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Fourier transform infrared spectrum of the radical cation of β-carotene photoinduced in photosystem II

Takumi Noguchi*, Tomoya Mitsuka, Yorinao Inoue

Solar Energy Research Group, The Institute of Physical and Chemical Research (Riken), Wako, Saitama 351-01, Japan

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Abstract A Fourier-transform infrared (FTIR) spectrum of the radical cation of β-carotene photoinduced in photosystem II (PSII) membranes was obtained at 80K under oxidizing conditions, by utilizing the light-induced FTIR difference technique. Formation of the β-carotene cation was monitored with the electronic absorption band at 993 nm. An FTIR spectrum of a chemically-generated β-carotene cation in chloroform was also measured and compared with the spectrum of PSII. Since the FTIR bands of carotenoid cation have characteristic features with strong intensities, they can be useful markers in studying the reaction of carotenoid in PSII.

Key words: Photosynthesis; Photosystem II; Fourier transform infrared spectroscopy; Carotenoid; Radical cation

1. Introduction

Carotenoids are known to play two major roles in photosynthetic apparatus: a light-harvesting role by absorption of light and subsequent singlet–singlet energy transfer to chlorophylls, and a photoprotective role by quenching triplet chlorophylls and singlet oxygen [1]. Besides these roles, in photosystem II (PSII), it has been known that a carotenoid functions as one of the redox components in the electron transfer chain under some special conditions; illumination of chloroplasts or PSII membranes produced the radical cation of a carotenoid on the electron-donor side of PSII either under oxidizing condition at cryogenic temperatures or in the presence of some kinds of phenolic herbicides or lipophilic anions [2–5].

The reaction mechanism of carotenoid cation formation in PSII has not been well understood partly because the detection method of carotenoid cation has been limited to the measurement of electronic absorption near 1000 nm [3–5], which is not available with most of conventional spectrophotometers. In addition, in ESR spectra the signal of a carotenoid radical cation at $g\sim2$ cannot be easily distinguished from that of a chlorophyll radical cation (Chl^+), which is another redox component on the donor side of PSII [6].

In this study, we measured the FTIR spectrum of radical cation of β-carotene photo-induced in PSII by utilizing the light-induced FTIR difference technique. For the band identification, we also measured the FTIR spectrum of a chemically-produced β-carotene cation in chloroform solution and compared with the PSII spectrum. The results showed that the FTIR spectrum of β-carotene cation exhibits characteristic band features with strong intensities, possibly useful for studying the reaction of carotenoid in PS II.

*Corresponding author. Fax: (81) (48) 462-4685.

Abbreviations: Chl, accessory chlorophyll of photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ESR, electron spin resonance; FTIR, Fourier transform infrared; IR, infrared; MES, 2-(*N*-morpholino)ethanesulfonic acid; P680, primary electron donor of photosystem II; PSII, photosystem II; Q_A, primary quinone acceptor; UVVIS-NIR, ultraviolet-visible-near infrared.

2. Materials and methods

BBY-type PSII membranes [7] were prepared from spinach according to Ono and Inoue [8] and suspended in a MES-NaOH buffer (400 mM sucrose, 20 mM NaCl, 40 mM MES, pH 6.5). Mn-depleted PSII membranes were prepared by NH_2OH treatment (0.5 mg chlorophyll/ml of PSII + 10 mM NH_2OH) and subsequent washes with a MES buffer. DCMU (0.2 mM) and then potassium ferricyanide (20 mM) were added to the PSII suspension (0.5 mg chlorophyll/ml) before measurements.

All-*trans* β-carotene (synthetic) was purchased from Sigma. Chloroform (HPLC grade) was bubbled with N_2 gas at least for 1 h before use. For preparing the β-carotene cation, β-carotene dissolved in chloroform (10^{-2} M) was chemically oxidized by addition of I_2 (10^{-1} M) into the solution. In order to avoid isomerization of β-carotene, all the experimental procedures were performed under dim red light.

