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Photoelectrochemical study of Zn cytochrome-c immobilised on a nanoporous metal oxide electrode

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Transient optical spectroscopies and photocurrent action spectra are used to demonstrate photoinduced charge separation between zinc-substituted cytochrome c and a nanocrystalline TiO_2 electrode.

There is wide interest in the functionalisation of porous, nanocrystalline metal oxide electrodes by the adsorption of molecular species to their surface. The high surface area of such films makes them attractive for applications ranging from dye sensitised solar cells to electrochromic mirrors.[1] We have recently shown that the functionalisation of such nanocrystalline films may furthermore be extended to the adsorption of biological macromolecules such as proteins and have demonstrated that the high protein loading achieved by this approach is particularly attractive for a range of bioanalytical applications.[2–4]

Electron transfer reactions at protein/electrode interfaces are of fundamental importance both for bioelectrochemical studies of protein function and also for technological applications such as biosensors and bioelectrocatalytic systems.[5,6] Such interfacial electron transfer reactions are typically studied by electrochemical techniques. We demonstrate here that the optical transparency and high protein loading achieved with nanoporous TiO_2 results in such films being particularly amenable to photoelectrochemical studies of interfacial electron transfer processes, and moreover opens up the possibility of the development of optically driven bioelectrochemical devices.

Transient optical techniques have been widely employed to resolve ultrafast electron injection from photogenerated dye excited states into the conduction band of the metal oxide.[7] However, with the exception of a report by McLendon and coworkers,[8] such techniques have not previously been applied to studies of protein/electrode electron transfer events.

The strategy employed in this study is illustrated in Fig. 1. The protein employed is zinc-substituted cytochrome-c (Zn Cyt-c). Zinc-substituted Cyt-c is employed due to its long singlet excited state lifetime (~ 3.5 ns) relative to native Fe Cyt-c. Interfacial electron transfer is initiated by optical excitation of the Zn Cyt-c, resulting in electron injection into the TiO_2 conduction band.

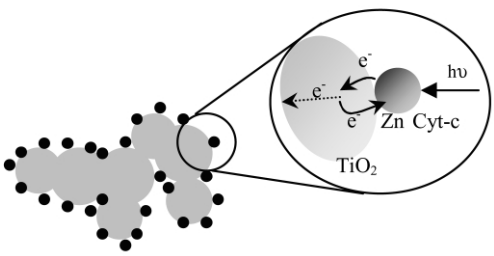

Fig. 1 Illustration of functionalisation of a nanoporous TiO_2 film with Zn Cyt-c. Optical excitation of the Zn Cyt-c results in electron injection into the semiconducting electrode.

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Anatase, nanocrystalline TiO_2 films were fabricated as reported previously[2] (nanoparticle diameter ~ 15 nm, film thickness 8 μm, pore volume fraction ~ 40%). Structurally analogous ZrO_2 films were fabricated by a sol–gel route as detailed previously,[9] these films were employed for control experiments as the higher conduction band edge of this metal oxide precludes electron injection from the Zn Cyt-c excited state. Zn Cyt-c was prepared[10] and immobilised[2–4] on the TiO_2 films according to the published procedures. The adsorption of Zn Cyt-c on the nanoporous TiO_2 films was confirmed by UV–visible absorption spectroscopy. Employing an extinction coefficient of 243 000 M^{-1} cm^{-1} at 423 nm for Zn Cyt-c,[11] we estimated a protein loading of 3.7 nmol cm^{-2}. The high protein loading, corresponding to a loading 160 times greater than monolayer coverage of a flat electrode surface (assuming a cross-sectional area for Zn Cyt-c of 7 nm^2), results from protein adsorption within the nanoporous film.

Fig. 2 shows a photocurrent action spectrum for a Zn Cyt-c/TiO_2 film. Data are plotted as the quantum efficiency for the conversion of incident photons to electrons flowing through the external circuit (IPCE). The Zn Cyt-c/TiO_2 IPCE spectrum exhibits maxima at 410 and 550 nm, in agreement with the UV–visible absorption maxima of the adsorbed Zn Cyt-c and consistent with this photocurrent deriving from photon absorption by the Zn Cyt-c followed by electron injection into the TiO_2 electrode.[11]

Time resolved emission spectroscopy was employed to investigate the quenching of the Zn Cyt-c singlet excited state due to electron injection into the TiO_2 electrode. Typical data are shown in Fig. 3. The emission decay for the control Zn Cyt-c/ZrO_2 film fitted well to a monoexponential decay with lifetime of 3.5 ns, in good agreement with the 3.2 ns lifetime of the Zn Cyt-c singlet excited state reported in previous solution phase studies of this protein.[8] Absorption of the Zn Cyt-c to the TiO_2 electrode results in clear quenching of the emission, with a monoexponential fit to the data yielding a lifetime of 0.9 ns (a biexponential analysis yielded a significantly better fit with

