Fourier-transform resonance Raman spectra of cation carotenoid in photosystem II reaction centres

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Abstract Resonance Raman spectra of the cation form of a redox-active carotenoid in photosystem II are presented. These results have implications for the nature of the carotenoid radical and in particular the localisation of the hole on this molecule.

Key words: β-Carotene; D1D2 particle; Carotenoid cation; Raman spectroscopy

1. Introduction

In photosystem (PS) II, electrons are transferred from water to plastoquinone molecules [1,2]. During the events following photon absorption by this protein, a series of highly reactive chemical species are formed, the role of which is ultimately to oxidise water. The first and most oxidising of these species is the cation form of the primary electron donor, P_{680}^{+}, the redox potential of which has been estimated at 1.1 V. Under normal conditions, P_{680}^{+} is reduced by a nearby tyrosine of the D1 polypeptide, called Tyr_Z, which is reduced in turn by the manganese (Mn) cluster, considered as the site for water oxidation. When the electron cascade from the Mn cluster to P_{680} is interrupted, light absorption could result in the creation of potentially damaging chemical species within PSII. Complex protection mechanisms exist in this protein, which are designed to minimise the photodamage induced by non-specific oxidation by P_{680}^{+} (see [3] for review). Amongst these, it has been shown in particular that P_{680}^{+} is able to obtain electrons from a series of electron donors other than Tyr_z and these side-path reactions have been studied at low temperatures where their products can be accumulated under continuous illumination. It was recently shown that illumination of PSII-enriched membranes at 20 K leads to nearly stoichiometric formation of cation carotenoid (Car), $Car^{+\bullet}$, while 77 K illumination results in formation of a mixture of chlorophyll $(Chl)^{+\bullet}$ and $Car^{+\bullet}$ [4]. We have conducted similar experiments in PSII reaction centres (D1D2 particles). In D1D2, illumination at 77 K leads primarily to the accumulation of $Car^{+\bullet}$, absorbing at 993 nm. By conducting Fourier-transform (FT)-Raman measurements at 77 K on these pre-illuminated D1D2 particles, we were able to observe resonance Raman spectra (excited at 1064 nm) of the $Car^{+\bullet}$ species bound to PSII.

2. Materials and methods

D1D2 particles binding approximately six Chls and two Cars per two phaeophytins were prepared essentially as described previously [5]. Full exchange to *n*-dodecyl-β,D-maltoside was monitored via the loss of the strong absorption by Triton X-100 below 300 nm. All manipulations were performed on ice and in the dark. Samples were incubated for 2–3 min in the presence of an oxygen trap (5 mM glucose, 0.1 mg/ml catalase, 0.1 mg/ml glucose oxidase) just prior to freezing to induce anaerobiosis. For generation of the $Car^{+\bullet}$ species, silicomolybdate (SiMo) (Pfaltz and Bauer) was added as electron acceptor and illumination with white light was performed (at 77 K) using a Flexilux fibroptic illuminator, as described previously [4].

Absorption spectra were collected in an SMC-TBT flow cryostat (Air Liquide, Sassenage, France), cooled with liquid helium to 20 K, using a Varian Cary E5 double-beam scanning spectrophotometer. Aqueous samples used for cation formation were absorbed onto a filter paper support, as glycerol caused precipitation of the SiMo used as electron acceptor. Illumination in the cryostat was performed after warming to 77 K and was continued until no further absorption changes occurred (∼20 min). Final absorption spectra were taken upon re-cooling of the sample to 20 K.

For low temperature FT-Raman experiments, the samples were concentrated to an optical density in the Q_y peak of around 200 cm^{-1} (∼2 mM Chl). FT-Raman spectra were recorded using 1064 nm excitation from a continuous Nd-YAG laser on a FT infrared spectrophotometer (Bruker IFS66) equipped with a Raman module (Bruker FRA106), as described in [6]. Raman measurements were obtained from samples kept at 77 K in the same cryostat as for absorption. The spectra shown are the result of 10 000 added interferograms. Resonance Raman spectra of the neutral, ground state of D1D2-bound Car molecules were measured at 77 K on a Jobin-Yvon U1000 Raman spectrophotometer equipped with a N_2-cooled, back-thinned CCD detector (Spectrum One, JobinYvon, France), as described previously [7]. 514.5 nm excitation was provided by a Coherent Argon laser (model Innova 100).

