxides with various functional groups on the polysaccharide. In this study we have characterized the influence of divalent

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cations and of pH on the adsorption behavior of fr2ps in order to further investigate the interactions involved in bonding of this adhesive polysaccharide at an aqueous/oxide interface.

MATERIALS AND METHODS

Isolation and Purification of fr2ps

The bacterium MHS-3 was isolated from shallow water sediments in Puget Sound, WA (24). Cultures of MHS-3 were grown in Marine Broth 2216 (37.4 g/L) (Difco Laboratories, Detroit, MI) at 25°C on a rotating shaker at 100 rpm. Teflon mesh (mesh opening, 1.8 mm, thread diameter, 0.5 mm, Tetko, Inc., Briarcliff, NY) was introduced into the culture vessels to provide greater surface area for attached growth. The attached cells and associated extracellular polymeric substance (EPS) were harvested from a culture in which the suspended cell population had just entered stationary phase. The medium was poured off, and the biofilm was removed from the Teflon mesh and the sides of the culture vessel walls by scraping.

EPS was extracted in two steps. The cell suspension was centrifuged for 20 min at 16,000*g*, and the EPS in the supernatant ("loosely bound") was precipitated with 4 volumes of ice-cold 2-propanol. The more "tightly bound" EPS was extracted from the cell pellet. The cell pellet was dispersed in a Waring blender in a minimum volume of 10 mM EDTA, 3% NaCl for 1 min at 4°C. The suspension was centrifuged for 15 min at 16,000*g* and the EPS from the supernatant was precipitated using 2-propanol as described above.

A crude EPS preparation was obtained by pooling the two EPS fractions and resuspending in a minimum volume of distilled water (dH₂O). They were then dialyzed for 12 h against dH₂O and lyophilized. Polysaccharide-enriched EPS (EPS_p) was prepared by a published protocol (28). EPS was dissolved in a minimum volume of 0.1 M MgCl₂, and DNase and RNase were added to a final concentration of 0.1 mg/ml, followed by incubation at 37°C for 4 h. Protease K was added to 0.1 mg/ml and incubated at 37°C overnight. Residual protein was removed with a hot phenol extraction, followed by a chloroform extraction. The preparation was dialyzed for 12 h against dH₂O and lyophilized. The EPS_p preparations were stored desiccated at room temperature.

Preparative high performance size exclusion chromatography (HPSEC) fractionation of EPS_p was performed using a Hewlett Packard 1090 liquid chromatograph equipped with a diode array UV-vis spectrometer (Shodex OHpack B-2004 column). The mobile phase was 0.5 M NaCl, 0.05 M ortho-PO₄ at pH 7.0 in dH₂O. EPS_p was dissolved in the mobile phase at a concentration of 1 mg/ml and filtered through a 0.22 μm Millipore filter. The injected sample volume was 1 ml. The column was run at room temperature at a flow rate of 0.9 ml/min. The adhesive polysaccharide fr2ps was isolated as the second peak in the chromatogram (26) and concentrated in 0.01 M NaCl using ultrafiltration

(Amicon 8010 stirred ultrafiltration cell, Amicon 5YM5 membrane). Aliquots were stored at -40°C.

Chemical Analysis

Neutral hexose content was determined using the phenol sulfuric acid assay with glucose as the standard (29). Protein content was estimated using the Lowry procedure with bovine serum albumin (BSA) as standard (30). Uronic acid content was determined by the *m*-hydroxydiphenyl method (31).

Surface Preparation

Single-crystal, cylindrical germanium (Ge) internal reflection elements (IRE) (Spectra Tech, Stamford, CT) were cleaned by ultrasonication in a base bath (saturated KOH in isopropyl alcohol) for 10 min. Following the base bath were two rinses in ultrapure water followed by a gentle scrubbing with undiluted Micro cleaning solution using cotton swabs. The cleaning solution was flushed off in a hard stream of ultrapure water. The IRE was then subjected to the following rinses which consisted of a 10 min ultrasonication in the liquid: ultrapure water (2×), and ethyl alcohol. After cleaning IREs were stored at 100°C for 12 h before being used for adsorption studies.

