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Communication

Flavin-Sensitized Electrode System for Oxygen Evolution Using Photo-Electrocatalysis

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Fabrication of bio-electrode systems decorated with redox active biomolecules, flavins, is demonstrated. Exploiting the photochemistry and electrochemistry of flavins, we explored the photo-electrochemical activity of flavin-functionalized electrode systems to assess their potential utility for sustainable energy production. As model systems, lumiflavin and flavin adenine dinucleotide were immobilized on carbon electrodes by dropcasting and covalent grafting techniques. Activity of these bio-electrodes towards generation of O_2 from H_2O in 0.5 M potassium phosphate buffer at pH 7.1 was demonstrated. Irradiation of the electrode system with visible light led to increased activity of the electrodes with a 3-fold enhancement of oxidation of H_2O.

Given ongoing depletion of economic fossil fuel reserves in conjunction with accumulating atmospheric greenhouse gases, it is mandatory that humanity develop efficient, renewable, environmentally-responsible energy sources.[1, 2] Nature employs *Photosynthesis* to satisfy much of its energy demand, inspiring considerable interest in designing an *artificial photosynthetic* system.[3-6] Water oxidation is considered to be the bottleneck of artificial photosynthesis because the sluggish kinetics and challenging thermodynamics of O-O bond formation limit the rate at which electrons can be extracted by the process.[7] Thus a catalyst is needed to lower the over-potential for H_2O oxidation and enable it to operate close to its thermodynamic value. Building on the first report of water oxidation catalysts (WOCs) by Meyer *et al*.[8] an impressive array of inorganic catalysts have been developed in the last five years.[9-15] In contrast, less effort has been devoted to organic water oxidation catalysts. Reports of C-based WOC describe nitrogen-doped graphite nano-material composites[16] and graphitic carbon nitride nanosheet–carbon nanotube composites[17] with encouraging performances comparable to those of noble metal catalysts. Very recently, N(5)-ethylflavinium ion was shown to be an active catalyst for water oxidation under electrochemical conditions.[18] N(5)-ethylflavinium ion is the only "metal-free" organic electrocatalysts reported so far, but it required a high overpotential of 0.78V vs NHE at pH 2.[18]

Flavins are cofactors of numerous enzymes that mediate oxidation or reduction of their substrates. The 7,8-dimethyl-isoalloxazine ring system that is the redox-active component of both flavin mononucleotide (FMN) and flavin adenine

dinucleotide (FAD) provides the reference point for the N(5)-ethylflavinium ion. These cofactors were named for their intense yellow color, due to strong absorbance near 450 and 370 nm in the visible region of the electromagnetic spectrum[19, 20] and the corresponding electronic transitions underlie well-known photochemistry and photosensitizing properties.[21-26]

While the electrocatalytic properties of flavins as WOCs were explored recently[18], exploitation of flavins' photo-electrocatalytic properties for water oxidation has not been reported to date. Recently Armstrong's group demonstrated the use of the flavoenzyme flavocytochrome c3 as an electrocatalytic reductant, able to support an artificial photosynthetic system employing other components as the photoactive ingredients.[27] We report complementary investigations wherein simple flavins serve as the photo-electrocatalysts, in the context of the water oxidation reaction. The objectives of our research were (i) to immobilize the simple flavins lumiflavin (**LF**) and flavin adenine dinucleotide (**FAD**, see Supplementary Figure S1) on graphite electrodes to assess the ability of this inexpensive organic material to serve as a photo-anode towards water splitting and (ii) to study the effect of light on flavin's water-oxidizing activity.

Commercial pencil graphite electrodes (**GE**) were used as the substrate to afford a high conducting surface area. Functionalization with the bio molecules was accomplished by either non-covalent or covalent immobilization approaches as described below. Lumiflavin-functionalized **GE** (**LF-GE**) were fabricated by drop-casting micromolar solutions of **LF** in water onto the **GE** followed by drying. The extensive π-system of **LF** interacts favourably with the graphitic planes through non-covalent π-π interactions,[28] binding the molecule to the electrode surface.[29] For **FAD**, a covalent immobilization technique was employed to counteract this molecule's greater solubility in water (Supplementary Scheme S1). The catalytic electrodes **LF-GE** and **FAD-GE** modified with **LF** and **FAD**,

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respectively, were then carefully rinsed with water to remove all loosely-bound bio-molecules until the rinsates were free of flavin (confirmed by absorption spectra). Similar immobilization was also carried out on glassy carbon (**GC**) electrodes to permit thorough characterization of the immobilized flavins.

