Direct electrochemical reduction of ferredoxin promoted by Mg^{2+}

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Ferredoxin 2[4Fe–4S] *Electrochemical reduction (direct)* *Redox-protein* *Electron-transfer promotion, by Mg^{2+}* *(Graphite electrode, pyrolytic)* (Clostridium pasteurianum)

1. INTRODUCTION

Direct (unmediated) electrochemical reduction of ferredoxins has been described by several authors [1–7]. Though both bacterial 2[4Fe-4S] and plant [2Fe-2S] proteins can be rapidly reduced at a mercury electrode, there are [4,5] associated problems of irreversibility and active site degradation. In contrast, Landrum et al. [6,7] have reported reduction and full reoxidation of spinach ferredoxin at a gold electrode modified with a polymeric form of methyl viologen. As part of an extensive investigation [8] of the direct electrochemistry of redox proteins, we now describe the rapid and reversible reduction of *Clostridium pasteurianum* 2[4Fe-4S] ferredoxin at a pyrolytic graphite electrode. A striking feature is the promotion of electron transfer by divalent metal aquo ions such as that of magnesium.

2. MATERIALS AND METHODS

Ferredoxin was isolated from *C. pasteurianum* wet cell paste (PHLS Centre for Applied Microbiology and Research, Porton, Wiltshire) using the procedure described by Thompson et al. [9]. The final solution (A_{390}/A_{280} = 0.81) was concentrated to ca. 2 mM and desalted using rapid diafiltration (Amicon model 8MC; UM2 membrane) and stored in liquid nitrogen in the form of small pellets. Solutions for electrochemistry were prepared by addition of buffer solution to aliquots of thawed pellets to yield desired final solution compositions. The concentration of ferredoxin was determined using an absorption coefficient ε_{390} = 30.6 (mM protein)$^{-1}$. cm^{-1} as determined for *C. acidi-urici* ferredoxin [10]. All reagents used were either of Analar or Aristar grades. Deionised (Millipore) distilled water was used throughout.

Cyclic voltammetry was carried out using an Oxford Electrodes potentiostat. All potentials reported are with respect to the Normal Hydrogen Electrode (NHE). The glass cell (ca. 300 μl capacity) incorporated a conventional three electrode configuration. The working electrode, a 3 mm diameter disc of pyrolytic graphite (General Electric Co., Detroit, USA) with the deposition layers (*a*-axis) parallel to the solution/electrode interface, was housed in a sheath of epoxy resin. Prior to experiments the disc face was polished using an alumina/water slurry on cotton wool and then rinsed. The counter electrode consisted of a piece of platinum gauze and the reference electrode was saturated calomel (SCE), E = +244 mV *vs* NHE at 25°C. Solutions of ferredoxin used for cyclic voltammetry (ca. 0.3 mM were made and maintained anaerobic by passing a slow stream of humidified O_2-free argon across the surface.

Bulk reduction and reoxidation were carried out at constant potential using the Oxford Electrodes potentiostat. The spectroelectrochemical cell, which will be described fully elsewhere [11], was of all-glass construction and featured two parallel horizontal pyrolytic graphite plates ca. 5 mm apart. The intervening solution (ca. 250 μl of a 37 μM ferredoxin solution) was stirred magnetically using a small sealed glass follower. A Cary Model 17 spectrophotometer was used for all optical experiments. The sample beam passed above the stirrer bar and below the upper electrode plate via an optical window in the otherwise opaque (black) cell face. The optical path length was 7 mm. The reference (SCE) and counter (platinum gauze) electrodes were positioned in two separate side

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arms with electrical conductivity maintained via a Luggin capillary and a porous plug respectively. To eliminate diffusion problems, argon-purged solutions of ferredoxin at sample concentration were placed in these side arms. Prior to experiments the sample solution was made anaerobic with several cycles of vacuum and argon equilibration and then transferred to the cell using a Hamilton gas-tight syringe. Oxygen contamination during electrolysis was minimised by passage of a stream of O_2-free argon above the solution. No attempt was made to cool the apparatus during electrolysis.