Adsorption Protocol

All adsorption experiments were performed in some modification of a synthetic seawater having the composition (w/v): 2.3% NaCl, 0.024% Na₂CO₃, 0.033% KCl, 0.4% MgCl · 6H₂O, 0.066% CaCl₂ · 2H₂O, pH adjusted to 8.4 with HCl. Modifications used for each experiment are explicitly given with the results. For adsorption experiments investigated using FT-IR the cylindrical IRE was positioned within a stainless steel flow chamber (Circle Cell, Spectra Tech, Stamford, CT). Details have been described elsewhere (32). A simple flow through system was used to deliver solutions into the flow chamber. Teflon valves (Cole-Parmer, Niles, IL) served to shuttle the appropriate solution into tubing leading to the flow chamber. All tubing leading into the flow chamber as well as the fittings were Teflon (0.08 cm I.D.).

For adsorption experiments frozen aliquots were thawed and diluted with the appropriate ionic buffer to produce a solution with the desired ionic composition and pH. Before each adsorption experiment the surface was exposed to the appropriately modified synthetic seawater for 20 min under flow. A vial containing 1 ml of approximately 0.025 mg/ml of fr2ps in this aqueous solution was inserted into the flow system, and the contents were immediately pumped through a short section of leader tubing and through the flow chamber for 125 s. (The estimate of concentration is based upon neutral hexose content of the frozen aliquots.) Flow was then stopped to allow adsorption for 60 min. Adsorption was performed under these static conditions to conserve purified polysaccharide. Flow was

then resumed, and the surface was rinsed with the modified synthetic seawater for 30 min.

For XPS analysis adsorption was onto 1 cm diameter Ge disks (Harrick Scientific Corp., Ossingen, NY). Ge disks were cleaned as described above. Samples were exposed to fr2ps in glassware cleaned with a H_2SO_4 -Nochromix mix (Godax Laboratories, New York, NY). The rinse was performed using fluid displacement. Samples were dried overnight in the FT-IR chamber before measurement.

FT-IR Spectroscopy

During the course of each experiment infrared spectra were acquired periodically using a Perkin-Elmer Model 1800 Fourier transform infrared (FT-IR) spectrophotometer. Experimental details are described elsewhere (32). FT-IR measurements were made at a FT-IR chamber temperature measured for each experiment at $23 \pm 0.5^\circ\text{C}$.

Estimation of Surface Coverage

Estimation of surface coverage was based on absorbance of the transmission spectrum of fr2ps, concentrated by freeze-drying and rehydrated to 50 mg/ml, obtained using a 15 μm transmission cell with CaF windows (Spectra Tech). A modification of a previously published derivation (25) was used. Briefly, it was assumed that

$$c_{B,E} = [(A_{W,T}/A_{W,E})(A_{S,E}/A_{S,T})]c_{B,T}, \quad [1]$$

where $A_{W,T}$ is the absorbance of water (band at 1640 cm^{-1}) in transmission, $A_{W,E}$ is the absorbance of water probed evanescently (ATR mode), $A_{S,E}$ is the absorbance of the substance of interest (fr2ps in this case) probed evanescently, $A_{S,T}$ is the absorbance of the substance in transmission, $c_{B,T}$ is the (known) concentration of the substance in the transmission cell, and $c_{B,E}$ is the bulk or volume concentration of the substance probed evanescently. Using glucose solutions as standards, Eq. [1] was found to predict $c_{B,E}$ with >90% accuracy. This calculated volume concentration is related to the (unknown) adsorbed amount by

$$\Gamma = (d_p/2)(c_{B,E}), \quad [2]$$

where d_p is the penetration depth of the evanescent (electric) field and Γ is the surface coverage (mass/area).

A spectrum of dehydrated fr2ps was obtained by placing 2 μl of a 50 mg/ml aqueous solution on the surface of the IRE and allowing it to dry in the FT-IR chamber until the water band centered at 1640 cm^{-1} disappeared.

