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BINDING OF METAL IONS BY EXTRACELLULAR POLYMERS OF BIOFILM BACTERIA

G. G. Geesey*, L. Jang**, J. G. Jolley***,
M. R. Hankins***, T. Iwaoka**** and
P. R. Griffiths****

*Department of Microbiology; **Department of Chemical Engineering,
California State University, Long Beach, CA 90840, U.S.A.

***Idaho National Engineering Laboratory/EG&G Idaho, Inc., Idaho
Falls, ID 83415, U.S.A.

****Department of Chemistry, University of California, Riverside, CA
92521, U.S.A.

ABSTRACT

Exopolymers which anchor sessile bacteria to metallic surfaces exhibit the capacity to bind copper ions with high affinity. Ionized carboxyl groups on the polymers appear to participate in cupric ion binding. Formation of complexes between the polymers and cupric ions results in the release of protons from the polymer molecule. Attenuated total reflectance Fourier transform infrared spectroscopy showed that polymers composed of acidic polysaccharides promote ionization and deterioration of metallic copper surfaces. X-ray photoelectron spectroscopy studies revealed that the ionic state of the surface-derived copper varied depending on the type of acidic polysaccharide that was in contact with the surface. The results suggest that exopolymers elaborated by adherent bacteria can enhance corrosion of the surfaces with which they are associated.

KEYWORDS

Exopolymers, Adherent bacteria, Corrosion, Copper

INTRODUCTION

Microbial biofilms develop on virtually every surface that comes in contact with water. Biofilms are formed after bacteria attach to and replicate on a surface. Exopolymers elaborated by the adherent bacteria anchor the cells to the surface and to each other. The exopolymers are often composed of acidic polysaccharides. Each adherent bacterial species may produce chemically- and structurally-unique acidic polysaccharides. Some exopolysaccharides exhibit a high-affinity toward certain metal ions (Mittelman and Geesey, 1985). The tendency of acidic polysaccharides to bind metal ions may, therefore, influence the stability of ground state metal atoms that are in contact with these microbial products. This paper presents the results of a study which implicate acidic polysaccharides in the corrosion of copper surfaces.

BINDING OF COPPER BY ACIDIC POLYSACCHARIDES

Metal binding assays with cupric metal ions and crude exopolymer isolated from a river sediment bacterial isolate, FRI, yielded a conditional stability constant of 7.3×10^8 which is comparable to that obtained between cupric ions and a humic acid preparation (Table 1). The binding site density or maximum binding ability (MBA) of the bacterial exopolymer for Cu^{2+} was more than an order of magnitude lower than for humic acids.

TABLE 1 Cu^{2+} Binding Characteristics of acidic polymers

Polymer	MBA (nmol/mg [dry wt.])	k_C^* ($\times 10^8$)
FRI exopolymer	37	7.3
Humic acids	807	6.6

*Conditional stability constant.

Fourier transform infrared spectroscopy of the FRI exopolymer in the presence and absence of cupric ions revealed differences in intensity of the C-O absorption bands centered around 1600 cm^{-1} (Fig. 1). These data suggest that carboxyl groups are present in the exopolymer and that more of these groups become ionized in the presence of cupric ions.

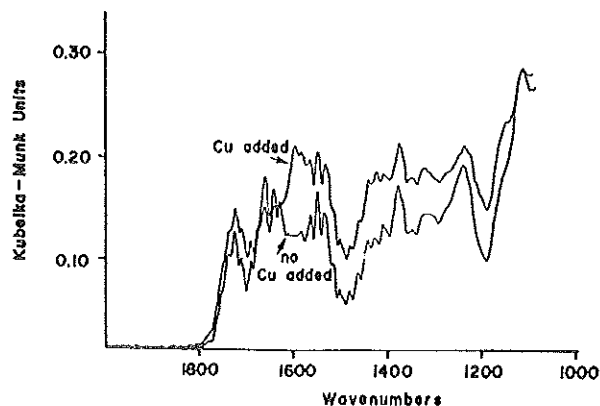


Fig. 1. Diffuse reflectance Fourier transform infrared spectra of FRI exopolymer in presence and absence of copper sulfate solution.

The pK_a of the FRI exopolymer in the absence of metal ions was found to be 4.90 (Fig. 2). Exposure of the exopolymer to cupric ions prior to potentiometric titration with base shifted the pK_a to 4.05. These results support the ir spectroscopic data indicating that copper binding occurs at an ionizable site such as a carboxyl group on the exopolymer.

ATR/FT-IR spectroscopy revealed that exposure of a copper-coated germanium internal reflection element (IRE) to an aqueous suspension of the FRI exopolymer resulted in a rapid decrease in the thickness of the copper film. This conclusion was based on an increase in the water absorption band at 1640 cm^{-1} (Fig. 3a). The intensity of the water (solvent) absorption band is directly proportional to the depth of penetration of the evanescent wave of ir radiation into the liquid phase which, in this case, is related to the thickness of the copper film. Preliminary studies showed that the intensity of the water absorption band was sensitive to film thickness changes as small as 2-3 atomic layers (Iwaoka *et al.*, 1986). Since no change in water absorption occurred when the copper-coated IRE was exposed to water alone, dissolution of the copper film appears to have been due to the presence of the exopolymer. Destruction of the thin copper film was also observed in the

presence of other acidic polysaccharides such as alginic acid and Gum Arabic (Fig. 3b). In the case of Gum Arabic, evidence was obtained by ATR/FT-IR which indicated that the polymer was adsorbed to the copper surface (Iwaoka et al., 1986). Some exopolymers such as those isolated from the marine bacterium, *Pseudomonas atlantica*, however, did not facilitate dissolution of the metallic copper film. These results may reflect differences in copper binding properties among these acidic polysaccharides.

