Electrochemical modulation of bioluminescence

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The electrochemical modulation of the bioluminescence of firefly luciferase is described, showing that the enzyme can be switched ON and OFF, as a function of both the magnitude and duration of the applied potential.

There is now a considerable literature concerning the communication between biological molecules and electronic materials, with research focused on the study of electron transfer between redox proteins and modified metal interfaces. For example, recent advances have been made in the understanding of the direct electrochemistry of proteins 'wired' to electrodes with inorganic redox centres,[1] as well as in the interactions between native (unmodified) biomolecules with functional self-assembled monolayers.[2,3] Beyond these examples, there are fewer opportunities for considering other modes of biological information processing at such surfaces *e.g.* using light. In one recent case, in a system analogous to mammalian vision, Willner and coworkers demonstrated optical modulation of biological electron transfer at a gold electrode by photo-isomerising a thiol based surface modifier, switching it ON and OFF.[4]

Here, we present an alternative configuration for electro-optical processing of biological information, in which the *sense* is reversed to the example described above,[4] *i.e.* we demonstrate the *electrochemical* modulation of the *optical* output from the bioluminescent enzyme, firefly luciferase. In describing the mechanism of the switch, we show that the phenomenon is dependent upon both the magnitude and duration of an applied potential. Finally, we show that the enzyme can be photo-patterned with a micrometre-scale resolution, using a novel technique that is appropriate for depositing biological material at lithographically fabricated microstructures.

The enzyme luciferase is responsible for bioluminescence in a variety of beetle species, including the firefly *Photinus pyralis*, catalysing the reactions between ATP, luciferin and oxygen to produce AMP, pyrophosphate, CO_2 and oxyluciferin and light.[5] At its optimum pH of 7.8, the peak emission wavelength of the bioluminescence is at λ = 562 nm. As a consequence of this unique activity, luciferases are of considerable commercial significance, being used widely both in ATP detection in the food hygiene industries,[6] and as a marker protein within molecular biology.[7] In both of these cases, luciferase is particularly applicable to the bioanalytical assays described, as the production of light gives a rapid method for signal transduction.

Likewise, in this work, we have found that bioluminescence provides an instantaneous measurement of activity, and can be used to show that the enzyme is being electrochemically switched ON and OFF. The effect is most apparent when the immobilisation layer is a very thin film, presumably facilitating the rapid diffusion of species either to or from the electrode surface. Under such circumstances, however, it is necessary to use a sensitive photomultipler to enable the low levels of light to be detected with a fast time resolution.

In all of the experiments investigating *photo-switching*, measurements were made in a single-compartment electrochemical cell using a conventional three-electrode configuration, with both working and counter electrodes defined photolithographically using metal evaporation and 'lift-off' to give a multilayer structure of 10/10/100 nm Ti/Pd/Au. The immobilisation film was cast from a 2% *m/v* solution of gelatin containing 0.025% Triton X100, to a thickness of *ca.* 1 μm. The modified electrode was bathed in a solution of 2.0 mg ml^{-1} luciferase which was subsequently immobilised at the film by exposure to UV light (using 100 W Zeiss fluorescence microscope light at a distance of 10 cm for 15 min). Currents and voltages were measured using a purpose-built potentiostat, with electrode potentials referenced against an Ag|AgCl electrode. Light intensity was monitored in the presence of the necessary substrate and cofactors using a photomultiplier operated in photon counting mode, with a 500–600 nm band pass filter to remove variations caused by changes in the emission spectrum of luciferase bioluminescence.

Fig. 1(*a*) shows the switching OFF of bioluminescence by applying a reducing potential of −1.2 V to the luciferase modified working electrode at the point labelled 'ON' in the diagram. The potential causes a decrease in bioluminescence (solid line) from its steady-state value of *ca.* 11000 cps to near background levels of light emission (τ = 38 s). Removal of the

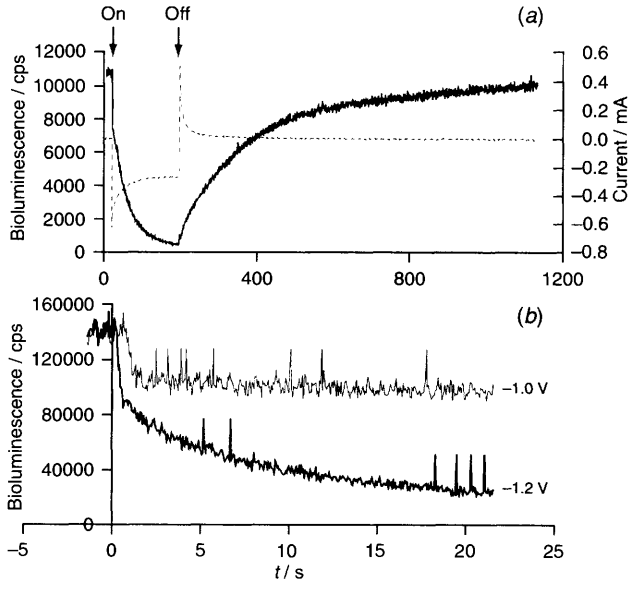

Fig. 1(*a*) The effect on the bioluminescence (——) of switching a potential of −1.2 V both 'ON' and 'OFF' at a luciferase-modified working electrode. Also shown is the measured current, resulting from electrochemical reactions at the electrode (·····), see text for details. (*b*) Effect of the magnitude of the applied potential, with two cases illustrating the dependence of light intensity at −1.0 and −1.2 V, as labelled. In all cases, bioluminescence was measured in 25 mM HEPES buffer, pH 7.8, containing 5 mM $MgCl_2$, 10 mM DTT, 200 μM ATP and 350 μM luciferin (ambient temperature = 18 ± 3° C, electrode area = 0.5 cm^{-2}).

potential (labelled 'OFF') led to a recovery of bioluminescence to >95% of the pre-pulse value (τ = 189 s). Also shown in Fig. 1(*a*) is the current passing through the cell (dotted line), resulting from either the cathodic reduction of oxygen, thus depleting the concentration of H^+, and leading to a shift in the water dissociation equilibrium to produce more OH^- ions, or from simple reduction of H^+ to dissolved hydrogen gas. Either case results in a rise in the local pH. By monitoring both the bioluminescence and the current simultaneously, it is possible to show that the switching effect is not observed at potentials above which these electrochemical reactions occur, such that enzyme modulation can be attributed, either wholly or in part, to a local change in pH.