XPS Analysis

XPS spectra were obtained with a Physical Electronics Model 5600 spectrometer (Physical Electronics, Eden Prairie,

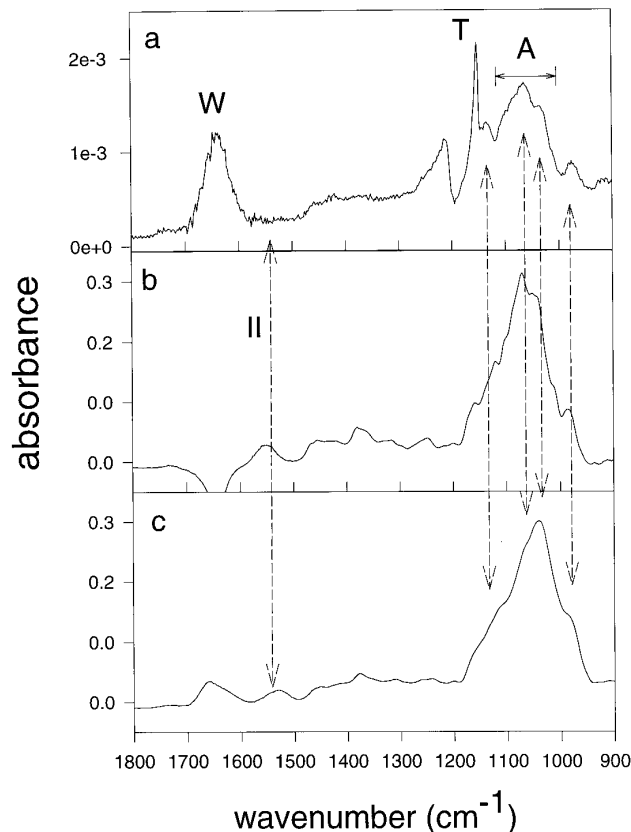


FIG. 1. FT-IR spectra of fr2ps: (top) adsorbed onto the Ge IRE (hydrated); (middle) transmission spectrum of hydrated bulk sample (50 mg/ml); (bottom) sample dried onto Ge IRE. W, region of strong water absorption at 1640 cm^{-1} ; T, absorbance from Teflon O-rings of flow chamber; A, composite band used to measure fr2ps adsorption; II, amide II band. Broken lines are to aid the eye in comparison and for discussion.

MN). A 5 eV flood gun was used to offset charge accumulation. A 800 μm diameter area was analyzed using a monochromatized $\text{AlK}\alpha$ X-ray source at 300 W and a pass energy of 23.50 eV. The binding energy scale was referenced by setting the CHx peak maximum in the C1s spectrum to 285.0 eV. Curve fitting was performed using Peakfit (Jandel Scientific, San Rafael, CA).

RESULTS

Chemical Characterization

Colorimetric assays indicated that fr2ps consisted primarily of neutral hexose (97.8%) with small amount of protein (2.2% w/w) and a trace amounts of uronic acids (<0.1%).

FT-IR spectra of three different preparations of fr2ps are shown in Fig. 1: fr2ps adsorbed to the Ge IRE (hydrated) (Fig. 1a), a transmission spectrum of bulk, hydrated fr2ps (Fig. 1b), and a spectrum of fr2ps dehydrated on the Ge IRE (Fig. 1c). Since the dehydrated sample consisted of 0.1 mg spread over about 0.5 cm^2 ($200\text{ }\mu\text{g}/\text{cm}^2$) this can be considered to be a

