

Formation and Growth of Au Nanoparticles inside Live Alfalfa Plants

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ABSTRACT

In modern nanotechnology one of the most exciting areas is the interaction between inorganic quantum dots and biological structures. For instance gold clusters surrounded by a shell of organic ligands covalently attach to proteins or other biological substances and can be used for labeling in structural biology. In the present report we show the possibility of using live plants for the fabrication of nanoparticles. Alfalfa plants were grown in an AuCl₃ rich environment. The absorption of Au metal by the plants was confirmed by X-ray absorption studies (XAS), and transmission electron microscopy (TEM). Atomic resolution analysis confirmed the nucleation and growth of Au nanoparticles inside the plant and that the Au nanoparticles are in a crystalline state. Images also showed defects such as twins in the crystal structure, and in some cases icosahedral nanoparticles were found. X-ray EDS studies corroborated that the nanoparticles are pure gold. This is the first report on the formation of gold nanoparticles by living plants and opens up new and exciting ways to fabricate nanoparticles. It shows how it is possible to link materials science and biotechnology in the new emerging field of nanobiotechnology.

The field of nanotechnology is one of the most active areas of research in modern materials science. New applications of nanoparticles and nanomaterials are emerging rapidly.^{1–3} The synthesis of nanoparticles and their self-assembly is a cornerstone of nanotechnology. New methods to manufacture nanoparticles are constantly being studied and developed. Additionally, environmental metal contamination byproducts associated with metal production has become a great concern for environmental reasons.⁴ An example is the processing of valuable metal ores, which provides a route for toxic compounds to enter the environment. A paradigm of this is the current technology to extract gold, in which cyanide is used to release gold from the ore into a solution as gold cyanide [AuCN₂].

Currently, the AuCN₂ is still absorbed onto activated carbon and is subsequently stripped from the carbon followed by electrochemical reduction to gold(0), which is a very expensive process. Recently, it has been shown that several types of inactivated biomasses and living organisms have

the ability to remove high concentrations of gold(III) from solution and to reduce gold(III) to gold(0).^{5–7} These studies provide the possibility of an environmentally friendly method to remediate mining wastes.

Although it is well-known that inactivated biological systems interact with metal ions, the connection between metal ions and biological systems is more in depth. As is well-known, many elements at trace concentrations are essential for plant growth and propagation; however, these same elements at higher concentrations are toxic to some plants. More specifically it has been shown that many bacteria and plants can actively uptake and bioreduce metal ions from soils and solutions. A well-known example of bioreduction and nanoparticle production is the magnetostatic bacteria that can synthesize magnetic nanoparticles.² Another example of nanoparticle production using inactivated alfalfa biomass has shown that biomass can reduce gold(III) ions in solution to gold(0) nanoparticles.⁸ The use of inactivated biomass to recover metal ions from solution has been studied extensively. The possibility of using live bacteria for the remediation of metal-contaminated waters has shown the bacterial production of silver–carbon composite materials. Also, the formation of surface trapping of nanoparticles by fungus has been reported.^{3,9} These are examples that link biotechnology and material science (nanobiotechnology).^{3,9,10}

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It has been well-known for some time that plants can uptake gold from soils as they are used as bioindicators of gold deposits by mining companies.¹¹ Although plants are known to uptake gold from solution and soils, the oxidation state and the form of the gold has never been elucidated to the authors' knowledge.

In the present work we report the growth of Au nanoparticles inside a live plant. This has very important implications and possible applications, for example, the removal of gold or another materials from contaminated zones using plants to extract them. X-ray absorption spectroscopy (XAS) was used in this study to gain information about the uptake and formation of gold nanoparticles by live alfalfa plants.^{12,13} X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) are two components of XAS, which provide information about the oxidation state, coordination environment, and the nearest neighboring atom.

In this work XAS studies were performed on alfalfa plants grown on a gold rich agar system. XANES were used to observe the oxidation state of the gold in both the plants and the agar. EXAFS were used to investigate the nearest neighboring atom to the gold in the plants and the agar. Finally, studies were performed using X-ray EDS, TEM, and HRTEM to further investigate the formation of gold nanoparticles in the plants.

