A Bifunctional Monolayer Electrode consisting of 4-Pyridyl Sulfide and Photoisomerizable Spiropyran: Photoswitchable Electrical Communication between the Electrode and Cytochrome C

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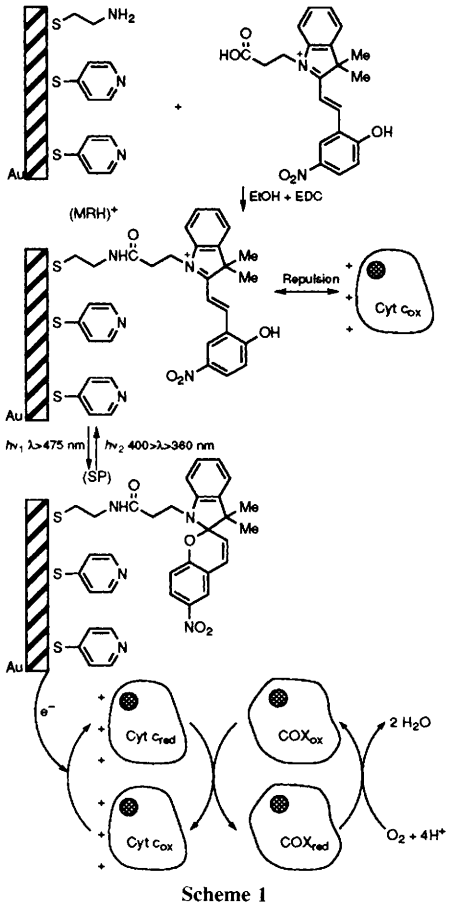
A bifunctional monolayer electrode, consisting of 4-pyridyl sulfide and photoisomerizable spiropyran, photostimulates the electrical communication of cytochrome c with the electrode and the inter-protein electron transfer between cytochrome c and cytochrome c oxidase.

Light-stimulated activation and deactivation of biomaterials provide the basis for bioelectronic devices.[1-5] The organization of biomaterials as nano-structured monolayers on electrode surfaces,[6,7] opens up the possibility of combining photoswitchable biomaterials and nanotechnology concepts to tailor novel bioelectronic devices.[8,9]

Here we report a novel method to reversibly control by light the electron-transfer communication between cytochrome c, Cyt c, and an electrode and to photostimulate inter-protein electron transfer and subsequent biocatalysed O_2-reduction using a hetero-component monolayer-modified Au-electrode composed of 4-pyridyl sulfide and photoisomerizable spiropyran units. The system reveals the element of amplification of the recorded optical signal by the transduced amperometric response.

Electron transfer communication of Cyt c with electrodes was accomplished by surface modification of the electrode with a molecular component such as pyridine.[10] It is believed that association of the protein to the molecular modifying agent orients the redox centre of the protein, in respect to the electrode surface, at sufficiently short distances to allow effective electrical communication. A bifunctional monolayer, consisting of 4-pyridyl sulfide and protonated nitromerocyanine, MRH^+, has been deposited into Au-electrodes according to the sequence of transformations outlined in Scheme 1. The monolayer exhibits reversible photoisomerizable properties.[8,9,11] Irradiation of the monolayer-electrode with visible light, $\lambda > 475$ nm, results in the nitrospiropyran, SP, monolayer state, while illumination of the SP-monolayer electrode, $360 < \lambda < 400$ nm yields the MRH^+-monolayer electrode. The surface density of the spiropyran units was evaluated electrochemically and corresponded to *ca.* 5×10^{-12} mol cm^{-2}, and the ratio of merocyanine units and pyridine components was *ca.* 1 : 10, respectively.†



Scheme 1

Fig. 1 shows the CV of Cyt c (from bovine heart, Sigma), in the presence of the SP-monolayer electrode [Fig. 1(*a*)] and the MRH^+-monolayer electrode [Fig. 1 curve (*b*)]. Effective electrical communication is observed with the SP-monolayer electrode, $k_{et} = 1 \times 10^{-3}$ cm s^{-1}, while electrical communication of Cyt c and the electrode is blocked in the MRH^+-monolayer electrode state. The organization of the bifunctional monolayer at the specific ratio is important in attaining electrical communication with Cyt c. A pure SP-monolayer electrode cannot communicate while increase of the SP component in the bifunctional monolayer adversely affects the electrical interaction of Cyt c with the electrode. That is, the pyridine units associated with the monolayer, bind Cyt c and orient the redox centre of the protein to distances allowing effective electrical communication with the electrode surface. The photoisomerizable co-immobilized units photostimulate the interactions of Cyt c with the monolayer. In the SP-monolayer state, the attractive interactions of Cyt c with

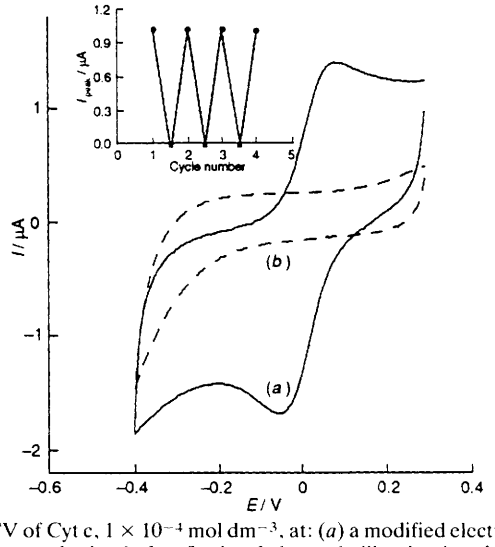


Fig. 1: CV of Cyt c, 1×10^{-4} mol dm^{-3}, at: (*a*) a modified electrode in the SP state, obtained after 5 min of electrode illumination, $\lambda > 475$ nm; (*b*) A modified electrode in the MRH^+ state after 2 min of UV-irradiation, $360 < \lambda < 400$ nm. Potential scan rate, 50 mV s^{-1}. (Inset) Reversible photostimulated peak currents for Cyt c in the presence of the monolayer-modified electrode. (●) The electrode in the SP state, (■) the electrode in the MRH^+ state.

