Direct Electron Transfer of Ferredoxins at an Indium Oxide Electrode in the Presence of Poly-L-lysine

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Received February 25, 1993 ; Accepted March 19, 1993

1 INTRODUCTION

Recently, electrochemistry of proteins has been extensively studied, because some small proteins have become *electroactive* at various functional electrodes¹⁻²). Ferredoxin is an important electron transfer protein in a photosynthetic process, having a non-heme iron sulfur cluster as a redox center and is a negatively charged acid protein (pI = ca. <4). Electrochemical studies of both plant [2Fe-2S] and bacterial [4Fe-4S] ferredoxins have been carried out at methylviologen modified Au minigrid electrode³), and at Hg and graphite electrodes in the presence of poly cations⁴), such as $\text{Cr}(\text{NH}_3)_6^{3+}$ and neomycin. Poly-L-lysine was also reported to be effective⁵) for the rapid response of ferredoxins at an Hg electrode. However, although ferredoxin is rather small protein in size (molecular weight of ca. 11,000), reported examples of rapid electron transfer at an electrode have still been limited, especially for plant ferredoxins.

In the present study, clear voltammetric responses of spinach and maize ferredoxins have been observed at an In_2O_3 electrode in the presence of a small amount of cationic poly peptides, such as poly-L-lysine and poly-L-arginine, where the formal heterogeneous electron transfer rate constant, k^0 , was estimated to be $>3 \times 10^{-3} \text{ cm s}^{-1}$, as one of the best responses so far reported.

2 EXPERIMENTAL

Spinach ferredoxin was extracted from spinach leaves, and purified chromatographically on DEAE cellulose and finally on Sephadex G75 columns at 4 °C. The purity of ferredoxin used was monitored by the ratio of absorbances at 424 and 277 nm, A_{424}/A_{277} , to be >0.5 . The concentration of spinach ferredoxin was determined spectroscopically using the molar extinction coefficient ($\epsilon = 9,680$)⁶) at 420 nm. All other reagents were of analytical grade and used as received.

Cyclic voltammograms were obtained at functional electrodes using a Toho-Giken 2020/2130 potentiostat with a function generator in tris-HCl or Britton & Robinson buffer solutions containing 0.33 M NaCl at various pHs. Other experimental details are similar to those described elsewhere²).

3 RESULTS AND DISCUSSION

Ferredoxin gave no electrochemical response in the buffer solutions used at In_2O_3 and other promoter modified electrodes, on which rapid electron transfer of cytochrome c can take place. When divalent metal ions, such as Mg^{2+} , Ba^{2+} and Ca^{2+} , were added at a concentration of ca. 0.2 M, not fully-reversible but well-developed voltammetric response of spinach ferredoxin appeared at In_2O_3 and 6-mercaputopurine modified Au electrodes. Interestingly, addition of small amounts of positively charged poly peptides, such as poly-L-lysine and poly-L-arginine, enhanced the ferredoxin electrochemistry to give well-defined diffusion-controlled redox

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Key words: Ferredoxin, Poly-L-lysine, Indium Oxide, Direct Electron Transfer, Modified Electrode

waves. Negatively charged poly-L-glutamic acid and neutral poly-L-tyrosine (either leucine or histidine) showed no positive effect on ferredoxin electrochemistry. Addition of poly-L-lysine (e.g., ca. 5 μM for a polymer of the mean molar mass of 3,600 g from Sigma) gave the best results at an In_2O_3 electrode.

Figure 1 shows a typical voltammogram of spinach ferredoxin together with simulated data. For purified spinach ferredoxin, the E^0 , D , and k^0 values of -0.60 V (vs. $\text{Ag}/\text{AgCl}/\text{sat. KCl}$), $1.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, and $3.3 \times 10^{-3} \text{ cm s}^{-1}$, respectively, were obtained in a 50 mM tris-HCl buffer solution containing 0.33 M NaCl (pH=7.5) at 10 $^\circ\text{C}$, from the voltammograms obtained after subtraction of the background current. The reversible reduction and reoxidation were confirmed by spectral changes. Also, no significant difference in voltammograms of ferredoxin was seen in the pH 6 to 9 region, where ferredoxin is stable in structure.

A poly-L-lysine modified pyrolytic graphite electrode was also prepared through the peptide linkage between amino groups of poly-L-lysine and carboxyl groups on the graphite surface; a well-defined voltammograms of ferredoxin similar to that shown in Fig. 1 was again observed.

These electrodes were also effective for maize ferredoxins from both photosynthetic and non-photosynthetic organs.

Ferredoxin acts as an electron-donating mediator for various enzyme reactions: For example, in the presence of ferredoxin-NADP⁺-reductase (FNR, E.C.=1.18.1.2), NADPH was monitored spectroscopically to be effectively produced in the current efficiency of near 100% from NADP⁺ through the reduction of ferredoxin at the electrode. By further conjugation with other enzyme reactions, where NADPH is a co-factor, various bioorganic reactions are easily designed.

Fig. 1 Cyclic voltammograms of ca. 60 μM spinach ferredoxin at an In_2O_3 electrode in the presence of 5 μM poly-L-lysine (M.W. 3600) in a 50 mM tris-HCl buffer solution containing 0.33 M NaCl (pH 7.5) at a scan rate of 20 mV s^{-1} with simulated data shown by circles (see the text for the data used for simulation). Electrode area: 0.175 cm^2 .

The partial financial support of this work by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan and from KAWASAKI STEEL 21st Century Foundation is gratefully acknowledged. The authors are indebted to Prof. S. Hase of Osaka University for the gift of maize ferredoxins.

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