Selective Resonance Raman Scattering from Chlorophyll Z in Photosystem II via Excitation into the Near-Infrared Absorption Band of the Cation

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Photosystem II (PSII) is a membrane-bound protein assembly responsible for photochemically catalyzing water oxidation. PSII utilizes photons to produce a charge-separated state in which the primary electron donor (P680) is rapidly reduced by the oxygen-evolving complex (OEC).[1] When the OEC is inactive, due to chemical inhibition or at low temperature, alternate electron donors such as chlorophyll Z (Chl_Z), cytochrome b_{559} (cyt b_{559}), and tyrosine D (Y_D) are oxidized to rereduce $P680^+$.[2] Chl_Z is thought to be a monomeric chlorophyll (Chl) located approximately equidistant from the lumenal and stromal sides of the membrane and outside the core of four Chls (one Chl dimer and two accessory Chls) in the reaction center.[2c] Oxidized Chl_Z (Chl_Z^+) is a potent quencher of fluorescence,[2d] and it may function to protect PSII against photoinhibition by dissipating excitation energy under conditions of high light.[2] Chl_Z^+ can be stably formed by illumination at low temperature in samples treated with a chemical oxidant to oxidize cyt b_{559},[2a] but studies of its molecular properties by optical spectroscopy have been limited by the difficulty of resolving Chl_Z from the array of other Chls in PSII. In this communication, we report near-infrared (IR) absorption[3] and near-IR-excitation resonance Raman (RR)[4] spectra of Chl_Z^+ in PSII isolated from *Synechocystis* PCC 6803.[5] Previous attempts to probe Chl cations by near-IR excitation have been plagued by large fluorescence background interference, but the quenching properties of Chl_Z^+ minimized this complication in our work. These studies identify the near-IR optical and RR signatures of Chl_Z^+ and reveal that high-quality RR spectra of a Chl cation can be selectively obtained by excitation into the near-IR absorption band of this species.

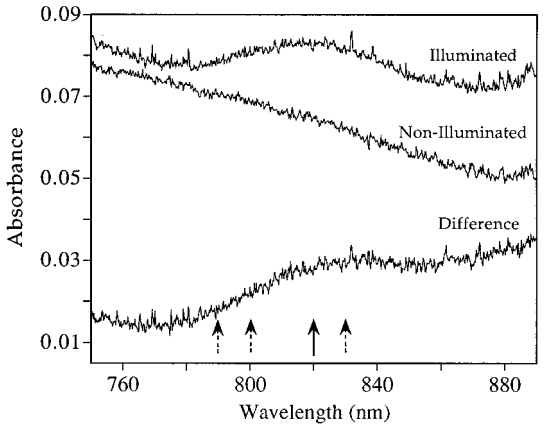

Figure 1. Near-IR absorption spectra of the PSII core complex at 77 K. The individual traces are discussed in the text.

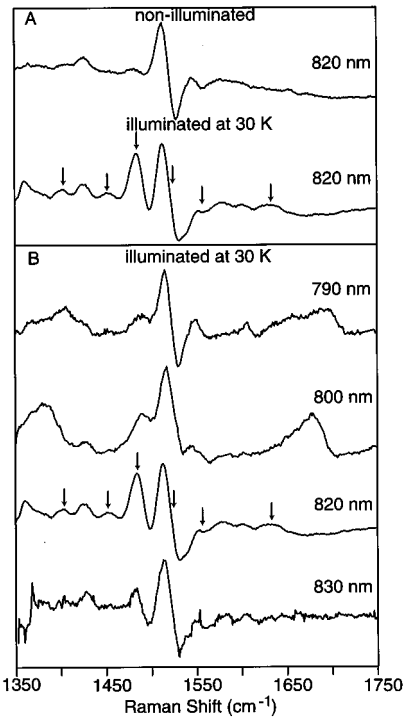

Figure 2. High-frequency (1350−1750 cm^{-1}) near-IR-excitation SERDS data obtained for the PSII core complex at 25 K. The individual traces are discussed in the text.