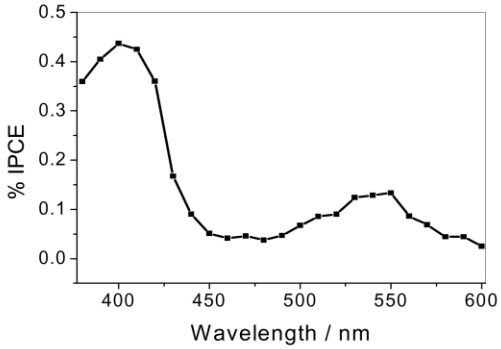

Fig. 2 Photocurrent action spectrum for a Zn Cyt-c/TiO_2 film incorporated as the working electrode of a three-electrode photoelectrochemical cell held at 0 V *vs.* a Ag/AgCl reference electrode, with an aqueous 2.2 mM $K_4Fe(CN)_6$ electrolyte, pH 7 and Pt mesh counter electrode. Negligible photocurrent was observed for TiO_2 electrodes in the absence of Zn Cyt-c.

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lifetimes (relative amplitudes) of 2.2 ns (13%) and 0.4 ns (87%)). The quenching of the Zn Cyt-c emission on the TiO_2 film is attributed to electron injection into TiO_2 film, consistent with photocurrent spectrum shown in Fig. 2. From the extent of emission quenching we estimate an approximate rate of electron injection of 0.8 s^{-1}, with a quantum yield of 72%. This rate of electron injection is similar to that reported previously for photoinduced electron injection from Zn Cyt-c into a SnO_2 electrode.[8]

The rate of electron injection reported here for the Zn Cyt-c/TiO_2 is 3–4 orders of magnitude slower than that reported for nanocrystalline TiO_2 sensitised by molecular dyes with similar excited state characteristics (*e.g.*: zinc tetra-carboxyphenyl porphyrin, $k_{inj} \sim 10^{12}$ s^{-1}).[11] This slower rate of electron injection is consistent with the expected greater physical separation of the heme group of the Cyt-c from the TiO_2 surface. Consideration of the surface charge of the TiO_2 and Cyt-c at the pH employed suggests that the Cyt-c will be immobilised with a patch of positive lysine residues adjacent to the heme pocket facing the (negative) TiO_2 surface. At this orientation, the distance between the edge of the heme group and the TiO_2 surface is estimated to be 3.3–3.5 Å. Employing the well established distance dependence of electron transfer ($k_{et} \propto \exp(-1.4r)$), this distance consistent with the observed retardation of the injection kinetics relative to those observed for molecular dyes directly adsorbed to the film surface. The biexponential decay observed for the Zn Cyt-c/TiO_2 is assigned to heterogeneous electron injection kinetics. Such non-exponential injection kinetics have been widely reported for electron injection from molecular dyes; we have recently suggested that such kinetics may derive from local inhomogeneties in the density of available acceptor states in the TiO_2 electrode.[11]

Transient absorption spectroscopy was employed to monitor the formation and decay of long-lived Zn Cyt-c cation species generated by electron injection into the TiO_2 electrode. Details of the experimental apparatus have been given previously.[2] Following optical excitation of the Zn Cyt-c/TiO_2 film at 549 nm, a broad absorption increase was observed with a maximum at ~650 nm, consistent with photoinduced absorption of the Zn Cyt-c cation / TiO_2 (e−) charge separated state. This assignment is supported by control experiments for Zn Cyt-c in solution and for a TiO_2 film in the absence of Zn Cyt-c, which both exhibited negligible transient signals. Typical data at a probe wavelength of 630 nm are shown in Fig. 4. The

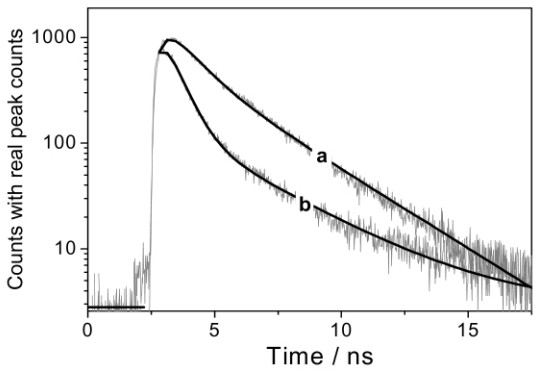

Fig. 3 Time resolved single photon counting decays for Zn Cyt-c immobilised on (a) a nanoporous ZrO_2 film and (b) a nanoporous TiO_2 film. Also shown are best fits to the data (dark lines). Excitation was at 404 nm (1 MHz, 0.2 mW cm^{-2}), the emission decay was monitored at 550 nm. The films were immersed in 10 mM phosphate buffer, pH 7, 25 °C. Control data on TiO_2 films in the absence of adsorbed protein exhibited negligible emission under these experimental conditions.

