Surface Selectivity in the Direct Electrochemistry of Redox Proteins. Contrasting Behaviour at Edge and Basal Planes of Graphite

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A comparison of the direct electrochemistry of cytochrome *c*, ferredoxin, and rubredoxin at edge and basal planes of pyrolytic graphite shows heterogeneous electron transfer to be catalysed at the edge surface indicating the importance of specific protein–electrode interactions.

It has recently been shown[1—5] that certain electrodes allow rapid direct electron-transfer to redox proteins. In a previous report[1] we described the electrochemistry of a range of redox proteins at polished basal plane pyrolytic graphite. The layer structure of pyrolytic graphite, however, provides two highly distinctive types of surface: the parallel (basal plane) face with, ideally, satisfied carbon valences and the edge face at which some additional surface structure must be present. The nature of such surface structure has been probed by E.S.C.A.[6—8] and by examination of chemical reactivity.[9] From these investigations it is clear that various oxidative pretreatment procedures, including polishing in the presence of air, generate a variety of C–O groups derived predominantly from edge carbon atoms. In this communication we demonstrate that direct electron transfer between redox proteins and pyrolytic graphite electrodes is critically dependent upon the orientation of the graphite and presumably the presence of surface oxidised functional groups.

Electrodes were prepared using 5 mm diameter discs cut from the edge or basal surface of standard pyrolytic graphite (Le Carbone Lorraine, Paris). For the basal surface the disc was mounted on a glass capillary tube with the edge surface masked by silicone rubber. Electrical contact was achieved through an internal Pt wire–Hg contact. A fresh basal surface, having a grey mottled appearance, was prepared by cleaving with a sharp cutting edge. The edge disc was sealed in a Teflon electrode housing and routinely polished using an alumina (0.3 μm particle size)–water slurry followed by extensive sonication in doubly deionised water. The surface of an edge disc polished in this way has a black reflective finish and is more readily wetted than a freshly cleaved basal surface. The electrochemical apparatus used has been previously described.[2]

The cyclic voltammograms of cytochrome *c* at edge and basal electrodes are quite different (Figure 1). At the edge electrode the response is stable and well defined; in contrast, the peak current recorded for the basal surface is at least 2-fold lower than at the edge. At a scan rate of 20 mV s^{-1} peak separations were typically 60 ± 5 and >150 mV respectively. A plot of cathodic peak current *vs.* square root of scan rate for the edge surface was linear up to 100 mV s^{-1} and gave a diffusion coefficient of 5.0×10^{-7} cm^2 s^{-1} based on the geometric area of the electrode surface. The redox potential calculated from the cyclic voltammogram was +272 mV *vs.* the normal hydrogen electrode (N.H.E.). Both these values are in good agreement with published values.[10,11] It is possible that some edge planes are exposed on cleaving pyrolytic graphite and these may contribute to the residual electrochemistry at the basal plane.

We have estimated[12] values for the standard heterogeneous rate constant for electron transfer (k_s) at the two types of surface. A typical value of k_s obtained for the edge surface is 4.5×10^{-3} cm s^{-1} while at the basal surface k_s is estimated to be lower than 2.5×10^{-4} cm s^{-1}.

We have observed that electron-transfer rates are similarly enhanced for *Clostridium pasteurianum* 2[4Fe-4S] ferredoxin and rubredoxin at an edge oriented electrode. As described previously,[1] for both these proteins there is an additional requirement for the presence of cation promoters. Typical peak separations observed at the edge in the presence of 4 mM $Cr(NH_3)_6^{3+}$ were 75 mV (ferredoxin) and 68 mV (rubredoxin) at 20 mV s^{-1}.

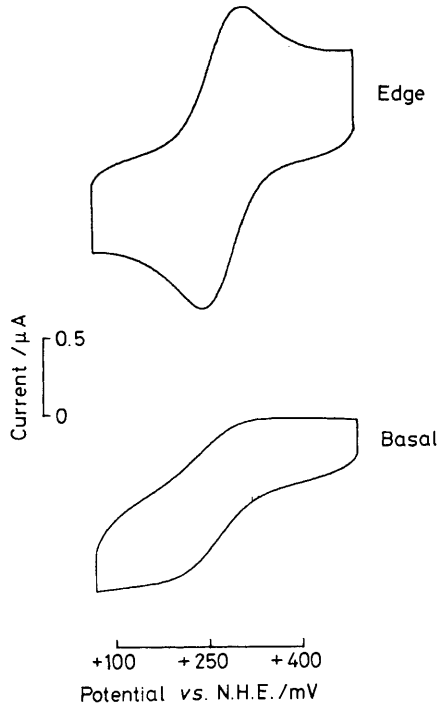

Figure 1. Steady-state D.C. cyclic voltammograms of cytochrome *c* at edge and basal plane pyrolytic graphite electrodes: 0.15 mM cytochrome *c* in 5 mM Tricine, 100 mM NaCl, pH 8.0, scan rate 20 mV s^{-1}.

Contrasting behaviour of edge and basal planes of pyrolytic graphite has been previously reported.[13—15] However, most investigations have focussed upon the basal surface and various attempts have been made to enhance the population of functional groups through chemical, electrochemical, thermal, and plasma treatments. Our results show that a reproducible oxidised surface, as characterised by supporting E.S.C.A. experiments,[6] may be generated merely upon polishing in air. The importance of surface oxidised functional groups localised at the edge plane, which are likely to impart considerable hydrophilicity and ionic character to the surface, is consistent with the hypothesis upon which these studies are based; to elicit the electrochemistry of redox proteins it is necessary that they bind at the electrode surface. In most cases this depends on electrostatic interactions between the electrode and regions on the proteins' surface.

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