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Pigment organisation in the membrane-intrinsic major light-harvesting complex of *Amphidinium carterae*: Structural characterisation of the peridinins and chlorophylls *a* and c_2 by resonance Raman spectroscopy and from sequence analysis

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ABSTRACT

The structures and environments of the protein-bound peridinins (*Pers*) and chlorophylls (*Chls*) a/c_2 in the membrane-intrinsic major light-harvesting complex of the dinoflagellate *Amphidinium carterae* (LHC_{Amph}) are characterised using resonance Raman (RR) spectroscopy with 11 excitation wavelengths, at 77 K. The excitation-dependent variation in the C=C stretching mode (ν_1) suggests the presence of three *Pers* with conjugation lengths over 8 double bonds (dBs), and one diadinoxanthin, between 413.7 and 528.7 nm. Two Per_{red} species are revealed on excitation at 550 and 560 nm. These Per_{red} species exhibit anomalously low ν_1 values, together with notable resonant enhancement of lactone ring-breathing and -deformation modes. To discern protein-specific effects, the RR spectra are compared to that of *Per* in polar (acetonitrile), polarisable (toluene) and polar-protic (ethanol) solvents. Resonantly enhanced lactone, ring-breathing (942 cm^{-1}) and ring-deformation (~650 cm^{-1}), modes are identified both in solution, and in the protein, and discussed in the context of the mixing of the S_1 and S_2 states, and formation of the intramolecular charge-transfer (ICT) state. In the *Chl*-absorbing region, two sets of *Chl* c_2's, and (at least) six *Chl a*'s can be differentiated. With a pigment ratio of 5–6 (*Chl a*):2 (*Chl* c_2):5–6 (*Per*):1 *Ddx* determined from the fit to the RT absorption and 77 K RR spectra, sequence comparison of LHC_{Amp} to LHCII, and the diatom LHC, fucoxanthin–chlorophyll-a/c–protein (FCP), a template for the conserved pigment binding sites is proposed, to fill the paucity of structural information in the absence of a crystal structure for LHC_{Amph}.

1. introduction

Marine organisms that use sunlight for their survival have adapted their photosynthetic apparatus according to their environment by, e.g., modifying the pigment content and organisation within their photosynthetic proteins. The membrane-intrinsic major light-harvesting complex from dinoflagellates (LHC_{DNF}) is a case in point, where carotenoids (Cars) rather than chlorophylls (Chls) are the primary light harvesters. The spectral range of the (sun)light available underwater is in the blue-green range, which is efficiently absorbed by carbonyl-containing carotenoids such as peridinin (*Per*), a Car present in both the dinoflagellate membrane-intrinsic LHC, and the membrane-extrinsic water-soluble peridinin–chlorophyll-*a*–protein (PCP). In contrast to PCP however, only a handful of studies have focussed on the spectroscopy of the membrane-bound LHC_{DNF} [1–6]. The current study adds to these contributions by providing structural information about the pigments in the LHC isolated from *Amphidinium carterae* (LHC_{Amph}).

In the absence of a crystal structure, structural information for the LHC_{DNF} has so far been based on CD and LD spectra and on its homology to LHCII [1,2]. These spectroscopic methods are linked to the electronic transitions of the pigments, whereas resonance Raman (RR) spectroscopy is a vibration technique that is sensitive to structural differences at the level of individual bonds and permits the distinction between different *Pers* and different *Chl* c_2s and *Chl a*s bound in LHC_{Amph}. Whereas vibrational studies on *Per* and PCP have been carried out at RT [7–10], the present RR study at 77 K has the advantage of decreasing inhomogeneous

Abbreviations: Cars, carotenoids; *Chls*, chlorophylls; dBs, double bonds; *Ddx*, diadinoxanthin; ET, energy transfer; FCP, fucoxanthin–chlorophyll-a/c–protein; *fwhm*, full-width half maximum; HOMO (LUMO), highest (lowest) occupied molecular orbital; HOOP, hydrogen out-of-plane; ICT, intramolecular charge-transfer; LHC_{DNF}/LHC_{Amph}/LHC_{Symb}, light-harvesting complex of the dinoflagellate/*Amphidinium carterae*/*Symbiodinium*; *Luts*, luteins; LN2, liquid nitrogen; PCP, peridinin–chlorophyll-*a*–protein; *Per*/Per_{Tol}/Per_{EtOH}/Per_{Acn}, Peridinin/in toluene/in ethanol/in acetonitrile; PES, potential energy surface; RR, resonance Raman; TA, transient absorption.