FTIR spectra were measured on a Jeol JIR-6500 spectrophotometer equipped with an MCT detector (EG&G Judson IR-DET101) with a spectral resolution of 4 cm^{-1}. For the measurement of PSII membranes, a PSII suspension was centrifuged for 30 min at 150,000 × *g*, and the resultant pellet was sandwiched between a pair of BaF_2 plates (13 mm φ). The sample temperature was adjusted to 80K or 210K in a cryostat (Oxford DN1704) with a temperature controller (Oxford ITC-4). In order to block the He–Ne laser beam partially leaking into the sample compartment, a Ge filter (OCLI LO2584-9) was placed in front of the sample. A light-induced difference spectrum was obtained by subtraction between the two spectra (300 scans; 150 s accumulation) measured before and after continuous-light illumination (5 s). Light illumination was performed by a halogen lamp (Hoya-Schott HL150) equipped with a heat-cut filter (FQHA-1(33T3.0)) and a red filter (> 600 nm) (R-60) with a light intensity of about 30 mW/cm^2 at the sample surface.

For FTIR measurements of β-carotene and its cation in chloroform (10^{-2} M), the solution was injected into the space between a pair of BaF_2 plates with an aluminum-foil spacer (≈30 μm in thickness), and was then quickly frozen with liquid N_2. Subsequently, the sample temperature was adjusted to 200K in the cryostat and FTIR spectra were measured. The FTIR spectrum of chloroform was separately measured and subtracted from the spectra of β-carotene and its cation.

UV-VIS-NIR spectra were measured on a Shimazu UV-3100PC spectrophotometer with a spectral resolution of 5 nm. A light-induced NIR difference spectrum of PSII membranes was measured in the same way as the measurement of light-induced FTIR difference spectrum except for using a buffer containing 50% glycerol. UV-VIS-NIR spectra of β-carotene and its cation in chloroform solutions (10^{-2} M) were measured at room temperature. A pair of quartz plates (1 cm × 4 cm) were dipped at their bottom in the solution stored in a quartz container. The solution rising between the two quartz plates afforded a very short light-path length (<5 μm) needed for measuring a dense carotenoid solution.

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3. Results

3.1. Light-induced spectra of PSII membranes

Fig. 1 shows a light-induced FTIR difference spectrum of PSII membranes measured at 80K in the presence of DCMU and ferricyanide. Since under this condition, the electron flow beyond Q_A is blocked and the charge recombination reaction is very slow, continuous-light illumination accumulates Q_A^- on the electron-acceptor side. In the spectrum (Fig. 1), the already-identified Q_A^-/Q_A bands [9,10], i.e. the positive band at 1481 cm^{-1} and most of the complex structures in 1750–1500 cm^{-1}, were observed, confirming that Q_A^- was in fact formed in PSII upon illumination.

On the donor side, oxidation of the Mn-cluster by $P680^+$ through the tyrosine residue (Y_Z) is completely blocked at 80K and some other redox components are oxidized [3,6,11]. In usual PSII, this redox component is Cyt b_{559} [6,11], but in the presence of ferricyanide, Cyt b_{559} is kept oxidized, so that the positive charge goes to a carotenoid or an accessory chlorophyll (Chl) molecule [3,6]. The light-induced NIR difference spectrum (Fig. 1 inset) showed a positive band at 993 nm, which Schenck et al. [3] previously assigned to a radical cation of carotenoid photoinduced in PSII, indicating that a carotenoid cation is formed in this PSII sample upon illumination. In the FTIR difference spectrum (Fig. 1), the strong positive bands that have not been identified so far were observed at 1465, 1441, 1148, 992 and 966 cm^{-1}. No corresponding negative bands with comparative intensities were observed in the 1500–900 cm^{-1} region. The primary candidate responsible for these positive bands is the carotenoid cation photo-produced in this PSII sample according to the result of NIR spectrum. Only with these data, however, the possibility of Chl^+ can not be excluded, because we do not know how much Chl^+ is formed under this condition.