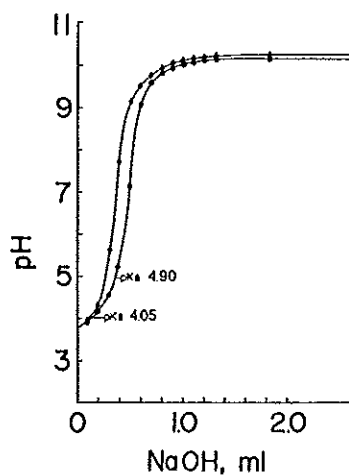


Fig. 2. Potentiometric titration of FRI exopolymer in presence (\blacklozenge) and absence (\bullet) of 1 μg of copper per ml. (From Mittelman and Geesey, 1985)

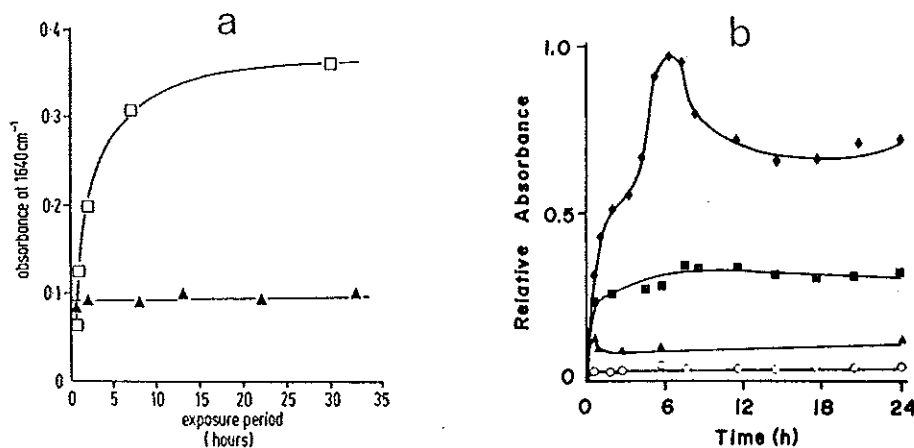


Fig. 3. Water absorption at 1640 cm^{-1} during exposure of Cu-coated Ge IRE to a) crude preparation of FRI exopolymer (\square) and to polymer-free water (\blacktriangle) b) alginic acid (\blacklozenge), Gum Arabic (\blacksquare), *P. atlantica* exopolymer (\blacktriangle), and polymer-free water (\circ).

X-ray photoelectron spectroscopy demonstrated that copper ionization had occurred on those surfaces that exhibited a decrease in thickness during exposure to the acidic polysaccharides (Fig. 4 and 5). The surface exposed to Gum Arabic produced Cu 2p photoelectron lines with a shake-up structure and Cu LMM Auger electron lines with a shoulder on the peak at 918.6 eV. This indicated a +2 oxidation state for the copper based on a comparison with a Cu_2O standard. When the surface was exposed to a culture extract containing exopolymer from the marine bacterium, LST, the Cu LMM peak was shifted to an even lower energy than Cu_2O and the Cu 2p photoelectron lines showed no shake-up structure. Based on results from a standard of CuO , it appears that the Cu was in a +1 oxidation state. The surface in contact with the *P. atlantica* exopolymer exhibited Cu 2p photoelectron and Cu LMM Auger electron peaks that were similar to those of Cu^0 . Thus, no ionization of copper was observed at the surface exposed to this exopolymer.

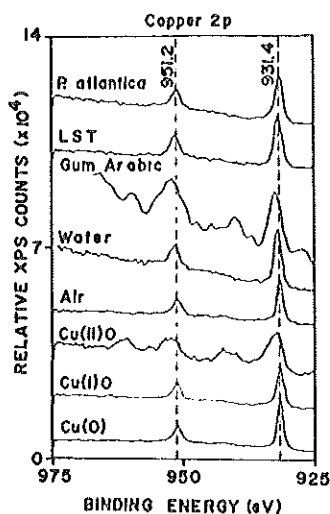


Fig. 4. Cu 2p photoelectron lines. (From Jolley *et al.*, in press)

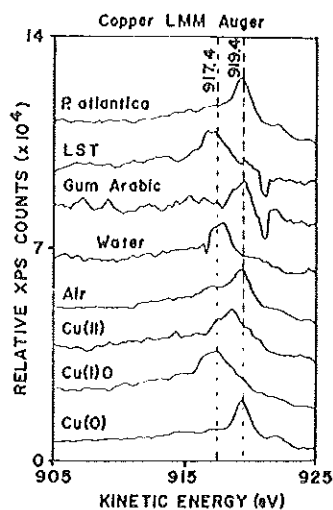


Fig. 5. Cu $L_{3}M_{4,5}M_{4,5}$ Auger electron lines. (From Jolley *et al.*, in press)

These results demonstrate the variable effect that acidic polysaccharides have on metallic copper surfaces. Ionic metal species were detected only at those surfaces which were exposed to polymers that caused a decrease in surface thickness. A polymer's ability to ionize a metal may be related to its affinity for a particular ionized metal species or to surface acidification resulting from the binding of metal ions by protonated sites on the polymer. The high affinity some bacterial exopolysaccharides have for cupric ions may reduce the concentration of the free ions (Cu^{1+} , Cu^{2+}) at the surface to such low levels that more metallic copper is dissolved (ionized) in order to establish thermodynamic equilibrium between the copper species at the boundary of the solid and liquid phases. Although the actual mechanism by which surface ionization occurs remains to be identified, it is apparent that acidic polymers of some biofilm bacteria facilitate corrosion of the copper surfaces with which they come in contact.

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