The nature of the bioluminescent response is explained by considering the flux of electrogenerated species into and out of the gelatin film. For example, the amount of OH^- within the film is dependent both upon the rate at which the ions are produced electrochemically, and upon the rate at which they are lost to the bulk solution. As stated, provided that the applied potential is < −0.8 V, there is always a decrease in intensity of bioluminescence, due to the locally occurring electrochemical reactions. Thereafter, the nature of the transient, shown in both Fig. 1(*a*) and (*b*), is dependent upon both the magnitude and the duration of the applied voltage. For example at −1.0 V in Fig. 1(*b*), following an immediate fall in the light intensity after electrode polarisation, the response approaches steady state, during which time the rate of production of OH^- is approximately equivalent to that of its loss from the film, with only a slight drift in bioluminescence. The same dynamic equilibrium would explain the response, if it were due specifically to changes in the local change of oxygen, rather than pH.

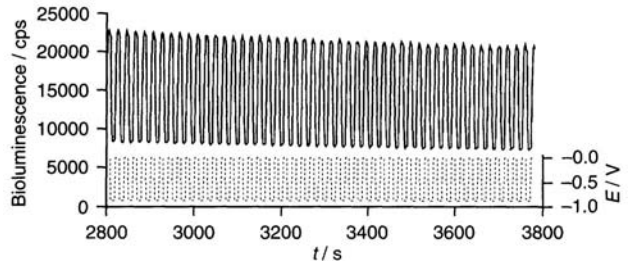

Fig. 2 Effect of switching the applied potential rapidly between −0.9 V (10 s) and 0 V (10 s), with the corresponding signal for the measured bioluminescence following 180° out of phase. Conditions were otherwise as described in Fig. 1.

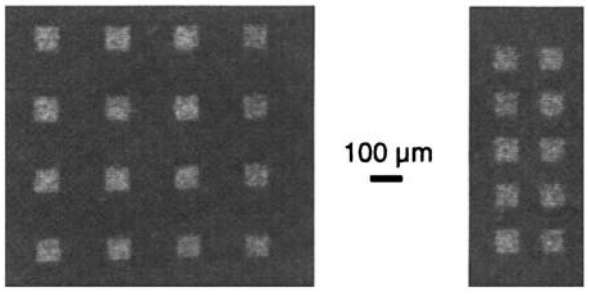

Fig. 3 A photo-patterned enzyme on a glass substrate. The gelatin film was spun to a thickness of 1 μm and bathed in a solution of enzyme (see text for details). The assembly was exposed to UV radiation through a photolithographic mask and enzyme was localised as squares of size 50 μm.

In contrast, at a potential of −1.2 V, Fig. 1(*a*) and (*b*), the rate of OH^- production is considerably faster, and the fall in the intensity of bioluminescence continues, ultimately reaching zero. In all cases, the observed time constants for both the ON and OFF transients, given above for Fig. 1(*a*), are correlated to the ion diffusion within the gel, which in turn depend on the magnitude and duration of the voltage. Consequently, times for photo-switching the activity of the immobilised enzyme are many orders of magnitude slower than those for the catalytic activation and deactivation of bioluminescence in solution.[8]

If the applied voltage lasts for a shorter period of time (*e.g.* 10 s) then the bioluminescent response not only will immediately decrease in intensity, as the local pH rises, but also will turn back ON again almost instantaneously, as the electrode potential returns to zero and the pH is re-established by diffusion of buffer around the enzyme. This is illustrated dramatically in Fig. 2, where the reducing potential is switched as a square-wave pulse between −0.9 and 0 V, and the bioluminescence follows 180° out of phase. Interestingly, over a period of 1 h, there is a drift in the initial maximum response, which can be attributed to ions accumulating within the membrane (*i.e.* again the ion flux is close to, but not in, steady state).

Finally, it was found that the described method for immobilisation was also compatible with a novel technique for photopatterning the enzyme with a μm-scale resolution, and providing localised bioluminescence. Fig. 3 illustrates this phenomenon, where the enzyme gelatin film has been bathed in the enzyme and then exposed through a Cr photo-mask, of the type used in the semiconductor industry. Areas that were irradiated gave a well defined enzyme pattern (with an edge resolution of 25 μm), either on glass, gold or silicon, with protein being immobilised by UV activation of the gel matrix (λ = 300–400 nm). It is particularly interesting that there are low levels of non-specific binding, and little significant short-wavelength mediated damage to the protein.

The method has the potential to be used to spatially control the deposition of the enzyme within an integrated microsystems device, providing a technique by which separately addressed miniaturised electrodes could be surface functionalised with a biologically active protein that can be switched ON and OFF. Indeed, previously there was a report that the enzyme alkaline phosphatase can be electrochemically modulated,[9] indicating that a wider range of protein activities beyond luciferase, and including enzymes, antibodies and ligand binding molecules, may exhibit similar pH dependent effects.

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