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Chl-*a* triplet quenching by peridinin in H-PCP and organic solvent revealed by step-scan FTIR time-resolved spectroscopy

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ABSTRACT

Triplet-state dynamics in Chl-*a*/Per mixtures in organic solvent and in native H-PCP were studied by means of step-scan FTIR spectroscopy. A single decay component of 10 μs was observed for the H-PCP triplet, the spectrum of which closely matches the 13 μs component of A-PCP [Alexandre et al., Biophysical Journal 93 (2007) 2118–2128], implying that in H-PCP, as in A-PCP, the peridinin triplet state is shared with Chl-*a*. In a mixture of Chl-*a* and Per in THF, TEET from ^{3}Chl-*a* to ^{3}Per proceeds in 3.5 μs followed by ^{3}Per decay in 7 μs. Using a target analysis procedure, ^{3}Chl-*a* and ^{3}Per infrared difference spectra were obtained. The specific carbonyl frequencies of ^{3}Per and ^{3}Chl-*a* in THF confirm our assignment of their co-existence in the infrared spectra of H-PCP and A-PCP.

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1. Introduction

Oxygenic photosynthesis is commonly summarized as carbohydrates synthesis using sunlight, water and carbon-dioxide, a phenomenon which greatly contributes to the abundance and diversity of life on earth. Photosynthetic organisms use antenna pigment-proteins to harvest light. Absorbed solar energy is transferred to the reaction centers (RC) where it is converted into an electrochemical gradient, which is used to synthesize ATP, powering the cell [1]. Together with (Bacterio)Chlorophyll ((B)Chl) carotenoids are the main pigments of photosynthesis. In addition to their structural involvement in the antenna architecture, carotenoids have a dual function namely light harvesting and photoprotection [2]. They harvest light in the blue–green spectral region where (B)Chl absorbs weakly thus increasing the absorption cross section of the light harvesting system. Carotenoid excitation is followed by ultrafast energy transfer to (B)Chl with a high efficiency [3–9]. (B)Chl intersystem crossing (ISC) may lead to triplet formation, the efficiency of which depends on the excited state lifetime. The long-lived (ms timescale) (B)Chl triplet reacts with ground state oxygen (which is a triplet state) to produce the highly reactive oxygen singlet ($^1O_2$) [10]. In light harvesting complexes (LHC), this process competes with fast (ns timescale) (B)Chl triplet excitation energy transfer to the carotenoid thereby avoiding formation of $^1O_2$ and ultimately the destruction of the light harvesting apparatus [10]. A good model to study this photoprotection mechanism is PCP (A-PCP) from the dinoflagellate *Amphidinium carterae*. The A-PCP monomer contains 2 Chl-*a* and 8 peridinins. In nature the peridinin-chlorophyll-*a*-protein (PCP) is found as a trimer and in each half of the A-PCP monomer one Chl-*a* is closely surrounded by 4 Per [11,12] leading to high efficiency for both light harvesting and photoprotection [13,14]. Following peridinin (Per) excitation, excitation energy transfer (EET) to Chl-*a* takes place with an efficiency of more than 90% [15]. In the isolated PCP complex, excited Chl-*a* undergoes ISC to the triplet state with a yield of about 10% which is entirely quenched by Per [16]. Per has a unique molecular structure constituted of an allene moiety and a lactone ring in conjugation with the π-electron system of the carotenoid backbone, an epoxy group with a secondary alcohol group on one beta ring and an ester group located on the opposite beta-ring with a tertiary alcohol group. The structural differences between Per and other carotenoids are related to its specific function in

Abbreviations: PCP, peridinin-chlorophyll-*a*-protein; A-PCP, PCP from *Amphidinium carterae*; H-PCP, PCP from *Heterocapsa pygmaea*; (B)Chl, (bacterio)chlorophyll; Chl-*a*, chlorophyll-*a*; Per, peridinin; LHC, light harvesting complex; ISC, intersystem crossing; EET, excitation energy transfer; TEET, triplet excitation energy transfer; T−S, triplet minus singlet; FTIR, Fourier transformed infra-red; EADS, evolution-associated difference spectrum; SADS, species-associated difference spectrum; ^{3}Chl-*a*, chlorophyll-*a* triplet excited state; ^{3}Per, peridinin triplet excited state; Chl-$a^{\cdot+}$, chlorophyll-*a* radical cation; $THF^{\cdot-}$, THF radical anion; Chl-$a^{\cdot+}$/$THF^{\cdot-}$, chlorophyll-*a* THF radical pair; ^{3}[Chl-a/Per], shared triplet excited state by Chl-*a* and Per.

