Voltammetry of Spinach Photosystem II in Lipid Films. Proton-gated electron transfer involving the Mn₄ cluster
Krisna Alcantara Bernard Munge, Zeus Pendon, Harry A. Frank, and James F. Rusling

SUPPORTING INFORMATION

PS II Characterization

1. Gel Electrophoresis

The proteins present in the native and Mn-depleted PS II samples were analyzed by gel electrophoresis. The proteins in the PS II complexes were first dissociated from one another, leaving negative charges on the proteins to enable them to migrate in the direction of the positively charged electrode. Methodology has been described previously.¹

SDS-PAGE was done in a Hoefer SE600 vertical electrophoresis unit (Amersham Pharmacia Biotech) with a 1.5 mm thick slab gel using 14% total acrylamide and bisacrylamide and 3% of the crosslinker, bisacrylamide as a uniform separating gel overlaid by a 4% T, 3% C, 2 cm stacking gel. The separating gel solution contains 14% acrylamide, 11% sucrose, 0.04% TEMED in 1 M Tris, pH 8.4. The stacking gel solution contains 4% acrylamide and 0.005% TEMED in 0.74 M Tris, pH 8.4.

The gels were polymerized separately, without prior degassing, by adding 0.05% ammonium persulfate. Sucrose in the separating gel solution prohibits mixing with the stacking gel solution during polymerization. The Anode buffer used for the experiment was 0.2 M Tris (pH 8.9) while the cathode buffer was a solution (pH 8.25) containing 0.1 M Tris, 0.1 M tricine, 0.1% SDS, 1mM EDTA. Samples containing 5 µg chlorophyll α were denatured in a buffer containing 2% SDS, 60 mM tris, pH 6.8, 10% glycerol, 2% mercaptoethanol, and 0.05% (w/v) bromophenol blue, by placing them in boiling water for 2 minutes. 30 µL of the solution were then placed in each channel of the gel. Moreover, 30 µL of the marker containing the molecular weight standards, phosphorylase b (94 kDa), albumin (67 kDa), ovalbumine (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa) were likewise placed in one or two channels.

The gels were run at a constant current of 30 mA at 4°C for about 22 hours, and then stained for 6 hrs in 30% methanol, 10% acetic acid, 0.1% Comassie brilliant Blue (R-350). Thereafter, de-staining was done using a solution containing 16% methanol, 7% acetic acid in water.

The gel electropherogram below shows the proteins found in the intact PS II and Mn-depleted PS II. Lane 4 shows the apparent molecular masses of the protein standards in kilodaltons. Two separate preparations of native PS II lanes show molecular masses at approximately 43, 30, 28, 27, 25, 22 and 17 kDa. The Mn cluster is destabilized and the protein with mass 17 kDa is lost in the Mn-depleted PS II. These results are characteristic of the known native and Mn-depleted PS II proteins from spinach.²⁴
Figure S1. SDS Polyacrylamide Gel electrophoresis of Mn-depleted PS II and two separate preparations of intact (native) PS II.
2. Oxygen evolving ability

Oxygen evolution of the PS II proteins was measured with a Clark-type oxygen electrode (Yellow Springs Instruments) in pH 6.0 buffer in solutions containing the photosystem II acceptor 2,5 dichlorobenzoquinone (DCBQ) or potassium ferricyanide (K₃Fe(CN)₆). A constant visible light source used to initiate the process.

The data output was measured with a chart recorder. A baseline current corresponding to about 1 cm was obtained at the time at which the light source turned on. An increase in height upon illumination indicated the evolution of oxygen. The chart recorder was calibrated to a rate of 3 cm/min and was stopped when a plateau was reached. The rate of oxygen evolution is calculated from the slope of current vs. time.

The oxygen evolution activity of the native PS II samples had an average value of 523 µmol O₂/mg Chl · hr, consistent with previous studies of spinach PS II. In the manganese depleted PS II, the oxygen evolution activity decreased to 93 µmol O₂/mg Chl · hour, indicating an 82% loss of oxygen evolution activity.

3. UV-Vis Spectra

Absorption spectra of films made on optically transparent indium-tin oxide coated (ITO) quartz slides were obtained with a Hewlett-Packard 8453 UV-Visible diode array spectrophotometer. Spectra (Figure S2) of dry films and the same films wet in pH 6.0 buffer solution gave characteristic strong absorption bands at 680 nm and 430 nm, and were nearly identical to the native PS II dissolved in pH 6.0 buffer. The strong background absorption due to the numerous antenna chlorophylls in PS II limits the identification of the characteristic absorption bands of the other cofactors in the PS II in the film. The spectra suggest, however, that the PS II reaction center complex in the PS II-DMPC films maintains its native state, and that the film is relatively stable on these slides after about 5 minutes in buffer.

![Figure S2](image-url)

**Figure S2.** UV-Vis spectra of films of dry PS II DMPC film, wet PS II DMPC film (pH 6 buffer) and PS II dissolved in pH 6.0 MES buffer
4. Voltammetry

**Figure S3.** Example of cyclic voltammetry before background subtraction for intact Spinach PS II RC in DMPC Film on PG Electrode in anaerobic pH 6.0 buffer containing 15 mM NaCl at 200 mV s\(^{-1}\).

**Figure S4.** Background subtracted Cyclic Voltammograms from Figure S3 for intact Spinach PS II RC in DMPC Film on PG Electrode in anaerobic pH 6.0 buffer containing 15 mM NaCl at 200 mV s\(^{-1}\). Background was subtracted as the CV of the DMPC film alone from each of the curves. Then, baseline correction was used by drawing a line from the baseline before and after the peaks so that the curves to remove the slope in the baseline. This did not change the values of the peak currents or potentials obtained after the background subtraction.
Figure S5. Forward and reverse square wave voltammograms without background subtraction for intact Spinach PS II RC in DMPC films on PG electrode in anaerobic pH 6.0 buffer containing 15 mM NaCl in pH 6 MES buffer. Pulse Height 25 mV, Step 1 mV.

Figure S6. Background subtracted forward and reverse square wave voltammogram of data in Figure S5 of intact Spinach PS II RC in DMPC films at PG electrode in anaerobic pH 6.0 buffer containing 15 mM NaCl; shown at 30 Hz in pH 6 MES buffer. Pulse Height 25 mV, Step 1 mV. Background was subtracted as the SWV of the DMPC film alone from each of the curves. Then, baseline correction (essentially a slope correction) was done using the feature by that name in the CHI software package. This did not change the values of the peak currents or potentials obtained after the background subtraction.
**Figure S7.** Background subtracted forward and reverse square wave voltammograms of native spinach PS II RC in DMPC films at different pH 8.2 HEPES buffer for (a) scan in negative direction, and (b) scan in positive direction. Frequency 5 Hz, step 1 mV, pulse height in mV labeled on curves.

**References**

(4) (a) Das, S. K.; Frank, H. A. Biochemistry, 2002, 41, 13087-13095. (b) BBY refers to preparations of PS II particles first described in reference 2.