Polypeptide-modified Indium Oxide Electrodes for Direct Electron Transfer of Ferredoxin

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Indium oxide, surface modified with a polypeptide, *e.g.* polylysine or polyornithine, is an effective promoter-modified electrode for direct quasi-reversible electron transfer of ferredoxin.

Protein electrochemistry has been a subject of extensive study in recent years.[1] However, many proteins still cannot undergo rapid electron transfer at an electrode. Here, cationic polypeptides are easily immobilized onto an indium oxide electrode to give effective and stable promoter-modified electrodes for ferredoxin electrochemistry.

Electrochemical studies of both plant |2Fe–2S| and bacterial |4Fe–4S| ferredoxins have been examined by several groups.[2–5] In the presence of such polycations as $Cr(NH_3)_6^{3+}$, polylysine and aminoglycosides in solution, ferredoxins have been reported to give quasi-reversible responses at Hg and carbon electrodes.[2–4] Recently, we have shown[5] that some cationic polypeptides are effective promoters, giving well-defined redox waves on an In_2O_3 electrode for both spinach and maize ferredoxins. However, no effective promoter-modified electrode for ferredoxin has so far been obtained, although methyl viologen modified gold minigrid[6] and gold electrodes on which bis(L-lysyl)-L-cystein was immobilized[7] providing cationic surfaces have been examined. Because very small amounts of cationic polypeptides gave well-defined voltammograms of ferredoxin at an In_2O_3 electrode and also because polylysine was reported to adsorb onto a tin oxide electrode,[8] preparation of a polypeptide modified In_2O_3 electrode would be promising.

Spinach ferredoxin was extracted from spinach leaves and purified by chromatography.[9] The surfaces of the In_2O_3 electrodes (*ca.* 5 × 5 mm, from Kinoene Optics Co., Japan or Donnelly Corp., USA) were cleaned by ultrasonication in a New Vista (the anionic surfactant, AIC Corp.) solution followed by washing in ethanol and then in water.[10] Polypeptide-modified electrodes were then obtained by immersing the In_2O_3 electrode for *ca.* ten minutes (or a little more) into a 10 mmol dm^{-3} tris-HCl buffer solution (pH 7.2) containing 0.33 mol dm^{-3} NaCl and 1 mg ml^{-1} polypeptide. Neither a longer modification time nor the modification at a low ionic strength improved the electrochemical response of ferredoxin. Cyclic voltammograms were obtained at functional electrodes using a Toho-Giken 2020/2130 potentiostat with a function generator in tris-HCl or Britton and Robinson buffer solutions containing 0.33 mol dm^{-3} NaCl at various pHs. An Ag/AgCl (saturated KCl) was used as a reference electrode, but potentials are given with respect to a normal hydrogen electrode (NHE) by adding the difference of 206 mV (at 10 °C) between these electrodes. Other experimental details are similar to those described elsewhere.[5,10]

Fig. 1 shows the typical cyclic voltammograms of ferredoxin obtained after repetitive cycles at polypeptide modified In_2O_3 electrodes with no promoter in solution. The peak currents obtained were proportional to the square root of scan rates (at least up to 0.3 V s^{-1}) and to the concentration of ferredoxin (at least up to *ca.* 200 μmol dm^{-3}) examined. The voltammograms were stable against repetitive cycles for more than 10 h. By washing the electrode with a strongly acidic (*e.g.* pH 1) solution, adsorbed polypeptide gradually left the electrode surface. Otherwise, the modified electrodes were rather stable and worked durably. Use of lysine and ornithine monomers gave no effective modified electrode. The degree of polymerization of the modifiers did not alter significantly when polypeptides having mean molar masses of more than 10 000 g were used to modify the electrode surface. When the polypeptides were in solution, the electrochemical responses of ferredoxin depended on both the amounts and the molar masses of the added polypeptides. Thus, the modified electrodes prepared are easy to use. Poly-L-arginine, which acts as an effective promoter when in solution, did not give a stable modified electrode. On carbon electrodes including the edge plane of pyrolytic graphite, no stable adsorption of polylysine and polyornithine was observed. Also, when poly-L-lysine, having a molar mass of more than 20 000 g in a strongly alkaline solution, in the α-helical form with no positive charge was immobilized onto an In_2O_3 electrode, the modified electrodes obtained were again effective, although the response was a little less-defined.