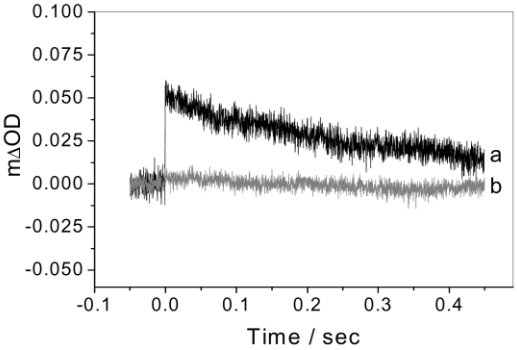

Fig. 4 Transient absorption data observed following pulsed laser excitation of a Zn Cyt-c/TiO_2 film immersed in (a) a pH 8 buffer solution while (b) shows control data for a TiO_2 film in the absence of Zn Cyt-c. Data collected at a probe wavelength of 630 nm with excitation at 549 nm (0.07 mJ cm^{-2}, 0.1 Hz).

remarkably slow timescale of this decay ($t_{1/2} \sim 0.2$ s) rules out possible contributions to the data from Zn Cyt-c triplet states, and further supports assignment of this transient signal to the Zn Cyt-c cation /TiO_2 (e−) charge separated state generated by photoinduced electron injection.

The decay of the transient absorption signal in Fig. 4 is attributed to charge recombination between electrons in the TiO_2 and the photoinduced Zn Cyt-c cations. The half time for this charge recombination ($t_{1/2} \sim 0.2$ s) is significantly slower than those reported for charge recombination to molecular sensitisers (typically ≤1 ms), consistent with the increased physical separation of the heme group from the electrode surface.

We conclude that we have demonstrated direct electron transfer from immobilised Zn Cyt-c into a nanocrystalline TiO_2 electrode on the nanosecond timescale. In contrast to many electrochemical studies of protein/electrode electron transfer, no electron mediators or promoters were required to facilitate the electron transfer reaction. The resultant photogenerated charge separated state is remarkably stable, with a $t_{1/2}$ of 0.2 s. These results demonstrate both the potential for using transient optical techniques to probe protein/electrode electron transfer and the potential to interface biological macromolecules with nanostructured electrodes for the development of light driven devices.

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Notes and references

1 A. Hagfeldt and M. Grätzel, *Acc. Chem. Res.*, 2000, **33**, 269.
2 E. Topoglidis, T. Lutz, R. L. Willis, C. J. Barnett, A. E. G. Cass and J. R. Durrant, *Faraday Discuss.*, 2000, **116**, 35.
3 E. Topoglidis, C. J. Campbell, A. E. G. Cass and J. R. Durrant, *Langmuir*, 2001, **17**, 7899.
4 E. Topoglidis, A. E. G. Cass, B. O'Regan and J. R. Durrant, *J. Electroanal. Chem.*, 2001, **517**, 20.
5 F. A. Armstrong and G. S. Wilson, *Electrochim. Acta*, 2000, **45**, 2623.
6 I. Willner and E. Katz, *Angew. Chem., Int. Ed.*, 2000, **39**, 1180.
7 Y. Tachibana, J. E. Moser, M. Grätzel, D. R. Klug and J. R. Durrant, *J. Phys. Chem. B.*, 1996, **100**, 20056.
8 L. H. Guo, S. Mukamel and G. McLendon, *J. Am. Chem. Soc.*, 1995, **117**, 546.
9 F. Nuesch, J. E. Moser, V. Shklover and M. Grätzel, *J. Am. Chem. Soc.*, 1996, **118**, 5420.
10 J. M. Vanderkooi and M. Erecinska, *Eur. J. Biochem.*, 1975, **60**, 199.
11 Y. Tachibana, I. V. Rubtsov, I. Montanari, K. Yoshihara, D. R. Klug and J. R. Durrant, *J. Photochem. Photobiol. A*, 2001, **142**, 215.