PROTEIN FILM VOLTAMMETRY OF A CYTOCHROME *C* NITRITE REDUCTASE

Julea N. Butt[1,2], Hayley C. Angove[2], David G. Moore[1], David J. Richardson[2] and Jeffrey A. Cole[3]

Centre for Metalloprotein Spectroscopy and Biology, [1]School of Chemical Sciences and [2]School of Biological Sciences, University of East Anglia, NR4 7TJ, UK
[3]School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

Protein film voltammetry provides a powerful tool with which to visualize and quantitate the activities of redox enzymes (1). In this technique the enzyme of interest is adsorbed as a catalytically active film on an electrode surface. Within the film the enzyme is engaged in direct exchange of electrons with the electrode and redox centers in the enzyme are swept between accessible oxidation states in response to defined variation of the applied potential. The flow of current recorded when the enzyme film contacts a solution of its substrate provides simultaneous visualization and quantitation of catalytic activity across the electrochemical potential domain.

The rapidly growing number of enzymes whose catalytic performance has been examined by protein film voltammetry show a predominance of 'complex' catalytic waveforms; waveforms which contain multiple boosts and/or attenuations of activity in response to variation of the applied electrochemical potential. Modeling the development of a catalytic waveform with increase of substrate concentration has suggested several mechanistic possibilities to account for such waveforms; for example, coupling of electrochemical and chemical transformations within the active center or redox transitions which serve to switch the enzyme between two kinetically distinct forms (e.g., 1-4). While interpretation of the voltammetry at a mechanistic level is indirect the waveforms provide unique insight into the catalytic mechanisms operating within redox enzymes which complements structural and spectroscopic studies aimed at defining reaction mechanisms at a molecular level.

The cytochrome *c* nitrite reductases perform a key step in the biological nitrogen cycle by catalyzing the six electron reduction of nitrite to ammonium, $E_{m,7}$ ~ 340 mV.

$$NO_2^- + 6e^- + 8H^+ \rightarrow NH_4^+ + 2H_2O$$

These soluble enzymes contain five *c*-type hemes. Four hemes have bis-histidine iron coordination, the fifth at the site of nitrite reduction exhibits novel Lys-water/ hydroxide coordination (5-7). The heme groups are packed in structural motifs common to a number of proteins and all nearest neighbor Fe-Fe distances lie below 13 Å. Graphite and gold electrodes painted with *Escherichia coli* cytochrome *c* nitrite reductase exhibit large, catalytic reduction currents during cyclic voltammetry to potentials below 0V in solutions containing nitrite (Fig.1). These catalytic currents were not observed in the absence of cytochrome *c* nitrite reductase and were shown to originate from an enzyme film engaged in direct electron exchange with the electrode. Protein film voltammetry has visualized distinct fingerprints of catalytic behavior during the reduction of nitrite and hydroxylamine, the latter being reduced to ammonium in a two-electron process. The voltammetry indicates underlying similarities but also clear differences in the handling of these alternative substrates by *E. coli* cytochrome *c* nitrite reductase.

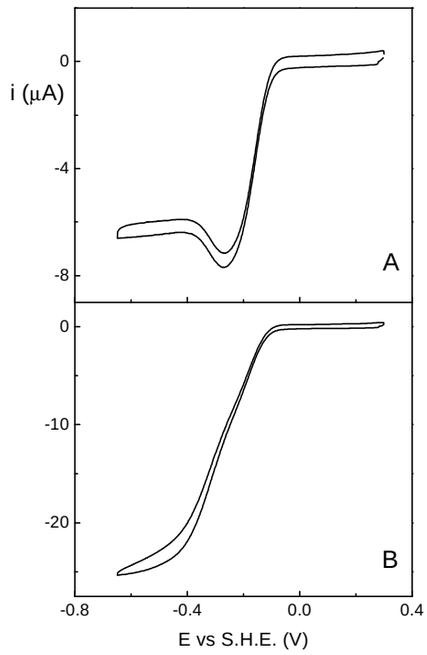

Fig. 1. Cyclic voltammetry from a film of *E. coli* cytochrome *c* nitrite reductase in (A) 14 and (B) 130 μM nitrite. Buffer-electrolyte, 2 mM $CaCl_2$, 50 mM Hepes, pH 7.0 20 ^oC, scan rate 20 mV s^{-1} with electrode rotation at 3000 r.p.m.

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