3. RESULTS AND DISCUSSION

The effect of magnesium ions on the direct electrochemistry of *C. pasteurianum* ferredoxin is clearly illustrated in fig.1. No electrode processes are evident in sodium chloride electrolyte, fig.1A, but in 40 mM magnesium chloride the protein shows well-defined electrochemistry, fig.1B. Bulk electrolysis as followed spectrophotometrically, fig.2, confirms that facile reduction and reoxidation of the [4Fe-4S] centres can be accomplished. We note, however, that over the course of these experiments some degradation, as indicated by ca. 7% general absorbance increases, does occur. After ca. 2 h a sample was removed for UV–Vis analysis. The ratio A_{390}/A_{280} had decreased from 0.81 to 0.75. It is likely that traces of oxygen contamination and the effects of above-ambient temperature (ca. 25°C) are contributory factors. Coulometric results using a 0.5 mM ferredoxin sample were consistent with one-electron reduction: 0.96 Faradays (mole [4Fe-4S])$^{-1}$ were passed for a current decrease of 88%. Similarly for reoxidation (37 μM ferredoxin sample), the total charge passed was consistent with one-electron oxidation: 0.8 Faradays (mole [4Fe-4S])$^{-1}$.

The appearance of the cyclic voltammograms, fig.1B, of which the cathodic and anodic peaks are of a similar shape and magnitude, indicates that the electrode reaction is reversible. For a diffusion-controlled one-electron process [12], a peak separation $\Delta E_p \sim 60$ mV independent of scan rate is expected. We note that ΔE_p as shown in fig.1B increases with scan rate, the smallest value being 80 mV at 10 mV s^{-1}. The half-wave potential, $E_{1/2}$, is −370 mV *vs* NHE under these conditions. A similar value was obtained using the differential pulse

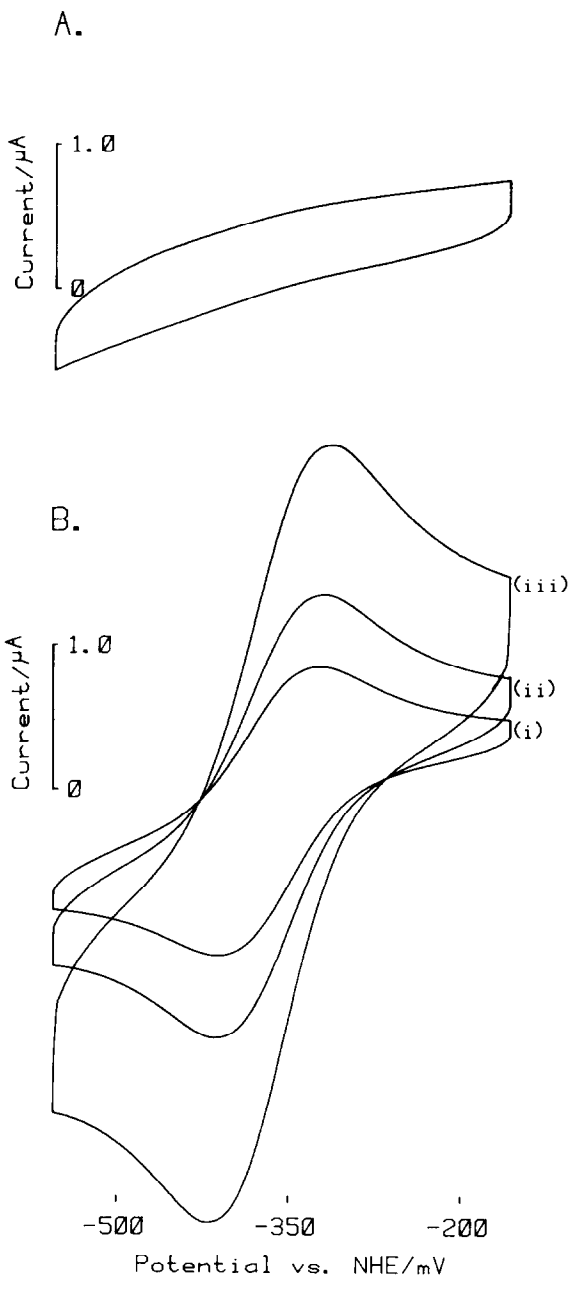

Fig.1. Steady state DC cyclic voltammograms of *C. pasteurianum* 2[4Fe-4S] ferredoxin at pyrolytic graphite. Ferredoxin 0.3 mM in 20 mM tricine buffer (pH 8.0), containing as supporting electrolyte: (A) 120 mM NaCl. Scan rate 20 mV s^{-1}. (B) 40 mM $MgCl_2$. Scan rates of (i) 10; (ii) 20; and (iii) 50 mV . s^{-1}.

