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## Imaging Nanometer Metallocatalysts Formed by Photosynthetic Deposition of Water-Soluble Transition-Metal Compounds

James W. Lee,<sup>†</sup> Ida Lee,<sup>‡</sup> and Elias Greenbaum\*,<sup>†</sup>

Oak Ridge National Laboratory, Chemical Sciences Division, P.O. Box 2008, Oak Ridge, Tennessee 37831-6194, and Department of Electrical Engineering, University of Tennessee, Knoxville, Tennessee 37991

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A method of imaging nanometer metallocatalysts formed by photosynthetic precipitation of the water-soluble transition-metal compounds  $[PtCl_6]^{2-}$  and  $[RuCl_6]^{2-}$  is reported. Hexachloroplatinate and hexachlororuthenate can accept up to four electrons from Photosystem I (PSI) reaction centers in photosynthetic thylakoid membranes, thereby converting  $[PtCl_6]^{2-}$  and  $[RuCl_6]^{2-}$  anions to either metallic platinum (Pt) and ruthenium (Ru) and/or partially oxidized nanometer catalysts at the reducing sides of PSI molecules. Use of this method can potentially create nanometer-sized Pt and/or bimetallic catalysts (such as Pt–Ru) on biomembranes and molecules at pH 7 and room temperature with preservation of the biological function of the molecules.

Oxygenic photosynthesis is the key biological process that converts the electromagnetic energy of sunlight to chemical energy via the reduction of atmospheric carbon dioxide  $(CO_2)$ to carbon fixation compounds using electrons from water oxidation. Photosynthesis supports virtually all life on Earth. However, it is also possible to use electrons from the photosynthetic process to create nanometer-sized catalysts at certain biomolecular interfaces, such as the reducing side of a Photosystem I (PSI) reaction center using water-soluble transitionmetal compounds including hexachloroplatinate [PtCl<sub>6</sub>]<sup>2-</sup> and hexachlororuthenate  $[RuCl_6]^{2-}$ . Figure 1A presents a schematic illustration of the photosynthetic thylakoid membrane, which can be isolated from green plants such as spinach leaves. The key components in the thylakoid membrane are the two photosynthetic reaction centers, Photosystems I (PSI) and II (PSII), both of which are well-organized chlorophyll-protein complexes  $\sim 10$  nm in size. Upon actinic illumination, water is oxidized to protons  $(H^+)$  and molecular oxygen  $(O_2)$  at PSII, which is driven by the P680 photochemical charge separation. Electrons acquired from water splitting are transferred vectorially from P680 to the Q<sub>B</sub> side of PSII, where they are accepted by the plastoquinone (PQ) pool in the thylakoid membrane and then transferred through a series of electron carriers, including the cytochrome (Cyt) b/f complex and plastocyanin (PC), to PSI, where the electrons are energized again by the PSI photochemical charge separation reaction. In normal photosynthesis, electrons emergent from PSI are used to reduce carbon dioxide to produce sugars through a collection of enzymatic reactions known as the Calvin cycle. It is also known that the electrons emergent from PSI can be used to reduce the common Hill reagent ferricyanide ([Fe(CN)<sub>6</sub>]<sup>3-</sup>) to ferrocyanide



**Figure 1.** Photosynthetic deposition of nanometer catalysts at the reducing ( $F_{AB}$ ) side of Photosystem I (PSI) reaction centers in the thylakoid membrane. Panel A shows the chemistry of the nanometer-scale catalyst deposition by photosynthetic reduction of water-soluble transition-metal compounds, whereas panel B depicts the catalytic activity demonstrated by the observed simultaneous photoevolution of O<sub>2</sub> and H<sub>2</sub> from water in the photosynthetically deposited catalyst thylakoid membrane.

 $([Fe(CN)_6]^{4-})$  as first demonstrated by Robert Hill over 60 years ago.<sup>1</sup> Our previous work indicated that the Hill reaction could be extended to reduce certain water-soluble transition-metal compounds (such as  $[PtCl_6]^{2-}$  or  $[RuCl_6]^{2-}$ ) to generate H<sub>2</sub> photoevolution, as demonstrated by the observation that illumination of the thylakoid membranes in the presence of 2 mM  $[PtCl_6]^{2-}$  or  $[RuCl_6]^{2-}$  can lead to simultaneous photo-

<sup>\*</sup> Author to whom correspondence should be addressed. Telephone: 865-574-6835. Fax: 865-574-1275. E-mail address: greenbaum@ornl.gov.

