

Photoreduction of Catalytic Platinum Particles Using Immobilized Multilayers of Photosystem I

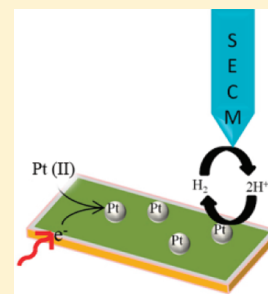
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S Supporting Information

ABSTRACT: Using the abundance of available electrons generated by immobilized multilayers of the photoactive protein complex Photosystem I (PSI), we have photoreduced platinum particles that are catalytically active for the H_2/H^+ redox couple. The resulting platinized PSI films were optimized using electrochemical measurements and then characterized using X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and scanning electrochemical microscopy (SECM). These results demonstrate a novel method for generating immobilized platinum catalysts that are readily available on the surface of a photoactive PSI multilayer.



INTRODUCTION

Photosynthesis is the essential process by which solar energy is converted to chemical energy through the use of numerous proteins and enzymes. One of these protein complexes, Photosystem I (PSI), is found in the thylakoid membranes of plants, algae, and cyanobacteria. Using a system of chlorophylls inside this protein complex, PSI transfers the energy of photons to the P700 reaction center. The excited P700 is then quickly oxidized as the electron is shuffled down the electron transport chain to an iron–sulfur cluster (F_B^-) located on the stromal side of the protein complex.¹ The efficiency of electron movement through PSI is near unity and thus has generated interest for the use of this protein in nonbiological systems.^{2–6} In natural systems, electrons are transferred from the F_B^- site of PSI to NADP^+ reductase via ferredoxin; however, the system can be manipulated in such a way that the electrons are utilized for alternative purposes.

One of the earliest applications of PSI in a nonbiological role was to precipitate platinum to the stromal side of the protein via a previously reported mechanism.⁷ Previous accounts have suggested that the source of the electrons for this reaction can be molecular hydrogen⁸ or the irradiated PSI reaction center itself.⁹ The extended lifetime of the excited electron at the F_B site of PSI¹ makes this location ideal for platinum photoreduction. Blankship et al.¹⁰ have outlined an ideal semiautomatic device for H_2 bioproduction utilizing the power of photosystems. Recent publications have demonstrated the utility of platinum-PSI particles for hydrogen production,^{11–14} enhanced photoactivity,¹⁵ protein orientation,⁸ and ordered multilayering capabilities.¹⁶ However, in all of these methods, the photoreduction of platinum occurs prior to or without immobilization of PSI. Our group^{17–20} and others^{21–23} have demonstrated that PSI can be immobilized on a substrate in a variety of ways while maintaining its functionality. In this work, we report the

photoreduction of platinum onto a previously immobilized multilayer assembly of unmodified PSI from spinach. Additionally, in order to demonstrate the catalytic ability^{24,25} of these photoreduced platinum particles, the surface generation of H_2 was imaged using scanning electrochemical microscopy (SECM).

EXPERIMENTAL SECTION

A. Materials. Substrates for electrochemical measurements were prepared by thermally depositing ~ 125 nm of gold (J&J Materials) onto a silicon support (Montico Silicon, $\langle 100 \rangle$ orientation). Unmodified silicon supports were used for all nonelectrochemical measurements. Buffered solutions (50 mM) of either potassium biphthalate (pH 4) or potassium phosphate monobasic-sodium hydroxide (pH 7) were purchased from Fisher Scientific. 2-Aminoethanethiol was purchased from Sigma as were sodium hexachloroplatinate(IV) hexahydrate and potassium tetrachloroplatinate(II). Triton X-100 and L-ascorbic acid sodium salt were purchased from Acros. Dialysis was performed using 10 000 MWCO dialysis tubing (Spectrapore).

