Reversible electrochemistry and catalysis with *Mycobacterium tuberculosis* catalase-peroxidase in lipid films

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Reversible voltammetry was achieved ($E^{\circ\prime}$ = −0.13 V *vs.* NHE at 25 °C at pH 7.1) for the Fe^{III}/Fe^{II} redox couple of mycobacterial KatG catalase–peroxidase in films of dimyristoyl phosphatidylcholine, which also catalyzed electrochemical reductions of hydrogen peroxide and oxygen.

The mycobacterial KatG catalase–peroxidase[1,2] oxidatively activates the prodrug isoniazid (isonicotinic acid hydrazide) used to treat tuberculosis. *Mycobacterium tuberculosis* catalase-peroxidase is a dimer of 80 kDa subunits, both of which contain an iron heme. Oxidative activation of isoniazid may occur through several possible pathways. The Fe^{III} form of the enzyme can react with peroxides to form an unstable intermediate peroxidase compound I that oxidizes isoniazid.[3] The catalase–peroxidase can oxidize isoniazid in the presence of dioxygen and a reductant, similar to cytochrome P450 catalysis.[4] The Fe^{III} enzyme receives one electron forming Fe^{II} enzyme, to which dioxygen binds to give an Fe^{II}–O_2 intermediate that is the precursor of a high-valent active oxidant.[5,6] Oxidation of isoniazid by the catalase–peroxidase can also be initiated by superoxide.[7] Knowledge of redox chemistry, oxidation kinetics, and reduction of H_2O_2 with the catalase–peroxidase is critical for elucidation of pathways for isoniazid oxidation. Here we report the first example of direct, reversible electron transfer between the Fe^{III}/Fe^{II} redox couple of the catalase–peroxidase from *M. tuberculosis* in thin films of dimyristoyl phosphatidylcholine (DMPC) on electrodes. These enzyme–lipid films catalyzed electrochemical reduction of H_2O_2 and of dioxygen.

Films of stacked lipid bilayers provide a biomimetic environment facilitating direct electrical communication between electrodes and redox sites of incorporated heme enzymes.[8,9] Fig. 1 shows a reversible cyclic voltammogram (CV) for the Fe^{III}/Fe^{II} redox couple of *M. tuberculosis* catalase–peroxidase (enzyme obtained as in ref. 3) in a DMPC film in pH 6.0 buffer. Films were made by spreading 10 μL of an aqueous vesicle dispersion of 1 mM DMPC and 0.05 mM enzyme evenly onto a basal plane PG disc electrode (area = 0.2 cm^2) and drying overnight.[10] The film thickness was *ca.* 0.5 μm.

Catalase–peroxidase/DMPC films in oxygen-free buffers gave symmetric CV peaks with equal reduction and oxidation peak heights that depended linearly on scan rate from 0.01 to 1 V s^{-1}. Peak widths at half-height were *ca.* 200 mV, and reduction–oxidation peak separations were *ca.* 50 mV, exceeding the 90/*n* mV peak width and 0 mV peak separation, respectively, predicted by theory for ideal thin layer voltammetry. Integration of CVs gave enzyme surface concentration of 2.1×10^{-10} mol cm^{-2}. Results are typical for non-ideal thin film protein voltammetry.[9] Formal potentials ($E^{\circ\prime}$) of the catalase–peroxidase obtained as midpoint potentials of reversible CVs are compared with other enzymes in Table 1.

Recently Wengenack *et al.*[11] reported irreversible voltammetry for the KatG catalase–peroxidase on graphite electrodes. Cyclic voltammograms with the enzyme in solution showed a Fe^{III} reduction peak, but no reverse oxidation peak, and reversibility of square-wave voltammograms was unsubstan-

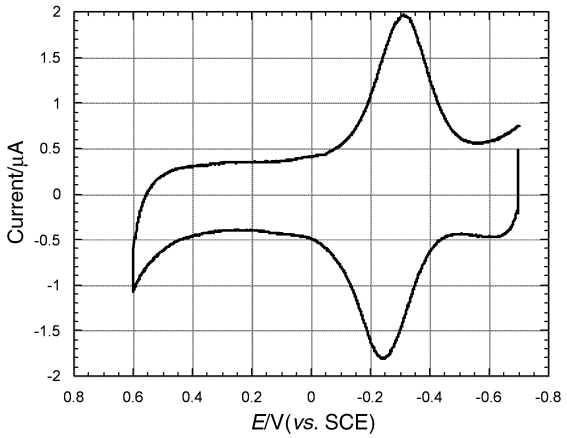

Fig. 1 Background subtracted cyclic voltammogram (CV) at 100 mV s^{-1} and 25 °C of catalase–peroxidase–DMPC film on basal plane PG electrode, in pH 6.0, 20 mM phosphate buffer purged with purified nitrogen for 20 min before CV. Note that *E*/V (*vs.* NHE) = 0.244 + *E*/V (*vs.* SCE).