Figure 1 shows the near-IR absorption spectra of PSII before and after illumination. The illuminated-minus-nonilluminated difference spectrum (bottom trace) reveals an absorption band around 820 nm, which is attributed to Chl_Z^+; the arrows indicate the excitation wavelengths used for the RR experiments. Near-IR-excitation RR spectra of the PSII core complex are shown in Figures 2 and 3. Because the RR signals are relatively weak and ride on a moderate fluorescence background, the RR spectra were acquired by using the shifted-excitation Raman difference spectroscopic (SERDS) method.[6] The SERDS method and its application to RR studies of photosynthetic proteins are discussed

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(3) The near-IR absorbance measurements were collected on a Perkin-Elmer Lambda 6 UV−vis spectrophotometer with a Plexiglas flat cell immersed in liquid nitrogen. The samples (∼2 mg Chl/mL in 40% v/v glycerol) were frozen to form an uncracked glass at 77 K.

(4) (a) The RR measurements were made on highly concentrated (∼10 mg Chl/ml) glassy samples (40% v/v glycerol) at 25 K contained in 1 mm i.d. capillary tubes. The sampling accessories, spectrometer, and laser systems have been previously described.[4b,c] The acquisition time for a complete SERDS[6] data set (single spectral window) was 4 h. The spectral resolution was ∼2 cm^{-1}. The laser power was ∼5 mW. (b) Palaniappan, V.; Aldema, M. A.; Frank, H. A.; Bocian, D. F. *Biochemistry* **1992**, *31*, 11050−11058. (c) Palaniappan, V.; Martin, P. C.; Chynwat, V.; Frank, H. A.; Bocian, D. F. *J. Am. Chem. Soc.* **1993**, *115*, 12035−12049.

(5) (a) PSII core complexes were purified[5b] and depleted of Mn[5c] as previously described. Chl_Z^+ was generated by illuminating a chemically oxidized sample [1 mM K_2IrCl_6 (absorbance); 250 μM $K_3Fe(CN)_6$ (RR)] with a white light source [100 W quartz/halogen lamp (absorbance); focused 200 W quartz/halogen lamp (RR)] at cryogenic temperatures [10 min at 77 K (absorbance); 15 min at 25 K (RR)].[2a,d] The EPR spectrum of Chl_Z^+ in the samples used in these studies was identical to that observed previously.[2] (b) Tang, X.-S.; Diner, B. A. *Biochemistry* **1994**, *33*, 4594−4603. (c) Tamura, N.; Cheniae, G. *Biochim. Biophys. Acta* **1987**, *890*, 179−194.

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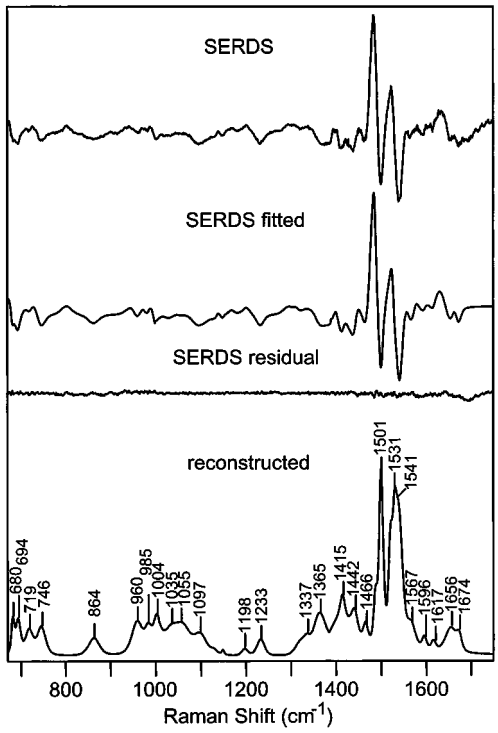

Figure 3. Near-IR-excitation (λ_{ex} = 820 nm) RR spectrum of Chl_Z^+ obtained at 25 K. The individual traces are discussed in the text.

in detail elsewhere.[6,7] Figure 2A compares the high-frequency (1350−1750 cm^{-1}) raw (unsmoothed) SERDS traces obtained for illuminated versus nonilluminated samples with λ_{ex} = 820 nm. Figure 2B compares the raw (unsmoothed) SERDS traces obtained for illuminated samples with λ_{ex} = 790, 800, 820, and 830 nm. Figure 3 (top trace) shows the difference data set (illuminated minus nonilluminated) obtained with λ_{ex} = 820 nm. The difference SERDS trace was constructed from the high-frequency data shown in Figure 2A plus SERDS data obtained in three additional ∼450 cm^{-1} wide overlapping spectral windows. The total range spanned by the four spectral windows was 150−1750 cm^{-1}. The spectral data obtained below 600 cm^{-1} are not shown because no features were observed. The second trace in Figure 3 is the fit of the difference SERDS data; the third trace is the SERDS residual (observed minus fit); the bottom trace is the RR spectrum reconstructed from the SERDS spectrum. The relatively small residuals compared with the SERDS intensities are indicative of the excellent fidelity of the fits.

