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ARTICLE

Antibacterial action of copper ions on food-contaminating bacteria

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ABSTRACT Silver and copper ions are widely used as antibacterial agents but the basic molecular mechanism of this effect is still poorly understood. The analysis of our investigation gives clear indications that Ag^+ do react with the bacterial cells and do not stay as silver in the system. Significant lower silver cysteine content coupled with higher silver histidine content in Gram-positive cells indicate that the peptidoglycan multi-layer could be buffering the biocidal effect of silver for the Gram-positives at least in part. Interaction with DNA or proteins can occur through Ag-N bonding. The formation of silver cysteine can be confirmed for both bacterial cell types which thus supports the hypothesis that enzyme catalyzed reactions and the electron transport chain within the cell is disrupted. The antibacterial property of copper is attributed mainly to adhesion with bacteria because of their opposite electrical charges, resulting in a reduction reaction at the bacterial cell wall. Nanoparticles with a larger surface-to-volume ratio might provide more efficient means for antibacterial activity. First results suggest that copper ions do not react compared to silver ions.
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KEY WORDS

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The antimicrobial properties of silver and copper ions were known since ancient times, but although silver ions (e.g. silver nitrate) and silver nanoparticles are already widely used for various antibacterial purposes, the exact antibacterial mechanism has not yet been elucidated. An *in situ* tool for speciation analysis on an atomic/ molecular level such as X-ray absorption near edge structure (XANES) spectroscopy is the method of choice. XANES allows not only the determination of the valence of an excited atom but also gives information about type of neighboring atoms. In a previous study (Bovenkamp et al. 2013) the antibacterial effect of silver on three types of bacteria, *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes* was investigated. Ag L_{III}-edge XANES spectra of the different bacteria confirm that a reaction occurs after the application of silver ions in solution (e.g. from silver nitrate). Silver bonding to Ag-S in cysteine and Ag-N or Ag-O bonding in histidine, alanine, and DL-aspartic acids was detected using synthesized silver-amino acids as reference compounds for linear combination fitting (LCF) analysis. The aim of the present study is to analyze the molecular reactions of selected food-relevant bacteria on copper ions. The antibacterial properties of copper are attributed mainly to adhesion on bacteria because of their opposite electrical charges, resulting in a reduction reaction at the bacterial cell wall.

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Materials and Methods

Materials

Bacterial strains: *Staphylococcus aureus* DSMZ 2569, *Escherichia coli* DSMZ 1103, and *Listeria monocytogenes* DSMZ 20600.

Medium: Yeast-Peptone-Dextrose broth (Difco Becton Dickinson, Franklin Lakes, NJ, USA).

Reference compounds/ chemicals: copper-I-acetate, copper-II-acetate, and copper nitrate were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cultivation of the bacteria and sample preparation

S. aureus DSMZ 2569, *E. coli* DSMZ 1103, and *L. monocytogenes* DSMZ 20600 were grown in a shaker (100 rpm) for 24 to 48 h at 30°C in YPD. Bacteria were washed with sterile deionized water and centrifuged twice. The bacteria were diluted to 10^6 CFU/mL with sterile deionized water (to avoid chemical reactions of silver or copper ions with medium contents). Then 50 µL of a 0.1 M stock solution (e.g. copper acetate) was added to 1 mL cell culture (about to 10^6 CFU/mL). The samples were incubated at 20°C for 10 min

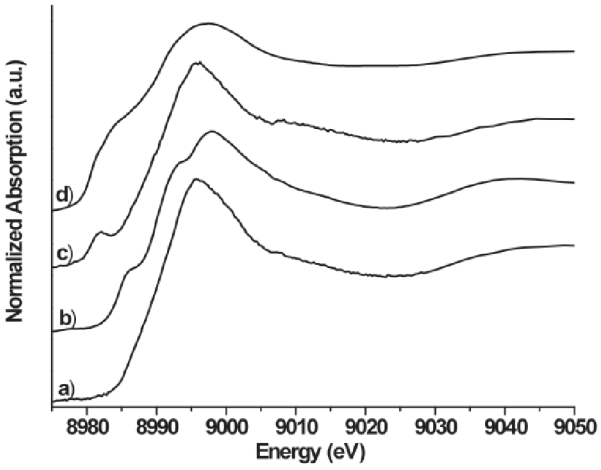

Figure 1. Copper *K*-edge XANES spectra of copper reference compounds a) copper-II-acetate solution, b) copper-II-acetate, c) copper-I-acetate solution, d) copper-I-acetate.

