Characterization of Interfacial Phenomena Occurring during Exposure of a Thin Copper Film to an Aqueous Suspension of an Acidic Polysaccharide

GILL G. GEESEY,*,[1] TEIKA IWAOKA,† AND PETER R. GRIFFITHS†

*Department of Microbiology, California State University, Long Beach, California 90840; and
†Department of Chemistry, University of California, Riverside, California 92521

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A Fourier transform infrared spectrometer equipped with a cylindrical internal reflection element was used to sample the solid–liquid interface of a metallic copper film submerged in an aqueous acidic polysaccharide solution. The presence of a polysaccharide absorption band at 1050 cm^{-1} in a water-subtracted spectrum supported previous spectroscopic data indicating polymer accumulation at the surface of the film. Firm binding of the polysaccharide to the surface after a 17-day exposure period was demonstrated by the retention of the 1050-cm^{-1} absorption band in spectra obtained after gentle rinsing of the film surface with polymer-free water. The sampling technique also provided evidence which suggests that acidic polysaccharides, including the firmly bound exopolymers produced by adherent cells of a freshwater sediment bacterium, promoted deterioration of the copper film. Internal reflection Fourier transform infrared spectroscopy appeared to be a useful, nondestructive method to study the sorption of biomolecules to a solid metal surface submerged in an aqueous medium. © 1987 Academic Press, Inc.

INTRODUCTION

Internal reflection spectroscopy has been employed in the past to obtain infrared spectra at solid–liquid boundaries. Jakobsen (1) described how internal reflection elements (IRE) could be coated with thin metal films to study reactions at a liquid–metal interface. IREs have also been used with Fourier transform infrared spectrometers (FT-IR) to determine the sequence in which blood proteins deposit on foreign substances implanted in a carotid–jugular shunt in animals (2, 3). Water can be accurately computer-subtracted with Fourier spectrometers to obtain useful spectra of substances in a hydrated state. The geometry of the rectangular surface face of the IREs used in most internal reflection work, however, does not efficiently capture the round beam of FTIR spectrometers. In addition, the flat-surfaced IREs are not well suited for depositing surface films of uniform thickness.

Cylindrical internal reflection (CIR) is an innovation in internal reflection spectroscopy that overcomes these geometric limitations (4). Recently Iwaoka *et al.* (5) described a method to deposit thin metal films of uniform thickness on a CIR sampling element. These developments provided the opportunity to observe, in a nondestructive manner, interfacial changes that occur when biomolecules concentrate on or near a solid metal surface immersed in an aqueous environment.

Microorganisms elaborate exopolymers which promote attachment to surfaces. In aquatic environments, filaments of acidic polysaccharides commonly extend from the surface of sessile bacterial cells to many different types of submerged substrates, including metallic surfaces (6). Very little is known, however, of the chemical changes that occur at the solid–liquid boundary when hydrated biopolymers sorb to a submerged surface. Quite often, sampling difficulties have hampered progress in understanding interfacial phenomena.

This report describes how an FT-IR spectrometer equipped with a CIR cell was used to follow changes that occurred at the surface

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[1] To whom correspondence should be addressed.

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of a copper-coated germanium crystal exposed to a suspension of Gum Arabic. This commercially available, acidic plant polymer was selected to simulate exopolymers which anchor aquatic bacterial cells to solid surfaces because of the chemical similarities it shares with bacterial exopolysaccharides.

MATERIALS AND METHODS

Gum Arabic (Sigma Chemical Co., G-9752) was prepared as a 10% (wt/wt) aqueous suspension using glass, double-distilled water. The suspension was filtered through a membrane (0.45 μm pore size) to remove undissolved aggregates and the pH was adjusted to 6.5 prior to use. Samples for diffuse reflectance (DR) infrared Fourier transform spectroscopic analysis were prepared as dispersions of dried Gum Arabic (25%) in dried spectroscopic-grade KCl. The sodium salt of alginic acid (Sigma Chemical Co., A-2158) and a crude exopolymer preparation from an unidentified freshwater sediment bacterial isolate (7) were prepared in a manner similar to that of Gum Arabic except that the final polymer concentrations were 2 and 1%, respectively. Protein concentration in the Gum Arabic suspension was determined by the method of Lowry *et al.* (8).

