Integration of Photosystem I with Graphene Oxide for Photocurrent Enhancement

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Photosystem I is a photoactive membrane protein used in nature to photo-excite electrons with nearly unit internal quantum efficiency, sparking interest in using this biomaterial for solar energy conversion. Films of PSI deposited on p-doped silicon have previously demonstrated significant photocurrents with an electrochemical mediator; however, improvement in electron transfer is needed. Here, it is investigated how PSI can be combined with graphene oxide (GO) or reduced graphene oxide (RGO) to generate composite films capable of improved photoelectrochemical performance. It is found that both composite films outperformed the PSI film alone, and the PSI-GO composite film is found to perform the best. The enhancement is attributed to the decreased impedance and shift in the onset potential of the composite films.

1. Introduction

Photosystem I (PSI) is an ~500 kDa protein complex found in the thylakoid membrane of higher order plants and is responsible for the excitation of an electron from the chlorophyll dimer, known as P700, to a terminal iron sulfur cluster known as F8. The energy for this excitation comes from photons of light collected by antennae chlorophylls, with internal quantum efficiency near 100%. This extraordinary efficiency, coupled with the abundance and nano-dimensional size of PSI, has resulted in the investigation of PSI in photovoltaic,[2] electrochemical,[3] and electronic systems.[4] Several developments have enabled dramatic improvements in the photo-generated currents that can be produced by PSI-electrode based devices.[5] These improvements include novel PSI immobilization methods on gold electrodes,[6-8] embedding PSI within polymer matrices,[9] the use of thick protein films,[9] and the use of semiconductor electrode materials.[10,11]

The integration of PSI with carbon nano-materials, however, has been limited. The work of Carmeli and co-workers demonstrated how PSI could be covalently attached to carbon nanotubes.[12] Additionally, our research group demonstrated how PSI could be interfaced as a monolayer on a graphene electrode.[13] The use of carbon nanostructures such as graphene and carbon nanotubes to develop nano-composite materials has become an area of great research interest due to the unique properties of these materials.[14]

The time-consuming and challenging preparation of graphene, however, has resulted in the increased interest in graphene oxide (GO).[15] GO, the oxidized form of graphene, is easily generated through the oxidation and subsequent exfoliation of graphite. The oxygen functional groups enable GO to be dispersed in polar solvents (i.e., water) and further functionalized. However, because the conjugation of the system is disrupted, the conductivity of GO is five orders of magnitude lower than graphite.[16] To regain conductivity, GO is commonly reduced to generate reduced graphene oxide (RGO).[17] Both GO and RGO have been incorporated with various materials to develop new functional composites.[18,19]

The addition of GO and RGO is particularly attractive for PSI-based devices for a number of reasons. GO and RGO are both water-soluble, making the integration of these materials with PSI straightforward. Furthermore, the low cost and facile synthesis of GO and RGO make these materials ideal candidates for making inexpensive composite materials. The functional groups present on GO make possible the interaction with both the polar groups of PSI as well as the electrochemical mediator. However, the improved conductivity of RGO can facilitate electron transfer between the PSI complexes and the underlying electrode. Here we describe how incorporating either GO or RGO with PSI can improve the photoelectrochemical properties of p-doped silicon.

2. Experimental Section

**Extraction and Isolation of Photosystem I:** Photosystem I complexes were isolated from commercially available baby spinach leaves as described previously.[12] Briefly, the baby spinach was
deveined and macerated followed by centrifugation at 8,000 g to isolate the thylakoid membranes. The resulting pellet was then resuspended using a buffer with a high surfactant concentration to break open the thylakoid membrane. The PSI complexes were purified by centrifugation at 20,000 g followed by column chromatography using a chilled hydroxyapatite column. Excess surfactants and salts were removed using dialysis at 1:2000 extract to water ratio. The concentration of the resulting PSI solution was $7 \times 10^{-6}$ M with a chlorophyll concentration of 0.3 mg mL$^{-1}$ as characterized by the methods of Porra and Baba et al.

**Preparation of Reduced Graphene Oxide:** Highly concentrated graphene oxide (graphene-supermarket.com) with an average flake size of 0.6–5 μm was diluted to a concentration of 0.1 mg mL$^{-1}$. The GO was then reduced using ascorbic acid (Sigma) at 95 °C until the UV-vis absorption peak shifted from 230 nm to 260 nm. Additionally, Raman spectroscopy (Thermo Scientific DXR) demonstrated a significant increase in the D/G intensity ratio after the GO was reduced.