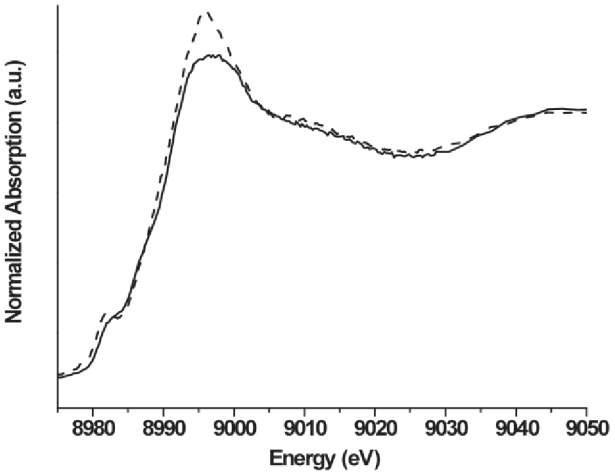

Figure 2. Copper *K*-edge XANES spectra of Cu^{+}-acetate solution (•••) and *L. monocytogenes* cells treated with Cu^{+}-acetate (—).

in darkness and then washed again with 0.5 mL deionized water to remove unreacted silver or copper compounds. About 20 µL cell material was put on filter paper and attached to Kapton tape. These samples were dried in darkness for 1 to 2 h. A control set was prepared as described above without adding copper-containing solutions. Samples for XANES analysis were prepared and handled according to Prange et al. (2002).

Experimental XANES spectroscopy

Cu *K*-edge XANES spectra were recorded at the Double Crystal Monochromator (DCM) beamline of the Center for Advanced Microstructures and Devices (CAMD), Louisiana State University, Baton Rouge, LA, USA (Hormes et al. 2006) and at the beamline of the Angströmquelle Karlsruhe (ANKA). The monochromators were equipped with Ge(220) and Ge(422) crystals, respectively. Measurements of the bacterial samples were performed in fluorescence mode to record the fluorescence photons and an ionization chamber for the incident photons with ambient pressure at the Cu *K*-edge inside the ionization chambers and sample chambers. Measurements of reference compounds were performed in transmission mode. For energy calibration of the copper spectra, the spectrum of elemental copper was used as a standard. Data were normalized and analyzed with the ATHENA program of the IFFEFIT package (Ravel and Newville 2006). The error of the percentage contributions for the compounds in the linear combination fitting (LCF) results can be estimated to ± 10%.

Results and Discussion

Cu *K*-edge XANES spectra of bacteria without the application of copper ions reveal that there is a small amount of copper (10-100 ppm) present in all bacteria samples (data not shown). This is not suprising since copper is an essential nutrient for all life. After the application of Cu-I-acetate solution or Cu-II-acetate solution (reference spectra see Figure 1) the Cu *K*-XANES spectra of the bacterial samples (data not shown) do not always show clear differences to the initial solution which was the case after the application of silver ions (Bovenkamp et al. 2013). For example the Cu *K*-XANES spectra of *L. monocytogenes* treated with Cu-I-acetate in comparison with the spectra of pure Cu^{+}-acetate solution show some differences (Pre-edge and White Line) which confirm that a reaction occurs (Fig. 2). However, the spectra are quite similar in the energy positions of peaks and shape resonances. The reason for the high similarity of the most spectra of bacteria to the solutions can be a reaction of copper with water molecules (complex formation).The $Cu_c(OH)_y$ complex might passivate the reactivity of copper. The high reactivity of Cu with water (also depending on the pH) is well known (Kruck and Sarkar 1973) and studied with XANES (Mesu et al. 2006). This also complicates the synthesis of copper amino acids as reference compounds. The application of copper ions using copper nitrate solution on the other hand resulted in much more pronounced differences of bacterial cell spectra and spectra of initial solution (will be published later). The mechanism of action of the copper ions is not yet fully understood. Detailed research and comparative study of strain-specific variability is required to determine the bactericidial efficiency.

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