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Photoinduced production of NAD(P)H from an activated fluorescein–DNA monolayer†

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A fluorescein (Fl)-labeled DNA monolayer on Au was formed such that under applied potentials of −750 mV (*vs.* Ag/AgCl) and incident irradiation (473 nm, 4 mW cm^{-2}) a photocurrent was generated that ultimately reduced NAD(P)$^+$ to biologically active NAD(P)H.

In biological photosynthesis, an electron is ejected from chlorophyll by absorption of a photon. In photosystem I (PSI), two electrons are used to reduce NADP$^+$ to NADPH. There have been many attempts to replicate this process by attaching chromophores to electrodes but few approach the efficiency of the biological reactions.[1] Previously, several groups have addressed the issue of converting photon energy into electronic signals and include fullerene,[2] porphyrin,[3] ferrocene,[4] Ru(bipy)$_3$,[5] and pyrene.[6] Recently, a photosynthesis mimic was described involving photoinduced electron transfer from chlorophyll *a* to vitamin K$_1$.[7] The efficiencies of such constructs range from 1000 photons electron^{-1} in a porphyrin SAM,[8] to 100 photons electron^{-1} in a multilayered pyrene construct on a gold surface,[6] and 13 photons electron^{-1} to 2 photons electron^{-1} in a C$_{60}$ SAM system.[9] Such constructs require two inputs, photonic and electronic, for the reaction to proceed. Serendipitously, in related experiments analyzing electron transfer in DNA, we discovered a method for simultaneous production of NAD(P)H (*i.e.* either NADH or NADPH).

Here, we describe the construction and properties of self-assembled monolayers (SAMs) of the chromophore fluorescein (Fl), attached through a 20 base-pair duplex DNA linker to a gold microelectrode (Fig. 1a). These monolayers have been extensively characterized previously.[10] Rather than eject an electron from a ground state chromophore, we have elected to transfer the electron first, to form the stable radical anion of the chromophore and then excite the chromophore with radiation (473 nm). Fl was chosen as the chromophore because it forms a stable radical anion at a modest reduction potential (−750 mV *vs.* Ag/AgCl) with a large absorption coefficient (ε_{473} = 43 000 M^{-1} cm^{-1}). The DNA spacer prevents the excited-state Fl from being quenched by close proximity to the electrode surface[11] but at the same time, the semi-conductive properties of DNA allow electron transfer from the electrode to the chromophore.[12] At the same applied potential, Fl is reduced to the Fl$^{\bullet -}$ as shown by EPR spectroscopy.[13] In order to allow a continuous current to flow, an electron acceptor is required; NAD(P)$^+$ was chosen because it possesses a high reduction over-potential and the expected product, NAD(P)H, is the biological reductant from PSI. Control experiments involving the use of an Fl-linked C$_{11}$-alkyl spacer group in place of the DNA yielded no photocurrent. Therefore, the semi-conductive properties of DNA are required for the multiple reduction cycles of the Fl radical anion (ESI†).

As shown in Fig. 1b, irradiation of the microelectrode at 473 nm with a 4 mW cm^{-2} laser produces a sustained current and there is little reduction in the magnitude with multiple irradiations. No current is observed with red laser light (632 nm, 10 mW cm^{-2}) at which wavelength Fl$^-$ does not absorb (Fig. 1c). Note that no photocurrent was produced when a monolayer of non-Fl-labeled DNA was used and that both NAD$^+$ and NADP$^+$ produce equal quantum yields of photocurrent. A linear relationship was found between the intensity of the photon flux and the current output in the presence of NAD(P)$^+$. As well, the current reaches a plateau at a reductive potential of −750 mV, which is evidence that the Fl must first be reduced to its radical anion before irradiation and subsequent electron transfer (see ESI).

Under Fl-excitation conditions, a photocurrent density of 450 nA cm^{-2} was obtained for a Fl-DNA labeled microelectrode at the above applied potential. Assuming that the molar absorption coefficient of Fl-DNA on the electrode surface is the same as that in solution, the efficiency was calculated to be 4 ± 1 photons electron^{-1} (equivalent to a quantum yield of about 25%).[14] Little decrease in photocurrent was observed by repeated laser exposure (ESI). Furthermore, a continuous current of 450 nA cm^{-2} indicates that one Fl must undergo thousands of cycles of reduction without photodegradation.

In order to confirm the production of NAD(P)H, the experiment was repeated on a larger scale with a gold mesh electrode so that the solution could be monitored by UV–vis spectroscopy. As shown in Fig. 2a, a UV–vis peak at 340 nm, which is characteristic of NAD(P)H (ε_{340} = 6220 M^{-1} cm^{-1})[15] appears upon irradiation.