The alfalfa seeds of the Mesa variety were used and prepared similar to that of Peralta et al.¹⁴ The seeds were soaked to avoid fungal contamination in 3% formaldehyde for 15 min and washed three times with deionized water. Approximately 100 seeds were transferred to a mason jar of one-pint volume already autoclaved for a sterile environment. Each jar contained 200 mL of nutrient solution made with $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (3.57×10^{-4} M), H_3BO_3 (2.31×10^{-5} M), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2.14×10^{-3} M), KH_2PO_4 (9.68×10^{-4} M), KNO_3 (2.55×10^{-4} M), MgClO_4 (1.04×10^{-3} M), FeCl_3 (6.83×10^{-5} M), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (7.69×10^{-6} M), MoO_3 (1×10^{-5} M), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (4.3×10^{-5} M), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.6×10^{-6} M), and agar-agar, 1 g per 200 mL. Gold(III) from potassium tetrachloroaurate was used at concentrations of 0, 5, 10, 20, 40, 80, 160, and 320 ppm. All jars were covered with plastic wrap after alfalfa seeds were spread out to avoid contamination from the environment and to allow light to pass through. Four replicates of each treatment were prepared at the optimum pH of 5.8, for quality control and quality assurance purposes. The study was performed in a growth chamber under a 12 h photoperiod, and 25/18 °C day/night temperature variations. The alfalfa plants were harvested after 2 weeks of growth, and the alfalfa plants were washed three times with deionized water. For the XAS experiments the washed alfalfa samples and the agar samples were frozen in liquid nitrogen for 40 min and then placed in a Freeze-Dry System for 2 days to remove any water within the samples.

XAS were conducted at the Stanford Synchrotron Radiation Laboratories (SSRL). The Au $L_{(\text{III})}$ edge (11.918 keV) was used for collecting the sample spectra using standard operating conditions of 3 GeV and 60–100 mA.¹⁵ The gold(III)-loaded alfalfa plant samples were run at ap-

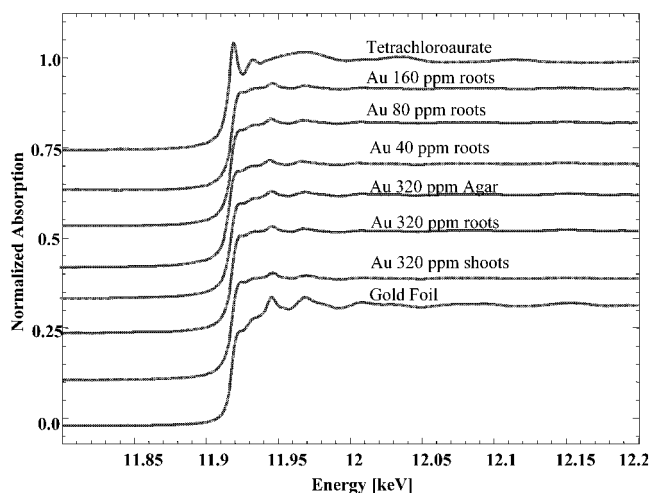


Figure 1. XANES of the gold alfalfa roots and shoots and gold-enriched agar samples, gold(0) foil, and the tetrachloroaurate model compound. These data show that the gold present in the alfalfa and agar samples is present as gold(0)

proximately 20 K using a liquid helium cryostat to reduce interference in the spectra of the sample due to thermal disorder. The fluorescence spectra of the alfalfa samples were acquired using a 13-element array germanium detector. The transmission measurements for the gold model compound were taken using argon-filled ionization chambers. The model compound tetrachloroaurate was ground and diluted using boron nitride to achieve a composition to give a change of 1 absorbance unit across the absorption edge. The samples and model compound were packed into 1 mm aluminum sample holders with X-ray transparent tape. A Si^{220} double crystal monochromator with an entrance slit of 1 mm was used for the measurements. The monochromator was detuned by 50% to reject higher order harmonics. All spectra were calibrated against the edge position of a gold foil (11.918 keV).