Table 1 Electrochemical results for enzymes in DMPC films and water[a]

Enzyme	pH	$E^{\circ\prime}$[b]/V (*vs.* NHE)	Γ[c]/ mol cm^{-2}	Slope[d]/nA μM^{-1}	Rel. rates of H_2O_2 redn.[e]
Catalase–peroxidase film	6.0	−0.058	2.1×10^{-10}	1.3	0.06
Catalase–peroxidase film	7.1	−0.128	2.1×10^{-10}		
Catalase–peroxidase in water	7.6	−0.028	(from ref. 11, by titration)		
HRP film	6.0	−0.065	1.8×10^{-11}	1.8	1.0
Catalase film	6.0	−0.18	9.0×10^{-11}	0.11	0.01

[a] Average of at least two electrodes in 20 mM phosphate buffer at pH 6.0, 25 °C. Standard deviations were *ca.* ±5 mV for $E^{\circ\prime}$ and ≤15% for other quantities. [b] Estimated as midpoint potentials of reduction–oxidation peaks in CV. [c] Determined by integration of CV peaks at low scan rates and application of Faraday's law. [d] Calculated by linear regression of amperometric responses to various concentrations of hydrogen peroxide using rotating electrodes at 1800 rpm at 0.24 V *vs.* NHE. [e] From steady state currents as nA $nmol^{-1}$ enzyme at 1800 rpm at 0.24 V *vs.* NHE, proportional to turnover rate, relative to HRP = 1.

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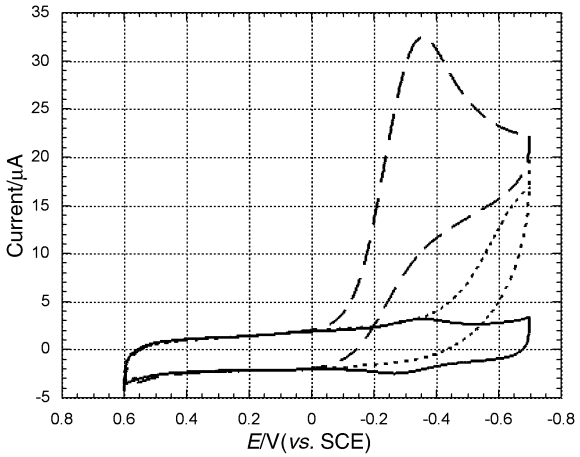

Fig. 2 CVs at 100 mV s^{-1} of KatG–DMPC films in pH 6.0 buffer saturated with air (– —) and without oxygen (—), and enzyme-free DMPC film in pH 6.0 buffer saturated with air (---).

tiated by the data.[11] The potentials of these irreversible redox peaks are controlled by kinetics and do not yield $E°'$ for the Fe^{III}/Fe^{II} couple of the enzyme.

The Soret absorbance band of catalase–peroxidase shifted slightly from 407 nm in pH 7 solution[12] to 409 nm in DMPC films, similar to myoglobin/DMPC films.[13] When the catalase–peroxidase was purposely denatured at pH 3.5, the Soret band disappeared and a new broad band at *ca.* 375 nm appeared. These results suggest that catalase–peroxidase in DMPC films at pH 6–7.5 is similar in secondary structure to the native enzyme.

If the Fe^{II} enzyme generated by reduction reacts with oxygen and product Fe^{II}–O_2 is reduced by the electrode, reduction current will increase due to the catalytic process, and the Fe^{II} oxidation peak will decrease or disappear. Fig. 2 shows such voltammetric behavior of catalase–peroxidase/DMPC in air-saturated buffer. Increased reduction current results from additional electrons injected into Fe^{II}–O_2, and the likely product is hydrogen peroxide as found for other iron heme enzymes.[9] This catalytic reduction is *ca.* 0.4 V more positive than direct reduction of oxygen on DMPC/PG electrodes (Fig. 2).

