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Auger Electron Spectroscopy and X-ray Photoelectron Spectroscopy of the Biocorrosion of Copper by Gum Arabic, Bacterial Culture Supernatant and *Pseudomonas atlantica* Exopolymer

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Thin films (3.4 nm) of copper on germanium substrates were exposed to 10% gum arabic aqueous solution, 1% bacterial culture supernatant (BCS) (aqueous and simulated sea water solutions) and 0.5% *Pseudomonas atlantica* exopolymer (aqueous and simulated sea water solutions). Pre- and post-exposure characterization were done by Auger electron spectroscopy and x-ray photoelectron spectroscopy. Ancillary graphite furnance atomic absorption spectroscopy was used to monitor the removal process of the copper thin film from the germanium substrate. Results indicate that the copper was oxidized by the gum arabic and BCS, and some was removed from the Cu/Ge interface by all three polymers and incorporated into the polymer matrix. Thus, biocorrosion of copper was exhibited by the gum arabic, BCS and *Pseudomonas atlantica* exopolymer.

INTRODUCTION

When a surface becomes exposed to a non-sterile environment, a series of chemical and biological events can occur, resulting in the surface microbial colonization and the propagation of microbial activities. The activity of micro-organisms on surfaces can be beneficial (such as biomining) or deleterious (such as biofouling and biocorrosion). Biologically induced corrosion has been shown to be responsible for a significant portion of materials failure.[1,2]

In an attempt to gain a better understanding of the biological reactions that occur on surfaces under biofilms and, perhaps, exploit microbial activity, investigators have directed their attention to the near surface region (μm range of interaction) using Fourier transform infrared/attenuated total reflection spectroscopy (FTIR/ATR).[3,4] This approach had led to the finding that bacterial exopolysaccharides which anchor the cells to surfaces also promote the destruction of the surface. X-ray photoelectron spectroscopy (XPS) and Auger electron spectroscopy (AES) make it possible to examine such surface (nm range of interaction) phenomenon.

In this work the gum arabic (GA), Bacterial Culture Supernatant (BCS) and *Pseudomonas atlantica* (*Pa*) exopolymer interactions with copper were investigated with XPS and AES to further elucidate the nature of the destruction of a copper surface exposed to exopolysaccharides from surface-colonizing bacteria and gum arabic from the Acacia tree.

EXPERIMENTAL

Sample preparation

Thin films (3.4 nm) of copper were deposited on germanium substrates (6 mm × 1 mm) and Pyrex microscope slides in an Enerjet magnetron sputtering system. Substrates were at ambient temperature. Target material consisted of 99.999% pure copper supplied by Kurt J. Lesker, Co. A pump-down pressure of 2×10^{-7} Torr was achieved before sputtering. Neon working gas (99.99% pure from Matheson Gas Products) was introduced into the sputtering chamber at a pressure of 30 milliTorr. The deposition rate was 0.12 nm s^{-1}. A quartz crystal oscillator, manufactured by R. D. Mathis Co. was used to monitor the Cu thin film thickness while graphite furnance atomic absorption spectroscopy (GFAAS) was used for analysis of the Cu thickness on the microscope slides for system calibration. A shutter assembly was used to allow target surface clean-up before sputter deposition. After two minutes of sputtering, the shutter was opened to allow deposition of the copper onto the Ge substrates.

Saturated polysaccharide suspensions were prepared from dehydrated preparations. Gum arabic (Aldrich, 26,077-0) was prepared as a 10% w:v solution in 18 megaohms nanopure water (obtained from a Barnstead NANOpure II water system), the pH adjusted to 7.0, filtered through a 0.45 μl pore size membrane and frozen at −70 °C prior to use. Exopolymer from a bacterial culture of *P. atlantica* was obtained by sedimenting the cells by centrifugation and treating the supernatant liquid with two parts 2-propanol at 4 °C overnight. The precipitated exopolysaccharide was

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Table 1. AQUIL (simulated) sea water composition

