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Directly probing redox-linked quinones in photosystem II membrane fragments via UV resonance Raman scattering

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ABSTRACT

In photosynthesis, photosystem II (PSII) harvests sunlight with bound pigments to oxidize water and reduce quinone to quinol, which serves as electron and proton mediators for solar-to-chemical energy conversion. At least two types of quinone cofactors in PSII are redox-linked: Q_A, and Q_B. Here, we for the first time apply 257-nm ultraviolet resonance Raman (UVRR) spectroscopy to acquire the molecular vibrations of plastoquinone (PQ) in PSII membranes. Owing to the resonance enhancement effect, the vibrational signal of PQ in PSII membranes is prominent. A strong band at 1661 cm^{-1} is assigned to ring C═C/C═O symmetric stretch mode (ν8a mode) of PQ, and a weak band at 469 cm^{-1} to ring stretch mode. By using a pump-probe difference UVRR method and a sample jet technique, the signals of Q_A and Q_B can be distinguished. A frequency difference of 1.4 cm^{-1} in ν8a vibrational mode between Q_A and Q_B is observed, corresponding to ~86 mV redox potential difference imposed by their protein environment. In addition, there are other PQs in the PSII membranes. A negligible anharmonicity effect on their combination band at 2130 cm^{-1} suggests that the 'other PQs' are situated in a hydrophobic environment. The detection of the 'other PQs' might be consistent with the view that another functional PQ cofactor (not Q_A or Q_B) exists in PSII. This UVRR approach will be useful to the study of quinone molecules in photosynthesis or other biological systems.

1. Introduction

Quinones play vital roles in biological energy transduction [1]. As an example, plastoquinones (PQs) serve as the terminal electron and proton mediators in photosystem II (PSII) [2–4], which is found in cyanobacteria, plants, and algae. PSII harvests sunlight with bound pigments, including chlorophyll (Chl) to drive a series of electron transfer reactions.

On the stromal side of PSII membrane, the electron transfer cofactor Q_A (a PQ) is reduced to Q_A^- in 400 ps by the primary electron acceptor $Pheo^-$ [5], and then Q_A^- reduces the neighboring Q_B (another PQ) in 200–400 μs [6]. Although Q_A and Q_B are chemically identical as PQ, their physiological redox processes are different. Q_A is only one-electron reduced, whereas Q_B accepts two electrons and two protons converting to plastoquinol PQH_2, which is then released from PSII [2,4].

As revealed by the X-ray crystallographic structure of cyanobacterial PSII (Fig. 1a) [7], cofactors Q_A and Q_B are situated symmetrically to the non-heme iron which binds with a bicarbonate and four histidine residues. The π-planes of their quinonoid rings are parallel with C═O pointing toward each other. The theoretical calculations based on the detailed crystalline structure suggest that the carbonyl groups of Q_B play important roles in proton coupled electron transfer [8]. Subtle structural changes imposed by the protein environment are not distinguishable from current X-ray crystallographic data.

To obtain more information about the structure and function of quinones, Raman spectroscopy is an incisive technique for studying aqueous biological samples giving fingerprint information on the chemical bond vibrations of target molecules [9,10]. Previous Raman investigations of benzoquinone (BQ), BQ anion radicals, and benzoquinols have shown that the C–O associated vibrations of these three species are distinguishable [11–13]. For example, reduction of BQ to BQ^- causes the ν8a mode (C═C/C═O stretch) shift from 1668 cm^{-1} to 1619 cm^{-1} [13]. Excitation at the wavelength of 441.6, 363.8, 351.1 or 325 nm, taking advantage of the large shifts in UV–visible absorption bands observed with reduction of quinones, shows clear resonance Raman

Abbreviations: UVRR, ultraviolet resonance Raman; PSII, photosystem II; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Tris–HCl, 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride; EDTA, tetrasodium ethylenediaminetetraacetic acid; MES, 2-(N-morpholino) ethanesulfonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PQ, plastoquinone; decyl-PQ, decyl-plastoquinone; OTG, n-octyl-β-D-thioglucoside; LHC, light harvesting complex; FTIR, Fourier transform infrared spectroscopy; HPLC, high-performance liquid chromatography

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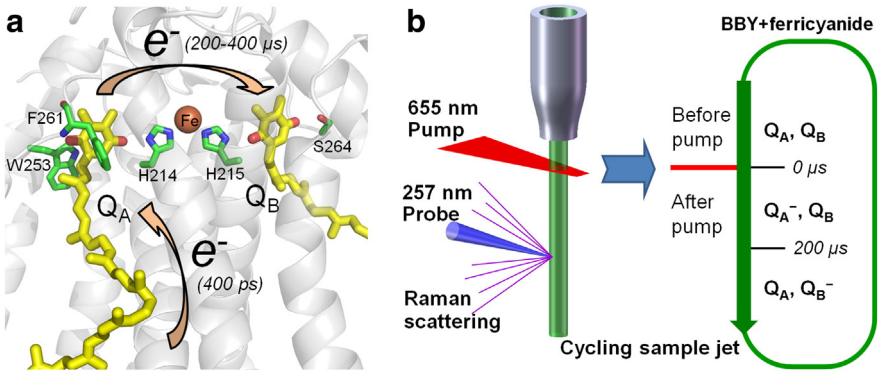