spectrum of bulk, dehydrated fr2ps. Comparison of the three spectra reveals differences in the band pattern. The spectrum for the adsorbed sample (Fig. 1a) is representative of spectra obtained under all the different adsorption conditions used. Close scrutiny of spectra of adsorbed fr2ps revealed no consistent discernable differences in band pattern for the different buffer conditions. The dehydrated sample (Fig. 1c) has bands at positions typical for proteins: amide I at 1660 cm^{-1} , amide II at 1528 cm^{-1} , as well as bands near 1460 , 1380 , and 1250 cm^{-1} which have been assigned to CH_3 , CH_2 , and amide III vibrational modes, respectively (33, 34). The amide II band is shifted, respectively, in the hydrated bulk sample (Fig. 1b) and notably absent in the adsorbed sample (Fig. 1a). The region where the amide I band should appear is obscured by residual bands from subtraction of the large water band centered at 1640 cm^{-1} (indicated by W in Fig. 1a) in the hydrated bulk and adsorbed samples. Careful examination of this region in the spectrum of adsorbed fr2ps revealed no trace of a component band at any other wavelength than 1640 cm^{-1} . Therefore, the FT-IR data indicate that the adsorbed component consists entirely of polysaccharide (within the limits of detection), while the bulk fr2ps sample has a significant, though small, protein component (corroborating the colorimetric analysis). This suggests that the polysaccharide portion of the bulk material is not bound up with the small protein component and out-competes it in the adsorption process. The protein content of fr2ps as estimated by FT-IR is higher than the estimate by the colorimetric assays (7.0% protein w/w).

The polysaccharide C–O stretch region extends from approximately 1200 to 900 cm^{-1} . Maxima of the most prominent component bands in this region are shifted, respectively, in spectra of the adsorbed sample and hydrated bulk sample (Fig. 1a and b); while spectra of both the adsorbed sample and dehydrated bulk sample have four bands in nearly the same positions (indicated in Fig. 1 by the dashed lines). Thus, with respect to position (not relative amplitude) of prominent component bands in this region of the spectrum, the adsorbed sample is slightly more similar to the dehydrated bulk sample than to the hydrated bulk sample. This suggests that interactions with the Ge substratum upon adsorption alter hydration of portions of the polysaccharide. Removal of interfacially bound water has been identified as a precondition for formation of strong adhesive bonds between biopolymers and a surface (35). It seems plausible that a binding mechanism involving displacement of interfacial water would also be likely to influence the hydration shell of the biopolymer. Following along with this interpretation, there are three shifts downward (1070 to 1065 cm^{-1} , 1040 to 1035 cm^{-1} , and 1000 to 994 cm^{-1}) and one shift upward (1119 to 1136 cm^{-1}) upon adsorption. In this region absorption bands arise primarily from C–C and C–O stretching modes (36) with a significant contribution from COH bending modes (37). The FT-IR data are thus consistent with a substratum bonding mechanism that involves pyranose ring carbons and oxygens.

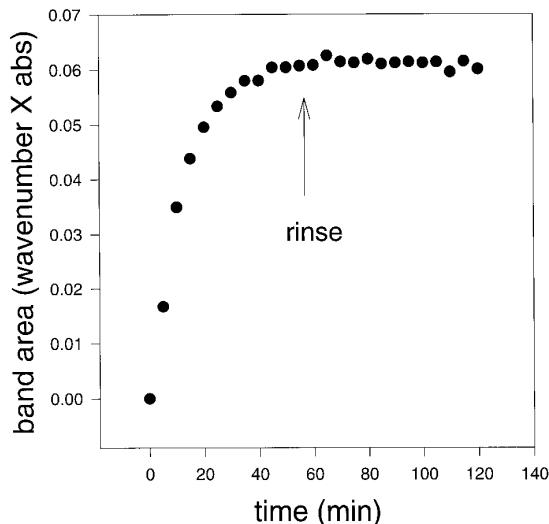


FIG. 2. Kinetics of adsorption of fr2ps onto the Ge IRE measured by computing band areas of the composite band indicated in Fig. 1. Time at which rinse was initiated is indicated. Band area has been converted to surface coverage in remainder of the figures using Eqs. [1] and [2].

Adsorption Studies

Adsorption and desorption of fr2ps to the Ge IRE was monitored by computing areas of the composite band indicated in Fig. 1a. A kinetic binding curve is shown in Fig. 2. Adsorption is typically irreversible in the empirical sense that the adsorbed component is not removed from the surface when the bulk solution is replaced with the rinse solution. All adsorption studies were performed at the same bulk concentration of fr2ps. This concentration was chosen because it yielded an easily detectable amount of adsorbed fr2ps, but was below surface saturation estimated from previous binding curves (Fig. 3) (26). Concentration of bulk fr2ps is given in terms of neutral hexose content of frozen aliquots as determined by the colorimetric assay.