Reagent	gm l^{-1} added
$CaCl_2 \cdot 2H_2O$	1.54
$MgCl_2 \cdot 6H_2O$	11.1
NaCl	24.53
KBr	0.10
$SrCl_2 \cdot 6H_2O$	0.017
NaF	0.003
KCl	0.70
H_3BO_3	0.03
Na_2SO_4	4.09
$NaHCO_3$	0.20

redissolved in nanopure H_2O, dialyzed against nanopure H_2O and then lyophilized (freeze dried). A 0.5% suspension of the lyophilized material was prepared as described above for the gum arabic except that AQUIL (simulated) seawater (pH of 7.6) was used in place of nanopure water. The composition for AQUIL simulated seawater is shown in Table 1. The supernatant fraction (BCS) from a stationary phase culture of an unidentified marine bacterial isolate, grown in liquid medium composed of brain heart infusion broth and 3% NaCl, was obtained by centrifugation and then lyophilized. BCS was rehydrated in either nanopure water or simulated seawater as a 1% suspension and filtered and frozen as above.

Copper deposited Ge discs were placed into analytically clean vials. One ml (pipetted by Eppendorf pipet) of each of the polymer solutions (nanopure H_2O was used as the control) was placed into the corresponding vial for copper/polymer exposure (24 h duration) at ambient temperature. Due to the rapid Cu removal exhibited by the gum arabic solution, it was necessary to use a one hour exposure in addition to the 24 h exposure for the XPS analysis. At the end of the exposure time period, the Ge discs were removed from the vials with analytically clean plastic hemostats, rinsed with nanopure H_2O to remove residual polymer solution (rinse was not placed into vials), excess H_2O 'wicked' off Ge discs via the side using Kimwipes and dried with N_2 gas stream from LN_2 boil-off. Within 30 min from disc removal, the Ge discs were placed in the sample introduction system of the AES/XPS analysis system. Exposed polymer and H_2O solutions were kept for subsequent GFAAS analysis.

X-ray photoelectron spectroscopy

Qualitative and semi-quantitative analyses of the Cu-coated Ge substrates were done on a Perkin–Elmer Physical Electronics XPS system which was equipped with Mg Kα x-ray source coupled with a double pass cylindrical mirror analyzer. High resolution spectra were taken using multiple scan, multiplexed pulse height counting methods. Energy resolution was 5 points eV^{-1} (the Cu/gum arabic 2p was collected at 2 points eV^{-1}). Pass energy was set at 25 eV. Chamber pressure during analyses was $<5 \times 10^{-9}$ Torr. Sample exposure to x-ray radiation was <two hours. The peak positions for the non-polymer exposed samples were referenced to the carbon 1s line (284.6 eV). The peak positions for the polymer-exposed samples were referenced to the germanium 3d line (29.8 eV) since the C 1s line was complex due to the polymer.[5] No peak position corrections were needed. The XPS spectra were smoothed once.

To evaluate the possibility of x-ray induced oxidation changes of the Cu, anhydrous $CuSO_4$ was exposed to x-ray radiation from the XPS analyzer for 11 h.[6,7] No noticeable changes in the Cu 2p and Auger lines were noted.

After the XPS analyses the samples were moved, using a carousel manipulator to the opposite side of the vacuum chamber for Auger depth profile analysis.

Depth profile analysis

Semi-quantitative depth profile analyses were done using a Perkin–Elmer PHI 590 Scanning Auger Microprobe and a Perkin–Elmer PHI sputter ion gun with argon working gas. Instrumental parameters consisted of a 3.0 keV electron beam energy, 200 nA electron beam current, 0.03 s time constant, 4 eV peak-to-peak modulation and 3 eV s^{-1} scanning rate. Derivative spectra were taken in the analog mode using a lock-in amplifier. A 1.0 keV, 25 µA cm^{-2} current density Ar$^+$ beam was used to sputter the samples with a 5 mm × 5 mm raster. Chamber pressure (back-filled with Ar) was 5×10^{-5} Torr. A 3.4 nm thick copper thin film on germanium standard was used with each sample set. The spectral ranges (monitored while simultaneously sputtering) were 240 to 290 eV (for carbon KLL @ 272 eV), 900 to 930 eV (for copper LMM @ 920 eV) and 1130 to 1160 eV (for germanium LMM @ 1147 eV). Peak-to-peak heights were measured and plotted versus sputtering time.[8]

Graphite furnance atomic absorption spectroscopy

The Cu thin films that were deposited on the microscope slides were dissolved with concentrated HNO_3. Nanopure H_2O was used for rinsing. The resulting solutions were quantitatively transferred to 10 ml volumetric flasks. Aliquots (0.020 ml) of solutions (diluted as required for linear absorbance region) were pipetted (using an Eppendorf pipet) into the Lvov platform equipped Perkin–Elmer HGA-400 graphite furnance which was installed in a Perkin–Elmer 703 atomic absorption spectrometer. The resulting concentration determinations were then used to calculate the Cu thin film thickness for the Ge substrates and quartz crystal oscillator response. The density of the Cu was assumed to be 8.93 gm cm^{-3}.