The WinXAS software package was used to analyze the experimental EXAFS.^{14–17} The EXAFS were extracted from the absorption spectra by subtracting a two-range spline fit to the preedge region from the total spectrum and normalizing the difference using a polynomial fit to the postedge region. Figure 1 shows the XANES spectra of the gold alfalfa root and shoot samples and the model compound. The edge energy or position of the plant and agar samples matches that of the gold(0) (11.918 keV) foil and not that of the initial reactant tetrachloroaurate. The position of the edge energy clearly shows that the gold present in the plant and agar is present as gold(0). Additionally, this shows that the alfalfa has the ability to actively uptake gold(0) from solid media. Furthermore, our results show that the gold(III) is being reduced to gold(0) in the agar solid media.

Figure 2 shows the EXAFS of the gold samples and the tetrachloroaurate (not phase and amplitude corrected).^{17,18} As observed the nearest neighbor in the tetrachloroaurate sample has a much shorter interatomic distance than the nearest neighbor in the gold alfalfa plant roots and shoots and gold-agar samples. But, the nearest neighbor in the gold(0) foil appears at the same distance as the nearest neighbor in the

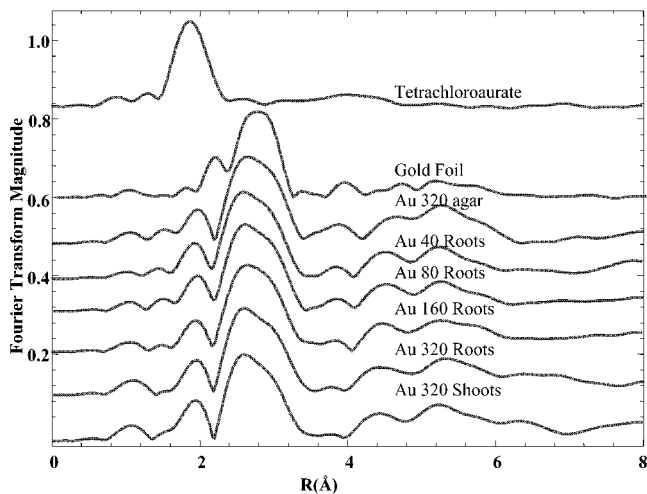


Figure 2. EXAFS of the gold alfalfa and roots and shoots and gold-enriched agar samples, gold(0) foil, and the tetrachloroaurate model compound. These data show that the nearest neighboring atom in the alfalfa and agar samples is gold.

gold plant and agar sample, thus confirming that the gold present in plant shoot, roots, and agar samples is gold(0). This result confirms that alfalfa has the ability to actively uptake gold(0) from a solid media. The Au–Au bond of the gold foil has a distance of 2.86 Å and a coordination number of 12. The model compound tetrachloroaurate has, as expected, a Au–Cl bond with a distance between them of 2.28 Å and a coordination number of 4. The remaining samples all have similar bond distances as the Au–Au (within ± 0.01 Å) but differ in their coordination numbers. The coordination number gives an indication that gold present in the biomass and agar samples are fractions of the total size of a full fcc Au particle.

For the TEM analysis new plant samples were embedded in a synthetic resin and dried in a furnace at 60 °C for 24 h. Thin slices (~ 50 nm thick) were cut using a microtome and put on Cu grids and placed on the sample holder of the TEM. The samples were not stained for the TEM analysis. As a consequence, the only possible sources of contrast are mass–thickness differences or diffraction contrast between different zones and the gold nanoparticles themselves. A JEOL 2010-F TEM equipped with a X-ray EDS elemental microanalysis system was used to study the nanoparticles within the plants.