After subtraction of background currents, voltammograms obtained were analysed using digital simulation. From the best-fit simulated voltammograms on the basis of the macroscopic model, the formal redox potential, $E^{o\prime}$, the diffusion coefficient, D, the formal heterogeneous electron transfer rate constant, $k^{o\prime}$, and the transfer coefficient, α, of ferredoxin at

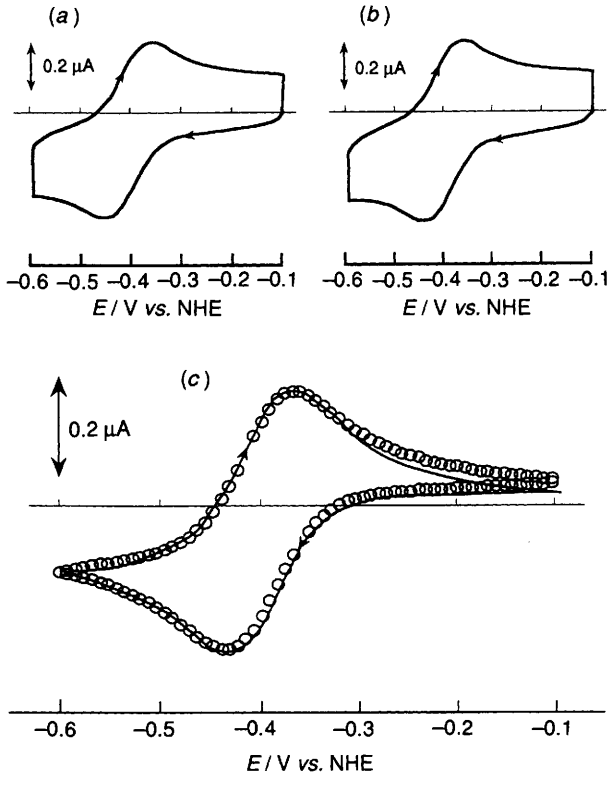

Fig. 1 Typical cyclic voltammograms of 50 μmol dm^{-3} spinach ferredoxin at In_2O_3 electrodes modified with (*a*) poly-L-lysine and (*b*) poly-L-ornithine having mean molar masses of 25 000 g and 23 000 g, respectively, in a tris-HCl buffer solution containing 0.33 mol dm^{-3} NaCl (pH 7.2) at 10 °C under a N_2 atmosphere, and (*c*) the background subtracted voltammogram of ferredoxin at a poly-L-lysine modified In_2O_3 electrode with simulated data shown by circles. Voltammograms shown were obtained after repetitive cycles and the simulation was made by using the values shown in the text. Scan rate: 20 mV s^{-1}.

the promoter-modified In_2O_3 electrodes were evaluated to be -0.395 ± 0.005 V (*vs.* NHE), $0.7 \pm 0.05 \times 10^{-6}$ cm^2 s^{-1}, $2.7 \pm 0.5 \times 10^{-3}$ cm s^{-1}, and 0.5 ± 0.05, respectively, in a 10 mmol dm^{-3} tris-HCl buffer solution containing 0.33 mol dm^{-3} NaCl (pH 7.2) at 10 °C. The $E^{\circ\prime}$ and D values obtained are in good agreement with those reported for ferredoxins.[2,6] The observed $k^{\circ\prime}$ value is the largest among those so far reported for ferredoxin using modified electrodes. No significant difference was observed for these values at either poly-L-lysine or poly-L-ornithine modified In_2O_3 electrode. Also, use of poly-L- and poly-D-lysine showed similar results to each other. At these electrodes, almost the same voltammograms of ferredoxin were obtained in the pH region of 6 to 9.5, where no structural change of ferredoxin was seen from UV–VIS and circular dichroism (CD) spectra.

Since the present electrodes have a rather wide optical window (> *ca.* 300 nm; the limit is due to the absorption of an In_2O_3 thin film on a quartz plate, polypeptides are optically transparent in this region), various spectroelectrochemical studies including CD and magnetic CD (MCD) measurements can be easily carried out. Also, these electrodes can be used for ferredoxins of various origins with different redox potentials, because such polypeptides themselves are electroinactive. Moreover, various bioelectrocatalytic reactions are designed with the aid of enzymes using ferredoxin as electron transfer mediator, like in photosynthetic processes.

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