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PCP. In contrast to most photosynthetic LHCs, the carotenoid (Per), and not Chl-*a*, is the main light-absorbing pigment in PCP. Per's unusual structure and especially its conjugated lactone carbonyl confers a high plasticity of absorptivity and reactivity on this carotenoid, for example by mixing CT states with the lower excited states [17–22].

In a recent investigation of the A-PCP triplet state using step-scan FTIR spectroscopy [13], we reported a significant involvement of the Chl-*a* triplet excited state (^{3}Chl-*a*) in the triplet excited state of Per (^{3}Per). This conclusion was based on a tentative assignment of both ^{3}Chl-*a* and ^{3}Per vibrational modes in the 13 and 42 μs decay phases of the A-PCP triplet state at RT. It was suggested that in A-PCP the triplet state was shared between Chl-*a* and Per. We proposed that the formation of the delocalized Per-Chl-*a* triplet state lowers the triplet energy and thus provides a general photoprotection mechanism for light-harvesting antenna complexes. A different dinoflagellate species *Heterocapsa pygmaea* uses a LHC closely related to A-PCP, H-PCP. H-PCP has the same pigment stoichiometry as A-PCP but its peptide unit is about half the size of that of A-PCP and contains only half the number of pigments [23–25]. The overall identity of the H-PCP monomer with that of the N and C terminal domains of A-PCP is about 70% [24]. 3D Modeling of the dimeric H-PCP sequence on the high-resolution X-ray structure of the monomeric A-PCP has shown only minor differences with the most significant being at the trimer interface [24]. Thus, the A-PCP monomer can be considered as the covalent equivalent of the H-PCP dimer and the pigment conformation in both systems should be very similar. This view is supported by the high similarity in the spectroscopic properties (absorption, CD, T−S spectra) of the two complexes [26].

It is of interest to investigate whether the same "triplet sharing" occurs in H-PCP as observed in A-PCP and to confirm our assignment of the ^{3}Per and ^{3}Chl-*a* features in the infrared spectra of PCP by observing these species in isolated molecules in organic solvent. Here, we have performed step-scan FTIR spectroscopy on H-PCP and on mixtures of Chl-*a* and Per in THF, thereby following triplet excitation energy transfer (TEET) from Chl-*a* to Per in THF solution. We observe a single decay component of 10 μs for the H-PCP triplet, the spectrum of which closely matches the 13 μs decay component of A-PCP, implying that also in H-PCP the triplet state has a mixed ^{3}Per and ^{3}Chl-*a* character. In addition, upon direct excitation of Chl-*a* in a mixture of Chl-*a* and Per in THF, TEET from ^{3}Chl-*a* to ^{3}Per proceeds in 3.5 μs followed by ^{3}Per decay in 7 μs. Using a target analysis procedure, ^{3}Chl-*a* and ^{3}Per spectra were obtained. The specific carbonyl frequencies of ^{3}Per and ^{3}Chl-*a* in THF confirm our assignment of their co-existence in the infrared spectra of H-PCP and A-PCP.

2. Materials and methods

2.1. Sample preparation

PCP samples of *H. pygmaea* have been isolated as previously described [23]. The FTIR sample was prepared by putting a droplet of 20 μL of PCP solution, containing 10 mg/mL PCP, 25 mM Tris.Cl (pH 7,5) buffer, 3 mM NaN_3, 2 mM KCl, on a CaF_2 window. The sample was concentrated under nitrogen flow and the resulting paste (3–5 μL) was spread between two tightly fixed CaF_2 windows. The same procedure was followed to prepare the FTIR sample of A-PCP [13]. Under our experimental conditions, H-PCP is dimeric.

