Direct electrochemistry of horseradish peroxidase adsorbed on glassy carbon electrode from organic solutions

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Using a 'solvent engineering' approach, the direct electrochemistry of catalytically active horseradish peroxidase (HRP) adsorbed on glassy carbon electrodes from HRP–DMSO and HRP–formamide solutions has been readily obtained in aqueous phosphate buffer (pH 7.0), with a surface formal potential of −0.365 V *vs.* Ag/AgCl and an apparent electron transfer rate constant of 0.655 s^{-1}.

Study of the direct heterogeneous electron transfer of proteins and enzymes is a convenient and informative means for understanding the kinetics and thermodynamics of biological redox processes.[1] Work on direct electron transfer involving biomolecules has largely focused on relatively small proteins,[2] most notably cytochromes, microperoxidases, ferredoxins and myoglobin. Direct electron transfer between the electrode and the prosthetic group of catalytically active protein-enzymes possessing a considerably greater molar mass is prevented by exceedingly large electron hopping distances, by improper orientation of the adsorbed enzyme, or by adsorptive denaturation.[3a] Mediators or promoters are generally employed to study the electron-transfer kinetics of large proteins.[3] We report here preliminary results on the use of 'solvent engineering' for the direct electrochemistry of *catalytically active* horseradish peroxidase (HRP). 'Solvent engineering' has attracted much interest in nonaqueous enzymology in the last decade.[4] Many interesting findings and advances have been made in these areas.[5] Here we demonstrate the feasibility of this approach in bioelectrochemistry.

Amperometric peroxidase-modified electrodes have been one of the most extensively studied biosensors for the detection of hydrogen peroxide, organic hydroperoxides, phenols, aromatic amines and hazardous compounds. Many of these sensors are reported to be based on an apparent direct electron transfer between the immobilized peroxidase and the electrode surface.[6] Only one observation of the direct redox process of HRP adsorbed on electrochemically activated carbon electrodes has been claimed,[7] providing only the redox potentials in the range of −0.33 to −0.25 V *vs.* SCE. Little is known of the rate of electron transfer of HRP at electrode surfaces. Using the 'solvent engineering' approach we have readily obtained the direct electrochemistry of HRP adsorbed on glassy carbon electrodes by cyclic voltammetry.

The glassy carbon electrodes (BAS, d = 1.6 mm) were polished with 0.05 μm Al_2O_3 and 1 micron diamond paste, respectively, sonicated in water, acetone and water successively, then dried in air at room temperature. Horseradish peroxidase solutions (2 mg ml^{-1}) were prepared by dissolving HRP (E.C. 1.11.1.7, 90 units mg^{-1}, Sigma) in pure DMSO or formamide (FA). The glassy carbon electrodes were soaked in these solutions for 15 min, then rinsed with copious amounts of phosphate buffer (pH 7.0, 20 mM) and ultrapure water. It has been demonstrated that enzymes (*e.g.* trypsin, chymotrypsin, lysozyme, ribonuclease) dissolved in pure organic solvents, such as DMSO and FA, do not inactivate irreversibly.[8] Nearly all original enzymatic activity is restored from organic solution not only upon immediate dilution with aqueous buffer but even after a 24 h incubation of the enzyme in organic solvents at 25 °C. Voltammograms were recorded with a BAS Electroanalyzer 100W/B interfaced to a 33 MHz Gateway 2000 computer. All the voltammetric experiments were performed with Ag/AgCl (3 M NaCl) reference and platinum auxiliary electrodes in 20 mM potassium phosphate buffer at pH 7.0.

Fig. 1 shows a typical cyclic voltammogram obtained for HRP adsorbed on a glassy carbon electrode from HRP–DMSO solution. The modified electrodes are quite stable, with little change in the voltammogram during cycling, as demonstrated by the first cycle and the fiftieth cycle shown in Fig. 1. The broadness of these peaks suggests an HRP adsorbate distribution[9] perhaps resulting from multiple conformations of adsorbed HRP from the organic solution or differential adsorption energies, and the full-width at half-maximum (E_{FWHM}) is 0.175 V. The redox potentials are in the range of −0.3 to −0.42 V with scan rate varying from 20 to 500 mV s^{-1}, and the surface formal potential [$E^{\circ\prime}$ = (E_{pc} + E_{pa})/2] was around −0.365 V. The position of the cathodic maximum is close to the value of the redox potential of the Fe^{2+}/Fe^{3+} electron transfer in the active centre of peroxidase obtained by potentiometric studies (−0.3 to −0.45 V).[1c,10] The peak current I_p was found to be proportional to scan rate (v), indicative of a surface-confined redox process. The surface coverage (Γ) was calculated to be 5.2×10^{-11} mol cm^{-2}, consistent with the high intensity of the nitrogen signal in the X-ray photoelectron spectra of the modified electrodes. From the variation of E_{pc} with v,[11] an apparent electron transfer rate constant k_s was calculated to be 0.655 s^{-1}.