<sup>&</sup>lt;sup>†</sup>Oak Ridge National Laboratory.

<sup>&</sup>lt;sup>‡</sup> University of Tennessee.



**Figure 2.** Scanning electron microscopy (SEM) images of  $[PtCl_6]^{2-}$  photoprecipitated and dried thylakoid materials at magnifications of  $30 \times$  (top) and  $5000 \times$  (bottom), using an electron beam with an excitation energy of 20 kV.

evolution of H<sub>2</sub> and O<sub>2</sub>, whereas the control sample (thylakoid membranes without the addition of 2 mM  $[PtCl_6]^{2-}$  or  $[RuCl_6]^{2-}$ ) could not.<sup>2–5</sup> This is a significant result, because it demonstrated a transformation of the photosynthetic molecular system to achieve a new type of photosynthesis-photoevolution of H<sub>2</sub> and O<sub>2</sub> from water (Figure 1B), instead of the usual CO<sub>2</sub> fixation activity. The observed photoevolution of H<sub>2</sub> represents a catalytic reduction of protons by the electrons from the oxygenic photosynthetic water-splitting process. Noble metals such as platinum are known to have low overpotentials for catalytic activity for electrolytic H<sub>2</sub> production. Therefore, the observed H<sub>2</sub>-producing catalytic activity could be explained if one assumes that the Hill oxidants  $[PtCl_6]^{2-}$  and  $[RuCl_6]^{2-}$  could accept four electrons consecutively from PSI, thereby converting [PtCl<sub>6</sub>]<sup>2-</sup> and [RuCl<sub>6</sub>]<sup>2-</sup> anions to lower-valence platinum (Pt) and ruthenium (Ru) at the reducing (FAB) side of PSI, according to the following reactions:

and

$$[PtCl_6]^{2^-} + 4e^- \rightarrow Pt + 6Cl^-$$
(1)

$$[\operatorname{RuCl}_6]^{2^-} + 4e^- \rightarrow \operatorname{Ru}_* + 6\operatorname{Cl}^-$$
(2)

Alternatively, photochemical reduction may not necessarily proceed completely to metal formation. In that case, the catalytically active species could be a partially oxidized insoluble nanometer structure. In either case, because of the complexity of the biological sample, the formation of the nanometer catalysts has never been physically confirmed by electron microscopy imaging. The specific difficulties in visualizing such nanometer catalysts in the biological samples are (i) the amount of catalyst is relatively small, in comparison with the complex matrix of the thylakoid material, and (ii) the thylakoid membranes are a soft and nonconductive material that cannot be



300nm

Electron Image 1



**Figure 3.** SEM analysis of nanometer-scale metallocatalysts formed by the photosynthetic precipitation of  $[PtCl_6]^{2-}$  with thylakoids. The SEM imaging (top) of the sample on the silicon substrate revealed individual nanocatalysts. The Pt peak in the SEM X-ray fluorescence (XRF) analysis (bottom) of the nanoparticles verified the identity of the platinum nanocatalyst. The Si and O peaks were from the silicon substrate. In each experiment, the SEM beam and the X-ray detector might be at slightly different locations with respect to the specimen. The detection of Al (base) and/or Cu (clips) peaks is dependent on the location of these components with respect to the SEM beam and the X-ray detector. These results represent the first time that photosynthetically precipitated platinum nanocatalysts were isolated and imaged via SEM.

easily imaged at high (nanometer-scale) resolution using scanning electron microscopy (SEM).<sup>6,7</sup> As shown in Figure 2, the thylakoid sample (dried) can be visualized by SEM only at very low resolution (with a magnification of  $\sim 30 \times$ ). When the magnification is increased to  $5000 \times$  with a 20-kV electron beam, significant electronic charge accumulates in the sample, typically resulting in a ring-like image (see bottom of Figure 2) that is reminiscent of the nature of the nonconductive materials that prevent visualization at the nanometer scale using SEM.