In order to examine the contribution of Chl^+ to the above bands, the light-induced difference spectra were measured at

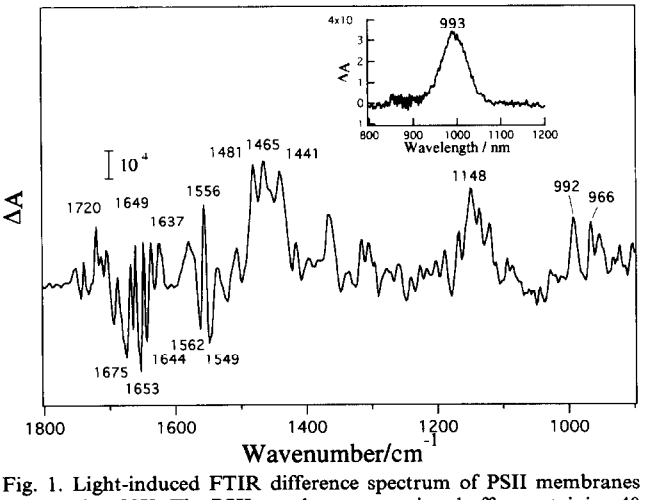

Fig. 1. Light-induced FTIR difference spectrum of PSII membranes measured at 80K. The PSII membranes were in a buffer containing 40 mM MES-NaOH (pH 6.5), 400 mM sucrose, 0.2 mM DCMU, and 20 mM potassium ferricyanide. The spectrum taken before continuous-light illumination was subtracted from that after illumination. Inset: Light-induced NIR difference spectrum measured under the same conditions as the FTIR spectrum except for the presence of 50% glycerol in the buffer.

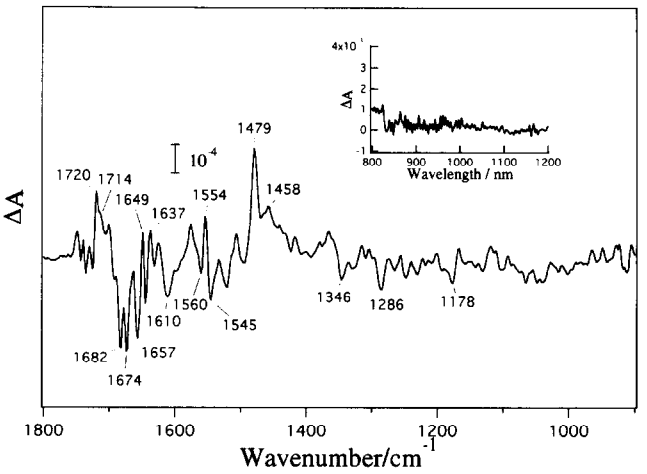

Fig. 2. Light-induced FTIR difference spectrum of Mn-depleted PS II membranes measured at 210K. Mn depletion was done by the NH_2OH treatment. The buffer contains 40 mM MES-NaOH (pH 6.5), 400 mM sucrose, 0.2 mM DCMU, and 20 mM potassium ferricyanide. Inset: light-induced NIR difference spectrum measured under the same conditions as the FTIR spectrum except for the presence of 50% glycerol.

210K (Fig. 2). In this PSII sample, Mn ions were depleted by the NH_2OH treatment so that the electron transfer from the oxygen-evolving center was blocked. Other conditions (i.e. the presence of DCMU and ferricyanide) were the same as those for the measurements at 80K. The typical Q_A^- band was observed at 1479 cm^{-1}, indicating that a negative charge was accumulated on Q_A and the charge separated state was formed in the reaction center after illumination. The light-induced NIR spectrum (Fig. 2 inset), however, showed that no carotenoid cation was formed at this temperature. Previously, Beck and Brudvig [12] observed a 10G wide signal at $g \sim 2$ upon illumination of the Mn-depleted (by the Tris treatment) PSII membranes at 210K (Cyt b_{559} is in the low potential configuration and is autooxidized in the dark), and suggested that Chl is an electron donor under this condition. In the present FTIR difference spectrum (Fig. 2), the positive bands at 1465, 1441, 1148, 992 and 966 cm^{-1} observed in the spectrum at 80K (Fig. 1), were altogether absent. This indicates that there is no contribution of Chl^+ to the above bands and supports the idea that those are ascribed to the carotenoid cation.

