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Characterization of the peridinin–chlorophyll *a*-protein complex in the dinoflagellate *Symbiodinium*

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Abstract

The water-soluble peridinin–chlorophyll *a*-proteins (PCPs) are one of the major light harvesting complexes in photosynthetic dinoflagellates. PCP contains the carotenoid peridinin as its primary pigment. In this study, we identified and characterized the PCP protein and the PCP gene organization in *Symbiodinium* sp. CS-156. The protein molecular mass is 32.7 kDa, revealing that the PCP is of the monomeric form. The intronless PCP genes are organized in tandem arrays. The PCP gene cassette is composed of 1095-bp coding regions and spacers in between. Despite the heterogeneity of PCP gene tandem repeats, we identified a single form of PCP, the sequence of which exactly matches the deduced sequence of PCP gene clone 7 (JQ395030) by LC–MS/MS analysis of tryptic digested PCP, revealing the mature PCP apoprotein is 312 amino acids in length. Pigment analysis showed a peridinin-to-Chl *a* ratio of 4. The peridinin-to-Chl *a* Q_y energy transfer efficiency is 95% in this complex.

Keywords

Photosynthetic antenna; Peridinin–chlorophyll *a*-protein; *Symbiodinium*; Peptide sequencing; Absorption spectroscopy; Fluorescence spectroscopy

1. Introduction

Peridinin–chlorophyll *a*-proteins (PCPs) are one of the major light harvesting complexes in photosynthetic dinoflagellates [1-3]. PCP is water-soluble and has the blue-green (470 nm to 550 nm) absorbing carotenoid peridinin as its primary pigments. *Symbiodinium*, the most commonly found endosymbiotic dinoflagellates in symbiosis with corals [4,5], also contain PCPs as their major light harvesting complexes [6-8]. A recent study [9] suggested that *Symbiodinium* PCP may play an important role in coral bleaching, which results from the loss of *Symbiodinium* cells from the coral host or the loss of photosynthetic pigments from *Symbiodinium* [4]. Because coral bleaching always follows severe photoinhibition [9-11], and moderate heat stress can induce the algal photosystem II (PSII) photoinhibition by damaging photosynthetic light harvesting complexes and thylakoid membranes [9,12], it is

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Appendix A. Supplementary data

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hypothesized that increased sea surface temperature is one of the major factors that trigger coral bleaching. The study [9] showed that the level of PCP in a cultured thermal-sensitive *Symbiodinium* strain dropped under light conditions when temperature increased, supporting the temperature-induced coral bleaching theory and suggesting the connection between *Symbiodinium* PCP and coral bleaching.

However, *Symbiodinium* PCP is poorly understood in the aspects of structure, spectroscopic properties, energy transfer, etc. In general, dinoflagellate PCP proteins are very diverse. The length, pigment content, sequence and spectroscopic properties of PCP can be distinct among different dinoflagellates or even in an individual dinoflagellate. In the aspect of length, there are two forms of PCPs: one is a homodimer with a monomeric molecular mass of ~15 kDa [13,14], the other is a monomer with a molecular mass of 32–35 kDa [3,13,14].The latter form was hypothesized to be the product of duplication and subsequent fusion of the PCP gene encoding the former form [8,15]. Two monomers of the homodimeric PCP probably form a dimer, whose structure could be very similar to that of the monomeric PCP monomer [3,16].In *Amphidinium carterae*, three copies of the monomeric PCP associate to form a trimeric complex [3,17]. The species specificity of the PCP quaternary structure has been reported [14]. Both monomeric and homodimeric forms were detected in *Symbiodinium microadriaticum*, while *Symbiodinium kawagutii* and *Symbiodinium pilosum* only possess a single form of PCP, monomeric and homodimeric forms, respectively [14]. In the aspect of pigment content of PCP, the molar ratio of peridinin and Chl *a* varies: the main form PCP complex (MFPCP) from *A. carterae* has the pigment composition of 8:2 [3]; the high-salt PCP (HSPCP) from the same species, eluted at higher salt concentration from an ion exchange column, has 6 peridinins and 2 Chl a molecules in one monomer [18]; Haxo et al. reported a 9:2 ratio for the PCP from *Amphidinium carterae* Plymouth 450 [1];PCP from *Alexandrium* cohorticula may consist of 12 peridinins and 2 Chl *a* molecules [19].