Germanium (Ge) and zinc selenide (ZnSe) IREs (3.2 mm diameter, 38 mm length) were mounted in a stainless-steel "open boat" cell positioned in a CIR beam condenser accessory (Spectra-Tech, Inc., Stamford, CT). A copper film was deposited on the Ge IRE by vacuum evaporation using copper wire of 0.1 mm diameter (99.999%, puratonic grade, Alfa Products, Danvers, MA). Uniformity of the copper film on the IRE was evaluated by transmission electron microscopic examination of carbon-coated copper grids positioned in the vacuum chamber with the IRE during the evaporation process. Details of the coating process have been presented elsewhere (5).

Aqueous samples were added to the CIR cell, the optical bench containing the sample chamber was purged with purified dry air, and the infrared spectrum was obtained at various time intervals. Samples were maintained at room temperature during incubation and spectral acquisition. Rinse treatment was conducted by replacing the polysaccharide solution in the open boat with three successive changes of distilled water. Care was taken throughout the rinse treatment to avoid dehydration of the IRE surface.

Samples for diffuse reflectance spectroscopic analysis were packed into a sample holder positioned in a DR accessory (Spectra-Tech "Collector"). The optical bend and sample chamber were purged before acquisitions of spectral data.

All spectra were measured at 4 cm^{-1} resolution using a Nicolet 60-SX FT-IR spectrometer equipped with a medium-range mercury cadmium telluride detector. DR spectra obtained were transformed into the Kubelka–Munk format so that relative band intensities were comparable to those measured in a conventional absorbance spectrum.

RESULTS

The spectrum of an aqueous suspension of the plant polysaccharide, Gum Arabic, was obtained by CIR FT-IR. Using either Ge or ZnSe IREs, which permit sampling to different depths in the aqueous phase, similar spectra were obtained for the polymer after water subtraction (Figs. 1a and 1b). The spectra remained unchanged with time. A Kubelka–Munk plot of the DR spectrum of Gum Arabic in dehydrated KCl matrix provided an absorption spectrum similar to that obtained for the aqueous polysaccharide suspension when sampled with the ZnSe and Ge IREs (Fig. 1c). Only small spectral differences around 1600 cm^{-1} were observed which probably reflect different ionization states for the carboxyl group on the glucuronic acid subunit of the polysaccharide. The characteristic polysaccharide absorption band centered at 1050 cm^{-1} was assigned to the C–O stretch in the pyranose subunits of the polymer. Although it is not evident in Fig. 1 (in which all spectra were expanded

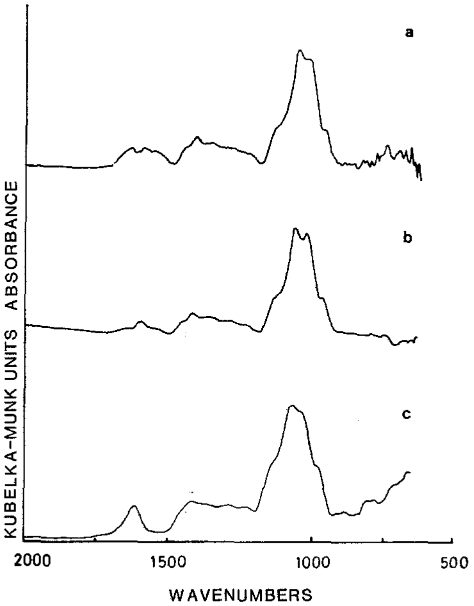

FIG. 1. FT-IR spectra of Gum Arabic: (a) absorption spectrum of aqueous sample obtained with an uncoated Ge IRE; (b) absorption spectrum of aqueous sample obtained with an uncoated ZnSe IRE; (c) Kubelka–Munk plot of diffuse reflectance spectrum in KCl matrix. All three spectra were expanded to fill the same ordinate scale.

to fill the full ordinate scale), the intensity of the polysaccharide absorption band was much stronger when sampled with the ZnSe IRE than with the Ge IRE.