B. Methods. **1. Photosystem I Extraction.** Photosystem I complexes were extracted from commercially available baby spinach as previously described.²⁶ Briefly, thylakoid membranes were separated from the spinach leaves via maceration and subsequent centrifugation following the method of Reeves et al.²⁷ with adaptations.²⁸ The PSI complex was then removed via additional centrifugation and purified using a chilled hydroxylapatite column.²⁹ The concentration of surfactant and salt in the resulting effluent was then reduced using dialysis.²⁶ The concentration of the resulting PSI solution used consisted of 3.7×10^{-5} M chlorophyll, with a chlorophyll *a/b* ratio of 3.0, and 9.4×10^{-7} M P700 as characterized by the methods of Porra³⁰ and Baba et al.²⁹ respectively.

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2. Multilayer Formation and Platinum Photoreduction on PSI Multilayer Films. PSI multilayer films were prepared on either an unmodified silicon substrate or a gold substrate that was modified via immersion in a solution of 2-aminoethanethiol (2 mM) to increase the hydrophilicity of the gold. The extracted PSI solution was placed on the substrate and vacuumed to dryness. This vacuum deposition process was repeated three times in order to generate thick multilayer films of PSI.²⁶ To photoreduce platinum onto the protein, the dried substrate was immersed in a solution of either 2 mM sodium hexachloroplatinate(IV) hexahydrate or 2 mM potassium tetrachloroplatinate(II), 8 mM sodium ascorbate, and 50 mM phosphate buffer at a pH of 7. The substrates in solution were illuminated for 2 h unless otherwise indicated using a Fiber-Lite fiber optic illuminator (model 190, Dolan-Jenner Industries). The resulting assemblies were rinsed with DI water and dried with N₂ gas before further analysis.

3. Instruments and Equipment. Electrochemical measurements were performed using a CH Instruments CHI 660a electrochemical workstation equipped with a Faraday cage. A custom built, three electrode cell was used where the modified gold sample was set as the working electrode, platinum mesh as the counter electrode, and Ag/AgCl as the reference electrode. An aqueous solution of potassium bipthalate buffer (50 mM) at a pH of 4 was used as the mediator. Illumination for photochronoamperometric measurements was provided by a 250 W cold light source (Leica KL 2500 LCD).

X-ray photoelectron spectroscopy (XPS) was performed on a Physical Electronics PHI 5000 VersaProbe spectrometer equipped with a monochromatized Al K α X-ray source (1486.6 eV). Samples were analyzed with an incident angle of 45°. All peaks were referenced to the Au 4f_{7/2} peak at 84.0 eV. XPS data analyses were performed using CasaXPS software with Gaussian–Lorentzian profiles using a Shirley background.

Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analyses were performed on a Hitachi S-4200 instrument. SEM images were collected with an accelerating voltage of 20 kV. EDX analyses were performed using an accelerating voltage of 20 kV and an emission current of 20 μ A.

Scanning electrochemical microscopy (SECM) experiments were performed on a CH Instruments CHI 900 electrochemical workstation. A 25 μ m platinum microelectrode was used as the tip electrode and a platinized PSI-gold sample (2 h photoreduction time) described previously as the substrate electrode. A 100 mM KCl solution adjusted to a pH of 4 using HCl was used as the mediator solution. The tip and substrate potential were set at their respective open circuit potential values. A step size of 10 μ m was used with the tip brought near the substrate electrode using a feedback approach curve technique.

RESULTS AND DISCUSSION

Our group has previously demonstrated that thick multilayer films of PSI are capable of producing large photocurrents.²⁶ Here we demonstrate that these immobilized multilayer films of PSI can be used to photoreduce platinum salt to catalytic particles. Upon illumination, electrons are excited from the P700 reaction centers present in these films. Our results show that these electrons then reduce platinum from its original oxidation state (II or IV) to 0, producing a catalytic particle. Importantly, the photoreduction of platinum was performed in an isolated chamber, while subsequent proton reduction to hydrogen gas was evaluated in an electrochemical cell after rinsing off any residual platinum salts. During the process of platinum photoreduction, electrons are resupplied to the P700 reaction center by a sacrificial electron donor (sodium ascorbate). Interestingly, multilayers of PSI are capable of photoreducing platinum(IV) to platinum(0) without a sacrificial electron donor (see the Supporting Information). Though less platinum(0) is produced due to the limited number of electrons present in the film, these data suggest that