The PCP protein sequence is not very conserved, leading to distinct pigment binding environments, which determine the spectroscopic properties of a protein [20]. The MFPCP and HSPCP from *A. carterae* have a sequence similarity of ~30%, resulting in peridinin loss and chlorophyll phytol chains rearrangement in HSPCP [18,20]. Besides, PCP isoforms with varying isoelectric points (pIs) [1,2,5,14,15] were observed in dinoflagellates. The major isoforms tend to be species-specific. *A. carterae* and *S. microadriaticum* mainly produce basic PCPs of pI 7.5 and pI 7.2–7.7, respectively [1,14], while *Gonyaulax polyedra* PCPs are mostly acidic of pI around 6 [15]. Small spectroscopic variations of PCP isoforms were reported in *S. microadriaticum*: the Chl *a* Q_y absorption maxima varied from 669.8 nm to 673.4 nm and the corresponding fluorescence emission maxima were from 673.0 nm to 676.5 nm [14].

In order to understand the source and significance of PCP isoforms, PCP protein structures and PCP genes have been explored since 1990s. Among different PCPs, the structures of MFPCP and HSPCP from *A. carterae* have been resolved to 2.0 Å and 2.1 Å, respectively [3,18]. A higher resolution of 1.5 Å was recently resolved in refolded PCP [21]. The X-ray crystal structure of MFPCP has revealed a trimer, in which each subunit folds in a twofold pseudosymmetry (a monomer has two pseudo-identical domains), holding 8 peridinins and 2 Chl *a* molecules. On the other hand, HSPCP crystallized as a monomer, which contains 6 peridinins and 2 Chl *a* molecules. The high degree of structural similarity was observed, although sequence variations led to the differences in the level of the protein scaffold, pigment composition, and interaction between the pigments and their binding environment [18]. The sequence analysis of PCP genes and transcripts based on the data from *A. carterae* [22], *G. polyedra* [15], *Heterocapsa pygmaea* [16,23] and *Symbiodinium* sp. [8,13,24] revealed that PCP genes are nuclear-encoded, intronless and exist in tandem arrays. PCP

gene arrays consist of coding regions and the spacers in between. The lengths of coding regions and spacers, as well as the copy number of PCP genes, are species-specific. The sequences of coding regions in PCP gene arrays are distinct among different dinoflagellate species and even in one individual dinoflagellate. Along with the observation of multiple PCP isoforms with varying pIs, the PCP gene heterogeneity is believed to be the source of the isoforms. However, except for two different forms of PCP proteins (MFPCP and HSPCP from *A. carterae*), which have been detected and determined at the protein sequence and structure level [3,16,18], there has been no report successfully relating cDNA or gDNA sequences to their corresponding protein sequences because of the complexity of PCP genes and the possible protein expression preferences.

In this study, we identified the PCP gene family in *Symbiodinium* sp. CS-156 [25], confirming the genetic diversity of PCP genes and the existence of PCP tandem arrays. We further related PCP gene sequence to its corresponding protein sequence and compared the spectroscopic properties of *Symbiodinium* PCP with those of *Amphidinium* PCP.

2. Materials and methods

2.1. Algal culture and PCP purification

Symbiodinium sp. CS-156 cells were cultured in f/2 media under a 14 h:10 h cycle of light:dark at 25 °C. Illumination was provided by a white color fluorescent lamp at an intensity of 80 μmol photon·m^{-2}·s^{-1}. The culture in late exponential phase was harvested by centrifugation at 8000 *g* for 10 min at 4 °C. Cells were resuspended in 50 mM tricine 20 mM KCI (pH 7.5), and broken by three passes through a French pressure cell at 8.3×10^7 Pa [22]. Cell debris and unbroken cells were removed by centrifugation at 20,000 *g* for 1 h. Solid ammonium sulfate was added to the resulting supernatant to achieve 70% saturation, followed by centrifugation at 8000 *g* for 15 min. The resulting supernatant was dialyzed against 20 μM Tris–HCl (pH 8.0), concentrated, filtered through a 0.2 μm filter, and applied to a HiLoad™ Superdex™ 200 prep grade column. The PCP fraction eluted by 20 mM Tris–HCl (pH 8.0), was then applied to a HiTrap™ Q Sepharose™ HP column and eluted with a linear gradient of NaCl from 0 to 0.5 M in the same buffer. SDS-PAGE was performed to confirm the size and purity of PCP [26].