Figure 1a compares the cyclic voltammogram (CV) of a **LF**-functionalized **GC** electrode with that of a bare **GC** electrode. As expected, no redox signal appeared for the bare electrode, so the signal centered at $E°'=-0.48$ V of the **LF-GC** electrode can be attributed to the immobilized **LF** (all potentials are vs. Ag/AgCl (1M KCl) electrode). The obtained value of $E°'=-0.48$ V ($E°'=(E_{pc}+E_{pa})/2$, where E_{pc} is the cathodic and E_{pa} is the anodic peak potential, vs. Ag/AgCl) is very close to the value of -0.44 V reported for the two-electron reduction of flavins in solution.[30] To learn whether electron transfer is governed by diffusion-controlled processes or an adsorption process, voltammetric scans were performed at different scan rates ranging from 20mV/s to 100 mV/s. A log-log plot of anodic current *vs.* the scan rate revealed a linear relationship with a slope of 0.95, which is very close to the value of 1 expected for electron transfer by electrode-adsorbed molecules (Figure 1b) and distinct from the slope of 0.5 expected for diffusion-controlled processes.[31, 32] This result clearly demonstrates that the **LF** was successfully immobilized on the electrode and that the flavin molecules were not released from the electrode surface over the course of the experiment. The separation between the peak potentials, ΔE_p ($\Delta E_p = E_{pa} - E_{pc}$), was approximately 46 mV, independent of the scan rate (Figure S2). In addition, the ratio of the cathodic peak current divided by the anodic peak current was close to unity at all scan speeds. This behavior suggests efficient electron transfer between the adsorbed flavins and the electrode. ATR-FTIR (attenuated total reflectance Fourier transform infra-red spectroscopy) was carried out to further assess the immobilization of flavins on the graphite electrodes (please see Supplementary Figure S3).

We quantified the success of our **LF** functionalizations via measurement of the surface coverage of the **LF**-functionalized electrodes using the following equation.[33]

$$\Gamma = (4RT/n^2F^2A) \text{ x Slope}$$

where, Γ is surface catalyst loading (mol cm^{-1}), R is the ideal gas constant (8.314 JK^{-1} mol^{-1}), T is 298 K, n is the number of electrons transferred (2), F is Faraday's constant (96485 C mol^{-1}), A is the electrode surface area, and 'Slope' is the slope of a plot of current (A) vs. scan rate (V s^{-1}) (Figure 1b). Surface concentrations of 0.13-0.89 nmol cm^{-2} and 3.5-7.8 nmol cm^{-2} were determined for **LF** on **GC** and **GE** electrodes respectively, based on at least three independent electrodes each.

Inspired by the recent demonstration of water oxidation by flavinium ions on a **GC** electrode[18] we explored the possibility of electrocatalytic water oxidation by **LF**-functionalized graphite electrodes. A sweep to positive potentials above 1.4 V generated catalytic currents attributable to water oxidation[18]
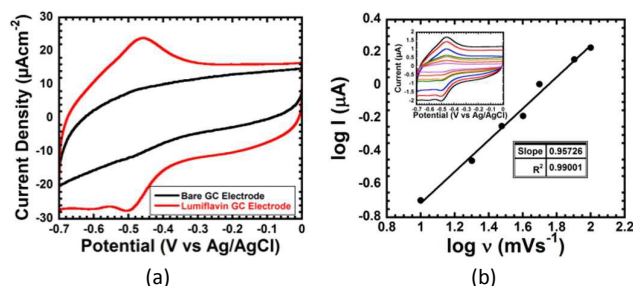

(a)   (b)

Figure 1. (a) CV of **LF** immobilized on a **GC** electrode (red line) compared with the CV of a bare **GC** electrode (black line). Conditions: scan rate, 100 mV/s; 0.5 M KPi (Potassium Phosphate) buffer, pH7.1. (b) Plot of log I_{pa} vs. log ν for CVs of a **LF** functionalized **GC** electrode (scans in the inset). Scans were conducted in 0.5M KPi pH 7.1 at room temperature.