No evidence of interaction of water with either uncoated or copper-coated IREs was observed, so that the 1640 cm^{-1} could be used as an internal standard. The intensity of the polysaccharide band at 1050 cm^{-1} increased relative to that of the 1640-cm^{-1} water band when the Ge IRE was coated with a thin film of metallic copper compared with the relative intensity when the solution was sampled with a bare Ge IRE. Evidence of polysaccharide accumulation near the surface of the copper film following a 20-min exposure period was obtained after subtracting the spectrum contributed by the bulk phase (based on compensation of the 1640-cm^{-1} water absorption) (Fig. 2).

With longer exposure times, the intensity of the polysaccharide absorption band increased (Fig. 3). Surprisingly, perhaps, the most conspicuous absorbance change was centered at 1640 cm^{-1} suggesting an increase in the effective path length of the evanescent wave through the solvent. This increase in water absorption with time did not occur when the copper-coated Ge IRE was exposed to double-distilled water which contained no Gum Arabic (5).

An increase in water absorption with time was also observed when the Cu-coated IRE was submerged in aqueous suspensions of other acidic polysaccharides. Exposure of the IRE to pH-adjusted suspensions of alginic acid or the exopolysaccharide from a freshwater sediment bacterium also led to a rapid increase in intensity of the 1640-cm^{-1} water band (Fig. 4). In fact, the initial rate of increase was greater in the presence of either of these two polysaccharides than with Gum Arabic.

Long-term (17 days) exposure of the Cu-coated Ge IRE to the 10% aqueous Gum Ar-

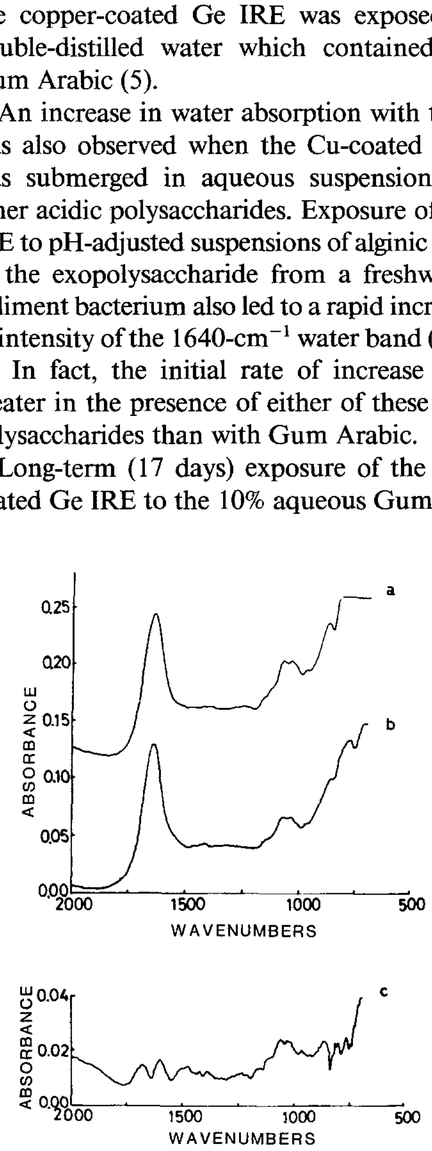

FIG. 2. (a) Spectrum of Gum Arabic solution in contact with Cu-coated Ge IRE for 20 min; (b) spectrum of Gum Arabic solution in contact with bare IRE, scaled by a factor (0.32) to make the absorbance at 1640 cm^{-1} equal to (a) above; (c) difference spectrum, (a) − (b). Note the weak polysaccharide band at 1050 cm^{-1} and evidence of protein adsorption from the bands at 1650 and 1550 cm^{-1}.