2.2. LC/MS analysis of PCP

The PCP protein sample was analyzed by a Synapt G2 Q-IM-TOF mass spectrometer coupled with a NanoAcuity UPLC (Waters Inc., Milford, MA) as previously described [27] with minor modifications. The protein sample was directly loaded onto a home-packed C18 column (Magic, 0.075 mm × 50 mm, 5 μm, 120 Å, Michrom Bioresources, Inc., Auburn, CA) by a six-port injection valve (IDEX Health & Science, Oak Harbor, WA). The gradient was delivered by NanoAcuity UPLC (0–2 min, 5% solvent B; 2–15 min, 5–95% solvent B. Solvent A: water, 0.1% formic acid; Solvent B: acetonitrile, 0.1% formic acid) at flow rate 1 μL/min. The protein spectrum was acquired at sensitive mode (“v” mode) with the capillary voltage of 1.8 kV, cone voltage of 30 V and source temperature of 100 °C.

2.3. Peptide sequencing of tryptic digested PCP

The Coomassie blue-stained band was excised and in-gel digested with trypsin as previously described [28] with minor modifications. After dithiothreitol reduction and iodoacetamide alkylation, 20 μg/mL trypsin (Sigma) in 36 mM NH_4HCO_3, 8.1% acetonitrile, and 0.1 mM HCl was added to the gel pieces. After incubation at 37 °C for 12 h and centrifugation, the supernatant was collected, and the gel pellet was extracted with 1% trifluoroacetic acid in 60% acetonitrile for 30 min. Then supernatants were combined and dried by vacufuge. The trypsin digested PCP sample was reconstituted in water with 0.1% formic acid (25 μL). The

sample was analyzed by LC–MS/MS using two instruments, a Waters Synapt G2 Q-IM-TOF and a Thermo LTQ Orbitrap (Thermo-Scientific, San Jose, CA). The data dependent mode was used in the LC–MS/MS experiment at Orbitrap as previously described [29] with minor modifications. The MS^E mode was used in the LC–MS/MS experiment on the Synapt G2 [30]. The tryptic digested PCP sample (5 μL) was loaded onto a homemade silica capillary column that was custom packed with C18 reverse phase material (Magic, 0.075 mm × 150 mm, 5 μm, 120 Å, Michrom Bioresources, Inc., Auburn, CA). The gradient was supplied by a Waters NanoAquity UPLC and run from 5% solvent B (acetonitrile, 0.1% formic acid) to 50% solvent B over 60 min, then to 95% solvent B for 2 min at 400 nL/min flowed by a re-equilibration step with 100% solvent A (water, 0.1% formic acid). The flow was directed by a nanospray source (Waters, Inc., Milford, MA). In the MS^E continuum mode, ions were dissociated in the trap region by ramping the trap collision energy from 14–40 V. Spectra from 50 to 2000 m/z were acquired with scan time 1 s for 70 min in positive sensitivity mode. The MS^E raw data were directly submitted to the ProteinLynx Global Server (V2.5, Waters Inc., Milford, MA) to search against the NCBI and UniProt database.

