of $\alpha_2\beta_1$ and $\alpha_1\beta_2$ relationships. We note that product terms are also involved in the E/C equation of Drago.[10] We have not attempted to relate our parameters to Drago's E and C values, partly because many of the acids listed by Drago are Lewis acids and are not hydrogen bond acids, but also because the E/C equation is specifically designed to correlate enthalpies of complexation, whereas our eq 3 is couched in terms of Gibbs energies of complexation (as log K).

In order to illustrate the power of the simple eq 3, we have calculated log K values for some acid-base combinations recently studied by Ruostesuo et al.[11] and published *after* our data base, leading to eq 3, had been set up. Observed and calculated log K values are given in Table II. The average deviation between observed and calculated values is 0.02 log units, and the standard deviation only 0.05 log units, the latter well within our value of 0.09 log units for 1312 complexation constants, eq 3.

Note that throughout this paper, all acidities refer to solute hydrogen bond acidity. In forthcoming publications we shall point out the large differences that exist between these acidities and acidities that refer to full proton transfer.

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Direct Electrochemistry of the Undecapeptide from Cytochrome c (Microperoxidase) at a Glassy Carbon Electrode

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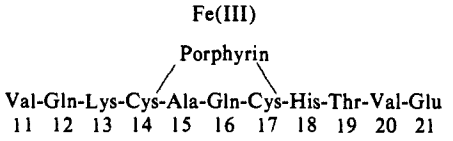
Application of cyclic voltammetry to the study of electron transfer of various metalloproteins has clarified several interesting aspects of the redox reaction of cytochrome c,[1,2] ferredoxins,[3,4] and plastocyanins.[5] In particular, direct electrochemistry of the redox process of these systems has been achieved in the presence of appropriate promoters, and some aspects of the mechanism of electron transfer between buried redox sites of the proteins and the electrode surface have been clarified.

We report here an electrochemical investigation of the undecapeptide obtained by hydrolysis of horse heart cytochrome c (called microperoxidase),[6,7] which contains iron protoporphyrin IX covalently bound by thioether bridges to Cys 14 and 17 (where the numbers refer to the amino acidic sequence of native horse heart cytochrome c).



This heme-peptide represents a good system in order to gain further information on (a) the factors controlling rapid electron transfer between a biopolymer and the electrode surface including the role played by electrostatic interactions and (b) the electrochemical behavior of the heme group when relatively unshielded by the protein but still covalently bound to cysteines and in a soluble state.[8]

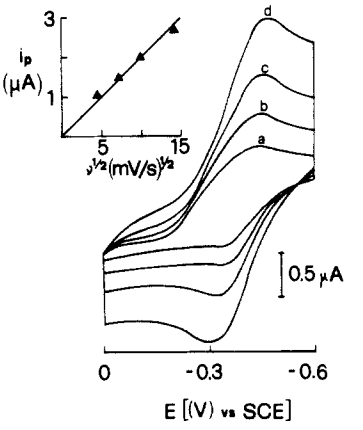


Figure 1. Dc cyclic voltammetry of microperoxidase (0.5 mg/mL) in $NaClO_4$ (0.1 M) and phosphate buffer (0.02 M) at pH 7.0, in the potential range 0 to -0.60 V vs SCE; sweep rate: (a) 20; (b) 50; (c) 100; (d) 200 mV/s. Temperature: 25 °C. Inset shows a plot of i_p vs (scan rate)$^{1/2}$.

The results reported below clearly indicate that the heme peptide undergoes rapid and reversible electron transfer at a glassy carbon electrode, with $E_{1/2}$ approximately -160 mV vs NHE (at pH 7.0 and 25 °C). The promotion of electron transfer by Mg^{2+} ions has also been investigated and found to be significant but not crucial.

Experiments were carried out in 20 mM phosphate buffer, pH 7.0, with either 100 mM sodium perchlorate or 25 mM magnesium perchlorate as supporting electrolyte. Microperoxidase was obtained from SIGMA (U.S.A.) or prepared in our laboratory following the method of Harbury and Loach.[7] An Amel 473 multipolarograph equipped with an Amel 863 recorder was used for voltammetric measurements.

Figure 1 shows the dc cyclic voltammograms of microperoxidase (0.5 mg/mL) in the presence of 100 mM Na^+. A well-defined electrochemistry is observed; the cathodic and anodic peaks are similar in shape and magnitude, with a i_{pa}/i_{pc} ratio close to unity. For a fully reversible one-electron-transfer reaction, a peak separation $\Delta E_p = 57$ mV (at 25 °C), independent of the scan rate, is expected.[9] In agreement with previous studies,[3,5] we found that ΔE_p increases with scan rate, the smallest value of about 90 mV being obtained at a scan rate of 20 mV s^{-1}. The calculated redox potential, $E_{1/2} = -160 \pm 8$ mV vs NHE, lies within the range of the potentiometric value reported by Harbury and Loach under similar conditions ($E_{1/2} \simeq -190$ mV vs NHE, at pH 7.0 and 25 °C).[8] As shown in the inset to Figure 1, i_{pc} is proportional to the square root of the scan rate; thus, the redox process of microperoxidase at the electrode surface is diffusion-controlled.[9] From the slope of the curve, a value of $D_0 = 2 \times 10^{-6}$ cm^2 s^{-1} for the diffusion coefficient was calculated.[10] Thus, the dc cyclic voltammetry shows that microperoxidase takes part in a rapid one-electron reaction at the glassy carbon electrode in the absence of mediators. In accordance with the procedure of Nicholson,[11] the rate constant for heterogeneous electron transfer was calculated to be $k_s = 3 \pm 1 \times 10^{-3}$ cm s^{-1}, based on $n = 1$, $\alpha = 0.5$ and $T = 25$ °C.

