Direct Electrochemistry of Cofactor Redox Sites in a Bacterial Photosynthetic Reaction Center Protein

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Received May 4, 1998

Conversion of light into chemical energy in photosynthesis is initiated in the reaction center (RC) protein. In photosynthetic bacteria, a series of one-electron steps occur in RC after photoexcitation of the primary electron donor (P), a dimer of bacteriochlorophylls (BChl). Electron acceptors include a bacteriopheophytin (BPhe) and a quinone (Q_A).[1] Herein we show that redox centers P, BPhe, and Q_A in an RC protein in layered films of phospholipid or polycations exchange electrons reversibly with an underlying electrode. Midpoint potentials measured by direct voltammetry are in excellent agreement with values from mediated optical redox titrations (Table 1). This ability to achieve direct electron exchange with electrodes without mediators provides for rapid monitoring of electrochemical properties of bound cofactors using only tiny amounts of protein.

Direct voltammetry may be inhibited by protein surface denaturation, lack of electrical access to prosthetic groups, or unfavorable orientation of proteins at electrodes. Thus, while pigments, BChl and BPhe, isolated from the reaction center (RC) protein displayed reversible voltammograms in solution,[2−6] to our knowledge, no direct voltammetry on the RC protein has been reported previously.

Various strategies can achieve direct electron exchange between electrodes and water-soluble proteins.[7−9] Studies of membrane proteins are rare, but voltammetry of cyt *c* oxidase in lipid films has been described.[10,11] We recently developed two film types which facilitate direct voltammetry of proteins. In the first, a mixture of lipid vesicles and protein are deposited onto a surface, yielding films ordered in multiple lipid bilayers.[9] In the second method, alternate layers of polyions and proteins are grown on a surface.[12,13] Both methods provided films which gave reversible electron transfer between electrodes and iron heme proteins or ferredoxins, whereas only very slow electron transfer occurred for these proteins in solution with bare electrodes.[9,13a]

In this work, we report direct reversible voltammetry for the photosynthetic RC from purple bacterium, *Rhodobacter (Rb.) sphaeroides* wild-type strain 2.4.1, incorporated into films of dimyristoylphosphatidylcholine (DMPC) on graphite or indium tin oxide electrodes, or sandwiched between polycation layers on gold electrodes. RCs from wild-type *Rb. sphaeroides* strain 2.4.1 were obtained with negligible amounts of quinone Q_B as described previously.[14]

DMPC−RC films were made by spreading 10 μL of 0.005 M DMPC vesicles and 0.25 mg mL^{-1} RC in pH 8 TRIS buffer onto basal plane pyrolytic graphite electrodes, and drying overnight at 4 °C in the dark. This procedure incorporates proteins within multiple lipid bilayers.[9]

Layered polycation−RC films were grown on Au treated with 3-mercaptopropanesulfonic acid (MPS) to provide a negative surface.[13a] Au−MPS electrodes were immersed into 2 mg mL^{-1} polydimethyldiallylammonium chloride (PDDA, MW 90 000) or poly(ethylenimine) (PEI, MW 70 000) to adsorb a polycation layer. Subsequently, electrodes were washed with water and immersed into pH 8 buffer containing RC (0.2 mg mL^{-1} + 0.06% Triton X-100), and a layer of negative protein was adsorbed. After washing, an outer polycation layer was added. Adsorption times were 30 min, and RC and outer polycation layers were grown in the dark at 4 °C.

Mass and thickness of individual layers were estimated on dry films with a quartz crystal microbalance, by growing layers on MPS−gold on a quartz resonator (9 MHz AT cut, USI, Japan).[13b] Average thicknesses were 0.5 nm for MPS, 0.7 nm for PDDA, 0.5 nm for PEI, 6.4 ± 0.6 nm for RC in PDDA−RC−PDDA, and 5.8 ± 0.5 nm for RC in PEI−RC−PDDA. Comparison with molecular dimensions of polyions[12] and RC protein (13 × 7 × 4 nm)[15] confirmed formation of monolayers at each step. The thickness of the RC layer suggests that the ellipsoidal protein lies on its side.