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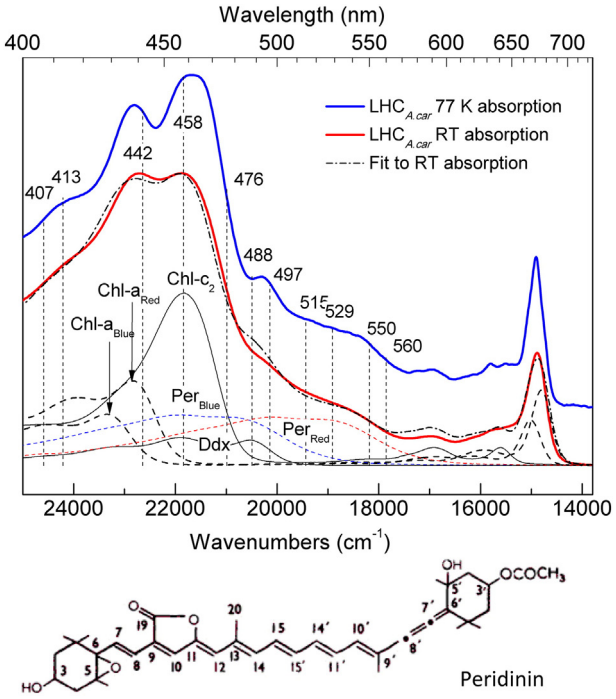
broadening and reducing thermal congestion. Moreover, the current multi-wavelength excitation, between 406.7 and 560 nm, selectively enhances the vibronic modes coupled to the electronic transition of the different pigments and permits the identification of *Per*, *Chl a* and Chlc_2 molecules that are localised in different environments within the protein. *Per* has been extensively studied in solution to better understand its role in light harvesting and energy transfer (ET) in both PCP and LHC$_{DNF}$, and the comparison of the protein RR spectra to that of *Per* in solution provides an added perspective about the effect of the protein environment on the pigment's functionality in vivo.

The potential energy surface (PES) of *Per* is described by analogy to the C_{2h} point group of symmetric polyenes, where the $S_0 \rightarrow S_2$ ($1^1A_{g-} \rightarrow 1^1B_u{}^{*+}$-like) transition is allowed, and the $S_0 \rightarrow S_1$ ($1^1A_{g-} \rightarrow 2^1B_u{}^{*+}$-like) transition forbidden. In addition, an intramolecular charge transfer (ICT) state identified in carbonyl-containing carotenoids such as *Per*, has been proposed to be the principal ET channel [11] to *Chl a*, e.g., in PCP [12] and in LHC$_{Amph}$ [3]. This intermediate ICT state has generally been considered to be very strongly coupled to the S_1 state [12,13], and was also (first) proposed to be linked to the S_2 state based on electroabsorption studies [14]. Indeed, a compelling argument, recently made by Wagner et al. [15], that the ICT state results from the mixing of the *ionic* $1^1B_u{}^{*+}$-like and *covalent* $2^1A_g{}^*$-like states, carrying mostly $1^1B_u{}^{*+}$ character while exhibiting "an unusually large doubly excited" character.

Notably, RR spectroscopy is sensitive to the variation in the (electronic) nature of the excited-state PES as it would concomitantly result in the enhancement of different vibronic modes. Experimentally, excitation into different regions of the absorption band of the pigment of interest would enhance different vibronic modes. Note that these differences would be more evident at 77 K. For example, in *Per*, red-edge excitation could show whether, and how, the lactone carbonyl group, which is proposed to be linked to the formation of the ICT state and ET [3,11], is affected. We note that the allene group is not really involved in the photophysics of *Per*, although conjugation may extend into the C8′═C7′ bond (Fig. 1). However, conjugation cannot extend into "the allene" moiety as a whole given that the central carbon of the allene (C7′) is sp hybridised and by definition the C8′═C7′ and C7′═C6′ π-bonds will be perpendicular to each other. Calculations actually show that the HOMO (and LUMO) is only marginally localised over C8′═C7′ [16] and that this bond does not lie in the same plane as the rest of the polyene backbone.