The previous inability to visualize any of the postulated formations of nanometer catalysts in the thylakoid samples directly poses a challenge to the aforementioned model of photosynthetic deposition of transition-metal compounds illustrated in Figure 1. Here, we report experiments that enable the first SEM visualization of the nanometer-sized metallocatalysts from the treated thylakoid samples.





**Figure 4.** SEM analysis of nonphototreated  $[PtCl_6]^{2-}$ -thylakoids as a control. The SEM image (top) shows that the organic materials of the applied thylakoid materials could be completely removed by passing the material through the sterilization flame. The Si and Al peaks in the XRF spectrum (bottom) are from the silicon wafer and the aluminum SEM sample holder. The absence of metallic platinum particles in this control sample indicated that the phototreatment was required for formation of nanometer-scale platinum particles in the photosynthetic thylakoid membrane.

This paper reports microscopic analysis and visualization of the nanometer-sized metallocatalysts formed by the photosynthetic deposition of water-soluble transition-metal compounds in thylakoid membranes at room temperature and neutral pH. The thylakoid materials were isolated from spinach leaves using the procedure described by Reeves and Hall,<sup>8</sup> and they then were suspended in Walker's assay medium9 and adjusted to a final chlorophyll (chl) concentration of 600  $\mu$ g/mL for photoprecipitation of catalysts for production of H<sub>2</sub> and O<sub>2</sub> with a reactor-flow detection system. The chl content was determined in 90% acetone extracts.<sup>10</sup> Photoprecipitation of metallocatalysts was performed in a helium atmosphere, using a reactor-flow detection system as described previously.5 Ten milliliters of thylakoid membrane suspension (600  $\mu$ g chl/mL) was placed in the reactor. The prospective Hill reagent (i.e., the transitionmetal compound species [PtCl6]2- and/or [RuCl6]2-) was added to the thylakoid suspension for a final concentration of 2 mM. The reaction vessel was water-jacketed and held at 20 °C with a temperature-controlled water bath (Lauda RM6, Brinkmann Instruments, Germany). The reaction medium (thylakoid suspension plus the transition-metal compound species) was gently stirred by a magnetic stirring bar and purged with a helium flow (50 mL/min) in the headspace above the liquid. The rate of helium flow was controlled by a gas-flow control system (Type 260, MKS Instruments, Inc., Andover, MA). Photoprecipitation of metallocatalysts was initiated by actinic illumination



700nm

Electron Image 1



**Figure 5.** SEM analysis of Pt–Ru nanometer metallocatalysts formed by photosynthetic coprecipitation of  $[PtCl_6]^{2-}$  and  $[RuCl_6]^{2-}$ . The SEM imaging result (top) of the bimetallic catalysts represents the first time that such bimetallic catalysts were isolated and observed via SEM. The Pt and Ru peaks in the XRF analysis (bottom) confirm the presence of platinum and ruthenium in the catalyst material. The Al and Cu peaks were from the sample holder. The Si and O peaks were from the silicon substrate.

(100  $\mu$ E m<sup>-2</sup> s<sup>-1</sup>, with a wavelength of 660 nm) provided by light-emitting diodes (QB1310CS-660, Quantum Devices, Inc., Barneveld, WI). Production of H<sub>2</sub> and O<sub>2</sub> was monitored in the effluent gas flow as detected by the tin-oxide hydrogen sensor and galvanic cell. After photosynthetic deposition of [PtCl<sub>6</sub>]<sup>2-</sup> with observation of H<sub>2</sub> and O<sub>2</sub> photoevolution for 12 h, the [PtCl<sub>6</sub>]<sup>2-</sup>-phototreated thylakoid materials were washed three times by resuspension in double-distilled water and subjected to pelletting by centrifugation; the supernatant was then discarded, to remove all water-soluble materials, especially residual [PtCl<sub>6</sub>]<sup>2-</sup>. A thin layer of the washed metallocatalystcontaining thylakoid material was deposited on a conductive silicon (P111) wafer and dried at 150 °C for 10 min. The organic (thylakoid) material was then removed by passing the sample plates through a sterilization flame. The samples were then imaged and analyzed using a Hitachi model S-4700 SEM system with an in situ X-ray fluorescence (XRF) analysis capability.

