ELECTROCHEMICAL STUDIES ON ELECTRON TRANSFER PROTEINS

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The electrochemistry of proteins containing a transition metal at the active center is a new exciting field which has attracted widespread interest, especially after the recent experiments conducted by Hill and co-workers[1] on the promotion of the electrochemical behaviour of cytochrome *c* (horse heart) at a modified gold electrode. Attention has been directed towards questions concerning the interaction between proteins and electrodes, and the kinetics of electron transfer. We have been developing a programme of work in order to study the electrochemical behaviour of redox proteins containing different catalytic metal arrangements: heme, and simple and complex iron-sulphur proteins.

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One of the most important features of the electrochemical behaviour of metal-proteins is their interaction with the electrode interface.

The electron transfer in biological systems between two proteins occurs within the assembly of the precursor complex formed by the proteins.

For successful electrochemical response of redox proteins the following events should occur:

1 - protein diffusion to the electrode surface

2 - protein association with the electrode in a favorable orientation

3- electron transfer

4 - protein dissociation from the electrode

5 - protein diffusion away from the electrode

Steps 3 and 4 are exclusive of redox proteins electrochemistry and are essential in the overall process of promoting the complexation/decomplexation with a biological partner for electron transfer.

So far different interfaces have been used, such as, semi-conductors (doped metal-oxides and pyrolitic graphite) and modified metal (gold, platin and silver) electrodes.

There are many modifiers used for promotion of the redox propreties of proteins. Genneraly they are bifunctional molecules, of the type X \/\/\/ Y, where the X group adsorbs on the electrode surface, and the Y group is directed towards the solution. Group X contains atoms such as N, S or P, and groups Y are carboxylate, phosphate, sulphonate or pyridil.

In our work we studied the electrochemistry of cytochrome *c* (horse heart), a mono-heme protein which has been the most widely investigated, since it is commercialy available.

Our results indicate a reversible behaviour of cytochrome *c* at a 4-4'-bipyridil modified gold electrode and the values of E^{o}= 45 mV *vs* SCE and $D=6\times10^{-7}$ cm^2s^{-1} obtained, agree with the literature values[1].

We have also been interested in the study of a di-heme (split-Soret) cytochrome and tetra-heme cytochrome c_3 (Mr 13.000) from *Desulfovibrio desulfuricans* ATCC 27774 and tetra-heme cytochromes c_3 *Desulfovibrio vulgaris* Hildenborough and *Desulfovibrio gigas*.

So far we have been unsuccessful in finding an appropriate interface for the electrochemical studies of de di-heme cytochrome (split-Soret), but with cytochrome c_3 from *D.vulgaris* we have obtained the cyclic voltamogram shown in Figure 1 when using a doped tin oxide electrode. The cyclic voltammetry data, presented in Table 1 suggests a quasi-reversible behaviour.

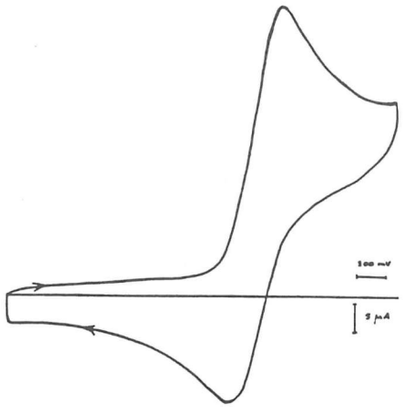

Figure 1. Cyclic voltammogram of a 0.62 mM solution of cytochrome c_3 *D.vulgaris* Hildenborough in phosfate buffer 0.1 M pH 7, at a doped tin oxide electrode. Potential limits +0.3 -1.2 V, scan rate 0.4 Vs^{-1}.

Table 1. Cyclic voltammetry data for cytochrome c_3 *D.vulgaris* Hildenborough at a doped tin oxide electrode. Same conditions as in Figure 1.

v (Vs^{-1})	i_p (μA)	ΔE (V)
0.02	10.2	0.09
0.05	19.	0.105
0.10	28.	0.108
0.20	36.	0.165
0.30	43.5	0.195
0.40	47.	0.205
0.50	40.	0.222

It is clear that further conclusions should only be drawn after coulometric measurements.

The cyclic voltammograms and the plot of peak current *vs* square root of scan rate obtained for *D.gigas* cytochrome c_3 at a pyrolitic graphite electrode are shown in Figure 2. Two cathodic and two anodic peacks are observed at potentials close to -0.465, -0.55, -0.49 and -0.435 V *vs* SCE. These peaks are probably related to the transfer of four electrons and further studies including deconvolution procedures are necessary in order to determine the standard potentials.

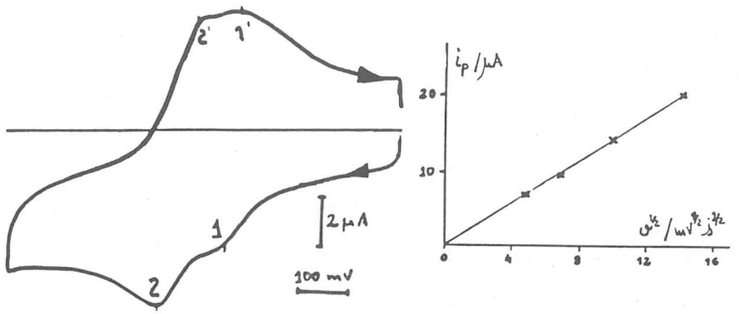

Figure 2. Cyclic voltammogram and plot of i_p *vs* $v^{½}$ for a 0.63 mM solution of cytochrome c_3 *D.gigas* in phosfate buffer 0.1 M pH 7, at a pyrolitic graphite "edge" clevead electrode. Potential limits -0.3 -0.9 V, scan rate 0.02 Vs^{-1}.

(1) F.A.Armstrong, H.A.O.Hill and N.J.Walton, Quarterly Review of Biophysics 18,3(1986)261.

ELECTROCHEMICAL BEHAVIOUR OF ARYLTHIOETHYL GROUPS IN CARBOXYL PROTECTION

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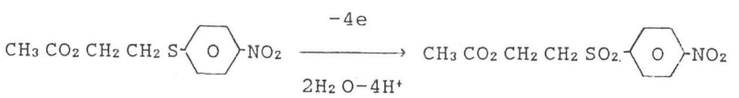
Electrolysis can provide an appropriate way to cleave protecting groups of interest in synthesis[1]. Arylthioethyl groups are suitable for the protection of the carboxyl function and are conventionally removed by treatment with alkali after conversion into the corresponding sulphone by catalytic oxidation[2]. This paper reports the investigation of an alternative route to the oxidation of the sulphide to sulphone by electrolysis. The model compound selected for study was the p-nitrophenylthioethyl ester of acetic acid and the desired reaction was



For these studies the anode was Pt or vitreous C and the medium CH_3CN or CH_3CN with small additions of water containing $NaClO_4$ or Bu_4NBF_4 (TBAB)

Cyclic voltammograms for the compound in CH_3CN/TBAB is shown in figure 1. Two well formed but totally irreversible oxidation peaks at + 1.8V and + 2.2V *vs* SCE can be seen.