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TOWARDS THE DESIGN OF ELECTROCHEMICAL INTERFACES WHICH PERMIT RAPID AND SPECIFIC HETEROGENEOUS ELECTRON TRANSFER TO REDOX ENZYMES

As a consequence of low electron-transfer probabilities across long distances, rates of redox processes involving biological molecules and membranes may depend upon the promotion of relatively long-lived intermolecular interactions. Selective lowering of activation requirements, through non-covalent stabilisation of molecular orientations which effectively minimise donor-acceptor separation, can provide the basis for recognition and regulation in electron transfer. We are currently approaching the study of these processes through the design of «model» electrochemical interfaces which permit the observation of direct (unmediated) protein electrochemistry. Pyrolytic graphite (PG) has proved to be an intriguing electrode material at which a wide range of redox proteins exhibit well-behaved quasi-reversible electrochemistry upon appropriate «tuning» of solution conditions. The various surfaces of this anisotropic material exhibit widely differing properties.

In a recent communication [1] we drew attention to the contrasting electrochemical reactivity of mitochondrial cytochrome *c* at polished «edge» and freshly cleaved «basal plane» surfaces of PG. Electrochemistry of cytochrome *c* at the hydrophilic «edge» is well-behaved, whereas at the hydrophobic «basal plane», a largely irreversible response is obtained. In subsequent surface analysis with ESCA we have been able to establish that a high (*ca.* 50%) surface coverage of various oxygen functionalities (probably including phenols and carboxyls) is readily generated at the «edge» upon polishing in air. By contrast the oxygen coverage at a freshly cleaved «basal» surface is very low.

We have now investigated the electrochemical effects of «edge» surface protonation equilibria through titrations of the faradaic current observed at constant overpotential using the rotating disc electrode technique. We have examined the responses of cytochrome *c* (overall positive charge) and spinach plastocyanin (overall negative charge) over the pH range 4-8 (addition of acid or base components to acetate-MES-Tris buffer with 1 mM NaCl background) and compared these with the response of a «marker» probe $Fe(CN)_6^{3-/4-}$. Results are shown in Fig. 1.

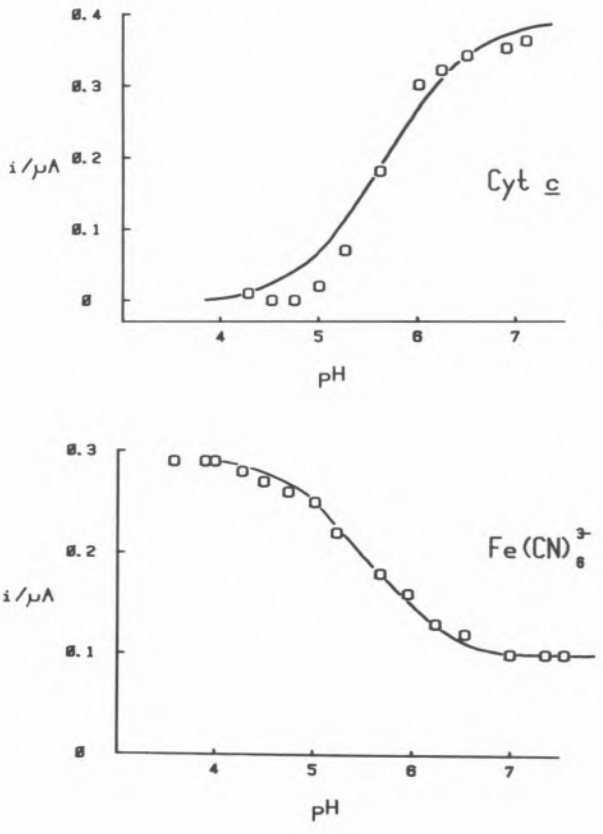

Fig. 1

Current, which registers fast heterogeneous electron transfer to cytochrome *c*, is clearly «switched off» as the pH drops below 6. By contrast, electron-transfer to plastocyanin or $Fe(CN)_6^{3-/4-}$ (which itself exhibits no intrinsic protonation

equilibria within the range pH 4-8) is «switched on». From these observations we conclude that productive interactions between protein and the hydrophilic «edge» surface may be regulated simply and effectively by electrode surface protonation.