We note that the Chl^+/Chl FTIR spectrum without a Q_A^-/Q_A contribution could be measured at 210K with the Mn-depleted PSII membranes in the presence of silicomolybdate and ferricyanide as exogenous electron acceptors, and the obtained spectrum exhibited large positive and negative bands at ~1714 and ~1680 cm^{-1}, respectively, assignable to the keto C = O stretching modes (unpublished data). It is seen in Fig. 2 that these bands are superimposed over the complex Q_A^-/Q_A spectrum. Also, negative bands at 1346 and 1286 cm^{-1} (Fig. 2) are characteristic of the FTIR spectrum of cation-minus-neutral chlorophyll *a*, previously measured in an organic solvent and P700 of photosystem I [13].

3.2. Spectra of β-carotene cations in chloroform solution

It has been known that carotenoids in organic solvents can be chemically oxidized by using I_2 [14,15]. Fig. 3 shows a UV-VIS-NIR absorption spectrum of β-carotene dissolved in

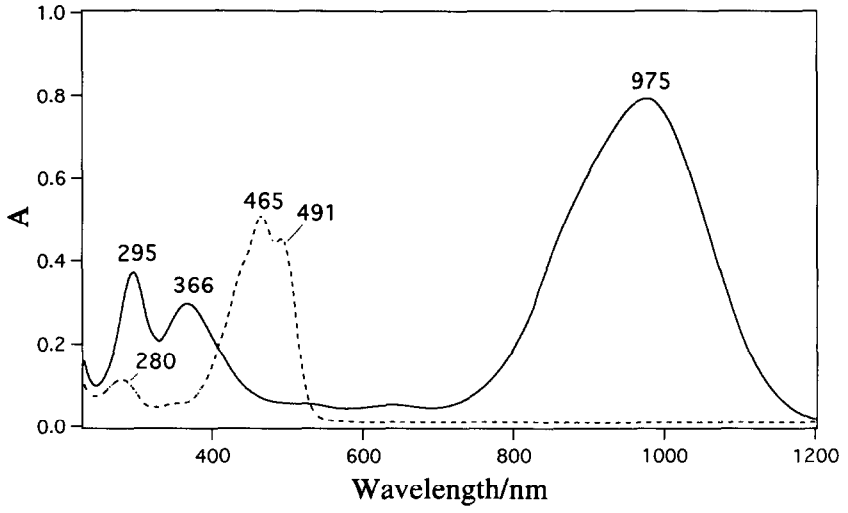

Fig. 3. UV-VIS-NIR spectra of β-carotene (10^{-2} M) dissolved in chloroform (dashed curve) and its cation produced by addition of I_2 (10^{-1} M) (solid curve). The spectra were measured at room temperature.

chloroform (10^{-2} M) (dashed curve) and that after addition of 10^{-1} M I_2 (solid curve). Upon addition of I_2 to the β-carotene solution, the absorption bands of neutral β-carotene in 500–400 nm completely disappeared and instead a large band at 975 nm and two bands at 366 and 295 nm appeared. The absorption band near 1000 nm is typical of a carotenoid cation, which has been extensively investigated by chemical oxidation [14,15], electrochemistry [16,17], pulse radiolysis [18], and flash photolysis [19]. The 366 and 295 nm bands were previously assigned to I_3^- that accumulated upon the oxidation of β-carotene with I_2 [14]. The above results indicate that under these conditions of solvent species and the concentrations of β-carotene and I_2, all the β-carotene molecules were oxidized to the cation form.

Fig. 4 shows the FTIR spectra of β-carotene in chloroform (dashed curve) and its cation produced by I_2 oxidation (solid curve). The samples were the same as those for the UV-VIS-NIR measurements in Fig. 3, except that the FTIR spectra were measured at 200K to avoid solvent evaporation. The most striking feature of the spectrum of β-carotene cation is that the band intensities were much stronger than those of the neutral species; In the spectrum of neutral β-carotene (dashed curve), the strongest band was the 966 cm^{-1} one, which has been assigned to the CH out-of-plane wagging mode [20], and all the other bands in 1600–1000 cm^{-1} had only weak intensities. By contrast, in the spectrum of β-carotene cation (solid curve), the three strong bands were observed at 1479, 1151, and 1001 cm^{-1}, and the intensities of these bands were significantly high even compared with the strongest 966 cm^{-1} band of the neutral β-carotene. This strong IR absorption of β-carotene cation may be rationalized by the increased dipole moments of some vibrational modes due to the presence of a positive charge in the polyene chain. It should be noted that similar three IR bands have been observed in an I_2-doped solid film of β-carotene [21,22].

