Redox Triggered Events in Cytochrome *c* Nitrite Reductase: A Voltammetric Study

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Abstract

Cytochrome *c* nitrite reductase (NrfA) is a dimeric decahaem-containing enzyme that catalyses the reduction of nitrite to ammonium. Protein film voltammetry (PFV) of NrfA has revealed how the enzymes activity may be modulated up or down by redox events at specific haems, raising questions as to the mechanisms through which these modulations occur.

PFV performed over a range of pH reveals how the enzyme shows greatest activity at acid pH (≤ 5), with activity decreasing as the pH is increased. In addition, the effect of pH is shown to induce significant changes in the shape and position of the current-potential profiles. PFV recorded under substrate-limited conditions reveals the onset of catalysis to be triggered by the two-electron reduction of two haems coupled to a single protonation. Further reduction of the enzyme at pH ~ 4 had no effect on the enzymes activity. However, at pH 5 an attenuation of activity is observed on lowering the electrode potential that becomes increasingly pronounced as the pH is raised. Proton-coupled electron transfer is thus shown to be a significant determinant of the catalytic waveshape observed from NrfA.

The electrochemical potential, via the oxidation state, also modulates the affinity for inhibitors. PFV has identified at least two groups of inhibitors of the enzymes activity, characterised by their contrasting effects on the catalytic waveshape. Azide binds with greatest affinity to the enzyme when the active site is oxidised, and with increased affinity to the free-enzyme as opposed the Michaelis complex. By contrast, PFV indicates the existence of two cyanide-binding sites, binding at which is with greatest affinity when the active site is reduced. PFV of the mono-cyano complex reveals it to retain catalytic activity, revealing catalytic waveforms distinct from the uninhibited enzyme.

In situ STM experiments of NrfA immobilised at Au(111) electrodes reveal a mixed population of NrfA monomers and dimers adsorbed at the electrode. This technique presents exciting opportunities for the integration of global (from PFV) and molecular descriptions of this, and other, protein films.