Incorporation and Direct Electron Transfer of Chlorella Ferredoxin in the Bilayer Films of Cationic Lipids on Electrodes

Masato Tominaga, Junko Yanagimoto, Alaa-Eldin F. Nassar,† James F. Rusling,† and Naotoshi Nakashima*
Department of Applied Chemistry, Faculty of Engineering, Nagasaki University, Bunkyo-cho, Nagasaki 852
†Department of Chemistry, Box U-60, University of Connecticut, Storrs, Connecticut 06269-4060, U.S.A.

(Received March 15, 1996)

Chlorella ferredoxin in solution was found to be incorporated into the cast films of ammonium lipids on basal plane pyrolytic graphite (BPG) electrodes *via* ion exchange between the lipid counter anion and negatively charged ferredoxin and was embedded in them without denaturation in a way to undergo direct electron transfer reactions with the electrode.

Redox protein-lipid films on electrodes provide suitable systems for studying not only the fundamental properties of the proteins by electrochemical and spectroscopic techniques, but also applications such as the construction of protein devices using lipid film interfaces.[1] Ferredoxins are water soluble iron-sulfur proteins of molecular weight *ca.* 12 k daltons that function as electron transfer carriers in the green plant chloroplast and in the photosynthetic bacteria. Despite the considerable interest in the electrochemistry of ferredoxin,[2-3] electrochemical behaviors of the protein immobilized in matrices such as lipid and polymer films on electrode surfaces has not yet been reported. In the present paper, we describe the incorporation of chlorella ferredoxin into the cast membrane films of ammonium lipids, $2C_nN^+Br^-$(n=16, 18) and $2C_{16}GluC_{11}N^+Br^-$ on electrodes *via* ion exchange and the direct electron transfer reactions of the embedded protein with the electrode. Poly(ion complexed)lipids, $2C_nN^+PSS^-$(n=16, 18), dipalmitoyl phosphatidic acid (DPPA) and $2C_{12}$gluco were also used as electrode modifiers to clarify the mechanism of the protein incorporation into the lipid films.

2Fe-2S chlorella ferredoxin (Wako Pure Chemical Industries) was used as received. The purity of ferredoxin was certified by the ratio of absorbances at 422 and 277 nm, A_{422}/A_{277}, to be > 0.5. Ammonium bromide lipids, $2C_nN^+Br^-$ (n=16, 18) were available from the previous study.[4] $2C_{16}GluC_{11}N^+Br^-$ and $2C_{12}$gluco were synthesized according to the method described elsewhere.[5] Biological lipid, DPPA from SIGMA was used as received. Basal plane of pyrolytic graphite (Union Carbide Co., the geometric area of a disk electrode surface is 0.20 cm^2), BPG, was used as electrodes. Lipid membrane-modified BPG electrodes were prepared by the following procedure. Ten microlitters of 0.1 mol dm^{-3} lipid in benzene was placed on a BPG electrode abraded by a #1500-grid SiC paper and then the solvent was evaporated slowly at room temperature. Cyclic voltammetric (CV) measurements were conducted with an electrochemical analyzer (Bioanalytical Systems, BAS 100B/W). An Ag/AgCl (saturated KCl) electrode and a platinum electrode were used as the reference and the counter electrode, respectively.

Prepared lipid film modified electrodes were immersed in 0.1 mmol dm^{-3} ferredoxin in a 50 mmol dm^{-3} tris-HCl buffer solution (pH 7.2). At ambient temperature (23 °C), oxidation/reduction current attributable to ferredoxin was found to increase gradually for electrodes coated with $2C_nN^+Br^-$ (n=16, 18) and $2C_{16}GluC_{11}N^+Br^-$ and reached steady states in about 4 h. Figure 1 (solid lines) shows typical cyclic voltammograms (steady state) of ferredoxin at BPG electrodes coated with the cast films of $2C_{18}N^+Br^-$ or $2C_{16}GluC_{11}N^+Br^-$. Direct electron transfers of ferredoxin with the electrodes are evident. $2C_{16}N^+Br^-$-modified electrode also gave similar voltammograms. These modified electrodes were gently rinsed with tris-HCl buffer solution (pH 7.2), and placed in a 50 mmol dm^{-3} tris-HCl buffer solution (pH 7.2) not containing ferredoxin. As shown in Figure. 2, well-defined redox waves were found to be retained. No significant change in the voltammograms were observed while keeping the coated electrodes in a buffer solution at 23 °C for 6 h. It was proved that the ammonium lipid membrane films on BPG provided suitable microenvironments for the immobilization of ferredoxin in a way to conduct direct electron transfer with the electrode.