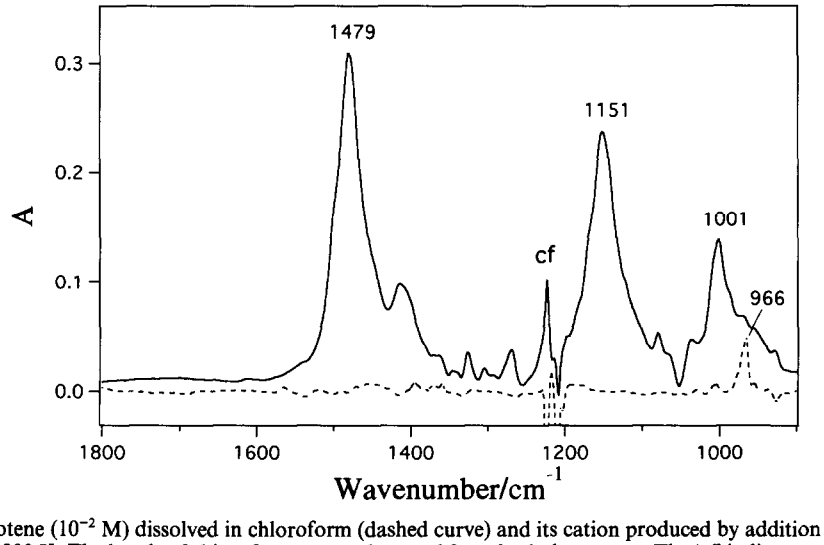

Fig. 4. FTIR spectra of β-carotene (10^{-2} M) dissolved in chloroform (dashed curve) and its cation produced by addition of I_2 (10^{-1} M) (solid curve). The spectra were measured at 200 K. The bands of chloroform were subtracted from both the spectra. The 'cf' indicates residual bands of chloroform after subtraction.

. Discussion

The FTIR bands at 1465, 1441, 1148, 992 and 966 cm^{-1} hotoinduced in PSII were assigned to the radical cation of arotenoid based on the NIR absorption measurement. Previusly, Berthomieu et al. [23] observed an FTIR difference spectrum similar to that of the present study by using Tris-treated 'SII membranes. Although they tentatively assigned the bands t 1466, 1439, and 1147 cm^{-1} in their spectrum to Chl^+, the resent study suggests that the bands in their spectrum is more kely attributed to the carotenoid cation.

The carotenoid oxidized upon illumination of PSII membranes is most probably the β-carotene molecule bound to the eaction center proteins adjacent to P680. In the absence of erricyanide, illumination of PSII membranes at 80K exhibited 'TIR bands due to oxidation of Cyt b_{559} by $P680^+$ [11]. However, in the presence of ferricyanide that keeps Cyt b_{559} in the oxidized form, the signals of Cyt b_{559} were replaced by the bands f the carotenoid cation. This means that a carotenoid is oxilized by $P680^+$ instead of Cyt b_{559}, because of the close location f the carotenoid molecule to P680.

Although the spectral features of the carotenoid cation as epresented by strong bands in three regions near 1450, 1150 nd 1000 cm^{-1}, were very similar between in PSII and in chlooform, some differences can be pointed out: The most promiient difference is that the cation spectrum of β-carotene in hloroform shows only a single strong band at 1479 cm^{-1} in the ighest frequency region, whereas in PSII membranes the coresponding band appears as a doublet with comparable intensiies at 1465 and 1441 cm^{-1}. Also, the 966 cm^{-1} band in PSII nembranes is much stronger than in chloroform solution. These observations mean that the structure of the β-carotene ation in the PSII reaction center somewhat differs from that n organic solvent.

In conclusion, we have identified the FTIR bands of the adical cation of β-carotene photoinduced in PSII. These cation ands are very strong and do not interfere with bands due to ther redox components, such as Q_A, chlorophyll and Cyt b_{559}. Hence, these bands can be useful markers in studying the reaction of carotenoid in PSII.

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