Catalase–peroxidase/DMPC films also catalyzed the reduction of hydrogen peroxide. Without H_2O_2, enzyme–DMPC films give similar cyclic voltammograms on quiet (*cf.* Fig. 1) and rotating electrodes [Fig 3(b),(d),(f)]. After adding H_2O_2, reduction current was greatly increased to give catalytic steady-state (limiting) currents for the catalase–peroxidase at 0.4 mM H_2O_2 (Fig. 3(a)] and for HRP at 0.05 mM H_2O_2 (Fig. 3(e)]. For catalase (bovine liver), partly reversible Fe^{III}/Fe^{II} redox peaks are still detected even at 1 mM H_2O_2 [Fig. 3(c)], and steady state was not achieved. The onset of catalytic current for reduction of H_2O_2 occurred at *ca.* 0.24 V *vs.* NHE for catalase–peroxidase, 0.14 V for HRP and 0.1 V for catalase. In no case was a direct Fe^{III} *oxidation* peak observed.

Amperometric responses for enzyme–DMPC films at 0.24 V *vs.* NHE to various concentrations of H_2O_2 were measured at rotating electrodes (Table 1). H_2O_2 presumably reacts with the Fe^{III} enzyme to give compound I,[3] which is reduced by the electrode in a catalytic cycle. Relative turnover rates from these steady-state currents[14] in nA $nmol^{-1}$ enzyme were in the order: HRP > catalase–peroxidase > catalase (Table 1).

Results presented above demonstrate direct, reversible electron transfer between electrodes and *M. tuberculosis* catalase–peroxidase in DMPC films. Catalytic reduction of oxygen involving the oxyferrous enzyme proceeded similarly to electrochemical catalytic cycles of cyt P450 enzymes.[8,9] For catalytic electrochemical reduction of H_2O_2, the catalase–peroxidase in DMPC films resembles HRP more than catalase.

Reversible voltammograms allowed estimation of a formal potential of −0.128 V *vs.* NHE in DMPC films at pH 7.1. An observed $E°'$–pH dependence of −53 mV pH^{-1} puts the value

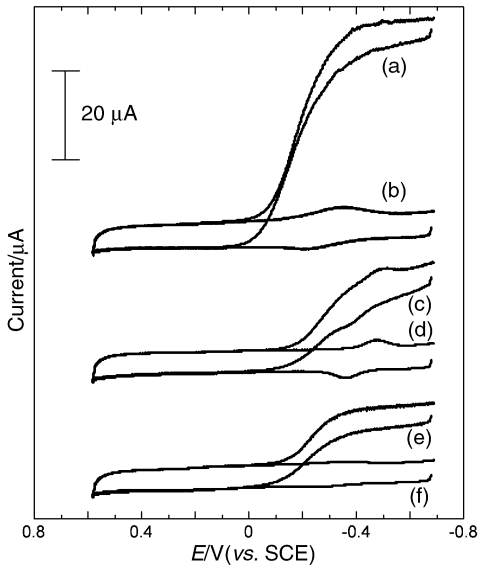

Fig. 3 Rotating disk CVs of enzyme–DMPC films on PG at 1800 rpm and 100 mV s^{-1} in pH 6 buffer illustrating catalytic electrochemical reduction of H_2O_2: KatG enzyme–DMPC films at (a) 0.4 mM H_2O_2, (b) 0 mM H_2O_2; Catalase-DMPC films at (c) 1.0 mM H_2O_2, (d) 0 mM H_2O_2; HRP-DMPC films at (e) 0.05 mM H_2O_2, (f) 0 mM H_2O_2. Buffer solution was purged with purified nitrogen for 20 min before CV; curves for different enzymes were offset for clarity.

at pH 7.6 in DMPC films at −0.155 V. This is significantly more negative than −0.028 V obtained recently in solution at pH 7.6 by spectroelectrochemical titration.[11] Results for other iron heme proteins suggest that $E°'$ values in DMPC films may differ from solution values by 100 mV or more as a result of lipid–enzyme and film–electrode interactions.[9] In comparison, HRP and the catalase–peroxidase have similar formal potentials in DMPC films (Table 1), even though the value for HRP in solution was reported at −0.278 V.[11] Thus, the previous conclusion[11] that the catalase–peroxidase $E°'$ is 200 mV higher than HRP may be modulated by enzyme environment.

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