Due to matrix effects (light scatter from polymer smoke) exhibited by the polymers, it was necessary to use an electrochemical separation technique to isolate the copper from the bulk of the polymer solution.[9] The polymer solutions were transferred to an analytically clean beaker, diluted to 80 ml with nanopure H_2O and acidified with 0.50 ml of concentrated H_2SO_4. Two analytically clean platinum electrodes were placed into the resulting solutions and a 2.6 volt potential was applied across the electrodes using a JQE 0-6V 0-10A Kepco power supply. The Cu which was plated onto the Pt cathode electrode was dissolved off using 10% HNO_3. The resulting solutions were analyzed by GFAAS as above. The resulting concentration determinations were then used to calculate the yield of Cu which was incorporated into the polymer solution from the Cu deposited upon the Ge substrates.

RESULTS AND DISCUSSION

X-ray photoelectron spectroscopy

The XPS binding and kinetic energies of the uncoated Ge disc, a metallic copper reference, copper reference compounds and samples are summarized in Table 2. The resulting spectra are shown in Figs 1, 2 and 3.

The Cu^0 was a standard left inside the AES/XPS vacuum chamber for instrumental calibration. Prior to analysis, Ar^+ sputtering was used to remove any oxide layer. The Cu $2p_{1/2,3/2}$ and LVV (LVV refers to $L_3M_{4,5}M_{4,5}$) positions were 951.2, 931.4 and 919.4 eV, respectively.

Cu_2O (Alfa Products) and CuO (Johnson Matthey) powders were mounted on indium foil and used as reference standards. The Cu $2p_{1/2}$ positions were 951.2 and 952.4 eV; the Cu $2p_{3/2}$ positions were 931.4 and 932.0 eV; and the Cu LVV positions were 917.4 and 918.6 eV, respectively. The Cu_2O had the characteristic Auger shift to lower energy while the CuO 2p showed the characteristic shake-up structure. The CuO LVV displayed a shoulder at 917.4 eV. These were in agreement with previous literature findings.[10,11,12,13,14]

The Cu/air sample had 3.4 nm of Cu sputtered upon a Ge disc which was exposed to ambient air for five minutes. The Cu $2p_{1/2,3/2}$ and LVV positions were 951.2, 931.2 and 919.2 eV, respectively. Due to the similarity of the Cu/air and Cu^0, it was determined that the thin film of Cu had undergone negligible oxidation and was metallic in nature. Kinetic investigations performed by Pinnel *et al.* indicate that the rate of copper oxide film growth at ambient conditions was $<10^{-4}$ nm/h^{-1}.[15] Thus, one would expect negligible oxidation.

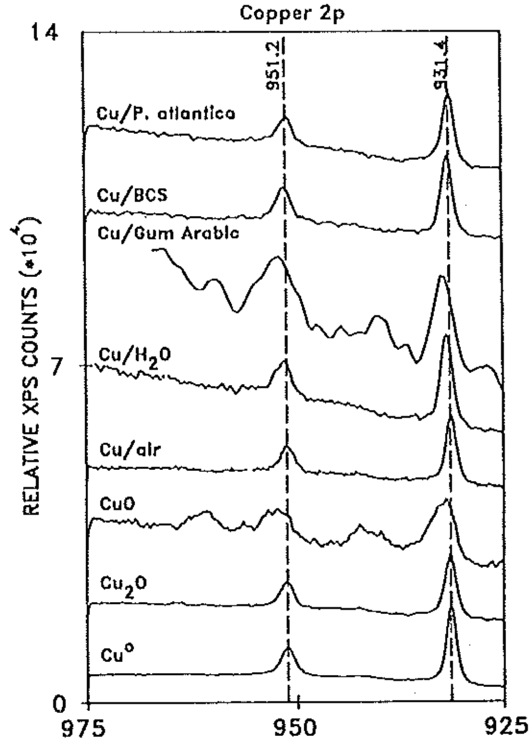

Figure 1. Cu 2p photoelectron lines.