We have previously shown [2] that direct electrochemistry of several negatively charged proteins could be promoted at polished PG by the addition of multivalent cations such as Mg^{2+} or $Cr(NH_3)_6^{3+}$. In order to define the relationship between protein, cation and surface more closely, we have examined the behaviour of «edge» graphite surfaces that have been covalently modified with a variety of Cr(III) complexes. As an illustration of this, voltammetric cycling through potential domains < -800 mV vs. SCE, which induces reduction of $Cr(NH_3)_6^{3+}$ in concentrated aqueous NH_3, results in the rapid formation of stable Cr(III)-surface complexes. The Cr(III) coverage with reference to carbon, as analysed by ESCA, is 5-10%. The resulting electrode surface is now *inactive* towards cytochrome *c* at pH 8. However, this electrode (but not one with which Cr(III) precycling has not been taken below -800 mV to activate Cr(II-III) «lock-on») shows *well-behaved* electrochemistry with plastocyanin. The roles of surface hydrophobicity and charge in the promotion of fast heterogeneous electron-transfer reactions of proteins are thus clearly evident. Our views on charge may be summarised in Fig. 2, in which a «charge-modified edge» surface (H^+ or discrete Cr(III) sites) is compared with the «native edge», with regard to the responses displayed by cytochrome *c* (positive charge around heme edge) and plastocyanin (negative charge conservatively localised at side of molecule).

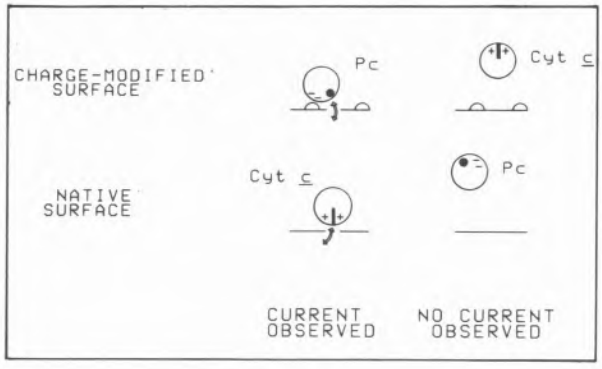

Fig. 2

Experiments currently in progress are directed towards a closer definition of these interactions and, in particular, a detailed understanding of specific charge and steric requirements for efficient and selective electron transfer.

REFERENCES

[1] F.A. ARMSTRONG, H.A.O. HILL, B.N. OLIVER, *J. Chem. Soc., Chem. Commun.*, 976, (1984).

[2] F.A. ARMSTRONG, H.A.O. HILL, B.N. OLIVER, N.J. WALTON, *J. Am. Chem. Soc.*, **106**, 921 (1984).

PS2.15 — TH

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EPR STUDIES ON THE FUNCTION OF THE 16, 24 AND 33 kDa SUBUNITS ON THE PHOTOSYSTEM II DONOR SIDE

The three extrinsic subunits (16, 24 and 33 kDa) of Photosystem II (PS II) do not contain any prosthetic groups, but are nevertheless necessary for the function of the oxygen-evolving complex (OEC). The effect of their selective removal from oxygen-evolving PS II particles was examined by monitoring the multiline manganese EPR signal and the decay time of the oxidized secondary donor, Z^+ (EPR Signal II). After washing with 1 M NaCl, which removes the two lighter proteins, only a small Signal II_f is observed. This indicates that rapid electron transfer from the OEC to Z still occurs, *i.e.* that some S state transitions are still functional, despite the loss of oxygen evolution. However, the multiline signal of state S_2 of