Effect of Divalent Cations

The binding curve presented in Fig. 3 was obtained in unmodified synthetic seawater. It was found that omission of the Na from this solution did not change the adsorbed amount significantly (see Fig. 3). The influence of variable amounts of K was minimal (data not shown). Carbonate is the primary buffer and is necessary in order to maintain the pH. With these results as a starting point a set of test solutions was chosen with invariant concentrations of K and carbonate, as in the unmodified synthetic seawater, and variable concentrations of Ca, Mg, or Sr. The pH was 8.4 for this set of experiments. The ionic strength was adjusted with NaCl so as to be the equivalent for all experiments. The effect of each of the three divalent cations on adsorption was tested in the absence of the other two.

The effect of Ca on the amount of adsorbed fr2ps remaining

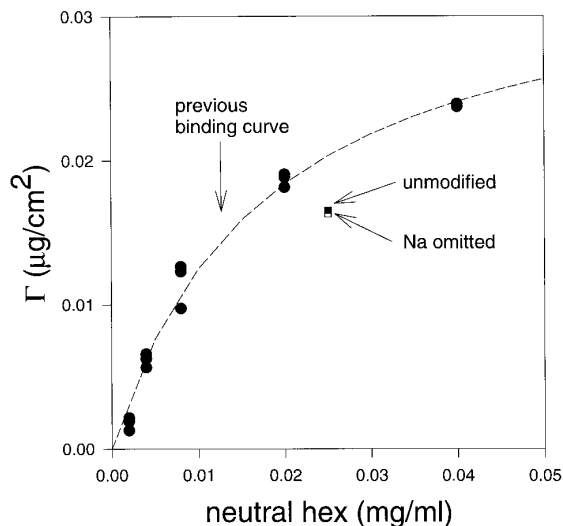


FIG. 3. Filled circles: binding curve and fit of Langmuir model (broken line) for fr2ps onto Ge IRE obtained with a previous preparation (26). Surface coverage was computed from band area as described in the text. Bulk concentration of fr2ps is given in terms of the total neutral hexose content determined by the colorimetric assay. Squares: results obtained with batch of fr2ps used for these experiments in unmodified synthetic seawater (filled) and synthetic seawater with Na omitted (unfilled).

at the end of the rinse is shown in Fig. 4a. There is a sharp increase in the adsorbed amount at a concentration of approximately 20 μM Ca. Fig. 4b shows the effect of substitution of Mg and Sr on the adsorption of fr2ps. Since the range of concentrations tested was relatively large they are exhibited on a log scale. (The Ca curve is included for comparison). It appears that Sr can, to some extent, replace Ca but that Mg is not nearly as effective in promoting adsorption. The influence of Ca and Sr on adsorption could not be tested at higher concentrations because a carbonate precipitate formed (without addition of fr2ps).

Effect of pH

In order to test the effect of pH on fr2ps adsorption a solution consisting of 22 μM Ca was chosen. This Ca concentration is at a threshold for influencing adsorption of fr2ps (Fig. 4a). Therefore, sensitivity to other solution components might be expected to be maximal. It was necessary to include 0.952 mM Mg in order to prevent a carbonate precipitate from forming in the more basic solutions. The influence of pH on adsorption of fr2ps is shown in Fig. 5, indicating that, in general, more basic conditions enhance adsorption. The exception to this trend at pH 8.0 is reproducible, suggesting a complex interaction between functional groups.