electrons from proteins within the thick PSI film can be transferred to surrounding PSI molecules. This provides further information for understanding why higher photocurrents are observed with increasing thickness of PSI films,²⁶ as the electrons could be passed from the F_B[−] site of one protein to the P₇₀₀⁺ site of another protein. Further experiments would be required to confirm this mechanism; however, this is beyond the scope of this work. In order to enhance and optimize the ability to produce photogenerated platinum particles that have catalytic characteristics, all the experiments described below used the sacrificial electron donor sodium ascorbate during the photoreduction of platinum salts on protein films.

In order to optimize the amount of time light was exposed to the protein film in the presence of the platinum salt solution, photochronoamperometric measurements in acidic buffer were utilized to analyze the photoreduction of protons at the Pt-modified PSI electrode. Photoreduction of platinum salt for 2 h was determined to provide the highest amount of hydrogen production as seen by the resulting reductive photocurrent (Figure 1a). Photoreduction of the platinum salt beyond this time frame resulted in a decrease in the measured photo-

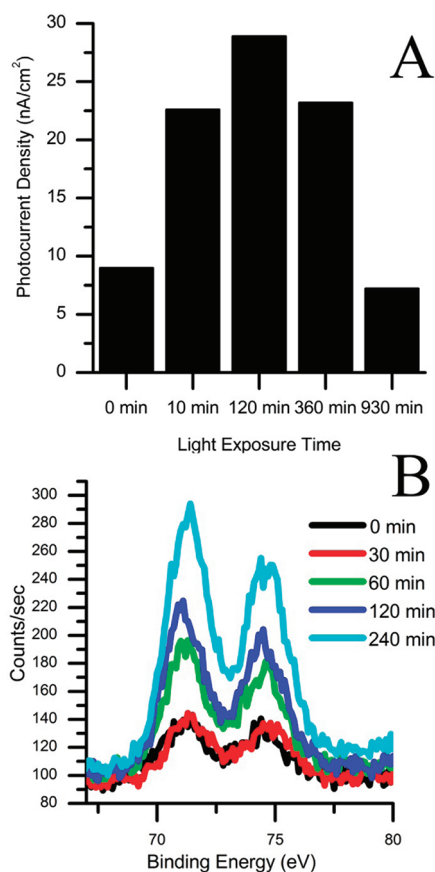


Figure 1. Optimization of the photoreduction of platinum onto PSI multilayer films (three deposition steps with a film thickness of approximately 700 nm). (A) Photochronoamperometric substrate reduction of protons measured for samples that underwent platinum photoreduction for various lengths of time. Values indicate the change in current following illumination from red light in a pH 4 buffered solution. The working electrode (sample) was set at the open circuit potential (~ 0.2 V vs Ag/AgCl) for these measurements. (B) XPS analysis of platinum that has been photoreduced on multilayer films of PSI for various lengths of time. Notice that the distinctive platinum 4f doublet increases in intensity with increasing light exposure time.

current; however, XPS analysis demonstrated that platinum salt continued to be photoreduced beyond 2 h of illumination (Figure 1b). The 4f doublet observed at 71.5 and 74.5 eV in the XPS spectrum corresponds to platinum in the 0 oxidation state that has either oxygen or chloride adsorbed to its surface.³¹ The binding energies are shifted from those expected for either Pt^{II} or Pt^{IV}. The small peaks present for the sample exposed to 0 min of illumination is likely due to residual platinum salt that was reduced by the ambient light in the lab, and thus provides a good baseline measurement to evaluate the other samples. Because multilayers films of PSI have been shown to remain active for at least 280 days,¹⁸ we attribute the decrease in proton reduction for illumination time of greater than 2 h to a shadowing effect. As the platinum photoreduces on the surface of the protein, it begins to shadow the protein, limiting the amount of light that can be absorbed by the protein film.