More generally, LHC$_{DNF}$ is closely related to the LHCs from diatoms, the fucoxanthin chlorophyll-*a*/*c* proteins (FCP), and LHCII from plants and green algae. The light harvesting and ET capabilities and differences between LHC$_{DNF}$, FCPs, and LHCII, are closely tied to the type and number of bound pigments. In contrast to LHCII, both LHC$_{DNF}$ and FCP possess Cars with conjugated-carbonyls, *Per* and *Fx*, respectively, and bind *Chl c* instead of *Chl b*. The *Chl a:Chl c:Fx/Per:Ddx* was determined to be 8:2:8:0.1 in FCP [17] and 7:4:12:2 in LHC$_{Amph}$ [1], versus 8*Chl a*: 6*Chl b*: 4Cars in LHCII [18]. The spectroscopic properties of LHC$_{Amph}$ and FCP are also quite similar, as highlighted in early studies on the ET dynamics in LHC$_{Amph}$ [3–6]. Transient absorption (TA) [3,5,6] studies identified red absorbing *Per*s in LHC$_{Amph}$ as the most efficient light absorbers and energy-transfer conduits to *Chl a*, estimated at 90% in the 530–550 nm region [3,11], similar to the red fucoxanthins (*Fx*$_{red}$s) in FCP, whereas ODMR and EPR measurements [4] drew parallels between the (blue absorbing) *Per*s and the luteins (*Lut*s) in LHCII. Two recent 77 K TA absorption studies, one on LHC$_{Amph}$ [5], and the other on the *Symbiodinium* LHC (LHC$_{Symb}$) [6], have further elucidated the spectroscopy of these LHCs and their similarity to the FCPs. The similarity of LHC$_{DNF}$ to LHCII, and to trimeric and oligmeric FCP, is also evident from the sequence analysis of their gene products. We have used this analysis to assign putative binding sites for the Chls, and locations for the Cars, identified from the RR spectra of LHC$_{Amph}$.



77 K	Per$_{MeTHF}$	Per$_{red}$	Per$_{green}$	Per$_{blue}$	Ddx
0-0	519	545	526	502	494
0-1	486	509	492	471	462
0-2	456	476	462	443	434

Fig. 1. The 77 K (blue) and room-temperature, RT (red), absorption spectra of LHC$_{Amph}$ are shown with their Q_y band areas scaled to the same area (~ 15300 to 14000 cm^{-1} in units) — the original OD of the Soret bands being ~0.5–0.7 OD. The fit to the RT spectrum (dash-dot line) is shown with the component pigment absorptions modelled as *Per*$_{red}$, *Per*$_{blue}$, *Chl c*$_2$, *Chl a*$_{red}$ and *Chl a*$_{blue}$ (Table 1). The vertical lines indicate the excitation wavelengths (406.7–560 nm) used to obtain RR spectra. The *x*-axis is linear in energy (cm^{-1} on top) and non-linear in wavelength (nm on bottom), and the *y*-axis is on an arbitrary scale. Below is shown the structure of *Per* numbered as per IUPAC nomenclature. For better comparison to the RR spectra measured at 77 K, the table lists the vibronic bands of the *Per*s based on the fit to the 77 K absorption of LHC$_{Symb}$ in Ref. [6].

2. Material and methods

2.1. Sample preparation

RR spectra of isolated Per in solution were obtained by dissolving *Per* in 1–2 ml of solvent – fresh, HPLC-grade toluene and ethanol (EtOH), and freshly distilled acetonitrile (Acn) – and then immersed in liquid nitrogen (LN2). RT absorption spectra of *Per* in these and other solvents can be found in the literature [19–21], and in glass-forming solvents at 77 K [14,19].

The major LHC$_{Amph}$ protein was isolated as in Ref. [2] and buffered in 25 mM Tris at pH 7.5, and 10 mM KCl. A concentrated solution ('slurry') of LHC$_{Amph}$ (absorbance of 300 OD at 460 nm estimated from the absorption spectrum of a 1000× diluted solution) was used to obtain RR spectra because the signals in the carbonyl stretching region (1550–1800 cm^{-1}) of both the *Per*s and the Chls are weak and noisy when using dilute samples.

2.2. Sequence analysis

Comparison of amino acid sequences of *lchb1* from spinach (PDB ID: 4LCZ), *fcp2* and *fcp5* from *Cyclotella meneghiniana* ([17]) and a single *lhc*$_{Amph}$ peptide from the putative polyprotein encoded in *Amphidinium* (GenBank AJ009670) was carried out using ClustalW.