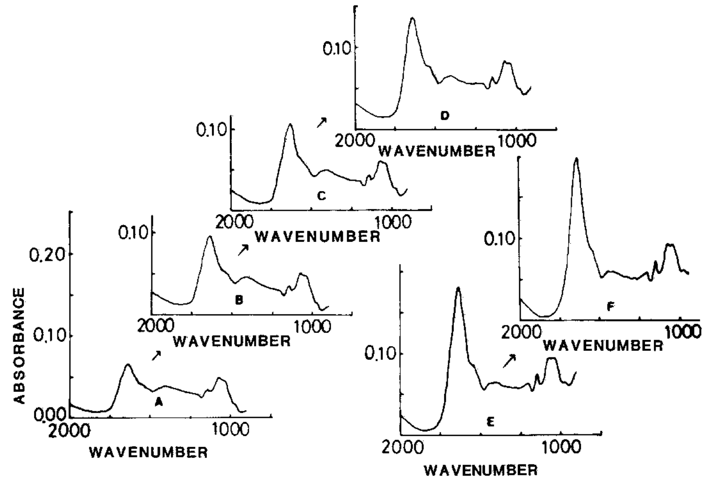

FIG. 3. Infrared spectrum at interface of copper-coated IRE and Gum Arabic solution after contact times of (A) 20 min, (B) 1 h, (C) 2 h, (D) 3.7 h, (E) 11 h, (F) 21 h. A subtraction factor of 1.00 was applied to all spectra.

abic solution resulted in a fourfold increase in intensity of the 1050-cm^{-1} band (Fig. 5a). Absorption bands centered at 1650 cm^{-1} (amide I) and 1550 cm^{-1} (amide II) were also detected at this time after water subtraction, indicating that protein, too, had accumulated at the surface of the Cu-coated Ge IRE.

Detection of protein was unexpected since care was taken to prevent microbial contamination. Analysis of the commercial preparation of the polysaccharide, in fact, revealed that it was contaminated with 1% (wt/wt) protein. That the protein was detected under these circumstances is evidence that it has a strong tendency to concentrate at the metal surface.

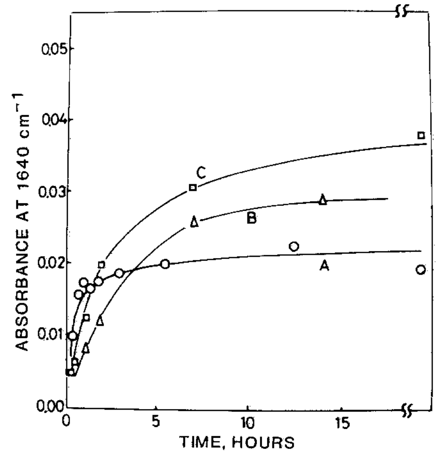

FIG. 4. Absorbance of a 1640-cm^{-1} water band as a function of exposure time of copper-coated Ge IRE to 2% alginic acid (○), 10% Gum Arabic (△), and 1% crude exopolymer from a freshwater sediment bacterium (□).

The mechanism responsible for the concentration of the polysaccharide at the copper surface was evaluated by rinsing the IRE after the 17-day exposure period. Three successive gentle rinses with polymer-free water resulted in a 50% decrease in the intensity of the 1050-cm^{-1} absorption band, indicating that approximately one-half of the Gum Arabic that had accumulated at the copper surface was firmly bound (Fig. 5b). In contrast, no polysaccharide was retained at the surface when the rinse treatment was performed after only a 2-day exposure period. The complete loss of the amide I and amide II bands following the rinse demonstrates that the protein which had accumulated in the aqueous boundary layer after 17 days was not firmly bound to the Cu surface (Fig. 5b).