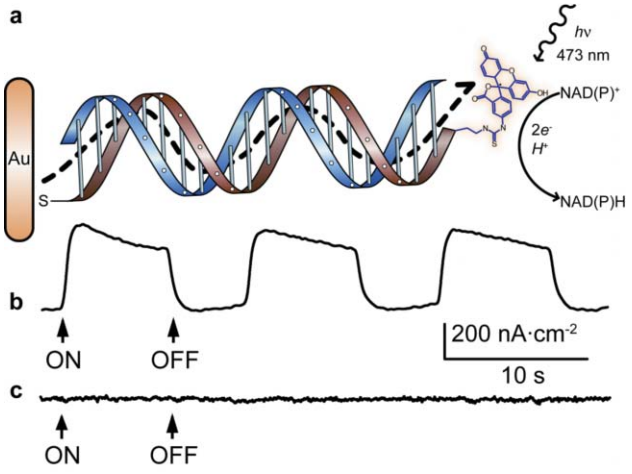

Fig. 1 a) A DNA monolayer that contains an electrochemically reduced Fl radical anion that is irradiated by *hν* at 473 nm, which further photo reduces NAD(P)$^+$ to NAD(P)H. b) The photocurrent response (473 nm, 4 mW cm^{-2}) in the presence of NAD(P)$^+$ at an applied bias of −750 mV (*vs.* Ag/AgCl). c) The photocurrent response (632 nm, 10 mW cm^{-2}) in the presence of NAD(P)$^+$ at an applied bias of −750 mV (*vs.* Ag/AgCl).

† Electronic Supplementary Information (ESI) available: Materials and methods, Fl-alkylthiol monolayer photocurrent, Fl-DNA EPR, incident intensity effect on photocurrent, bias effect on photocurrent, multiple irradiations of Fl-DNA monolayer, UV–vis and fluorescent electrospectra and quantum yield calculation parameters. See http://www.rsc.org/suppdata/cc/b4/b406689h/

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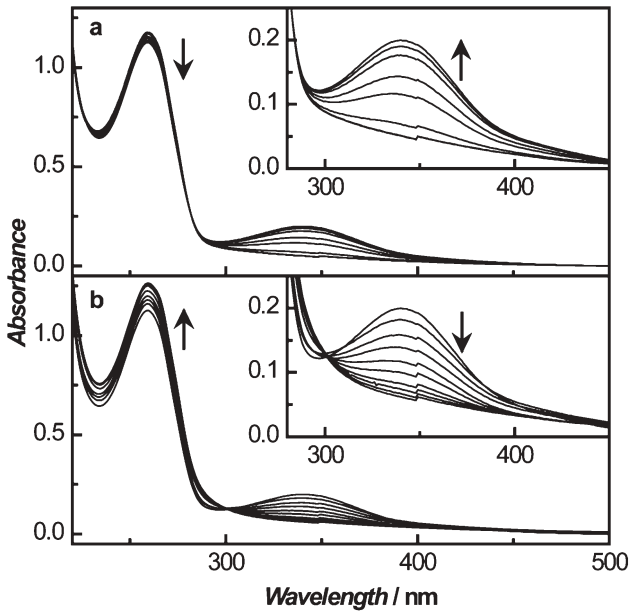

Fig. 2 a) The generation of NAD(P)H monitored by UV–vis (peak at 340 nm) using a Fl-labeled DNA monolayer on a Au mesh electrode at a applied potential of −750 mV (*vs.* Ag/AgCl) with incident 473 nm laser (4 mW cm^{-2}). There was a 5 min time interval between spectra. b) The enzymatic consumption (lactate dehydrogenase, acetaldehyde) of NAD(P)H monitored by UV–vis (peak 340 nm). There was a 1 min time interval between spectra.

However, nicotinamide coenzymes have been shown to form biologically inactive dimers from radicals produced by single electron reductions.[16] In order to show that the NAD(P)H was biologically active, alcohol dehydrogenase and acetaldehyde were added to the solution. As shown in Fig. 2b, the peak at 340 nm is gradually reduced demonstrating that the photoelectrochemically produced NAD(P)H can be used enzymatically to drive the conversion of an aldehyde to ethanol. The formation of non-biologically active $NAD(P)^+$ reduction products, which also have a peak at 340 nm,[17] was estimated to be less than 1%.

The mechanism of NAD(P)H synthesis is unclear but the absence of dimers tends to preclude step-wise electron transfer and suggests that a hydride equivalent might be transferred from the monolayer directly to the $NAD(P)^+$. Whether these two electrons and one proton originate from the excited-state $Fl^{\bullet -}$ is unclear. However, it is unlikely that the full reducing requirement comes from an individual excited state Fl radical anion. But two excited-state $Fl^{\bullet -}$ could disproportionate into a ground-state neutral species and a dianionic Fl,[18] which could drive the direct formation of NAD(P)H from one Fl. The proximity of neighbouring Fls on a tightly packed monolayer could predispose them to disproportionate. Alternatively, it is possible that the transfer could operate in a stepwise fashion with the $NAD(P)^+$ held in a confined environment by the DNA-monolayer thus, minimizing dimer formation. A cage complex might be formed between the base-pair stack of the DNA and the $Fl^{\bullet -}$* that contains a water molecule, which participates in the delivery of the two electrons and one proton. Experiments with other chromophores and simple pyridinium analogues of NAD^+ are currently under way.

In conclusion, we have constructed a simple self-assembling system which, upon irradiation, is able to transfer electrons from the excited-state chromophore and reduce $NAD(P)^+$ with a quantum yield of 0.25. Such a simple photoelectrochemical construct that leads to the formation of enzymatically-active NAD(P)H has not been demonstrated previously. In the long term, it may be possible to improve the efficiency and reduce the negative potential on the electrode by using alternate chromophores. Overall, these reactions loosely mimic the function of PSI with the gold electrode behaving as a source of electrons, like PSII.

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