Fig. 1. (a) The local environment of the cofactors Q_A and Q_B in cyanobacterial PSII (PDB ID: 3ARC, reference [7]). The time constants for electron transfer to Q_A, and from Q_A to Q_B, are indicated. (b) Schematic of the pump-probe approach for UVRR measurements of PSII samples. The liquid sample was forced through a nozzle, forming a jet. The continuous-wave laser with 655 nm wavelength was focused as a line cutting the sample jet to pump the light-driven physiological processes in PSII samples. The Raman scattering excited with a 257 nm continuous-wave laser was exploited to probe the molecular vibrations of the samples. On the right side, the states of Q_A and Q_B cofactors before and after pump are indicated.

signals of the reduced BQ species (BQ^-, BQH^- and BQ^{2-}) [12]. In contrast, excitation at a shorter wavelength of 245 nm gives the greatly responsive resonance Raman signal of BQ [11]. These data were obtained on model compounds in aqueous solution or organic solvents. Importantly, previous researches on model quinone derivatives with vibrational spectroscopy reveal that the carbonyl vibration of quinone is related to the induction effect and the redox potential [13–15].

Excitation with proper UV light enhances Raman scattering from aromatic molecules, and shifts the Raman signal to UV region therefore avoiding most fluorescence interference especially from pigment molecules in protein complex, such as PSII [16] and PSI [17]. Similarly, PQ possesses a strongest UV absorption band at around 255 nm [18,19], whereas, reduction of PQ shifts its maximum absorption to longer wavelengths as 288, 320, 412 and 436 nm [18–21]. To obtain more information concerning the chemical bonds of the redox-linked quinone cofactors of PSII, we exploited 257-nm ultraviolet resonance Raman (UVRR) spectroscopy with a sample jet technique to characterize the PSII enzyme (Fig. 1b), because 257-nm excitation is expected to resonantly enhance the spectral contribution of PQ. However, the signals of reduced PQ species (e.g., PQ^- and PQH_2) are not expected to be resonantly enhanced because their electronic transition absorption bands are far away from the Raman excitation wavelength of 257 nm [18–21].

2. Materials and methods

2.1. PSII samples

The PSII membranes (referred to as BBY) were isolated from fresh spinach leaves as described previously [22,23]. Their final Chl concentrations were in the range 2.2–2.7 mg Chl mL^{-1}, and oxygen evolution rates were over 800 μmol O_2 (mg Chl)$^{-1}$ h^{-1} [24]. The Mn-depleted PSII membranes (denote as Mn-depleted BBY), in which the manganese oxide cluster was removed as well as extrinsic PsbO, PsbP and PsbQ subunits, were prepared by incubation of BBY in an equal volume of a buffer containing 1.6 M Tris–HCl (pH 8.0) and 4 mM EDTA in light [25]. Their final Chl concentrations were ~2.0 mg Chl mL^{-1}. The PSII core complexes, in which LHCII, CP26, CP29 and part of PsbP and PsbQ subunits were removed, were obtained by the treatment of BBY with detergent OTG [26]. Their final Chl concentrations were ~0.26 mg Chl mL^{-1} and oxygen evolution rates were ~1360 μmol O_2 (mg Chl)$^{-1}$ h^{-1}. All these samples were individually suspended in an SMN buffer (400 mM sucrose, 50 mM MES-NaOH, 15 mM NaCl, pH 6.0), and were frozen at −70 °C until use. The polypeptide compositions of these PSII samples were confirmed by SDS-PAGE [27].

2.2. UV–visible spectra

To record the absorption bands corresponding to the electronic transitions of PSII complexes, UV–visible spectroscopy (spectrometer: Cary 50, Varian, USA) has been exploited. The PSII samples were diluted in the SMN buffer, and this buffer was the reference solution. The parameter settings were: scan speed, 120 nm min^{-1}; sampling interval, 1.0 nm; and averaging time, 0.5 s. These parameter settings were also applied to the measurement of decyl-plastoquinone (decyl-PQ) which was dissolved in ethanol.

2.3. UVRR setup

UV resonance Raman measurements were performed by using a home-built instrument. The continuous-wave laser line of 257 nm wavelength (LEXEL 95, Cambridge, USA) was selected as Raman excitation source. In a backscattering collection mode, the laser beam was defocused on the sample with a UV objective (OFR division of Thorlabs Inc., USA), and Raman scattering from the sample was collected by the same objective and passed through edge filters (Semrock, USA) that block Rayleigh scattering. The Raman signal was further focused into a monochromator with 500 mm focal length (SR-500, Andor Technology, UK), thus was dispersed onto a UV-enhanced CCD detector (Newton 940-BU2, Andor Technology, UK). In order to capture high signal-to-noise spectra, the UV objective with a numerical aperture of 0.32 and an 8.5 mm working distance was adopted for high signal collection efficiency and for sufficient space in holding the sample jet; a 3600 groove mm^{-1} ruled grating which blazed at 240 nm was used for the dispersion of Raman signal; the wavelength responses of all mirrors and lens are specific for UV light of ~250 nm. Prior to the UVRR measurements, the spectral linearity was calibrated with the spectral lines of a mercury lamp, and the accuracy was calibrated with the UV Raman signal of a diamond. The spectral parameters were determined to be as follows: spectral resolution, 3.5 cm^{-1}; data interval, 1.1 cm^{-1}; and spectral cut-off frequency, <300 cm^{-1}. The frequency reproducibility for measurements was better than 0.1 cm^{-1}, which can be indicated by successive measurements of cyclohexane (see Fig. S1). This good reproducibility was also confirmed with the difference spectrum of successive measurements of PSII samples (see Fig. S2).

2.4. UVRR measurements

UVRR measurements were conducted at room temperature. Samples were recirculated in a flow cell with a nozzle, forming a liquid