Chl-*a* was purchased from Sigma–Aldrich (Netherlands) and was used without further purification. Per was isolated from *Amphidinium carterae* thylakoids by the method of Martison and Plumley [27] and was purified using reverse phase HPLC using an Alltech C18 column. The samples were dried in vacuo and stored at −80 °C. Chl-*a* and Per were dissolved in THF and sonicated. Samples were flushed with nitrogen or air gas to ensure anaerobic and aerobic conditions, respectively. Samples in THF were injected into an infrared cell composed of two CaF_2 windows separated by a 100 μm spacer.

2.2. Time-resolved step-scan FTIR absorption measurements

The time-resolved interferograms were recorded using a step-scan FTIR spectrometer (IFS 66s Bruker) placed on an air-bearing table (Kinetic Systems). The experimental set-up included a globar IR light source and a fast pre-amplified photovoltaic MCT detector (20 MHz, KV 100, Kolmar Technologies). The IR light impinging on the sample was sent through 4000 cm^{-1} low-pass and 1850–1200 cm^{-1} band-pass filters, which blocked the laser light before the interferometer and the detector. The detector signal was recorded with the internal digitizer (200 kHz, 16-bit A/D converter) allowing measurements with an IRF of 5 μs. A 20 Hz, Nd:YAG laser (5 ns, 100 mJ at 355 nm, Continuum) was used to pump an optical parametric oscillator (Panther, Continuum), producing tunable visible light from 400 to 700 nm, with a pulse duration of 5–7 ns. This light was attenuated to 2 mJ/cm^2 at 530 nm for H-PCP, weakly focused to a spot of 4 mm in diameter and overlapped with the IR probe beam, which was slightly smaller. For samples in THF, direct excitation of chlorophyll using laser radiation at 670 or 625 nm resonant with the Q_y or the Q_x Chl-*a* transition, led to the same results (data not shown). Chl-*a* only and Chl-*a* mixed with Per experiments were performed at 625 nm excitation as more power and better pulse to pulse stability is obtained from the YAG laser /OPO system at this wavelength. A Stanford Research Systems digital delay generator (DCR 35) was used to vary the time delay between the pump laser pulse and the trigger of the detection electronics. Each three-dimensional IR interferogram has an instrument response of 5 μs and 660 points in the spectral region 1800 to 1200 cm^{-1}, giving a spectral resolution of 8 cm^{-1}. Every data set is an average of 20 time-resolved interferograms of which each point is a time slice that is the average of 9 co-additions. The time-resolved interferograms have been further Fourier-transformed into time-resolved IR difference spectra (OPUS software, Bruker Optics). All experiments were performed at room temperature.

2.3. Global and target analysis

The time-resolved IR difference spectra were analyzed using global and target analysis [28]. For the interpretation of the H-PCP and Chl-*a* in THF data we applied a parallel and sequential kinetic model, respectively, that simultaneously fitted the dynamics at every point of a spectral data set. This analysis leads to decay and evolution associated difference spectra (DADS and EADS), respectively, with associated lifetimes. To model the Chl-*a* mixed with Per in THF time-resolved data a specific Target analysis has been used reflecting the complex decay processes yielding species associated difference spectra (SADS) with associated lifetimes.

3. Results and discussion

3.1. The triplet state in H-PCP

In A-PCP, we previously observed the coexistence of Per and Chl-*a* carbonyl modes during the Per triplet lifetimes of 13 and 42 μs [13], which led us to conclude that in this triplet state, ^{3}Chl-*a* and ^{3}Per are mixed. Here, we investigated the H-PCP complex by step-scan time-resolved FTIR spectroscopy to assess the nature of its triplet state. Upon excitation of peridinin at 530 nm, the H-PCP triplet decay is satisfactorily fitted with a single time constant of 10 μs.

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