3. Results

Fig. 1 displays the absorption spectrum of D1D2 particles at 20 K before illumination in the range 350–750 nm, as well as (inset) the calculated light minus dark spectrum in the near infrared for a sample containing SiMo. Illumination at 77 K induces the appearance of an absorption peak centred at 993 nm, which is typical of a Car cation [8,9]. This peak is quite similar to that observed after illumination of larger PSII particles at a low temperature [4,10,11] and we attribute it to a cation radical of the redox-active Car, $Car^{+\bullet}$. Note that a less apparent absorption was seen around 830 nm from the chlorophyll cation, $Chl^{+\bullet}$, than for the more intact system at the same temperature [4], although the use of filter paper as a sample support in the present work has resulted in spectra of a much lower quality.

Fig. 2 displays the FT-Raman spectra (100–3500 cm^{-1}) of D1D2 particles recorded before and after illumination. It shows that build-up of the $Car^{+\bullet}$ absorption transition is ac-

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Abbreviations: Car, carotenoid; Chl, chlorophyll; FT, Fourier-transform; PS, photosystem; SiMo, silicomolybdate ($SiMo_{12}O_{40}^{4-}$)

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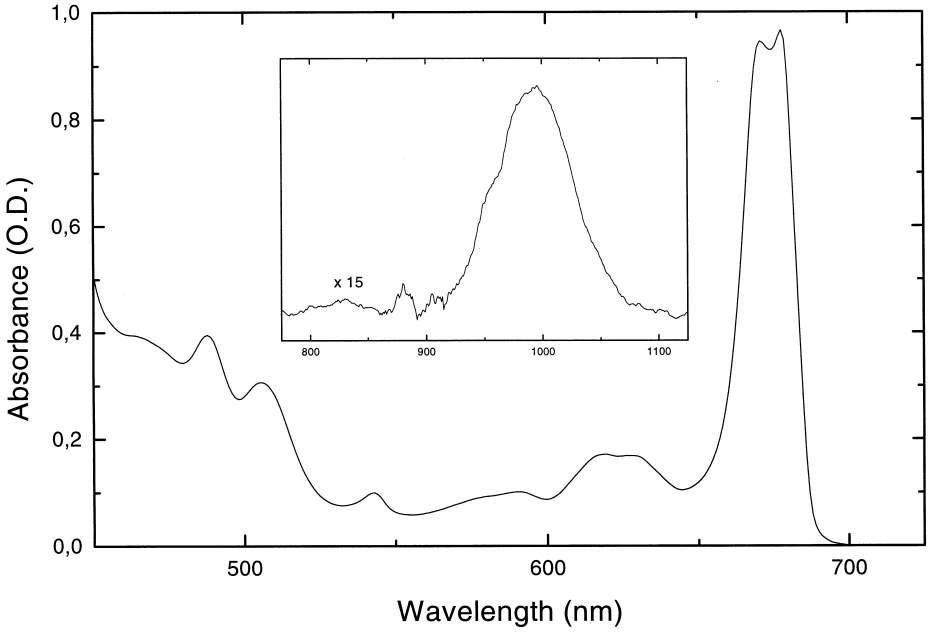

Fig. 1. Low temperature (20 K) absorption spectrum of the D1D2 particles in 60% glycerol, for the region 450–725 nm. Also shown (inset) is the calculated difference spectrum (light minus dark) in the near infrared (775–1125 nm) before and after 77 K illumination of a sample with 1.7 mM SiMo and oxygen trap but without glycerol, on a filter paper support. The same sample absorbance was used in each case.

companied by the appearance of a number of vibrational bands in the 200–1600 cm^{-1} region, as well as contributions between 2000 and 3300 cm^{-1} which are likely to be overtones and combinations of the lower frequency modes (indicating true resonance conditions: [12]). Shown in Fig. 3b is the calculated difference spectrum (light minus dark). This spectrum shares a number of bands with that of electrochemically generated $Car^{+\bullet}$ cation radicals [13] (see Table 1). It thus represents the vibrational contributions from the D1D2-bound β-carotene in its cation state. Of note is the huge increase in the contribution of low frequency modes (around 250 cm^{-1}) when compared with the neutral species (Fig. 3a), although the significance of this observation is not yet clear. Table 1 summarises the frequencies of the bands observed between 100 and 1600 cm^{-1} for both states of this molecule. Very few resonance Raman spectra of Car cations have been reported up to now and those only in vitro [13]. It is also the first time that such a spectrum has been obtained over such a large frequency range.

