Uptake of silver nanoparticles in *Chlamydomonas reinhardtii*

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Engineered silver nanoparticles (AgNP) are increasingly used in a variety of consumer products because of their bactericidal properties, thus increasing the likelihood for their entrance in aquatic systems. Other than animal cells, algae possess a cell wall that might constitute a primary site for interaction and a barrier for the entrance of silver nanoparticles into algal cells. Major components of cell walls are carbohydrates, linked to form a rigid complex multi-sheaths network, and proteins. Our working hypotheses are that sieving properties of cell walls are determined by the diameter of the pores that span through the thickness of walls and that adsorption of particles to algae is determined by the charge of the particles. So far, only few studies have examined pore sizes of algal cell walls but the available information indicate a size range between 5 and 20 nm. Thus, only NP smaller than about 20 nm are expected to reach the cell membrane.

To examine whether AgNP enter into the freshwater green algae Chlamydomonas reinhardtii, we have carried out the studies with AgNP previously characterized by dynamic light scattering and transmission electron microscopy (TEM), and ranging in size from a 2 to 20 nm. Silver associated with algae was quantified by ICP-MS. Various microscopic techniques have been used for particle imaging, including scanning electron microscopy, TEM and high angle annular dark field scanning transmission electron microscopy (HAADF-STEM). The elemental composition of visualized particles was analyzed by energy dispersive X-ray spectroscopy (EDX).

The samples were fixed in in cacodylate buffered 2% glutaraldehyde and postfixed in 1% OsO_4, dehydrated in ethanol series and embedded in Epon-resin. Unstained ultrathin sections of 70 nm were cut on a Reichert Ultracut S.

Short-term sorption experiments indicate a considerable level of Ag associated with algae after exposure to AgNP. Indirect evidence for the internalization of particles is given by SEM-EDX analyses which show that only a small amount of particles has sorbed to the cell walls of the algae. However, AgNP exposed algae show major ultrastructural changes particularly in the pyrenoid and starch content, as visualized by TEM analyses of algal ultrathin sections (Figure 1). Detection of particles in algae, and identification of Ag as component of many of these particles has been enabled by HAADF STEM coupled to EDX analysis: In the HAADF STEM image high brightness is an indication of either high sample thickness or of heavy atomic species. Membranes and vacuoles are bright due to the presence of osmium. Besides this, a large amount of small, very bright objects between the thylakoid membranes are observed. Preliminary results indicate that a large amount of these bright particles can be identified by EDX to be silver containing particles (Figure 2).

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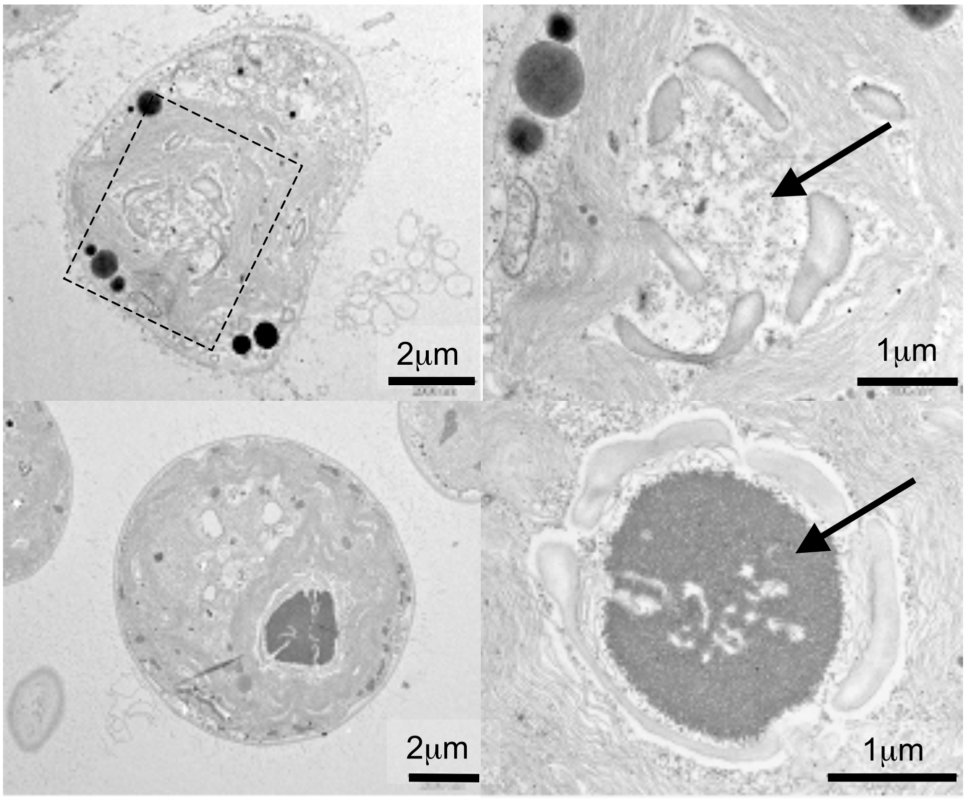

Figure 1. TEM-images illustrating the ultrastructural changes caused by the exposure of algae to the AgNP Top row: control (section displayed in higher magnification is marked), bottom row: algae exposed for 1h to 10µM AgNP; after exposure e.g. the pyrenoid is much more compact and shows a darker contrast (arrows; magnified image from different cell).

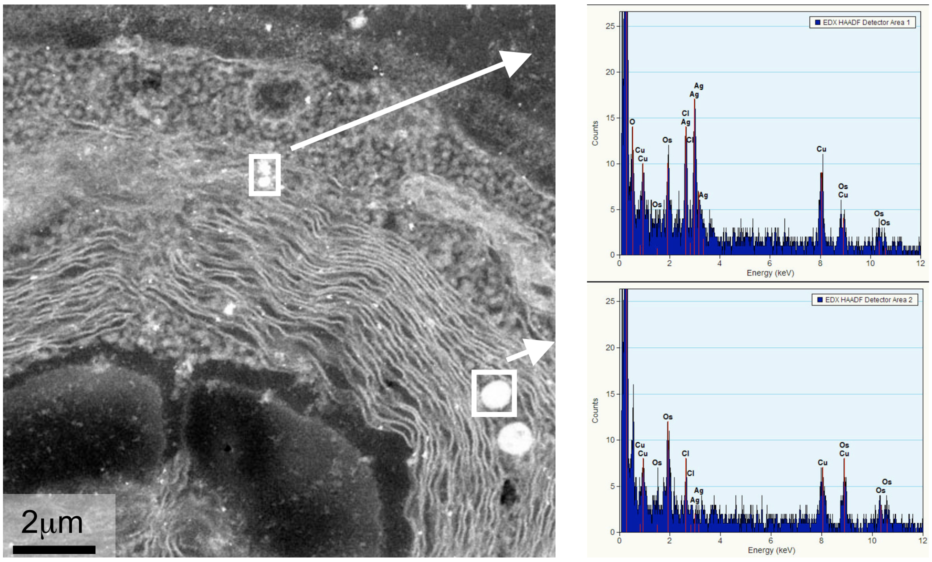

Figure 2. HAADF STEM image showing several areas of high brightness. In images taken with this technique, the image is bright for heavy material and thick sample areas. A high brightness is therefore an indication of material consisting of atoms with high atomic number. While the (bright) thylakoid membranes as well as especially the vacuoles are shown to contain Os (coming from the preparation protocol; bottom spectrum), many of the small bright areas are identified to contain silver (top spectrum).

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