Besides DMSO, direct electrochemistry of HRP has been also observed for electrodes modified in HRP–FA solution, with $E^{\circ\prime}$ = −0.348 V, E_{FWHM} = 0.190 V, Γ = 4.4×10^{-11}

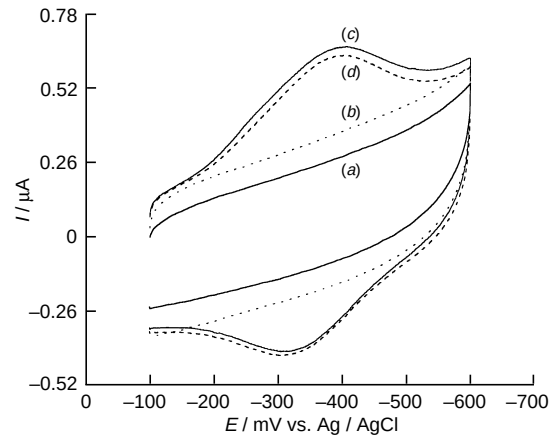

Fig. 1 Cyclic voltammograms of a glassy carbon electrode at a scan rate of 100 mV s^{-1} before and after soaking for 15 min in pure DMSO or in 2 mg ml^{-1} HRP–DMSO solution: (*a*) before soaking, (*b*) after soaking in DMSO, (*c*) the first cycle after soaking in HRP–DMSO, (*d*) the fiftieth cycle after soaking in HRP–DMSO. The supporting electrolyte is 20 mM potassium phosphate buffer at pH 7.0.

mol cm^{-2} and k_s = 0.60 s^{-1}. Considering the distinctly different characters of DMSO and FA toward proteins, that is, DMSO is destructive to a protein's structural integrity[12] while FA is a solvent similar to water where there exists solvophobic interactions,[13] we might expect to observe different conformations for HRP in DMSO and in FA, and it is surprising to find that the electrochemical performance of HRP adsorbed on glassy carbon electrodes from HRP–DMSO and HRP–FA solutions is nearly the same. However, considering the reversible inactivation of dissolved enzymes in organic solvent and the complete restoration of their original catalytic activities upon dilution, these results are reasonable and expected. It is reported that a higher degree of haem exposure lowers the redox potential of a haemoprotein,[14] and there is only a few millivolt difference in the redox potentials of HRP adsorbed from HRP–DMSO and HRP–FA solutions, indicating that the restored conformation of the adsorbed enzyme upon dilution with aqueous buffer is independent of the solvent from which it was adsorbed. Furthermore, the heterogeneous electron transfer rates for HRP adsorbed on a glassy carbon electrode from organic solutions are very close to that of HRP adsorbed on a graphite electrode from aqueous solution (0.66 s^{-1}), which was obtained as the rate of electron transfer of the electrocatalytic reduction of H_2O_2 in phosphate buffer by rotating disk technique.[15] This suggests that the enzyme conformation and catalytic activity might be the same as that when the enzyme is adsorbed from aqueous solutions. As expected, these modified electrodes show catalytic activity for the reduction of hydrogen peroxide, with an onset potential at around 0.0 V and levelling off at around −0.25 V. When the peroxide inhibitor KCN was introduced into the buffer solution, the peaks of the current–voltage curve disappeared and no catalytic activity was observed after H_2O_2 addition. As we noticed a small direct reaction occurs between peroxide and the naked electrode and cyanide poisons the electrode, but the current on the enzyme modified electrode is much higher and the biocatalytic process is inhibited substantially by cyanide. The facts above demonstrate that the redox process of the *active* enzyme's prosthetic group has been obtained by cyclic voltammetry using 'solvent engineering'. The exact role of the organic solvents is not clear at present. Proteins dissolved in organic solvents are at least partially unfolded, resulting in a more intimate contact of the proteins with the electrode surface, a shorter electron transfer distance and a favourable orientation for the restored conformation upon rinsing with aqueous buffer. That is, one of the significant advantages of this approach is the possibility of reorganization of the conformation of adsorbed proteins on electrode surface from their organic solutions. Another possibility is to operate protein modification in protein-dissolving organic solvents, allowing inner core modification on the unfolded proteins. The latter has yet to be exploited.

In summary, we have demonstrated the feasibility of using a 'solvent engineering' approach to study the electron transfer reaction of adsorbed HRP. Applications for the direct electrochemistry of other large proteins and enzymes, and the construction of mediatorless biosensors, can be readily envisaged.

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Footnote

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