4. Discussion

The possibility of obtaining resonance FT-Raman spectra of the $Car^{+\bullet}$ radical of the D1D2-bound β-carotene opens

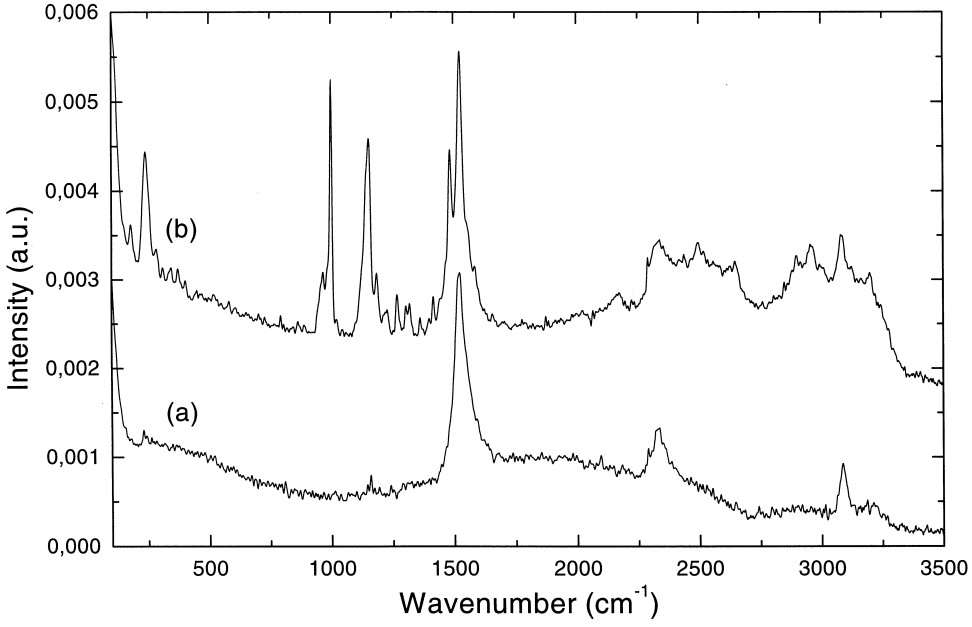

Fig. 2. 77 K FT-Raman spectra of D1D2 particles (a) and after illumination in the presence of 8.4 mM SiMo (b). Excitation was at 1064 nm.