Table 2. XPS photoelectron binding and Auger electron kinetic energies (eV)

Sample	Exposure time (h)	Cu[a] $2p_{1/2}$	Cu[a] $2p_{3/2}$	Cu LVV[b] Auger	Ge[a] 3d
Cu/*Pa*[e]	24	951.2	931.2	919.4	ND[d]
Cu/*Pa*[f]	24	951.0	931.2	919.2	ND
Cu/BCS[e]	24	951.6	931.6	916.6	29.8
Cu/BCS[f]	24	951.4	931.6	916.4	29.8
Cu/GA	1.0	952.0	932.5	919.4	29.8
	24	ND	ND	ND	29.8
Cu/H_2O	1.0	951.6	931.8	917.8	ND
Cu/air	0.08	951.2	931.2	919.2	ND
CuO	NA[c]	952.4	932.0	918.6	NA
Cu_2O	NA	951.2	931.4	917.4	NA
Ge	NA	NA	NA	NA	29.8
Cu^0	NA	951.2	931.4	919.4	NA

[a] Binding energy.
[b] Kinetic energy. LVV refers to $L_3M_{4,5}M_{4,5}$.
[c] Not applicable.
[d] Not detectable.
[e] Nanopure H_2O.
[f] Simulated sea water.

The Cu/H_2O sample was prepared in the same manner as the Cu/air sample and exposed to nanopure H_2O for one hour. The Cu $2p_{1/2,3/2}$ and LVV positions were 951.6, 931.8 and 917.8 eV, respectively. Due to the similarity of the Cu/H_2O and Cu_2O spectra, it appears

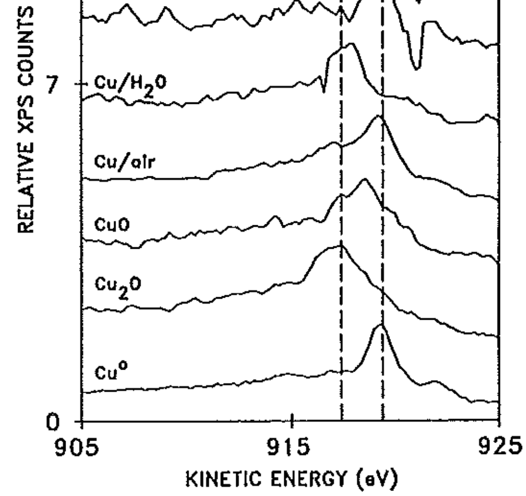

Figure 2. Cu $L_3M_{4,5}M_{4,5}$ Auger electron lines.

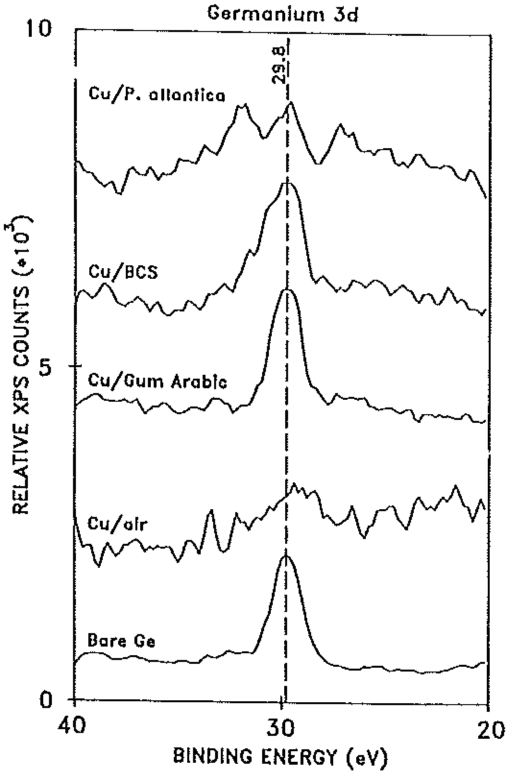

Figure 3. Ge 3d photoelectron lines.

that the Cu had oxidized to a +1 oxidation state. One would expect some oxidation since the oxygen concentration in ambient H_2O is approximately 6000 times larger than in ambient air, thus resulting in a greater interaction probability.