Figure 3 presents a typical SEM image and XRF analysis using the new method for visualizing nanometer-scale metallocatalysts formed by the photosynthetic precipitation of  $[PtCl_6]^{2-}$  with thylakoids. The XRF spectrum (see bottom of Figure 3) corresponded to the analysis of an individual nanometer-sized platinum particle, as marked in the SEM image (see top of Figure 3). The Pt XRF peak at ~2.1 keV is clearly above



Figure 6. Atomic force microscopy (AFM) image of Pt-Ru particles from the sample plate of Figure 5 after washing with acetone and methanol and drying with an argon gas stream.

the background noise. The Si and O peaks are from the substrate silicon wafer. The SEM images clearly illustrate the nanometer-scale platinum particles formed from the  $[PtCl_6]^{2-}$ -phototreated thylakoid sample. "Spectrum 14" is automatically assigned by the SEM machine as a label for the selected nanometer spot of the sample. The XRF spectrum (see bottom of Figure 3) was from this nanometer spot.

As an experimental control, a nonphototreated  $[PtCl_6]^{2-}$ -thylakoid sample was prepared in the same way as that of the  $[PtCl_6]^{2-}$ -phototreated thylakoid sample for SEM analysis. Figure 4 presents the SEM analysis result of a nonphototreated  $[PtCl_6]^{2-}$ -thylakoid sample. As shown in the SEM image (see top of Figure 4), the organic materials of the applied thylakoid materials were removed by passing the material through the sterilization flame, and no metallic platinum particles were observed in this control sample. The absence of any metallic platinum particles in the control sample indicated that phototreatment is indeed required, as expected, for formation of nanometer-scale platinum particles in the XRF spectrum (see bottom of Figure 4) are from the silicon wafer and the aluminum SEM sample holder.

We are now also able to visualize Pt and/or Ru metallic nanometer catalysts that are formed via photosynthetic coprecipitation of  $[PtCl_6]^{2-}$  and  $[RuCl_6]^{2-}$  at the reducing side of PSI in the thylakoid membrane. Figure 5 presents the SEM analysis of Pt and Ru nanometer metallocatalysts formed by photosynthetic coprecipitation of  $[PtCl_6]^{2-}$  and  $[RuCl_6]^{2-}$ . The SEM result (see top of Figure 5) of the two catalysts represents the first images of coprecipitated metallocatalysts by photosynthesis. The Pt and Ru peaks in the XRF analysis (see bottom of Figure 5) confirm the presence of platinum and ruthenium in the catalyst material. The Al and Cu peaks were from the sample holder. The Si and O peaks were from the silicon substrate. These observations provide direct evidence that both  $[PtCl_6]^{2-}$ and  $[RuCl_6]^{2-}$  can be photosynthetically reduced to microscopic particles with thylakoid membranes.

According to the SEM image (see top of Figure 5), residual organic (thylakoid) materials seem to remain on the substrate silicon wafer. Therefore, this sample (on a silicon wafer) was washed with organic solvents, including acetone and methanol, and then imaged under atomic force microscopy (AFM)<sup>11</sup> after the organic solvent was removed by a gas stream of ultrapure nitrogen (99.9997% pure). The AFM imaging (Figure 6) shows that clean particles can be obtained with the organic solvent treatment. The particles are in the 10–40-nm size range.

We conclude that these results of microscopic analysis and visualization provide direct evidence in support of the model of photosynthetic metallocatalyst deposition (see Figure 1). The results confirm that, unlike the common photosynthetic Hill reagent ferricyanide ([Fe(CN)<sub>6</sub>]<sup>3-</sup>), hexachloroplatinate and hexachlororuthenate are able to accept up to four electrons consecutively from PSI, thereby converting [PtCl<sub>6</sub>]<sup>2-</sup> and [RuCl<sub>6</sub>]<sup>2–</sup> anions to hydrogen-evolving platinum and ruthenium nanometer metallocatalysts at the reducing side of PSI. Use of this method can potentially create nanometer-scale metallic and/ or bimetallic (such as Pt-Ru) catalysts on biomembranes and molecules at pH 7 and room temperature that preserve the biological function of the molecules. Using photosynthetic deposition of platinum (or ruthenium) at the reducing side of PSI reaction centers, a novel biomimetic photosynthesis can be created for simultaneous photoproduction of H2 and O2 by lightactivated water splitting and metallocatalysis. The results reported here may be of both theoretical and practical importance.

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