XPS of Adsorbed fr2ps

XPS spectra of the $\text{Ge}2p_{3/2}$ region of fr2ps adsorbed at 4 and 40 μM Ca onto Ge and of a clean Ge substratum are shown in Fig. 6. The three samples were stored under identical condi-

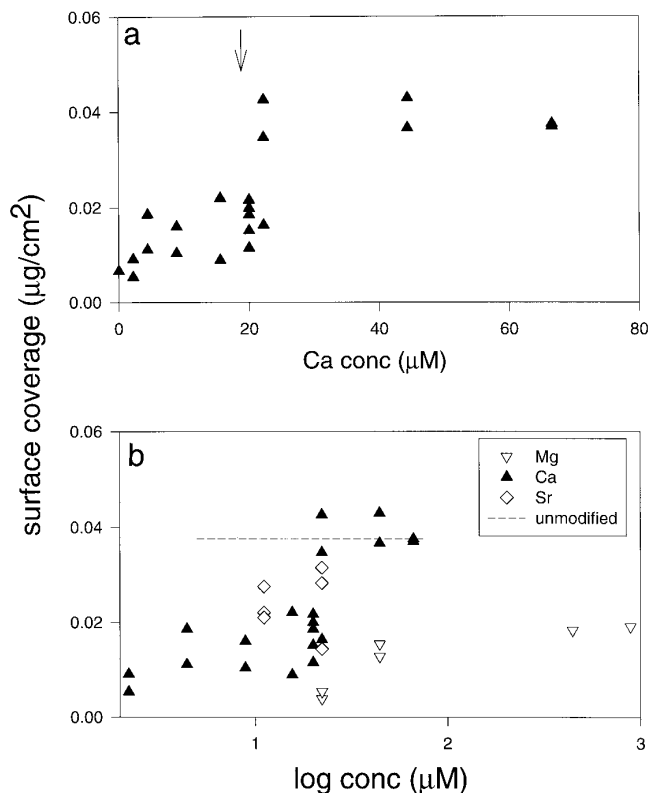


FIG. 4. (a) Effect of Ca on adsorption of fr2ps. Arrow indicates transition point. (b) Effect of Mg, Ca and Sr on adsorption of fr2ps. Level of adsorption for unmodified synthetic seawater is indicated (broken line). Note log scale in (b).

tions and loaded into the XPS instrument at the same time. The relative size of the Ge oxide peak (at higher binding energy) appears to become progressively smaller compared to the Ge peak as more fr2ps is adsorbed. The $\text{Ge}3d$ region exhibits the

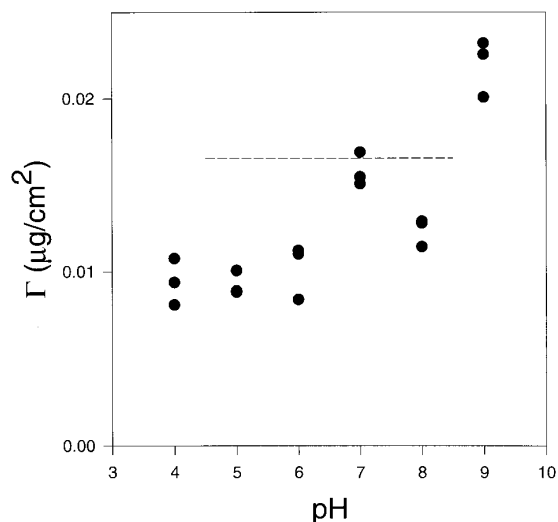


FIG. 5. Effect of pH on adsorption of fr2ps. Level of adsorption for unmodified seawater is indicated (broken line).

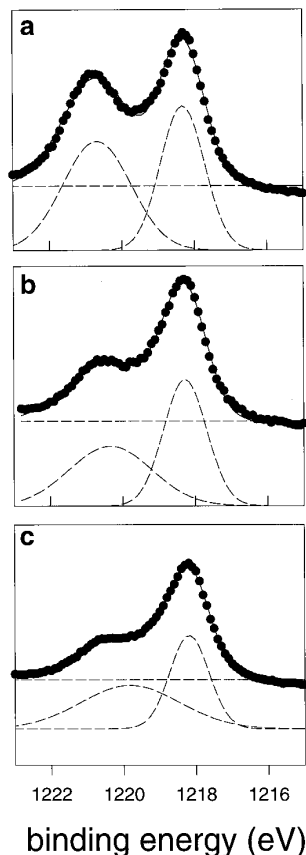


FIG. 6. XPS of Ge disks, Ge $2p_{3/2}$ bands; Ge oxide is on the left. (a) Clean Ge; (b) fr2ps adsorbed at 4 μ M Ca; (c) fr2ps adsorbed at 40 μ M Ca. Curve fits are indicated by lines, data by filled circles, and Gaussian curves constituting each fit by broken lines. Counts have not been included for simplicity of presentation.