Figure 3a shows a low magnification TEM image of the alfalfa shoots, confirming the existence of Au nanoparticles aggregated together. In this image the small black dots correspond to the gold nanoparticles. This image also indicates that the nucleation of the particles inside the plants occurs in preferential zones. The morphology in Figure 3a suggests an active transport of gold(0) atoms through the plants from the roots to the shoots. This active transport of the gold atoms is indicated by the positions of the particles throughout the entire region of the shoot in the micrograph. This confirms the EXAFS and XANES results since they showed that gold(0) was found in both the roots and shoots, although at lower concentrations in the stems, the signal was lost (data not shown). Figure 3b is a qualitative X-ray EDS spectrum corresponding to an arbitrary nanoparticle. The

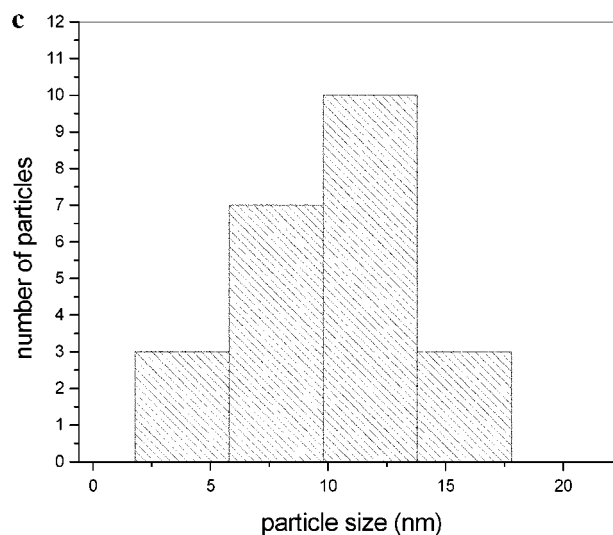
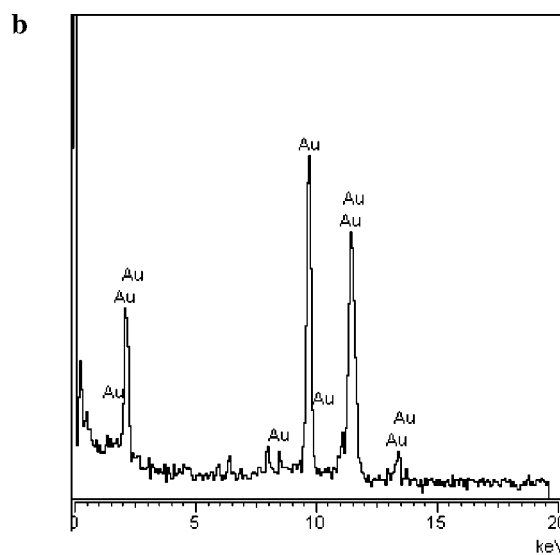
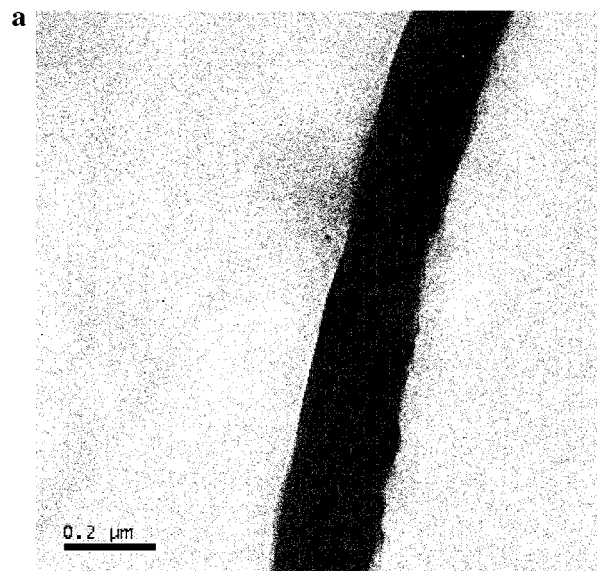


Figure 3. (a) Low-magnification TEM image of the alfalfa shoot showing aggregates of gold nanoparticles. The scale is shown in the image. (b) X-ray EDS analyses confirming that the nanoparticles are constituted by pure Au. (c) Histogram showing the Au nanoparticle size distribution in a typical alfalfa sample.

analysis confirms that the nanoparticle is pure gold. The same study was performed on many different nanoparticles, confirming that the only constituent element of each nanoparticle is Au. Figure 3c shows the particle size distribution found for all the samples. This figure shows a broad distribution with typical sizes between 2 and 20 nm in diameter. The difference in size is possibly due to the fact that the nanoparticles are being formed at different times. The limits on the nanoparticle size due to constraints related to the particles nucleating inside a plant cannot be discussed at this stage, as it requires further study. HRTEM can be used to give a better characterization of the nanoparticles, as this technique gives a resolution at the atomic scale.