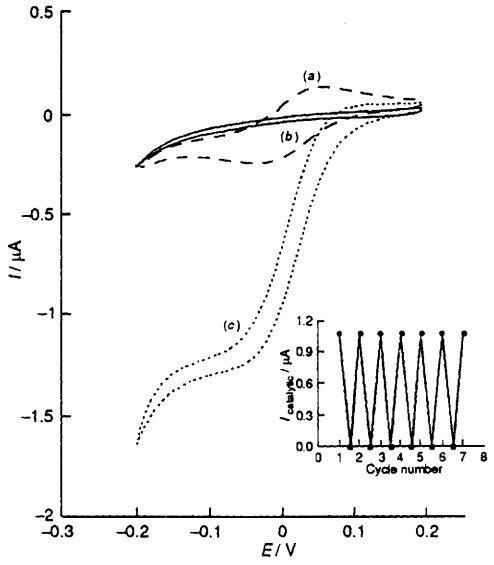


Fig. 2 CV at the photoisomerizable monolayer-modified electrode in the systems: (*a*) Cyt c + O_2 and electrode in SP state; (*b*) Cyt c + COX + O_2 and the electrode in MRH^+ state; (*c*) Cyt c + COX + O_2 and the electrode in the SP state. Potential scan rate, 2 mV s^{-1}; [Cyt c] = 1×10^{-4} mol dm^{-3}, [COX] = 1×10^{-6} mol dm^{-3}, [O_2] = in equilibrium with air. (Inset) Reversible photostimulated bioelectrocatalytic reduction of O_2 by the system consisting of Cyt c, (1×10^{-4}) and COX, (1×10^{-6} mol dm^{-3}): (●) the electrode in SP state, (■) the electrode in MRH^+ state.

the pyridine units prevail and effective electron transfer proceeds. In the MRH^+-monolayer state, electrostatic repulsion of Cyt c exhibiting positive charges[10] perturbs the associative interactions with the pyridine units and electrical communication between Cyt c and the electrode is blocked, Scheme 1. The reversible photoisomerizable properties of the monolayer allow the cyclic activation–deactivation of the electron-transfer communication between Cyt c and the electrode, Fig. 1 (inset).

Cyt c acts as electron-transfer mediator to Cyt c oxidase, COX (EC 1.9.3.1, from bovine heart, Sigma), that mediates the four-electron reduction of molecular oxygen to water.[12] The interprotein electron transfer and subsequent biocatalysed reduction of oxygen are controlled by the photoisomerizable monolayer electrode. Fig. 2 shows the amperometric responses of the Cyt c–COX system in the presence of O_2 using the photoisomerizable monolayer electrode. The reversible CV of Cyt c in the presence of the SP-monolayer electrode is shown in Fig. 2(*a*). This curve is not affected by O_2. Addition of COX to the Cyt c system under O_2 in the presence of the SP-monolayer electrode results in an electrocatalytic cathodic current at the Cyt c reduction potentials [Fig. 2(*c*)].‡ These results imply that electroreduced Cyt c mediates electron transfer to COX that biocatalyses reduction of O_2. Application of the MRH^+-monolayer electrode in the Cyt c–COX system under oxygen, results in background current only, and the biocatalysed reduction of O_2 is blocked [Fig. 2(*b*)]. Thus, the photostimulated electrical communication between the photoisomerizable electrode and Cyt c controls the inter-protein Cyt c–COX electron transfer and subsequent biocatalysed O_2-reduction, Scheme 1. Fig. 2 (inset) shows the reversible photostimulated catalytic cathodic currents observed in the Cyt c–COX system under O_2, upon reversible photoisomerization of the electrode between the SP and MRH^+ monolayer states. It should be noted that the 'OFF' position of electron-transfer communication represents a true zero-value amperometric response. Usually, photoswitchable biomaterial retains a residual activity even in the 'OFF' state.[1,3,4] The present biochemical assembly represents a unique example where the system responds to external optical signals through a zero amperometric response and a high-magnitude amperometric signal.

We therefore conclude that a photoisomerizable spiropyran monolayer electrode provides a means to control the electrical communication between Cyt c and the electrode as well as the subsequent inter-protein electron transfer in the Cyt c–COX system. Amperometric transduction of recorded optical signals is the basis for the development of bioelectronic information storage devices. Coupling of the photostimulated electrical communication of Cyt c with the electrode interface, to the secondary biocatalysed reduction of O_2, provides a means for the amplification of the transduced amperometric response.

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Footnotes

† The surface density of merocyanine groups was determined by following the charge associated with the reversible two-electron redox process of the intermediate nitroso product formed by the irreversible reduction of the nitro substituents. The 4-pyridyl sulfide–merocyanine ratio in the monolayer was determined by comparison of the MRH^+-surface density in the bifunctional monolayer to a full MRH^+-saturated electrode surface.

‡ No direct electrical communication between COX and the SP or MRH^+-monolayer electrodes was observed.

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