In order to gain a greater understanding of the size of the platinum particles present on the surface, SEM and EDX analyses were performed (Figure 2). As seen in the images, platinum particles are scattered over the surface of the sample (Figure 2a). Performing EDX directly on one of these particles revealed a strong platinum signal (Figure 2c). The presence of various sized platinum regions would indicate that the photoreduction begins with a small nucleation event, presumably at the F_B⁻ site, which then allows for larger platinum regions to form as electrons are continually fed to the platinum particles by the protein complexes. This method of particle growth is supported by the fact that smaller platinum particles are observed when photoreduction occurs in solution.¹¹ Furthermore, the darker regions of the SEM image produce a platinum signal under EDX analysis; however, the signal is greatly reduced in comparison to the bright areas in the image, suggesting the presence of smaller platinum particles. The ability for particle growth to larger sizes appears specific to this immobilized PSI photoreduction technique.

SECM experiments were then performed to demonstrate the catalytic ability of the photoreduced platinum present on the biohybrid electrode. A SECM image was collected using an ultramicroelectrode (UME) close to the substrate surface in order to electrochemically image individual platinum particles (Figure 3). To generate the image, the UME was rastered above the biohybrid working electrode in an acidic buffer. When the tip comes in close proximity to an area producing hydrogen, a feedback loop is produced between the tip and substrate electrode, resulting in "hot spots" of catalytic activity that can be observed as increases of current. From the image seen in Figure 3, it is obvious that there are large areas of increased current present on the surface of the protein film that correspond well with the size of platinum particles observed in the SEM images. "Tails" can be observed following each of these platinum particles due to scan direction of the tip.³² These "tails" are caused by the diffusion of electrochemical products away from the catalytic particles, enabling the feedback loop to continue beyond the physical dimensions of the particles themselves. We were also able to image the small platinum particles produced without a sacrificial electron donor using a similar method (Figure S3, Supporting Information); however, these particles did not have "tails", most likely due to the slower scan rate used to produce the image.

CONCLUSION

Platinum particles have successfully been photoreduced onto the surface of PSI that had been preimmobilized onto either a