HRTEM images of the gold nanoparticles are shown in Figure 4a–c for three different examples. The images were obtained at the optimum defocus condition. Different particles were imaged using the high-resolution mode, showing that the nanoparticles are in a crystalline state. The inset in the three images shows the corresponding fast Fourier transform (FFT). In the case of nanoparticles <5 nm thick, the FFT is similar to the diffraction pattern and therefore it can be used to study the interplanar distances.¹⁹ The shortest lattice parameter calculated from each image is in every case 0.23 nm. This value corresponds approximately to the interplanar spacing between (111) planes of gold. The particles have different atomic orderings and many show the presence of microstructural defects that are similar to gold nanoparticles obtained by synthetic techniques such as evaporation or colloidal methods.²⁰ The splitting of the points observed in the FFT confirms that the particles are inter-twinned. In Figure 4c a very small crystalline nanoparticle can be observed in the first stage of the nucleation process. A closer examination of the FFT pattern indicates that there are spots showing splitting of the particles. By measuring the angle of splitting and comparing with previous calculations, we concluded that the structure corresponds to an icosahedron tilted away about 18° out of the 3-fold orientation.²¹ Such a small particle with an icosahedron structure implies that the gold atoms arrange themselves into the minimum energy configuration even within the plant. Figure 5 shows the coalescence of the nanoparticles within the plant structure. The image shows atomic resolution in all the particles, suggesting an orientational relationship among the different particles. Additionally, within the same plant sample, it was possible to find small particles of approximately 4 nm and large coalesced particles ranging in size between 20 and 40 nm. This result clearly indicates that continuous growing and particle rearrangement processes occur within the plant structure. The nucleation of Au nanoparticles inside living plants is a remarkable phenomenon, which deserves a deeper study in relation with possible applications. It offers three interesting possibilities: first, it provides a new method for the synthesis of gold nanoparticles; second, it will generate new studies concerning the interaction between the plants, metals, and metal ions; finally, it may constitute a new method for the mining of gold from solution and soils as an inexpensive method for the purification of economically important elements.