The redox peak currents for the ferredoxin immobilized cast

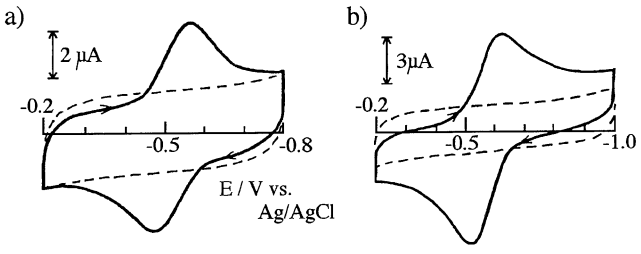

Figure 1. Typical CVs for BPG electrodes coated with cast films of $2C_{18}N^+Br^-$ (a) and $2C_{16}GluC_{11}N^+Br^-$ (b) in the presence (solid lines) and the absence (dotted lines) of 0.1 mmol dm^{-3} chlorella ferredoxin in a tris-HCl buffer solution (pH 7.2) at 23 °C under N_2 atmosphere. Scan rate: 100 mVs^{-1}.

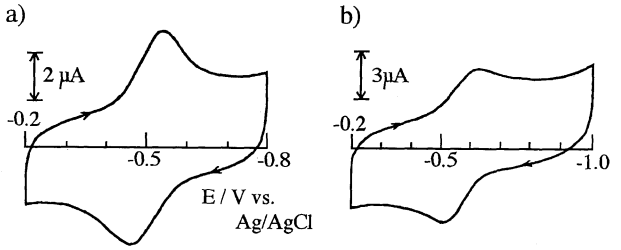

Figure 2. Typical CVs for the films of chlorella ferredoxin / ammonium lipids (a: $2C_{18}N^+Br^-$, b: $2C_{16}GluC_{11}N^+Br^-$) on the BPG electrodes in a tris-HCl buffer solution (pH 7.2) containing no chlorella ferredoxin at 23 °C under N_2 atmosphere. Scan rate: 100 mVs^{-1}.

films of the lipids showed thin layer electrochemical behavior; the peak currents were proportional to the square root of scan rate, v, above *ca.* 100 mVs^{-1}. At v below *ca.* 5 mVs^{-1}, more symmetric peaks proportional to v were observed. The formal redox potentials, $E^{\circ\prime}$, evaluated from the midpoint of anodic and cathodic peak potentials of the redox waves at $2C_{16}N^+Br^-$, $2C_{18}N^+Br^-$ and $2C_{16}GluC_{11}N^+Br^-$ modified electrodes were -0.50, -0.51 and -0.56 V *vs.* Ag/AgCl(saturated KCl), respectively. The formal potential of chlorella ferredoxin in solution at a polypeptide modified indium oxide electrode is reported to be -0.6 V.[6] There was no significant difference between the UV-Vis spectrum of ferredoxin dissolved in tris-HCl buffer solution (pH 7.2) and the specular reflectance spectra of ferredoxin immobilized in the cast films. Observed positive shift of the $E^{\circ\prime}$ compared to that in solution may reflect protein-lipid interactions or double-layer effects on the electrode potential.