DISCUSSION

Evidence implicating acidic exopolysaccharides as an adhesive which anchors aquatic

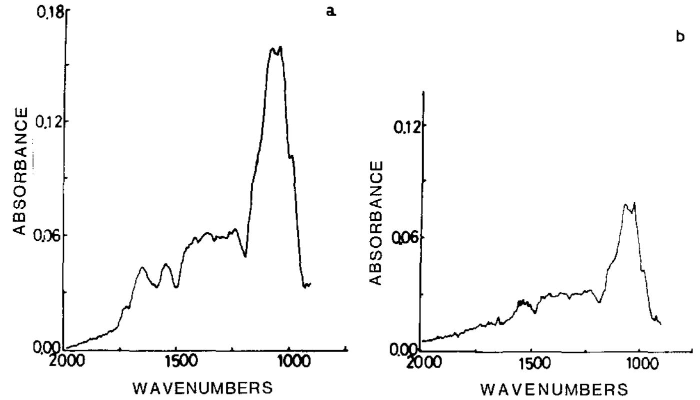

FIG. 5. Infrared spectrum of interface of copper-coated Ge IRE and Gum Arabic solution (a) after a 17-day exposure period and (b) after three water rinses of IRE exposed to Gum Arabic solution for 17 days. Suitable scaling factors were selected to subtract the water absorption from both spectra.

microorganisms to submerged inert surfaces has been derived mainly from electron microscopic studies which reveal ruthenium red-stained polymeric fibers extending from the cell surface to the substratum (10). These firmly bound exopolymers, however, are difficult to isolate and purify in quantities needed for CIR spectroscopic analysis (7). Substitution of the commercially available plant polysaccharide, Gum Arabic, for the microbial polymers proved useful in demonstrating, by spectroscopic means, the interactions acidic polysaccharides have with submerged metal surfaces.

The absorption spectrum of the polysaccharide solution obtained by CIR was similar to the DR spectrum of dehydrated preparations of the same sample. It is unlikely, therefore, that the absorption bands observed in this study were artifacts of the IRE sampling method. The stronger absorbance of polysaccharide and water observed when the solution was sampled with the ZnSe IRE reflects the fact that this element permits a greater depth of penetration (d_p) of the radiation into the bulk aqueous phase. The Ge IRE samples an aqueous boundary layer adjacent to the crystal surface of less than 1 μm thickness (5). That the relative intensities of the polysaccharide and water absorption bands were similar when either the uncoated Ge or ZnSe IREs were used suggests that the concentration of the polymer did not change with sampling depth. It appears that neither the ZnSe nor the Ge IREs promoted accumulation of Gum Arabic at their surface.

By itself, the intensity of the 1050-cm^{-1} absorption band contributed by Gum Arabic provided little information on the concentration of the polymer at the liquid–solid interface. However, the ratio of the intensity of a characteristic absorption band of the polymer to that of a unique band contributed by the solvent (water) should be proportional to polymer concentration. Iwaoka *et al.* (5) found that the bulk liquid displayed a ratio ($\mathrm{abs}_{1050\,\mathrm{cm}^{-1}}/\mathrm{abs}_{1640\,\mathrm{cm}^{-1}}$) of 0.2 for a 10% aqueous suspension of Gum Arabic. While this same ratio was obtained at the surface of a bare Ge IRE, a significantly higher ratio was demonstrated at the surface of a Cu-coated Ge IRE. It was proposed that a ratio greater than 0.2 reflected a higher polymer concentration at the metal surface than that in the bulk aqueous phase.