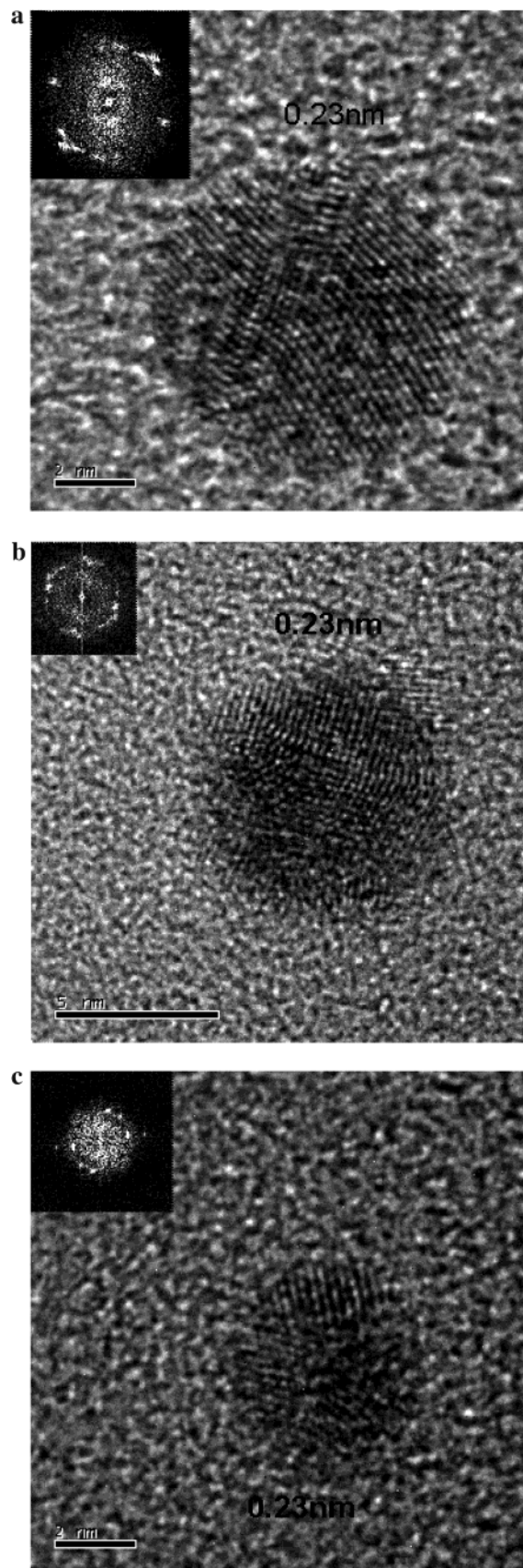


Figure 4. HRTEM images of three different nanoparticles. The inset in the upper left corner of the micrograph corresponds to the FFT. (a) and (b) show different crystalline arrangements. The FFT confirms that the structures are twinned. (c) shows a very small icosahedron tilted 18° from the 3-fold orientation.

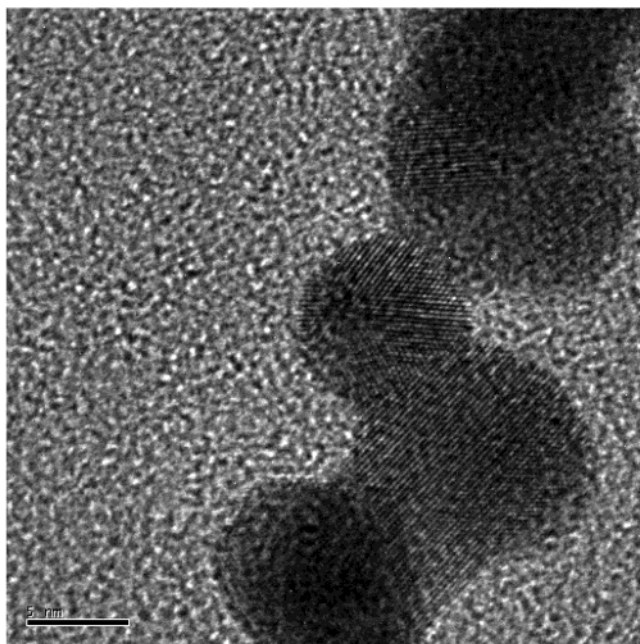


Figure 5. Image showing the coalescence of nanoparticles inside the alfalfa plant. Nanoparticles show atomic resolution or at least fringes indicating an orientation relationship between them.

In summary, through the use of XANES, EXAFS, X-ray EDS, TEM, and HRTEM we have shown that living alfalfa plants have the ability to uptake zero valence gold from solid media. The XAS spectroscopy studies showed that the gold present in the agar and alfalfa plant samples was elemental gold. The fittings performed using an ab initio code FEFF (v.810) showed that gold in the samples is in a structure that is only a fraction of the size needed for a complete Au(0) fcc gold particle. X-ray EDS and the XANES studies confirmed that the only element in the nanoparticles is gold. TEM, and HRTEM confirmed the results of the EXAFS that the gold present in the samples was in a structure that was smaller than that required for a complete fcc gold particles or nanoparticles. The gold particles with an approximate size of 4 nm had an icosahedron structure, which is the lowest energy configuration for gold nanoparticles. Further analysis of the HRTEM showed the gold particles have a fcc twinned structure when the particle size ranges between 6 and 10 nm. Furthermore, when the particles coalesced, the fcc structure was observed. The results indicate that live alfalfa plants may be viable cost-effective means of phytomining gold from soils at low and high concentrations. It is also a promising road to synthesize quantum dots using biological techniques.

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