2.4. PCP gene identification

Symbiodinium genomic DNA was extracted by Qiagen® DNeasy Plant Mini Kit. *PCP* genes were cloned by a method previously developed for *Symbiodinium* with some modifications (Fig. 1) [24]. Briefly, primer-1 and primer-2 amplified a ~200 bp fragment of *PCP* gene; primer-3 and primer-4, designed according to the sequence of the ~200 bp fragment [24], amplified a ~1.9 kb fragment containing partial PCP genes and a linker; primer-5 and primer-6 were designed according to the possible start and stop codon sites in the 1.9 kb fragment. The PCR products amplified by primer-5 and primer-6 were cloned and analyzed. Primers used for PCR are listed in Table 1. The purified PCR products were cloned and sequenced. Sequences were analyzed by NCBI BLAST and Vector NTI®.

2.5. Spectroscopic characterization

2.5.1. Steady-state absorption spectroscopy—The PCP complex was suspended in 10 mM Tris–HCl (pH 8.0) containing 60% glycerol. Steady-state absorption spectra at both room temperature and 77 K were recorded using a Perkin-Elmer Lambda 950 UV–vis spectrophotometer. The OptistatDN (Oxford Instruments, UK) liquid nitrogen cryostat was used to cool the protein sample to 77 K.

2.5.2. Fluorescence spectroscopy—The PCP complex was suspended in the same buffer as described in 0. The fluorescence emission and excitation spectra at both room temperature and 77 K were obtained using a Photon Technology International fluorometer. The cryostat described in 0 was used in 77 K spectra measurement. Emission and excitation monochromator slits were set to a bandpass of 4 nm. The fluorescence excitation spectrum was corrected using a calibrated reference photodiode. The fluorescence excitation spectrum shown is the average of 10 individual spectra.

2.6. Pigment determination

The purified PCP complex in 20 mM Tris–HCl (pH 8.0) was dried in darkness by vacufuge. 200 μL methanol was added to the dry PCP, followed by brief vortexing and centrifugation. The resulting supernatant was collected, and the methanol extraction was repeated 4 times. All the supernatants were combined, then well mixed. Peridinin and Chl *a* were extracted by methanol from *Symbiodinium* whole cells, and separated by Agilent 1100 HPLC on a Zorbax Eclipse XDB-C18 reverse phase column (250 mm × 4.6 mm) [31]. Peridinin and Chl *a* fractions eluted from the column were dried in darkness by vacufuge and redissolved in methanol. The steady-state absorption spectra of the PCP methanol extract, peridinin and

Chl *a* were recorded using a Perkin-Elmer Lambda 950 UV–vis spectrophotometer. The extinction coefficients used for the pigments ratio calculation were:

Chl *a* (methanol): $\varepsilon_{665\ nm}$=71.43 L·mmol^{-1}·cm^{-1} [32]

Peridinin (methanol): $\varepsilon_{469\ nm}$=85.80 L·mmol^{-1}·cm^{-1} [33,34]

The molar ratio was calculated according to the Beer-Lambert Law:

A=ε·c·l (A: absorbance, c: molar concentration, l: optical path length).

3. Results and discussion

3.1. PCP identification

The PCP of *Symbiodinium* sp. CS-156 was purified by ammonium sulfate precipitation, gel filtration chromatography and ion exchange chromatography. The purity and size of PCP were examined by SDS-PAGE and LC/MS (Fig. 2). SDS-PAGE revealed a single band at a mass of 33 kDa, which was determined to be 32.7 kDa by LC/MS. No protein peak around 14–16 kDa was observed by LC/MS, indicating that the PCP of *Symbiodinium* sp. CS-156 is of the monomeric form. Peptide sequencing identified multiple peptides that are part of PCP putative preproteins in UniProt database. The one with most hits is a PCP (the UniProtKB/Swiss-Prot entry: P51874) from *Symbiodinium* sp., which is a cDNA-translated protein sequence [8]. This PCP preprotein contains a transit peptide, and the predicted mature protein is composed of 2 almost identical repeat units. The theoretical molecular weight of the mature P51874 PCP gene product is 32585.18 Da.