Cyclic voltammograms performed at different concentrations of microperoxidase (0.1-1 mg/mL) gave results similar to those shown in Figure 1. Therefore, no indication of gross effects related

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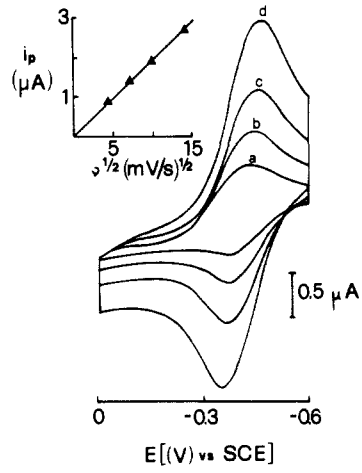

Figure 2. Dc cyclic voltammetry of microperoxidase (0.5 mg/mL) in $Mg(ClO_4)_2$ (0.025 M) and phosphate buffer (0.02 M) at pH 7.0, in the potential range 0 to -0.60 V vs SCE; sweep rate: (a) 20; (b) 50; (c) 100; (d) 200 mV/s. Temperature: 25 °C. Inset shows a plot of i_p vs (scan rate)$^{1/2}$.

to aggregation of oxidized or reduced microperoxidase was obtained.

It has been reported[3,5] that Mg^{2+} acts as a promoter for the electron-transfer process by forming a transient bridge between a negatively charged macromolecule and the electrode surface. Since both microperoxidase at neutral pH (pI = 4.7) and the glassy carbon surface, under the experimental conditions employed,[12,13] are negatively charged, the role of Mg^{2+} has been investigated. Dc cyclic voltammograms of microperoxidase in the presence of 25 mM Mg^{2+}, shown in Figure 2, indicate a reversible electrode process. Under these conditions, both the cathodic and anodic waves are better resolved with respect to the experiments carried out in 100 mM Na^+, and the electron-transfer reaction is reversible, ($i_{pa}/i_{pc} \simeq 1$). As expected, these results demonstrate some role of the electrostatic interactions in controlling the formation of a favorable transient complex between the electrode surface and the heme-peptide; given the negative charge of microperoxidase at neutral pH, the presence of divalent ions (e.g., Mg^{2+}) surely facilitates electron transfer. Even under these conditions, the process is diffusion-controlled (see inset of Figure 2); the calculated diffusion coefficient and rate constant have values similar to those obtained in the presence of 100 mM Na^+. Thus, Mg^{2+} affects only some aspects of the electrochemistry of microperoxidase, such as the reversibility, while the electron-transfer rate remains unaffected.

It has been reported[8] that the microperoxidase–imidazole complex exists in solution as monomer, independent of pH, while unliganded microperoxidase tends to aggregate at alkaline pH. Therefore, cyclic voltammograms were also obtained in the presence of 50 mM imidazole, which coordinates as a ligand of the heme iron. The results (not shown) in the presence of Na^+ or Mg^{2+} were very similar to those illustrated in Figures 1 and 2, respectively; moreover, in accordance to what was reported by Harbury and Loach in their potentiometric studies on the same system,[8] a more negative value for $E_{1/2}$ (-200 ± 5 mV) was obtained.

Microperoxidase, the undecapeptide of cytochrome *c* with molecular mass of about 1900, represents a good system to study the reversibility of the reaction at the electrode surface, and it is a source of information on the electrochemical behavior of the heme iron when unshielded by a large polypeptide, as it happens in the case of all hemoproteins. For metalloproteins, rapid electron transfer at the electrode is best achieved in the presence of appropriate promoters, such as 4-4′ bipyridyl for cytochrome *c*,[2] which increase the electron-transfer rate by favoring correct orientation of the metalloprotein. Cyclic voltammetry of microperoxidase shows that rapid, reversible electron transfer at the electrode is achieved even in the absence of mediators or promoters. Of course, this behavior is to be associated with the simpler and disordered conformation of microperoxidase in which the heme, more exposed to the solvent, is facilitated to exchange electrons. Thus, these results strongly support the notion that, in metalloproteins, a buried metal site is the major hindrance to a rapid reversible electrochemical behavior. Under appropriate conditions, the exposed heme of microperoxidase may serve the role of a specific promoter between the electrode surface and metalloproteins in solution.

The redox potentials determined for microperoxidase are in good agreement with previous data obtained by Harbury and Loach[8] by potentiometry. Comparison with these results shows that cyclic voltammetry is extremely rapid, does not require the use of mediators or stepwise addition of reductants, and yields additional information on reversibility and rates of electron transfer.

The negative $E_{1/2}$ values of microperoxidase provide additional evidence for the significance of the degree of exposure of the heme to the solvent in affecting its redox potential. In fact, a higher degree of heme exposure lowers the redox potential of a hemoprotein,[14] as recently confirmed by an electrochemical investigation on carboxymethylated cytochrome *c*, whose redox potential ($E_{1/2}$ = -250 mV vs NHE) was found to be approximately 0.5 V lower than that of native cytochrome *c* ($E_{1/2}$ = +250 mV).[15]

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Potential Building Blocks for Molecular Ferromagnets: $[Mn_{12}O_{12}(O_2CPh)_{16}(H_2O)_4]$ with a $S = 14$ Ground State

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Several groups are currently trying to prepare ferromagnetic organic,[3-8] organometallic,[9] and inorganic[10-12] materials. Mataga

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