(blue line, Figure 2a). During the anodic scan the immobilized **LF** undergoes oxidation at approximately 1.4 V to generate the reactive species, presumably the previously-proposed transient flavin radical cation,[34] capable of water oxidation. Upon attack by water/OH$^-$ this transient species may rapidly transform to a hydroxyflavin radical which upon further oxidation could be susceptible to attack by another water/OH$^-$. Similar O-O bond formation by water attack on metal-hydroxo or metal-oxo intermediates during water oxidation by inorganic catalysts is well documented.[9, 35] Alternatively, flavinium has been proposed to react with O sites on the electrode[18] No corresponding peak near 1.4 V is evident in the cathodic scan indicating that sequelae to the anodic reaction are irreversible. This is consistent with reduction of the flavin radical cations by water (see below) before the oxidized species can be re-reduced by the cathodic scan. The fact that cathodic peaks at -0.5 V persist even after several scans up to 2 V, as does the current at high positive potentials, indicate that flavin remains immobilized and acts as a catalyst rather than a reagent during the reaction. The aforementioned behaviors were absent from similar scans of bare graphite electrode under similar conditions (Black line, Figure 2a).

The stability of **LF-GEs** was investigated through multiple CV scans and post-operation FT-IR analyses. The **LF-GEs** displayed persistent activity towards water oxidation for more than 10 CV cycles (details and data in Supplementary Information) after which gradual loss of surface-bound **LF** was observed, which may be partially attributable to oxidation and exfoliation of graphitic material at high electrode potentials.[36]

We investigated the possibility that strongly oxidizing hydroxyflavin radical reacts with H_2O to form H_2O_2 which can disproportionate to produce half an equivalent of O_2. However, any H_2O_2 formed was below the detection limit of the horseradish peroxidase-ABTS assay (0.2 μM) even under conditions of bulk electrolysis at 1.8V with **LF-GE**. This is in accordance with mechanisms proposed for most inorganic catalysts and the recently reported N(5)-ethylflavinium ion where there was no accumulation of H_2O_2.[17, 18, 29]

Recognizing flavins' strong absorbance of visible light, we investigated the effect of visible light on the catalytic activity

of immobilized **LF**. The CV of **LF-GE** illuminated for 20 minutes displayed significantly higher current than that observed in scans collected in darkness (red vs. blue lines, respectively, Figure 2a). The light-dependent increase in the intensity of the peak at 1.4 V suggests photo-stimulated formation of the oxidizing species, likely flavin radical cation. As a control, the bare electrode was also irradiated with visible light. In this case however, the CV traces were indistinguishable from the scans in the absence of light (green line Figure 3a). Thus the effect of illumination can be attributed to the immobilized flavins.
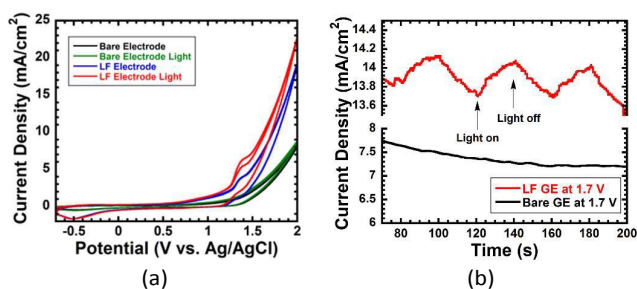

Figure 2. (a) Cyclic voltammograms of **LF-GE** showing the effect of illumination on **LF** electrochemistry. Conditions: scan rate, 20 mV/s; 0.5 M KPi buffer, pH7.1 at room temperature. (b) i-t amperometric response to light of **LF-GE** (red) and bare electrode (black) at 1.7 V (vs Ag/AgCl). '+Light' data were collected beginning 20 min. after the onset of illumination.

To assess the effect of illumination we monitored current vs. illumination time via *i*-t amperometry. Figure S5 displays the current generated by the **LF-GE** at different constant potentials of 0.4 V, 1.0 V, 1.5 V, 1.7 V. Our data show that at potentials below 1.0 V there was very little current, independent of illumination. However significantly higher currents were observed at potentials of 1.5 V and 1.7 V when the reactive species can be formed, and they were responsive to illumination. Figure 2b shows a comparison of current from a bare **GE** (black line) vs. current from **LF-GE** (red line) both at a potential sufficient for water oxidation to take place. The traces show that when the **LF-GE** is illuminated with visible light the current rises and when the light is switched off the current drops, whereas the current from the bare **GE** is indifferent to light (also Figure S7). Thus we confirm that the effect of light is mediated by the flavin. The slow growth of the illumination effect can be understood in terms of accumulation of a species that contributes to water oxidation (Figure S8).