same trend (data not shown). In a separate experiment a drop of bulk fr2ps was dried on Ge which was cleaned by the protocol given above. XPS measurements indicated no detectable Ge oxide peak (data not shown). Ge exposed to the solution containing 40 μ M Ca (with no fr2ps) exhibited an oxide peak larger than that of clean Ge.

The XPS data in Fig. 6 was fit with a set of Gaussian curves. This provided a better fit than Lorentzian, Voigt, or mixed Gaussian–Lorentzian curves by both the criteria of the R^2 value and the functionality exhibited by the residuals. The simplest fit is obtained with two Gaussian curves as shown for the Ge $2p_{3/2}$ spectra. The ratio of the areas of the Gaussian curves which correspond to the Ge and Ge oxide peaks is 0.85, 1.11, and 1.87 for peaks in Fig. 6a, b, and c, respectively, corroborating the qualitative appraisal.

High resolution spectra were also obtained for the C1s region, the O1s region, the five Ca regions (2s, $2p_{1/2}$, $2p_{3/2}$, 3s, and $3p_{1/2}$), and the N1s region. Ca and N (an indication of protein) were not detected. The C1s region and O1s regions of the adsorbed fr2ps displayed satellite bands with shifts to higher binding energies consistent with the presence of C–O–H

or C–O–C bonds (1.4 eV, O1s; 1.5 eV, C1s and C=O bonds (3.2 eV, C1s) (data not shown) (38). There were no chemical shifts in this region which could be interpreted as originating from formation of bonds with the Ge oxide surface.

DISCUSSION

It has been proposed that a capsular extracellular polysaccharide with the appropriate extension and functional groups could serve to bridge an electrostatic barrier that prevented a bacterium from approaching closer than 10–20 nm from a surface (39). Divalent cations, especially calcium ions, have been found to play a role in adhesion of bacteria (40, 41) and algae (42) to inert surfaces. It is obvious that divalent cations have the appropriate oxidation state to mediate ionic bonds between a negatively charged surface and anionic functional groups of an extracellular polysaccharide. Although this interaction has not yet been demonstrated in the published literature, preliminary data indicate that it makes a significant contribution to binding of some polysaccharides (personal communication with Georges Belfort, Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, NY). Many types of hydrogen bonds are possible between mineral oxides and organic molecules (27). This implies that hydrogen bonding may contribute significantly to the adsorption of polysaccharides onto mineral surfaces in seawater.

A number of previous investigations have indicated that fr2ps serves as an adhesion for the marine bacterium MHS-3 and that it adsorbs to Ge with a comparatively high affinity (24–26). Ge is not a common element of marine sediments; however, it is in the same group as silicon, and its oxide film is likely to have some of the general characteristics of mineral oxides. Although proteins generally adsorb strongly to Ge, this is not the case for polysaccharides. We have tested alginate (carboxylate functionality), hyaluronic acid (*N*-acetyl and carboxylate functionalities), and cellulose (glucose subunits), and all of these adsorb only slightly to Ge. This suggests that fr2ps has some special molecular characteristics which enhance its adsorption onto oxide films.