The Cu thin films on the Ge substrates were prepared in the same manner as the Cu/air sample and then exposed to gum arabic, BCS (nanopure H_2O and simulated sea water) and *P. atlantica* exopolymer solutions (nanopure H_2O and simulated sea water) respectively for 24 h. The Cu film was also exposed to the gum arabic solution for a one hour period due to negligible detection of copper in the 24 h experiment (see Table 2 and Figs 2 and 3 for data). The Cu/gum arabic Cu 2p showed a shake-up structure and a shoulder on the Cu LVV peak at 918.6 eV. This indicated a +2 oxidation state for the Cu. The nanopure H_2O and simulated sea water versions of the Cu/BCS and Cu/*P. atlantica* were essentially identical in appearance and peak position. Thus, for clarity, only the simulated sea water versions were plotted. The Cu/BCS Cu LVV peak was shifted to an even lower energy than the Cu_2O. The Cu 2p showed no shake-up structure. It appears that the Cu oxidation state is +1. Due to the similarity of the Cu/*P. atlantica* and the Cu^0 it was determined that the thin film of Cu had negligible oxidation and was metallic in nature. Apparently, the *P. atlantica* exopolymer had inhibited oxidation of the Cu.

The semi-quantitative evaluations of the Cu removal by the polymers are shown in Fig. 3 and summarized in Table 2. The Ge 3d was essentially not detectable in the Cu/air and Cu/*P. atlantica* samples, whereas the Cu/gum arabic (24 h exposure) and the Cu/BCS had a strong signal at 29.8 eV. These spectra indicate that some of the Cu had been removed by the gum arabic solution and BCS (both nanopure H_2O and simulated sea water) solutions, while the Cu removal was not detected by XPS in the *P. atlantica* (both nanopure H_2O and simulated sea water) experiments.

Auger depth profile

The sputter depth profiles are shown in Fig. 4. The Cu/air sample was used for the Copper/Ge depth profile. The Cu, C and Ge maximum relative Auger signals were 6.3, 2.7 and 6.6, respectively. The Ge started at a minimum of 1.5. The FWHM values were 2.6, 3.0 and 3.3 minutes, respectively.

The Cu/gum arabic sample (24 h exposure) was used for the gum arabic/Cu/Ge depth profile. The Cu, C and Ge maximum relative Auger signals were 1.5, 5.5 and 6.6, respectively. The Ge started at a minimum of 1.5. The FWHM values were 6.8, 1.2 and 3.3 minutes, respectively. The increase in the C signal was probably due to a residual thin film of the polymer in addition to the ambient carbon contamination. The small initial Cu signal (76% less than the Copper/Ge value) and the larger starting Ge signal indicated that some of the Cu thin film had been removed by the polymer solution prior to analysis. The depth profile time was almost double as compared to the other depth profiles. Instrumental settings were verified and the experiment run in triplicate with the same results. The ion current was cut in half on a fourth run and the depth profile time was almost 40 min. The postulate at this time is that one would expect an increase in the depth profile time due to the fact that the C starting signal was approximately double that of the other depth profiles and the sputtering yield of Cu is approximately four times greater than C.[16] Nevertheless, the objective of this experiment was to verify whether or not the polymer solution was able

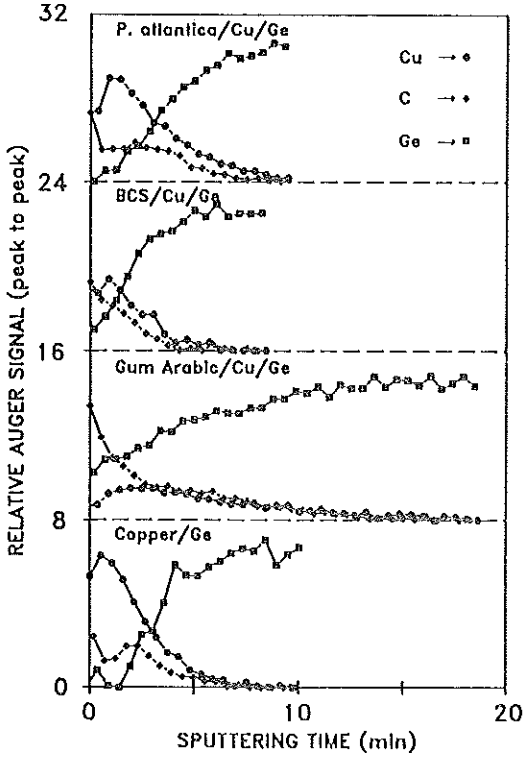

Figure 4. Auger sputter depth profiles.