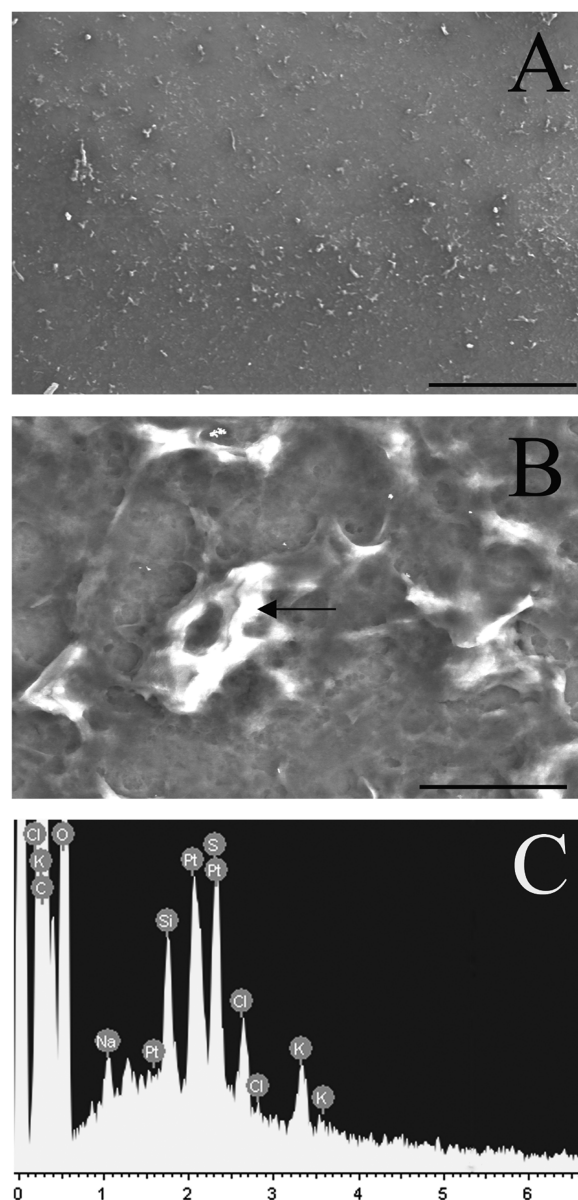


Figure 2. SEM and EDX analysis of an optimized sample. Images were collected with an accelerating voltage of 20 kV. (A) SEM image demonstrating both the abundance and various sizes of platinum particles that have been photoreduced for 2 h onto the surface of three multilayer films that were previously immobilized on a silicon substrate (scale bar 750 μ m). (B) SEM image depicting the geometry and size of platinum particles (scale bar 49.5 μ m). (C) EDX spectrum performed at the point indicated by the arrow in Figure 2B with an accelerating voltage of 20 kV and an emission current of 20 μ A.

modified gold substrate or an unmodified silicon substrate. There was found to be a balance between the amount of photoreduced platinum and the amount of hydrogen production possible. To the best of our knowledge, this is the first report of imaging the catalytic activity of photoreduced platinum particles on the surface of a photoactive protein film using SECM. The size of these catalytic particles is much larger than that of previously reported particles photoreduced in PSI solution.^{8,11} Our results provide an increased understanding of these biohybrid catalysts that may be later employed for enhanced utility of these systems in current and future applications. Because the multilayer protein film can be

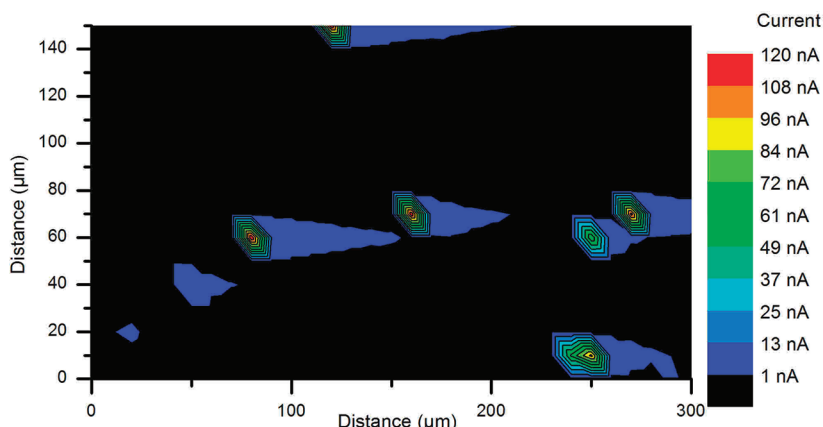


Figure 3. SECM analysis of individual platinum particles. Tip image produced using a 25 μm platinum UME as the tip electrode, a gold electrode modified with three multilayer films of PSI and photoreduced platinum particles (platinum salt photoreduced for 2 h) as the substrate electrode, and a pH 4 mediator solution containing 100 mM KCl as a supporting electrolyte. Green to red areas indicate “hot spots” generated by the electrochemical feedback loop between the tip electrode and the catalytic platinum particles present on the surface of the protein film. The blue “tails” are caused by the diffusion of electrochemical products away from the catalytic particles and follow the scan direction of the tip electrode.

immobilized on any substrate of choice, this method can be easily modified for incorporation into functional devices.

■ ASSOCIATED CONTENT

Supporting Information

Additional data and discussion concerning the photoreduction of platinum particles without the aid of a sacrificial electron donor. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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