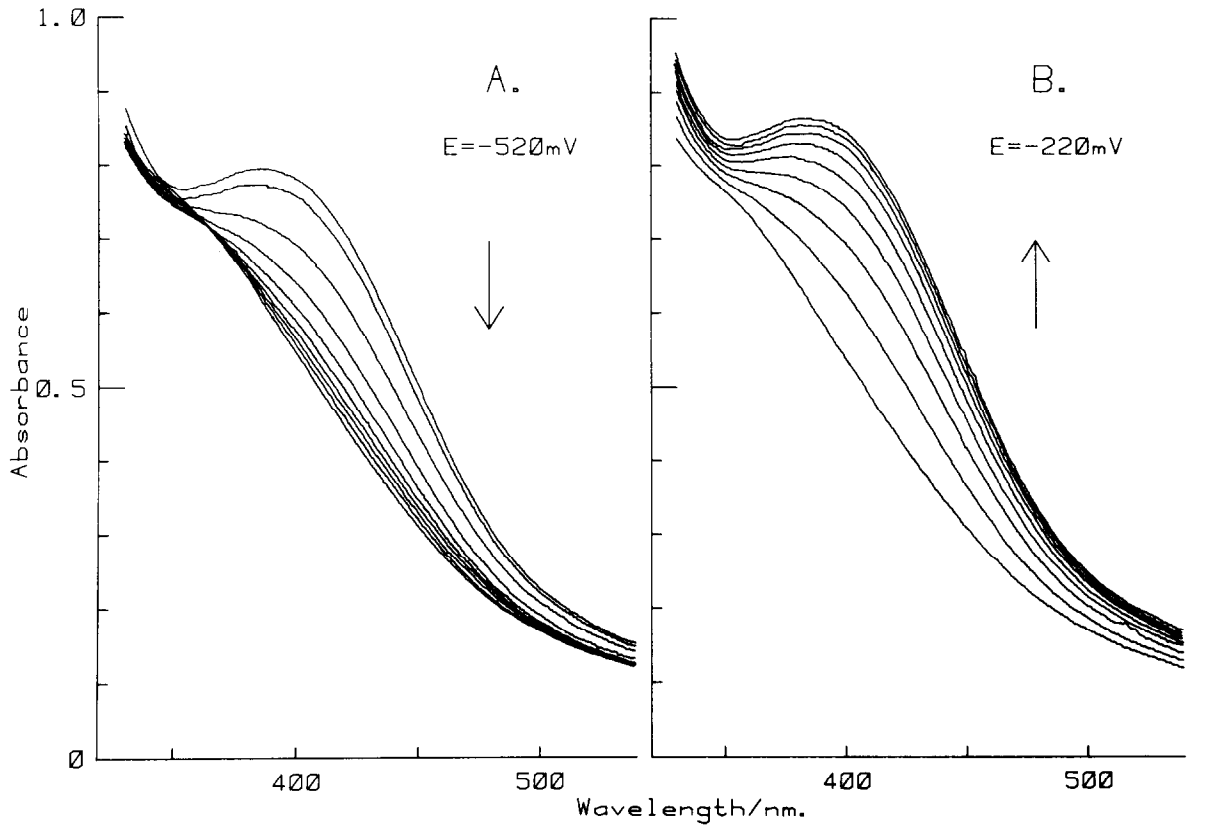

Fig.2. Direct electrochemical reduction (A) and reoxidation (B) of *C. pasteurianum* ferredoxin (37 μM; 40 mM $MgCl_2$; 20 mM tricine, pH 8.0) at pyrolytic graphite, electrode potentials as indicated. Successive scans (period 7 min) were obtained in situ using the optical 'graphite-sandwich' electrochemical cell described.

voltammetry technique. While this lies within the range of reported potentiometric values [13–19] we find that it is somewhat dependent upon magnesium ion concentration, becoming more positive as this is increased at constant pH. Experiments carried out with Ca^{2+} and Mn^{2+} showed these also to be effective electron transfer promoters.

We are currently pursuing a detailed study of the electrochemistry of ferredoxins and other proteins. We have proposed [8] that, in order to effect electron transfer between redox proteins and electrodes, binding of the protein to the electrode surface is essential. In this example it is likely that Mg^{2+} promote electron transfer by forming a bridge between the highly negatively charged ferredoxin molecule and carboxylate groups on the graphite surface. Promotion of electron transfer by divalent metal aquo ions may be important in other biological systems [20] and it is possible that our findings may illuminate hitherto little-studied aspects of in vivo homogeneous and heterogeneous electron transfer processes.

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