to remove some of the Cu thin film. The Cu signal and especially the starting Ge signal provided this verification.

The Cu/BCS simulated sea water (the nanopure H_2O and simulated sea water had similar depth profiles) was used for the BCS/Cu/Ge depth profile. The Cu, C and Ge maximum relative Auger signals were 3.3, 3.1 and 6.6, respectively. The Ge started at a minimum of 0.5. The FWHM values were 3.2, 1.7 and 1.4 minutes, respectively. The exposure to the BCS solution appeared to have little effect on increasing the C starting signal. The lowness of the Cu signal (48% less than the Copper/Ge value) and the highness of the starting Ge signal indicated that some of the Cu thin film had been removed by the solution prior to analysis.

The Cu/*P. atlantica* simulated sea water (the nanopure H_2O and simulated sea water had similar depth profiles) was used for the *P. atlantica*/Cu/Ge depth profile. The Cu, C and Ge maximum relative Auger signals were 4.9, 3.1 and 6.6, respectively. The Ge was N.D. (not detectable) at the start. The FWHM values were 3.6, 4.7 and 3.3 minutes, respectively. The exposure to the *P. atlantica* exopolymer solution appeared to have little effect on increasing the C starting signal. Since the Cu maximum signal was approximately 22% less than the Copper/Ge reference and the Ge signal started to rise one minute earlier, it appears that the *P. atlantica* exopolymer solution did remove some of the Cu thin film.

Graphite furnance atomic absorption spectroscopy

The quantitative evaluation by GFAAS of the removal of Cu for the Cu/H_2O, Cu/gum arabic, Cu/BCS (in simulated sea water) and Cu/*P. atlantica* (in simulated sea water) experiments are summarized in Table 3. Less than 0.1% of the total available Cu was removed from the substrate and solubilized into the H_2O during 262 h of exposure to nanopure H_2O. This indicated that the Cu thin film is relatively stable while being exposed to nanopure H_2O.

Of the total available Cu, 36, 22 and 6% were removed by the gum arabic solution, BCS solution and *P. atlantica* exopolymer solution, respectively, from the substrate and incorporated into the solution. The data indicates that the three polymer solutions were able to remove some of the deposited Cu in the order of gum arabic > BCS > *P. atlantica*.

Table 3. Graphite furnance atomic absorption spectroscopy yield

Sample	Exposure time (h)	per cent Cu removed
Cu/*Pa*[a]	24	6
Cu/BCS[a]	24	22
Cu/GA	24	36
Cu/H_2O	262	<0.1

[a] Simulated sea water.

SUMMARY

The XPS, AES depth profile and GFAAS data indicate that the 10% w:v gum arabic solution was able to oxidize the Cu thin film to a +2 oxidation state and also remove some of the Cu from the substrate and incorporate it into the solution.

The XPS, AES depth profile and GFAAS data indicate that the 1% w:v BCS solution (in simulated sea water) was able to oxidize the Cu thin film to a +1 oxidation state and also remove some of the Cu from the substrate and incorporate it into the solution.

The XPS data for the 0.5% w:v *P. atlantica* exopolymer solution interaction with the Cu thin film indicated metallic Cu with no detectable removal. Since the nanopure H_2O had oxidized the Cu to +1 oxidation state, it appears that the polymer solution may have inhibited oxidation of the Cu. The AES depth profile and GFAAS data indicate that a small amount of the Cu was removed from the substrate by the polymer.

Thus, biocorrosion of copper was exhibited by gum arabic, BCS and *P. atlantica.* The overall rates of Cu removal by the saturated solutions were in the order of gum arabic > BCS > *P. atlantica.*

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