**Preparation of Silicon Substrates:** Boron doped silicon wafers <100> with a resistivity of 1–10 Ω cm were purchased from University Wafer. The silicon substrates were etched using a 2% hydrogen fluoride solution to remove the native oxide layer. (Caution: hydrogen fluoride is extremely corrosive and dangerous. Proper protective equipment and procedures should always be observed.) The etched silicon substrates were rinsed with deionized water and dried with N$_2$. Electrochemical masks (Gantry) with an exposed area of 0.283 cm$^2$ and a thickness of 0.17 mm were used to define the deposition area on the silicon substrate.

**Modification of Silicon Substrates:** Solutions of PSI, GO, RGO, or combinations thereof were prepared by mixing these solutions at various ratios as described in the figure captions. Films of these solutions were then deposited onto the silicon substrates following the procedures previously developed in our lab. Briefly, 50 μL of solution was deposited on the exposed area of the substrate and vacuum was applied until the film was dried.

**Electrochemical Measurements:** A CH Instruments CHI 660A electrochemical workstation equipped with a Faraday cage was used to perform photocathodoamperometric and linear voltammetry measurements. Electrochemical Impedance Spectroscopy (EIS) was performed using a Gamry Instruments CMS300 Impedance system. A custom-built, three-electrode cell was used to analyze the bihybrid electrodes. The silicon substrate was used as the working electrode, Ag/AgCl used as the reference electrode, and a platinum mesh used as the counter electrode. The electrochemical mediator consisted of 100 mM KCl (Sigma) and 2 mM methyl viologen (Sigma).

EIS was performed using a 10 mV AC voltage over a frequency range of $10^{-4}$ to $10^5$ Hz, with 10 points per decade recorded. Photocathodoamperometric experiments were performed at the experimentally determined open circuit potential for each system. The photocurrent values reported in Figure 3 and 5 were determined by taking the difference between the current after 10 seconds of illumination and the current prior to illumination. The full data sets can be found in the supporting information. Linear voltammetry in Figure 4 was performed from 1 V to −1 V vs Ag/AgCl with a scan rate of 0.01 V s$^{-1}$.

Illumination was generated using a 250 W cold light source (Leica KL 2500 ICD) equipped with a 633 nm high pass filter, generating a light intensity of 0.19 W cm$^{-2}$. Comparison of this illumination with 1 sun illumination was performed using a Scientech SF150B solar simulator with AM1.5G filter (class A spectral match, class B non-uniformity). The lamp was calibrated to 1.00 full sun with an NREL certified silicon reference diode. Results from this experiment demonstrated roughly a 15% decrease in photocurrent density when compared to the illumination used in the manuscript.

**3. Results and Discussion**

Previously, our research group achieved dramatic improvements in the measured photocurrent of PSI based systems by using a p-doped silicon substrate in conjunction with a methyl viologen mediator. We hypothesized that interfacing this photosynthetic protein with a water-soluble, carbon-based material would improve the organization of the film and improve the conductivity of the PSI film. We began by preparing aqueous solutions of the various materials and depositing them on silicon substrates. A scanning electron micrograph of the PSI-GO composite cross-section demonstrates relatively ordered layering (Figure 1). This ordering has previously been observed with amylloid-GO composites and was attributed to the hydrophilic interactions between the protein and GO. The luminal and stromal ends of the PSI protein, which contain large amounts of hydrophilic lysine residues, generate a strong interaction with the oxygen functionalities present in GO and RGO. In order to determine the effect of the GO and RGO on the impedance of the PSI film, we performed electrochemical impedance spectroscopy (EIS) in the presence of methyl viologen, an electrochemical mediator (Figure 2). In the dark (Figure 2A), PSI behaves as an insulating film and provides resistance against the transport of aqueous mediator to the silicon surface. The resulting impedance spectrum contains two time constants: one for the PSI film and one for the silicon interface (space-charge layer, oxide, etc.). In the light (Figure 2B), the PSI/p-Si electrode behaves as a single interface where electrons can be exchanged with the mediator either at the silicon surface or
at the active sites of the PSI complexes within the film. Thus, the time constant exhibited by PSI in the dark disappears from the spectrum, revealing a single time constant. Because there are more sites for electron exchange with the mediator in the PSI system, the impedance at low frequencies in the light is reduced below that of the uncoated p-Si electrode, which is consistent with higher photocurrents observed for PSI modified electrodes versus p-Si controls. The addition of conductive elements (RGO) or charged structuring elements (GO) does not significantly affect the impedance response of the PSI film in the dark, suggesting that these components do not hamper the mediator transfer into the film. However, in the light, two interesting effects are observed. First, the GO-PSI and RGO-PSI films exhibit only a single time constant, suggesting that the entire composite acts as a single interface as observed for the PSI film alone. Second, we observe a significant decrease in resistance with the addition of GO or RGO. At high frequencies the resistance decreases as PSI > PSI-PSI-RGO, which is consistent with the expected conductivities of these films. This resistance is the uncompensated resistance, which is a function of both the solution conductivity and the electrode conductivity. The impedance at low frequencies decreases as PSI > PSI-PSI-RGO. This impedance element reflects the actual electron exchange with the mediator, showing that the addition of either GO or RGO improves the interfacial electron transfer.

