USING CYCLIC VOLTAMMETRY TO DISCERN PROTEIN MECHANISM AND CORRELATE STRUCTURE WITH FUNCTION.

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The development of electrochemical biosensors has hinged on the ability to obtain and sustain an active layer of the desired protein at a suitable electrode. This layer must be stable under a wide range of conditions and demonstrate a high degree of specificity for the substrate. Over the past few years, we have explored a range of surface modification techniques that result in stable protein films, enabling direct electrochemical experiments that have led to novel mechanistic information.

We have observed that both the nature of the promoter and the electrode surface play a crucial role on the electrochemical properties of immobilised plastocyanin, evidenced by significant shifts in midpoint potential[1]. This study identified edge-oriented pyrolytic graphite (PGE) modified with poly-L-lysine as a superior film in terms of stability, reproducibility and general applicability. The first direct electrochemistry of human adrenodoxin was also obtained in this way. A persuasive correlation was observed between peak separation and pH, suggesting optimum protein activity could be determined in this way[2].

During the past decade, alkanethiol monolayers (SAM's) have been extensively used to modify gold surfaces for electrostatic and covalent protein immobilisation. However, quality reports of direct protein electrochemistry have been limited; cytochrome c and azurin being notable exceptions[3]. We have observed reproducible cyclic voltammograms of the multicopper oxidase, laccase immobilised using a 3-mercapto propionic acid SAM followed by activation with EDC/NHS and coupling to free amines on the laccase surface (Figure 1). A comprehensive study of the electrochemical properties has indicated a concerted 4-electron transfer at slow scan rates (v) under anaerobic conditions, involving all four copper atoms within the protein, as assessed by peak current ratios (ipa/ipc), Figure 2. Addition of micromolar concentrations of the known laccase inhibitors N_3^- and F^- resulted in a significant reduction in peak current ratio, indicative of binding to the type 3 (T3) copper atoms. This study seems to indicate that azide binds in a bridging manner to both T3 copper atoms, preventing observation of the electrochemical signal at higher v. Under unbuffered conditions, catalytic currents were observed in the presence of the biological substrate oxygen, emphasising the retention of physiological function throughout the immobilisation process.

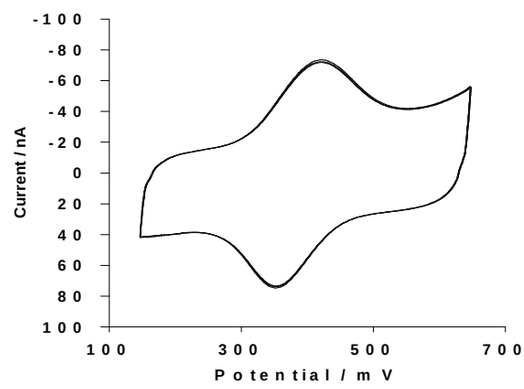

Figure 1. Representative CV of laccase immobilised on an MPA-modified gold electrode. Scan rate 500µV/s, pH 7.0, supporting electrolyte 0.1M NaCl. Potentials corrected to NHE.

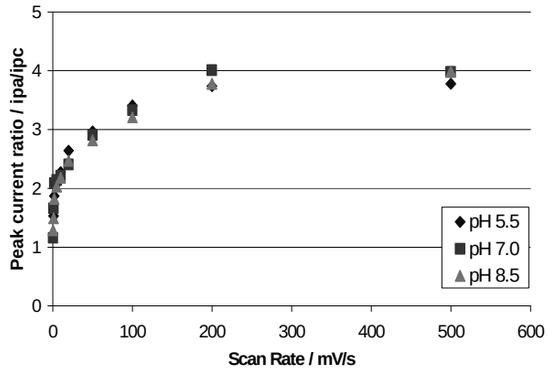

Figure 2. Influence of scan rate on the peak current ratio.

[1] Johnson D. L., Maxwell C. J., Losic D., Shapter J. G. and Martin L. L. *Bioelectrochemistry* **58** 137-147 (2002).
[2] Johnson D.L., Norman S., Tuckey R.C. and Martin L.L. *Bioelectrochemistry* (2002) accepted.
[3] For example see: a) Chi Q., Zhang J., Andersen J. and Ulstrup J. *J. Phys. Chem. B* **105** 4669-4679 (2001); b) Song S., Clark R.A., Bowden E.F. and Tarlov M. J. *J. Phys. Chem.* **97** 6564-6572 (1993).