To clarify the mechanism of the incorporation of the redox protein into the lipid films, we used $2C_nN^+PSS^-$(n=16, 18), DPPA and $2C_{12}$gluco instead of the ammonium bromide lipids and found that no redox wave of ferredoxin was observed both at temperatures of 23 and 50 °C. Incorporation of solution species into lipid films are usually enhanced at temperatures above phase transition of bilayers. However, even at temperatures above the crystal phase-to-liquid crystal phase transition temperatures[7] ferredoxin incorporation into the lipid films was electrochemically and spectroscopically undetectable. The protein incorporation into the cast films of the ammonium bromide lipids, on the contrary, occurs at temperatures lower than phase transition. These results together with the fact that ferredoxin is a negatively charged protein suggest that the incorporation of negatively charged ferredoxin (pI 3.8) into the cast films of the cationic lipids on electrodes is governed by the ion exchange between bromide anion of the lipids and the negatively charged ferredoxin. The ion exchange mechanism was supported by electrolyte concentration dependence experiment; *i.e.*, ferredoxin incorporation into the ammonium lipid films was found to be completely inhibited at electrolyte (NaBr) concentration higher than 0.5 mol dm^{-3}. $2C_nN^+PSS^-$(n=16, 18) are poly(ion complexed)lipids, where electrostatic interaction between the positively charged ammonium ions and the negatively charged polyanion (PSS^-) is stronger than that for $2C_nN^+Br^-$(n=16, 18). Thus, inhibited incorporation of ferredoxin into the poly(ion complexed)lipid films *via* ion exchange is understandable. Salamon and Tollin[3] also pointed out the importance of electrostatic interaction in the incorporation of ferredoxin into the mixed cast films of phosphatidylcholin and dimethyldioctadecylammonium chloride on gold although the voltammograms measured in the presence of the protein in solution are not evident.

We conclude that ferredoxin is incorporated into the cast films of the ammonium lipids on the electrodes *via* ion exchange and is stably embedded in them without denaturation in a way to undergo direct electron transfer reactions with the electrode. A detailed study of electron transfer kinetics are currently under investigation.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan and the Toray Science Foundation and by U.S. PHS grant No. ES03154 from NIH awarded by the National Institute of Environmental Health Sciences.

References and Notes

1 a) J. K. Cullison, F. M. Hawkridge, N. Nakashima, and S. Yoshikawa, *Langmuir*, **10**, 877 (1994); b) J. F. Rusling and A.-E. F. Nassar, *J. Am. Chem. Soc.*, **115**, 11891 (1993); c) A.-E. F. Nassar, Y. Narikiyo, T. Sagara, N. Nakashima, and J. F. Rusling, *J. Chem. Soc., Faraday Trans.*, **91**, 1775 (1995); and references cited therein.

2 a) C. D. Crawley and F. M. Hawkridge, *J. Electroanal. Chem.*, **159**, 313 (1983); b) M. A. Harmer and H. A. O. Hill, *J. Electroanal. Chem.*, **170**, 369 (1984); c) F. A. Armstrong, H. A. O. Hill, B. N. Oliver, and N. J. Walton, *J. Am. Chem. Soc.*, **106**, 921 (1984); d) J. Haladjian, P. Bianco, and L. Asso, *Electrochimica Acta*, **31**, 1531 (1986); e) P. D. Barker, K. D. Gleria, H. A. O. Hill, and V. J. Low, *Eur. J. Biochem.*, **99**, 3154 (1990).

3 Z. Salamon and G. Tollin, *Bioelectrochem. Bioenerg.*, **27**, 381 (1992).

4 N. Nakashima, H. Eda, M. Kunitake, O. Manabe, and K. Nakano, *J. Chem. Soc., Chem. Commun.*, **1990**, 444; and references cited therein.

5 N. Nakashima, S. Asakuma, J.-M. Kim, and T. Kunitake, *Chem. Lett.*, **1984**, 1709.

6 a) I. Taniguchi, Y. Hirakawa, K. Iwakiri, M. Tominaga, and K. Nishiyama, *J. Chem. Soc., Chem. Commun.*, **1994**, 953; b) I. Taniguchi, K. Hayashi, M. Tominaga, R. Muraguchi, and A. Hirose, *Denki Kagaku (J. Electrochem. Soc., Jan.)*, **61**, 774 (1993); c) I. Taniguchi, K. Iwakiri, A. Miyahara, K. Nishiyama, and T. Matsubayashi, *Rev. of Polarography*, **40**, 77 (1994).

7 Phase transition temperature are: 29, 46 and 20 °C for $2C_{16}N^+PSS^-$, $2C_{18}N^+PSS^-$ and $2C_{12}$gluco, respectively.