In this study, concentration of Gum Arabic in the liquid boundary layer adjacent to the copper film was demonstrated by spectral subtraction. The absorbance produced over the 2000- to 750-cm^{-1} range was scaled by a factor selected to reduce the water absorption band centered at 1640 cm^{-1} to zero. Since the intensity of water absorption depended on the thickness of the copper film (5), the value of the subtraction factor generally varied each time the IRE was coated for a new experiment. The thickness of the copper film could not be controlled to the extent that the same water band intensity was achieved after each coating process.

Subtraction of water also reduced the absorbance contributed by substances in the water by an amount proportional to their concentration in the bulk aqueous phase. Thus, the water-subtracted spectrum is bulk phase solute-subtracted, as well. Therefore, detection of the polysaccharide absorption band in the water-subtracted spectrum in Fig. 2c suggests that a higher polysaccharide concentration developed in the boundary layer sampled by the copper-coated Ge IRE than that in the surrounding bulk aqueous phase. These data are consistent with our previous results based on absorbance ratios and provide additional evidence that Gum Arabic concentrates on or near submerged copper surfaces (5). Efforts are currently being made to assign concentration values to the absorbance data obtained from these spectra.

While polymer concentration at the copper surface could, over short exposure intervals, be demonstrated by the above spectral subtraction and absorbance ratio methods, the increase in water absorption makes it difficult to use these methods to detect changes over longer periods of time. The increase in water absorption is probably the result of polymer-induced dissolution or spalling of the copper film since a similar increase was achieved by exposing IREs with copper films of decreasing thickness to polymer-free water (5). In spite of the change in the water band intensity, an appropriate subtraction factor could be chosen to yield reasonable water-subtracted spectra of events which occurred over a 17-day period. That the intensity of the polysaccharide band increased even during deterioration of the copper film suggests that the polymer accumulated at the copper–water interface at a more rapid rate than the copper dissolved.

The fact that 50% of the absorbance at 1050 cm^{-1} from the 17-day sample was retained by the IRE after rinsing suggests that a substantial portion of the polysaccharide concentrated at the interface was firmly bound to the copper surface. The time dependence of this interfacial phenomenon indicates that sorption of these biomolecules to a copper metal surface does not proceed at a rapid rate.

That IRE contact with water alone produced no change in water absorption with time indicates that properties specific to the solvent were not responsible for dissolution of the copper film. Destruction of the copper film by the polysaccharides may have been caused by interactions between acidic functional groups on each polymer chain and cupric ions at the metal surface. The bacterial exopolysaccharide, in fact, has been shown to exhibit high affinity binding of cupric ions (12). Furthermore, binding of cupric ions was found to result in the release of protons from the bacterial exopolymer. Preliminary studies using diffuse reflectance FT-IR spectrometry suggests a cupric ion interaction with carboxyl groups of all three acidic polysaccharides used in these studies (unpublished results). Polymer interactions with copper ions at the film surface may, therefore, promote ionization of metallic copper.

In a similar study, Jakobsen *et al.* (11) reported that addition of lauric acid to an iron film led to the formation of an iron laurate salt on the surface of the film. The organic acid was believed to have induced ionization of the metal.

The basis for the different rates and extent of film dissolution recorded in the presence of each polysaccharide is not presently known. The variation could not have been due to differences in initial solution pH since each was adjusted to 6.5. It is likely that each polymer's affinity and binding site density for the cupric ion was unique and, as a result, promoted a different rate and extent of copper ionization at the film surface.

The internal reflectance measurements described here confirm our previous observations and electron microscopic evidence that acidic polysaccharides bind to metallic copper surfaces submerged in an aqueous environment. Spectroscopic analysis also revealed the destructive effects the polysaccharides exerted on the copper film, a phenomenon which indicates the involvement of biopolymers in the initial events of surface corrosion. The fact that these techniques were successful in demonstrating the accumulation of Gum Arabic on the submerged copper film indicates that cylindrical internal reflectance will be suitable for investigating the adhesion characteristics of various microbial exopolymers.

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