Cyclic voltammograms (CVs) of polyion−RC and DMPC−RC films gave oxidation−reduction peaks (Figure 1a) which were reproducible during 1 month of storage in buffer at 4 °C. Reversible, fast electron transfer in thin films is characterized in CV by symmetric oxidation−reduction peaks of equal height at nearly the same potential.[16] Midpoint potentials (E_m) are estimated as the average of oxidation and reduction peak potentials. For PDDA−RC−PDDA films, reversible peak pairs occurred at 0.46 V (all vs NHE), with smaller reversible peak pairs at −0.05 and −0.53 V. Peaks remained reversible up to 100 mV s^{-1}. Similar peaks were found for RC lacking bound carotenoid. Comparisons with midpoint potentials from mediated optical redox titrations (Table 1) suggest that the peaks represent redox chemistry of bacteriochlorophyll dimer (0.5 V), bacteriopheophytin (−0.53 V), and quinone Q_A (−0.05 V). Controls employing polymer-coated or bare gold electrodes in buffer or RC solutions gave no significant peaks (Figure 1a).

The potentials of peaks I and II shifted −55 mV/pH from pH 5−8.5. Proton-coupled electron transfer for RC centers has also been observed in redox titrations and other experiments, and may involve protonation of amino acids.[18,19]

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(13) (a) Lvov, Y.; Lu, Z.; Schenkman, J. B.; Zu, X.; Rusling, J. F. *J. Am. Chem. Soc.* **1998**, *120*, 4073−4080. (b) For our resonator electrodes, layer thickness (*d*) and QCM frequency shift (ΔF) are related by d (nm) = 0.016ΔF (Hz), shown by calibration using SEM cross sections of dry protein−polyion films using 12 different proteins. This equation comes from the Sauerbrey equation ΔF (Hz) = (1.83 × 10^8)M/A (mass/area) using a layer density of 1.3 g cm^3 and a measured Au surface area 20% larger than the geometric area. See ref 12.
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S0002-7863(98)01521-2 CCC: $15.00 © 1998 American Chemical Society
Published on Web 07/09/1998

Table 1. Comparison of Midpoint Potentials for RC Redox Couples

sample	E_m, P/P$^+$ V vs NHE (ref)	E_m, Q_A/Q_A^- V vs NHE (ref)	T, °C	method[a]/pH	E_m, BPh/BPh$^-$ V vs NHE (ref)
RC/soln	0.505 (22)	−0.05 (25)	22	ORT/8.0	(−0.60) (4)[d] free in CH_2Cl_2
RC/soln	0.485 (23)		8	ORT/7.4	
RC/LB film	0.414 (17)	−0.064 (17)	22	ORT/8.0	
RC/soln	0.453 (24)		20	ORT/8.0	
RC/soln		−0.045 (26)	−196	ORT/7.0	
Au−MPS−PDDA−RC−PDDA	0.455 ± 0.005	−0.050 ± 0.005	1	CV[b]/8.0	−0.53 ± 0.01
Au−MPS−PDDA−RC−PDDA	0.458 ± 0.005	−0.047 ± 0.005	1	SWV[c]/8.0	−0.52 ± 0.01
Au−MPS−PEI−RC−PEI	0.462 ± 0.005	−0.055 ± 0.005	1	CV[b]/8.0	−0.55 ± 0.01
Au−MPS−PEI−RC−PEI	0.465 ± 0.005	−0.049 ± 0.005	1	SWV[c]/8.0	−0.54 ± 0.01
PG−DMPC/RC	0.475 ± 0.005	−0.045 ± 0.005	1	SWV[c]/8.0	−0.50 ± 0.01

[a] ORT = mediated optical redox titration. Data from present work from CV = cyclic voltammetry and SWV = square wave voltammetry. [b] Av for 10−100 mV s^{-1}. [c] Av for 50−120 Hz and pulse height 50−150 mV. [d] From CV on free pigment not bound to RC protein.