To interpret the currents in terms of O_2 production, we used a Clark-type O_2-sensing electrode to quantify O_2 produced in the course of controlled-potential bulk electrolysis at pH 7.1 in 0.5M KPi buffer at 1.8 V (Figure S8). Our results confirm production of O_2 only at applied voltages above 1.4V. Moreover significantly more O_2 was generated in the presence of light than in the dark with the same **LF-GE** electrode and under the same experimental conditions, indicating that the larger currents observed in light can be interpreted in terms of photo-stimulated water oxidation.

To further test oxidized **LF**'s capacity to act as an electrochemical catalyst in solution-phase O_2 evolution, controlled-potential bulk electrolysis was carried out with stepwise addition of **LF** with the working electrode held at 1.8 V. The amount of **LF** was varied from 3.18 µM to 12.38 µM and O_2 evolution was monitored using a Clark-type O_2-sensing electrode (data not shown). Production of O_2 increased with increasing **LF** concentration consistent with an electrocatalytic role for **LF**. Moreover significantly more O_2 was produced in light than in darkness, during solution-phase electrocatalysis as for the on-electrode systems.

Given the encouraging catalytic properties of **LF-GE**, we also generated **FAD**-functionalized **GE**, to assess the generality of the phenomena observed and their dependence on the method of immobilization. Graphite electrode surfaces were first modified with carboxyphenyldiazonium salt to install phenyl carboxylate functionalities. The well-established EDC-NHS coupling methodology was then employed to form amide linkages between the adenine of **FAD** and the newly-installed carboxyl functionalities (scheme S1). The **FAD**-**GE** were then characterized by CV and FT-IR (Figure S4). As in the case of **LF-GE**, a log-log plot of anodic current *vs*. the scan rate yielded linear behavior with a slope of 0.98 confirming successful immobilization of **FAD** (Figure S9). A surface concentration of 0.96-1.2 nmol cm^{-2} was determined for our **FAD**-**GE** (based on three independent electrodes). Similar to the **LF-GE**s, **FAD-GE** generated modest but reproducible catalytic currents due to water oxidation and evolved O_2 at potentials above 1.4 V. The former is shown in Figure 3. During the anodic scan the immobilized **FAD** undergoes oxidation at around 1.4V and generates reactive species able to execute water oxidation.

Upon irradiation with visible light the current increased with changes in the CV trace suggesting increased formation of the active species mediating water oxidation. However the **FAD**-**GE** suffered from unsatisfactory durability as they lost activity after a few cycles (See Supplementary Information). This could reflect modification or loss of the **FAD** from the electrodes in the course of repeated scans. **FAD** immobilization by drop casting was also of limited success due to the high solubility of **FAD** in aqueous solution. In contrast, the lower solubility of **LF** in water is likely an important factor in the high and long-lived attachment of **LF** to our electrodes.

In conclusion, we have successfully immobilized biologically relevant flavins on carbon surfaces including glassy carbon and graphite, and demonstrated their ability to catalyze water oxidation to O_2 that is stimulated by light. To the best of our knowledge, this is the first demonstration of light-stimulated water oxidation catalyzed by flavins or any other organic molecular catalyst. Both consumption and production of O_2 must include a mechanism for reconciling the S=1 spin of O_2, thus most of the biological cofactors able to reduce O_2 include at least one transition metal ion. Flavins are organic cofactors able to catalyze reduction of O_2, so our finding that flavin-functionalized electrodes can oxidize water to O_2 has
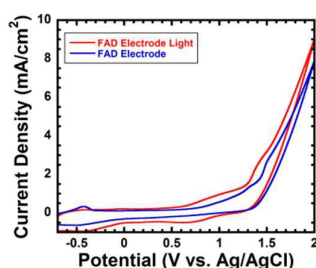
Figure 3. Cyclic voltammograms of **FAD**-functionalized **GE** showing catalytic water oxidation. Conditions: scan rate, 30 mV/s; 0.5 M KPi buffer, pH7.1, at room temperature.

mechanistic precedent in biological chemistry. However given the intimate interaction between **LF**'s π system and that of the electrode graphite[29] we speculate that other conjugated multi-ring redox dyes may also prove able to catalyze light-stimulated water oxidation when immobilized on electrodes. Although practical deployment of **LF** as a photoelectro-catalyst is limited by the high cost of this particular compound, it provides an exciting proof of concept and a new chemical lead to accelerate the search for organic WOCs for sustainable energy generation, including photogeneration of energy.

Acknowledgements

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