Rapid Modification of Graphite Electrodes by Surface-bound Chromium Complexes: Preparation of an Electrode for Direct (Unmediated) Electrochemistry of the 'Blue' Copper Protein, Plastocyanin

Fraser A. Armstrong,* P. Anthony Cox, H. Allen O. Hill, B. Nigel Oliver, and Adrian A. Williams

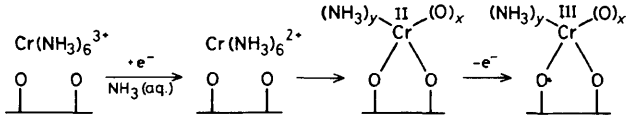
Inorganic Chemistry Laboratory, Oxford University, South Parks Road, Oxford OX1 3QR, U.K.

The contrasting substitutional reactivities of chromium(II) and chromium(III) have been exploited electrochemically to incorporate stable chromium(III) complexes at the surface C–O functional groups of 'edge' oriented pyrolytic graphite: the modified electrode binds the 'blue' copper protein plastocyanin reversibly, promoting persistent and well-behaved *direct* electrochemistry.

While redox proteins do not generally exhibit well-behaved unmediated electrochemistry at conventional electrodes, recent studies[1—3] have demonstrated the ease with which this may be achieved through appropriate design of electrode surfaces and electrolyte conditions which permit rapid and reversible protein–electrode interaction. The polished 'edge' surface of pyrolytic graphite contains a high coverage of C–O functionalities[4] which can engage the lysine-rich haem-edge region of mitochondrial cytochrome *c* and accordingly promote electrochemistry. For proteins bearing negatively-charged interaction domains, binding is promoted by free multivalent cations including Mg^{2+} and $[Cr(NH_3)_6]^{3+}$.[1—3] In our quest to understand more closely the inter-relationship between protein, electrode, and cations, we have sought to confine cationic centres to the electrode surface. Here we report a novel method for the rapid surface modification of edge-oriented graphite with chromium(III) species which permits persistent, and well-behaved, *unmediated* electrochemistry of the negatively-charged photosynthetic 'blue' copper protein, plastocyanin.

The basis of the modification technique is the rapid formation (*via* the electrochemical generation of reactive, substitution-labile Cr^{II} species) of substitution-inert Cr^{III} complexes incorporating electrode surface C–O groups. A projected reaction, Scheme 1, is outlined, where surface functionalities and co-ordinated H_2O are represented generally as –O. The complex $[Cr(NH_3)_6]^{3+}$ is used,[5] thus permitting surface modification to be carried out in concentrated aqueous ammonia without complications due to insoluble Cr^{III} hydroxy species. The use of aqueous ammonia ensures deprotonation of acidic surface groups, and the competition of NH_3 with H_2O (OH^-) for the remaining chromium co-ordination sites is expected to minimise extensive formation of lower-charged polymers through olation and oxolation.[6]

Electrode discs (5 mm) were cut from standard pyrolytic graphite (Le Carbone, Portslade, Sussex) with the *a–b* (basal) plane perpendicular to the disc face, and sealed in a Teflon electrode sheath. For parallel studies in which modification was examined by *X*-ray photoelectron spectroscopy, a 9 mm disc was mounted in a PVC girdle and electrical contact was achieved by insertion of a stainless steel needle into the intersection. Spectra were obtained with an ESCALAB 5 spectrometer (VG Scientific, U.K.) with a Mg-$K_{\alpha(1,2)}$ excitation source (1253.6 eV). The electrochemical apparatus was as described elsewhere.[3]



Scheme 1

At an unmodified electrode, and with low background electrolyte levels, *e.g.*, 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES)–1 mM KCl, no electrochemical response from spinach plastocyanin is detectable at pH 7; voltammograms are identical to those of buffer alone.[3] This observation is consistent with the expected presence of unshielded coulombic repulsion between protein and electrode. With increased electrolyte levels, *e.g.* 0.1 M KCl, a plastocyanin response is observed but this is poor and short-lived.

Following electrode pretreatment by single-scan reductive cycling in a concentrated aqueous ammonia solution of $Cr(NH_3)_6Cl_3$ (typically 10 mM), stable and well-behaved plastocyanin electrochemistry is observed at room temperature, Figure 1(a). The half-wave potential $E_{1/2}$ is +385 mV *vs.*