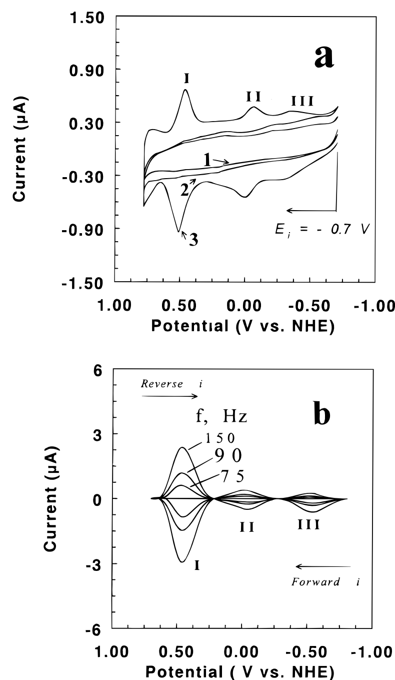

Figure 1. Voltammetry in argon-saturated pH 8.0 TRIS buffer in the dark at 1 °C. (a) CV at 5 mV s^{-1}: (1) Au−MPS−PDDA-Triton X-100−PDDA electrode; RC-free 0.06% Triton X-100 solution used to assemble film, (2) bare Au electrode in 30 μM RC, (3) Au−MPS−PDDA−RC−PDDA electrode in RC-free buffer. (b) Background-subtracted SWV in the dark of Au−MPS−PEI−RC−PEI film in RC-free buffer; pulse height 25 mV and frequencies 75, 90, and 150 Hz. (Oxidation current in negative direction; reduction current in positive direction.)

Square wave voltammetry (SWV)[20] is a potential pulsed method with superior sensitivity and resolution to CV. Background-subtracted SWV provided improved resolution of the RC cofactor peaks (Figure 1b). Scans at 75−150 Hz (pulse widths from 13 to 7 ms) showed that electron-transfer involving the three redox couples remained reversible (i.e., fast) on a time scale of 7−10 ms.

Midpoint potentials from SWV and/or CV for the films agree quite well with values from optical redox titrations (Table 1). Results suggest the following peak assignments: (**I**) P $\rightleftharpoons$ P$^+$ + e$^-$; (**II**) Q_A^- $\rightleftharpoons$ Q_A + e$^-$; and (**III**) BPhe$^-$ $\rightleftharpoons$ BPhe + e$^-$.

For the PDDA−RC−PDDA films, 8.4 × 10^{-12} mol cm^{-2} RC was estimated by QCM, compared to 8.1 × 10^{-12} mol cm^{-2} from integration of CV peak I using Faraday's law. For PEI−RC−PEI films, 7.5 × 10^{-12} mol cm^{-2} was found by QCM, compared to 6.8 × 10^{-12} mol cm^{-2} by CV. These data show that nearly all the RC in the polyion films communicates with the electrode.

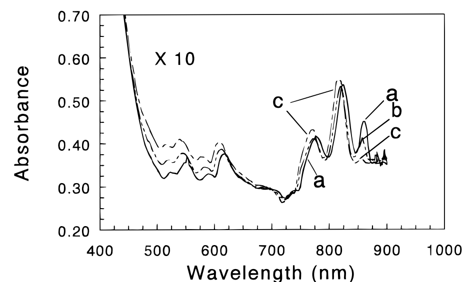

Figure 2. UV−visible spectroelectrochemistry of RC−DMPC film on transparent ITO electrode with electrolysis at 0.6 V vs NHE for (a) 0 min, (b) 10 min, and (c) 20 min. Following this, electrolysis at 0.0 V for 10 and 20 min gave spectra identical to (b) and (c), respectively.

RC−DMPC films on transparent indium-doped tin oxide (ITO) electrodes were made with sufficiently large optical absorbance to demonstrate reversible electrochemical interconversion between P and P$^+$ by spectroelectrochemistry. Figure 2 shows that the intial spectrum of RC in this film is nearly identical to that in solution, with relative peak intensities and wavelength maxima agreeing well with those in aqueous dispersions.[21] Electrolysis at 0.6 V in a spectroelectrochemical cell converted the initial spectrum of P to the spectrum of P$^+$ in 20 min (Figure 2). Following this experiment, electrolysis at 0.0 V for 20 min converted the P$^+$ spectrum back to the original P spectrum.

Results above show that redox states of electron-transfer components in the membrane-bound RC of *Rb. sphaeroides* can be interconverted reversibly by direct, nonmediated voltammetry in layered films of polycations or lipids. Midpoint potentials from CV and SWV are in good agreement with those obtained by more tedious, time-consuming optical redox titrations. Further, CV and SWV show peaks for BPhe, for which redox titrations in RC have not been reported to our knowledge. More generally, the methods described should be applicable to many other membrane-bound proteins.

Acknowledgment. This work was supported by grants from National Institute of Environmental Health Sciences, NIH, to J.F.R. (ES03154) and National Institute of General Medical Sciences, NIH, to H.A.F. (GM30353).

JA981521D

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