The XPS data indicate that fr2ps interacts strongly with the Ge oxide. Theoretically, the expected proportion of Ge to Ge oxide photoelectron intensities would be invariant for a simple three layer film (fr2ps/Ge oxide/Ge bulk material), with variable thickness of the fr2ps overlayer. This ratio has been used to estimate the oxide film thickness (43): as the thickness of the oxide decreases, the relative ratio (Ge oxide to Ge) decreases. Ge forms an amphoteric oxide that could presumably be dissolved in either acidic or basic solutions. Thus one possibility is that acidic or basic functional groups on fr2ps, which are concentrated near the surface upon adsorption, result in a local pH which degrades the Ge oxide layer. Since fr2ps has been demonstrated to adsorb strongly to Ge relative to other biopolymers (26), it seems unlikely that it is dissolving the surface it adheres to, especially since the oxide provides the

most obvious chemistry for forming bonds with functional groups. A second possibility is that the Ge oxide is patchy and that fr2ps adsorbs preferentially to islands of Ge oxide, thus decreasing the XPS signal from these portions of the surface. This also seems unlikely. The Ge is single crystal and thus homogeneous, and the exposure to KOH during cleaning should create a virgin Ge surface for uniform formation of the oxide film upon exposure to air. A third possibility is that the apparent relative decrease in the Ge oxide band is actually the result of a shift and/or widening of component bands. The Ge oxide band originates from a shift in the measured binding energy of the Ge core electrons caused by electron withdrawal from the Ge shell by the electronegative oxygen. Interactions which lowered the oxidation state of the oxygen would decrease its electronegativity and might shift a portion of the oxide band to lower energy. This would result in an apparent increase in the proportion of Ge to Ge oxide in the XPS spectrum.

The data show a clear participation of Ca in adsorption of fr2ps to Ge. The effect is not simply one of charge shielding, since ionic strength was invariant. In addition, the interaction between the divalent cation and fr2ps which promotes adsorption appears to be sensitive to elemental properties (probably size) other than the oxidation state, since Ca cannot be replaced with Mg. This type of interaction with divalent cations is responsible for the gelling behavior of alginate in solutions containing divalent cations, and has been explained by the presence of a cavity formed by alginate that can accommodate some divalent cations better than others. In the model which is most accepted, the Ca^{+2} forms a coordination complex with two carboxylate functionalities and several hydroxyl groups of the pyranose ring (44).

There is no clear evidence for the importance of hydrogen bonding of fr2ps to Ge. The trend is that, as the pH is raised, the amount of fr2ps adsorbed becomes greater. Deprotonation of functional groups would be expected to decrease hydrogen bonding, since the proton donors would be replaced by negatively charged groups, thus increasing the electrostatic repulsion. The data for pH 8 suggests involvement of interactions of multiple functional groups with different pK values.

The abundance of carboxylate functionalities in fr2ps is very slight according to the chemical characterization. In addition, no trace of S or P was found in fr2ps (XPS) which might indicate other negatively charged functionalities. In addition, no Ca was detected in adsorbed films of fr2ps (XPS). Therefore, if fr2ps employs anionic functionalities and Ca^{+2} to form a complex with the Ge oxide the density of binding sites must be low relative to the number of monomers. The level of detection of the XPS was limited to >1% (relative elemental composition) by the Ge background: i.e., for every region in which a Ca band appeared, a small Ge band was also present. If it assumed that there is one uronic acid (UA) residue (carboxylate functionality) for every 200 residues of fr2ps then the elemental percent

of Ca would be between 0.03 and 0.003 for 1:1 binding ($\text{Ca}^{+2}:\text{UA}$), assuming between a 5 and 0.5 nm mean free path of the photoelectrons. It would make sense that a polysaccharide designed to bridge an electrostatic double layer would be nearly neutral and be only sparsely populated with anionic functionalities. Pinning of the polysaccharide via these rather sparsely located points might enable other bonds to form.

SUMMARY

The bacterial polysaccharide (fr2ps) binds strongly to Ge substrata. The mechanism by which it forms bonds has been probed by measuring the influence of ionic composition and pH on adsorption, and the influence of fr2ps adsorption on the Ge substratum. The data support a mechanism involving divalent cationic bridging between functional groups on fr2ps and the Ge oxide overlayer. The importance of atomic size of the divalent cation suggests that the interaction involves coordination of a number of fr2ps functional groups. Involvement of pyranose ring atoms is implicated. There is evidence for displacement of water contained in the fr2ps hydration shell upon adsorption.

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