Novel Method for the Investigation of the Electrochemistry of Metalloproteins: Cytochrome c

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Summary The d.c. and a.c. cyclic voltammetries of horse heart ferricytochrome c have been investigated and it is shown that, in the presence of 4,4′-bipyridyl, the electrochemistry corresponds to a quasi-reversible one-electron process, from which an E° value of $+0{\cdot}25$ V *vs.* normal hydrogen electrode can be derived.

OF all the methods used to investigate the redox properties of metalloproteins those involving electron transfer from an electrode to the metalloprotein would appear to be the most direct. Unfortunately it has proved difficult[1] to overcome a number of problems, particularly adsorption of the protein on the electrode and the slow rate of electron transfer. Recently, in attempts to avoid these problems much effort has been expended[2] on the construction of chemically modified electrodes. In the belief that it should be possible to modify the double layer on the electrode by the addition of an effector for electron transfer,

we investigated the use of 4,4′-bipyridyl, a molecule known[3] to promote electron transfer between transition-metal complexes, on the electrochemistry of horse heart ferricytochrome c.

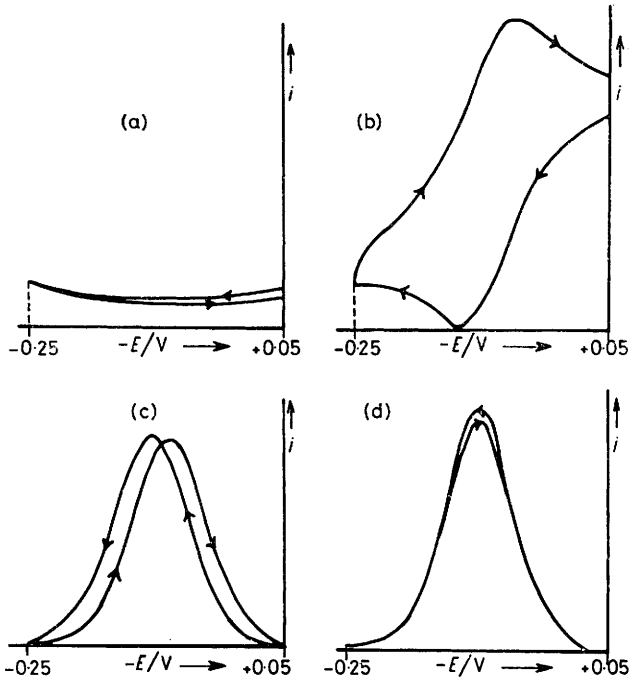

FIGURE. Cyclic voltammograms of an aqueous solution of horse heart ferricytochrome c (2 mM), containing $NaClO_4$ (0·1 M), and Na_2HPO_4 (0·02 M), pH 7, at a planar gold disc electrode in the potential range + 0·05 to −0·25 V *vs.* 0·1 M KCl–calomel ($E°$ + 0·336 V *vs.* NHE). (a) A.c. cyclic voltammogram at a scan rate of 10 mV s^{-1} in the absence of 4,4′-bipyridyl; (b) d.c. cyclic voltammogram at a scan rate of 10 mV s^{-1} in the presence of 4,4′-bipyridyl (5 mM); (c) a.c. cyclic voltammogram at a scan rate of 10 mV s^{-1} in the presence of 4,4′-bipyridyl (5 mM); (d) a.c. cyclic voltammogram at a scan rate of 2·5 mV s^{-1} in the presence of 4,4′-bipyridyl (5 mM).

The a.c. cyclic voltammogram of horse heart ferricytochrome c is shown in the Figure (a) and, like its d.c. equivalent, is indistinguishable from background. On addition of 4,4′-bipyridyl to the solution both the d.c. (Figure, b) and the a.c. (Figure, c and d) cyclic voltammograms of the resultant solution show the presence of an electroactive species. 4,4′-Bipyridyl itself is not electroactive in this potential region. At low scan rates the peak-to-peak separation in the d.c. voltammogram (Figure, b) of 60 mV is that expected for a reversible one-electron process. The more demanding criteria[4] of reversibility for a.c. cyclic voltammetry, *viz.* that the forward and reverse sweeps should overlap and that the width at half peak height should be 90 mV, are almost satisfied at slower scan rates (Figure, d). Therefore in the presence of 4,4′-bipyridyl electron transfer between cytochrome c and the electrode is fast. We assume that 4,4′-bipyridyl modifies the double layer by interacting with the electrode and/or the protein though, if the latter, not by co-ordinating to the iron. Using 0·1 M aqueous KCl–calomel as reference electrode and 0·1 M KCl as supporting electrolyte it was possible to obviate any junction potential and the $E°$ value determined for cytochrome c was +0·25 V *vs.* normal hydrogen electrode (NHE), which compares very well with values obtained by other methods.[5]

Amongst other possible effectors investigated only 1,2-bis-(4-pyridyl)ethylene was as effective as 4,4′-bipyridyl, 1,2-bis-(4-pyridyl)ethane having no effect. The effect of the pH on the electron transfer was also investigated. It was found that the electroactivity diminished as the pH value was increased towards the isoelectric point (pH 10) of the protein, suggesting that the protein charge may affect the efficiency of electron transfer at the electrode.

Preliminary results indicate that the method is applicable to the multi-heme protein cytochrome c_3 from *Desulfovibrio desulfuricans* (*Norway*) and the copper proteins, bovine erythrocyte superoxide dismutase, azurin from *Pseudomonas aeruginosa*, and laccase from *Rhus vernicifera*.

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