To test the photocurrent production of these composite electrodes, we performed photochronoamperometric and linear sweep voltammetry measurements on films of PSI, PSI-GO, and PSI-RGO containing the same quantity of PSI. As expected from the impedance measurements, the addition of either GO or RGO to the PSI results in a significant photocurrent improvement at the open circuit potential due to the improved interfacial electron transfer (Figure 3). Interestingly, the PSI-GO composite has a significantly higher photocurrent density than the PSI-RGO composite. To understand this unexpected result, we performed linear sweep voltammetry (Figure 4). Two interesting observations can be made from this data. First, the PSI-GO composite shifts the photoreduction potential of the mediator system more positive. Thus, more current is expected at the open circuit potential (−0.347 ± 0.007 V vs Ag/AgCl). The PSI-GO composite film and the PSI-RGO composite film demonstrated a positive 75 mV and a 45 mV shift, respectively, in peak current compared with the PSI film alone. Second, the increased peak current density observed for the PSI-GO composite suggests that the film is able to increase the local concentration of the electrochemical mediator. We attribute this effect to the charged nature of the GO when compared to RGO. This

![Figure 3. Bar graph with the average photocurrent density (n=4) for each sample after 10 s of illumination with red filtered light (633 nm high pass filter).](https://www.advenergymat.de)

**Figure 3.** Bar graph with the average photocurrent density (n=4) for each sample after 10 s of illumination with red filtered light (633 nm high pass filter). Photochronoamperometric analysis of PSI and PSI-composites was performed on p-doped silicon substrates in 2 mM methyl viologen mediator solution. The concentration (mg mL⁻¹) ratio of chlorophyll to RGO or GO was 3.
observation correlates well with our impedance measurements for this film.

In order to determine the ideal mixture of PSI with GO or RGO, we varied the ratio of chlorophyll to GO or RGO and measured the resulting photocurrent (Figure 5). As expected, the PSI-GO composites outperformed the PSI-RGO composites at all ratios. This provides further evidence towards the hydrophilic interactions between PSI and the oxygen functional groups present in GO. Additionally, relatively little PSI is required to generate significant improvements to the photocurrent. The highest performing ratio, 1.5 mg chlorophyll to 1.0 mg GO, roughly corresponds to 1 PSI protein complex per 76 nm² of GO. Since a single PSI protein complex has an area footprint of roughly 80 nm², this result suggests that each PSI complex would interact with the graphene oxide surface in a homogeneous film. The absence of PSI, however, results in a dramatic reduction in photocurrent. The low cost and global availability of these materials makes the scale-up of this technology possible.

4. Conclusions

We have successfully generated composites composed of PSI and graphene-based materials. Depositing films of these composites on p-doped silicon provides a significant improvement in the photocurrent generated at the open circuit potential. The improvement was attributed to the decreased impedance and shift in the onset potential of the composite films. Additionally, the composites containing GO outperformed those with RGO because of the increase in charged functional groups which generate a higher local mediator concentration. Analysis of the chlorophyll to GO or RGO ratio demonstrated that lower ratio values performed best. These results demonstrate the advantage of integrating inexpensive carbon-based materials with PSI. More broadly, this study demonstrates how the use of graphene oxide can be used to enhance interaction between an enzyme and mediator. This benefit can be utilized in other systems for either increased sensitivity to a mediator or the ability to use less mediator in the case of a high cost or hazardous mediator. An investigation into other additives that may improve electron transfer both within the PSI film and between the non-biological electrodes is currently underway.

Supporting Information

Supporting Information concerning the photocurrent measurements and calculations performed in order to determine the area of graphene oxide per PSI complex are available from the Wiley Online Library or from the author.

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