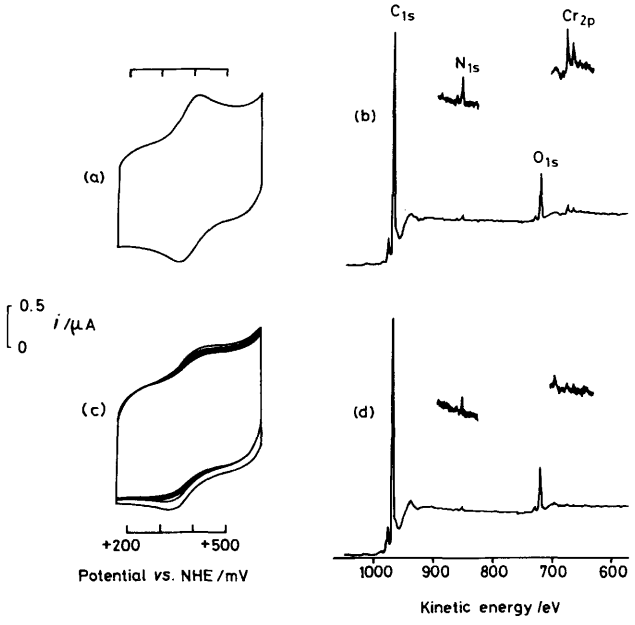


Figure 1. (a) DC cyclic voltammogram (20 mV s^{-1}) of plastocyanin at a Cr-modified electrode. Pretreatment consisted of a single voltammetric cycle (−400, −1200 mV) in $[Cr(NH_3)_6]^{3+}$/aqueous NH_3, followed by 4 min sonication. For clarity, only the fifth scan is shown. Protein concentration 30 μM in 0.1 M KCl, 5 mM HEPES, pH 7.0. Temperature 20 °C. (b) Wide scan ESCA spectrum of an edge graphite disc treated identically to (a). Scale expansions (× 5) of Cr_{2p} and N_{1s} spectral regions are shown. (c) DC cyclic voltammogram (20 mV s^{-1}) of plastocyanin at an electrode with which $[Cr(NH_3)]^{3+}$/aqueous NH_3 voltammetric cycling has been restricted (−400, −800 mV). Conditions are as for (a), scans 1—5 are shown. Result is similar to that observed under identical conditions with a routinely polished electrode. (d) Wide scan ESCA spectrum of an edge graphite disc treated identically to (c). Scale expansions (× 5) of Cr_{2p} and N_{1s} spectral regions indicate incorporation of small amounts of NH_3 but signals in the Cr_{2p} region lie within the noise level.

normal hydrogen electrode (NHE) at 20 °C, close to potentiometrically determined values,[7] and peak separations are typically 60 mV at a scan rate of 20 mV s^{-1}. This result is reproducibly obtained upon $[Cr(NH_3)_6]^{3+}$/aqueous NH_3 modification, either by voltammetric cycling [typically one cycle at 20 mV s^{-1}, between limits of *ca.* −400 and −1200 mV *vs.* saturated calomel electrode (SCE)] or by poising at a potential of −1000 mV or below for *ca.* 1 min, and is unaffected by sonicating the modified electrode for 90 min. Parallel investigations with ESCA confirm the incorporation of Cr (and N) as further shown in Figure 1(b). The Cr_{2p} intensity (relative to carbon) is typically consistent with a surface coverage of *ca.* 10%.†

Control experiments show that restricting potential cycling to the region positive of −1000 mV, or cycling in aqueous ammonia alone, gives only an impersistent and rather irreversible plastocyanin response, similar to that obtained for a routinely polished electrode, Figure 1(c). The corresponding ESCA spectrum, as shown in Figure 1(d) accordingly shows negligible incorporation of Cr, although there is a small amount of N, most likely representing adsorbed NH_3. The potential threshold for Cr modification, as judged by ESCA and plastocyanin electrochemical activity, lies in the region of −1000 mV *vs.* SCE. This is close to reported values of the polarographic reduction potential[8] for $[Cr(NH_3)_6]^{3+}$ and corresponds with the appearance of a small cathodic peak at *ca.* −1050 mV in the reduction cycle. We suggest that, as outlined in Scheme 1, reduction of $[Cr(NH_3)_6]^{3+}$ produces labile Cr^{II} species, a proportion of which co-ordinates to surface C–O functional groups and undergoes rapid reoxidation.

† The surface coverage of Cr with respect to C was calculated from the attenuation of photoelectron flux with depth expected for an overlayer of Cr, formed on an array of C–O groups, at a periodic 'edge' lattice structure. An analysis, using estimated ESCA cross-sections and mean-free paths, was used to derive the approximate coverage.

Plastocyanins (*M* 10 500) from higher plants transport electrons between Photosystems II and I. As a consequence of the significant overall (and conservatively localised) negative charge, physiological[9] and electrochemical[3] activity is highly sensitive to coulombic interactions. We note that even with low background electrolyte levels, *e.g.* 5 mM HEPES–1 mM KCl, the chromium-modified electrode is active toward plastocyanin. By contrast, examination of cytochrome *c* electrochemistry shows clearly marked *inhibition* of heterogeneous electron transfer as compared with the unmodified edge surface. The response at the Cr-modified electrode is thus consistent with the surface now bearing positively-charged domains which function to promote *reversible* binding of plastocyanin prior to electron transfer.

We thank Mr P. Tavener for assistance with ESCA experiments and the S.E.R.C. for research studentships to B. N. O. and A. A. W. and financial support. F. A. A. is a Royal Society University Research Fellow.

Received, 13th March 1985; Com. 338

References

1 F. A. Armstrong, H. A. O. Hill, and B. N. Oliver, *J. Chem. Soc., Chem. Commun.*, 1984, 976.
2 F. A. Armstrong, H. A. O. Hill, B. N. Oliver, and N. J. Walton, *J. Am. Chem. Soc.*, 1984, **106**, 921.
3 F. A. Armstrong, H. A. O. Hill, B. N. Oliver, and D. Whitford, *J. Am. Chem. Soc.*, 1985, **107**, 1473.
4 F. A. Armstrong, P. A. Cox, H. A. O. Hill, B. N. Oliver, and A. A. Williams, unpublished work and see for example: R. Schögl and H. P. Boehm, *Carbon*, 1983, **21**, 345.
5 A. L. Oppegard and J. C. Bailar, *Inorg. Synth.*, 1961, **3**, 153.
6 C. L. Rollinson, 'The Chemistry of Chromium, Molybdenum and Tungsten,' Pergamon Press, Oxford, 1973.
7 K. O. Burkey and E. L. Gross, *Biochemistry*, 1981, **20**, 5495.
8 N. Tanaka and G. Sato, *Nature*, 1963, **197**, 176.
9 T. Takabe, H. Ishikawa, S. Niwa, and S. Itoh, *J. Biochem. (Tokyo)*, 1983, **94**, 1901.