Inspection of Figure 2 reveals that illumination of the PSII core complex generates a number of spectral features in addition to those observed in the nonilluminated sample. The intensity of the features generated by illumination grows (relative to the features present prior to illumination) as the excitation wavelength is tuned toward the red from 790 nm, maximizes near 820 nm, and begins to fall off at 830 nm. The fact that the spectral features are stably generated by low-temperature illumination and exhibit an intensity pattern that tracks the absorption band contour of Chl_Z^+ indicates that these new features are RR signals from the oxidized cofactor.[8] The remaining features in the spectrum are attributed to preresonance Raman scattering from the large number of neutral Chl cofactors in the PSII core complex. Although the neutral Chl chromophores absorb maximally near 680 nm, Raman scattering is observed even far off resonance[9] because the ratio of neutral Chl to Chl_Z^+ in the PSII core complex is ∼40:1.[5b] The absence of any of the light-induced signals in the dark spectra indicates that there was no actinic effect of the exciting beam; the excitation energy was probably too low to produce charge separation.

To date, the only RR spectra available for Chl cations were obtained with Soret excitation.[10] Comparison of the near-IR excitation spectra of Chl_Z^+ in the PSII core complex with Soret-excitation spectra previously reported for Chl cations in solution reveals many differences, both in the modes that gain resonance enhancement and in the intensity enhancement pattern. In this regard, neutral Chl also exhibits different RR scattering patterns with near-IR versus Soret excitation.[11] A significant difference between the near-IR excitation RR spectra of neutral Chl versus Chl_Z^+ is in the intensity enhancement pattern observed for the low-frequency modes (below 600 cm^{-1}). These modes exhibit essentially no RR enhancement in Chl_Z^+ but significant enhancement in neutral Chl.[11] [It should be noted that the absence of *preresonance* Raman scattering from the low-frequency modes of the neutral Chl chromophores in the PSII core complex is not surprising because the preresonance Raman intensities scale as ν^2.[12]] It is not clear whether weak enhancement of low-frequency modes is a general property of near-IR excitation RR scattering from Chl cations or whether this effect is unique to Chl_Z^+. For example, ultrafast dephasing processes, which may contribute to the damping of fluorescence in the PSII core complex by Chl_Z^+,[2d] might selectively attenuate the RR cross sections of the low-frequency modes of Chl_Z^+. Exploration of such issues must await further studies.

Finally, the significance of this work is 2-fold. First, the accessibility of RR scattering from Chl cations by near-IR excitation has been demonstrated. Second, the RR technique has been shown to be a selective molecular probe of a unique Chl in the PSII core complex. These methods are likely to be useful for future studies because there are several important Chl cations that are photochemically generated within PSII and PSI. For example, near-IR excitation RR studies could be extended to aid in the definitive assignment of P680 as either a "special pair"-like Chl dimer or a monomer Chl.[13] Similarly, the electronic and vibrational properties of $P700^+$, the room temperature-stable Chl cation in PSI, might be characterized to develop a better molecular picture of this cofactor.

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(8) The illumination-induced RR features exhibit diminished intensity on the red side (λ_{ex} = 830 nm) of the near-IR absorption band of Chl_Z^+ despite the fact that there appears to be appreciable absorption in this region. The absorption due to Chl_Z^+ is in fact also diminished in this region and only appears to be appreciable because the near-IR band rides on a baseline that slopes positively toward low energy.

(9) The Raman intensities of the neutral Chl cofactors are approximately constant with excitation in the 810−830 nm range because the resonance denominator in the Raman cross section, $((\nu - \nu_{ex})^2 - \Gamma^2)^{-1}$, changes little over this relatively small tuning range far off resonance. Thus, the intensities of the Raman bands of the neutral Chl cofactors serve to a very good approximation as internal intensity standards.

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