Electrochemical studies of heme enzymes and proteins: bacterial peroxidases and cytochromes c

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The bacterial cytochrome *c* peroxidase from *Nitrosomonas europaea* is examined using the technique of protein film voltammetry. Sub-monolayers of the bacterial diheme enzyme at a pyrolytic graphite edge electrode give catalytic, reducing voltammetric signals in the presence of the substrate hydrogen peroxide. The resulting waveshapes indicate that C*c*P is bound non-covalently to the electrode in a highly active configuration. The native enzyme has been shown to possess two heme groups of low and high potential (-260 and +450 mV vs. hydrogen, respectively), and here we find very similar potentials of the C*c*P hemes upon the graphitic surface. In the presence of substrate, the catalytic waves of the *N. europaea* enzyme have a mid-point potential of >500 mV, and all catalytic data have been fit to a n_{app}=1 process. The signals increase in magnitude with hydrogen peroxide concentration, revealing Michaelis-Menten kinetics, and K_m = 55 μM. The mid-point potentials shift with substrate concentration, indicating the electrochemically active species observed in our data corresponds to species linked to catalysis. Similarly, upon inclusion of cyanide, azide or fluoride, inhibitors cause a shift in the observed catalytic midpoint potential. These data and the pH dependence of the enzyme indicate an electrochemical mechanism of peroxide reduction described by two distinct, 1-electron transfer steps. Comparisons of the *N. europaea* C*c*P with other bacterial peroxidases and cytochromes *c* will also be described.