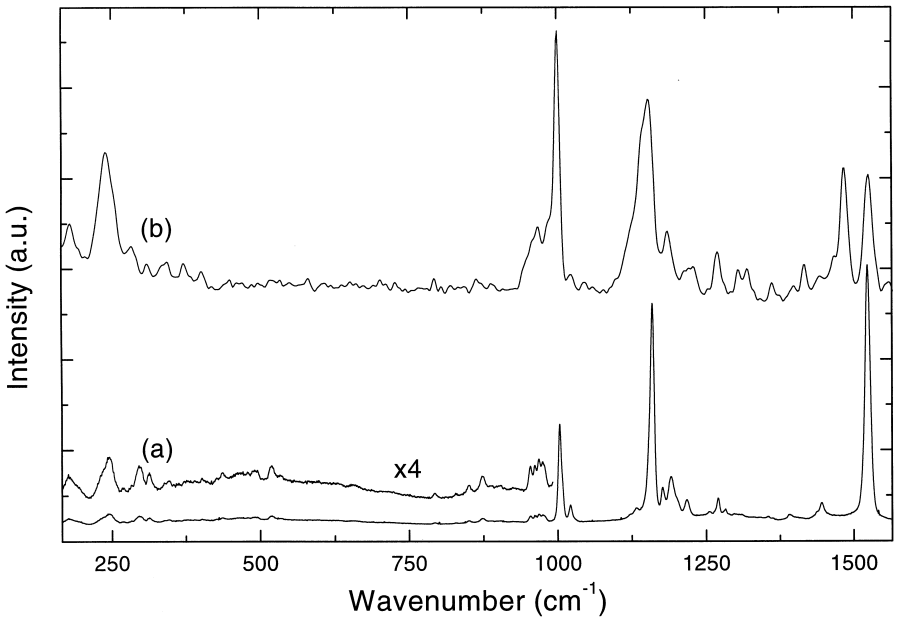

Fig. 3. Resonance Raman spectra of the D1D2-bound Car molecules. (a) Neutral, ground state Car excited at 514.5 nm. (b) $Car^{+\bullet}$ excited at 1064 nm, calculated from Fig. 2.

Table 1
Frequencies of resonance Raman bands in spectra of D1D2-bound β-carotene

β-Carotene		8′-Apo-β-carotene-8′al	Canthaxanthin
Neutral	Cation	Cation	Cation
176	179		
245	240		
296	283		
312	310		
343	344		
	372		
439	402		
519	581		
793	793		
850			
873	864		
953	946		
961	958		
965	968		
975			
1 003	1 001		
1 021	1 023		
1 121	1 124		
1 132	1 145		
1 159	1 154	1 157	1 162
1 176			
1 190	1 186	1 199	1 193
1 217	1 219	1 231	
1 254			
1 269	1 270	1 281	1 274
1 282	1 305		
	1 320		
	1 362	1 369	1 365
1 391	1 396		
	1 417		
1 445	1 445		
	1 484	1 481	1 507
1 524	1 525		
1 582	1 560		
1 594	1 586	1 545	1 542

Also shown for comparison are the bands reported for Car cations in vitro, taken from [13].

new possibilities for characterising this photo-induced species. Table 1 compares the frequencies observed for $Car^{+\bullet}$ molecules in solution and when bound to D1D2. In vitro, only the main bands of the spectra could be characterised (see [13]). Amongst those, one of the most intense, located at circa 1540 cm^{-1}, is not observed in our spectra. It seems likely that this is due to differences in resonance conditions between the two sets of experiments. Indeed, the in vitro experiments were conducted in post-resonance with the 993 nm absorption band (752.6 nm excitation) while our spectra were obtained in pre-resonance conditions (1064 nm excitation). As pointed out by Jeevarajan et al. [13], the intensity of the 1540 cm^{-1} may vary, depending on the precise position of the excitation line used. In vitro, it was observed that the formation of the Car cation induced a large downshift of the ν_1 frequency (around 1530 cm^{-1}), with little effect on ν_2 (at circa 1157 cm^{-1}). Spectra of cation β-carotene bound to D1D2 are in fair agreement with these findings (Table 1). It was observed that the decrease in frequency of ν_1 depended on the length of the conjugated polyene chain. For 8′-apo-β-carotene-8′-al (eight conjugated double bonds), this band downshifts by as much as 46 cm^{-1}, whilst for canthaxanthin (11 conjugated double bonds), it moves by only 11 cm^{-1} [13]. This was interpreted as reflecting the delocalisation of the hole over the entire conjugated region of the molecule. In the case of β-carotene bound to D1D2, for which the extent of the conjugated polyene chain is similar to canthaxanthin, we observed a 41 cm^{-1} downshift of this mode. In the frame of this hypothesis, this could indicate that the hole in this case is more localised than observed in vitro, possibly because of intermolecular interactions with the surrounding macromolecules. A recent EPR study showed that although the g value of $Car^{+\bullet}$ in vitro and in Mn-depleted PSII particles is the same (2.0024), the linewidth of these signals differs, being 13.4 and 9.5 gauss, respectively [4]. Current work in our laboratory has been undertaken to characterise the resonance Raman

contributions of $Car^{+\bullet}$ in larger PSII particles, to determine how the integrity of the PS influences the structure of this radical.

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