PCP genes were cloned and sequenced. Among 11 PCP gene clones (see Supplementary Data), only 2 of them are identical in nucleotide sequence (GenBank ID: JQ395029 to JQ395038), indicating that a variation of PCP genes exist at the gene level as was found for free-living dinoflagellates in previous studies [23,24]. The comparison of 11 genomic DNA sequences with *Symbiodinium* PCP cDNA sequences in previous studies [8,13,24], showed no evidence of introns. Each clone encodes a 365 amino acid preprotein, which corresponds to a 1095 bp coding region of the PCP gene. The predicted preproteins have identities ranging from 96.4% to 100% when aligned with each other. The observed identities are close to the reported values based on the analysis of PCP cDNA clones [24], which are from 96.2% to 99.7% identical. The small variations of PCP genes increase the genetic diversity, introducing small differences in deduced preprotein mass (by ExPASy Compute pI/Mw tool, from 32633.52 Da to 32868.63 Da) but relatively large differences in their predicted isoelectric points (pIs from 5.34 to 7.91).

Sequencing the 1.9 kb clones shows that each clone contains open reading frames that can be translated to PCP at both 5′ and 3′ ends, and a spacer region in the middle of the two ORFs. This confirms that *PCP* genes of *Symbiodinium* sp. CS-156 are organized in tandem arrays as was found in other *Symbiodinium* strains [24]. As anticipated, the spacer sequences are highly varied, containing several insertions and deletions (data not shown).

In the LC–MS/MS peptide sequencing experiment, peptides from PCP protein were fragmented in the mass spectrometer by collision-induced dissociation. The protonated peptides fragment along the peptide backbone to form b and y production ions. The peptide sequences were elucidated from the match between in-silico digested protein sequence and sequence product ions from experiment [35]. All detected peptides were processed by MassMatrix Database Search Engine [36,37]. The comparison of the translated PCP sequences with peptide sequencing by LC–MS/MS, revealed that the mature protein starts at Asp53. Protein sequence alignment of five PCP proteins (which exist at the protein level,

UniProt entries: P51873 [22], P51874 [8], P51889 [19], P80483 [22] and P80484 [3]) and translated sequences in this study shows that all mature PCPs begin at Asp53, and except for the HSPCP of *A. carterae*, other proteins share a sequence of DEIGDAAK at the N terminus. The search results also show that the sequence of the trypsin digested sample exactly matches the deduced sequence of clone 7(Fig. 3). The calculated MW of the putative PCP mature protein deduced from clone L4 is 32.8 kDa, which is in agreement with the MW of 32.7 kDa obtained by LC/MS. Compared to other protein sequencing methods, e.g. Edman degradation [8,13] and protein microsequencing [15], the LC–MS/MS protein sequencing obtained a full coverage, which is the highest to our knowledge for any PCP.

We explored more characteristics of PCP based on DNA and protein sequence. As expected, the first half of the mature PCP apoprotein (Asp53–Ala208) has 61% identity with the second half (Pro209–Gln364). An identity of 54% was observed in previous studies [8,15]. Along with the two-fold symmetry of PCP X-ray crystal structure [3,18], our finding supports the hypothesis that the monomeric form of PCPs arose by duplication and fusion of gene(s) encoding the homodimeric form [8,15]. The transit sequence (under-lined sequence in green in Fig. 3) adjacent to the mature PCP sequence has two distinct hydrophobic regions when analyzed by ExPASy ProtScale. This transit peptide (TP) shares an identity of 43% with the *G. polyedra* PCP transit peptide [15,38], which contains an additional hydroxylated amino acids (S/T)-rich region flanked with two distinct hydrophobic regions. The first region could guide the nuclear-encoded protein translocation from cytosol to chloroplast, and then be cleaved off, generating an intermediate preprotein; the second region could lead the intermediate to thylakoids where a thylakoidal processing peptidase (TPP) recognizes the A–X–A motif in TP, cleaves TP off and generates the mature apoprotein [39-41]. The second hydrophobic regions of both *G. polyedra* and *Symbiodinium* sp. TPs are rich in alanine, a typical feature of several TPs of nuclear-encoded thylakoid proteins [40]. The TP sequence analysis is in agreement with the co-crystallization of PCP and DGDG [3], which is mostly found in the inner thylakoid membrane [42], supporting that PCP is located in the thylakoid lumen [8,15].

To our knowledge, this is the first report that relates PCP gene sequence to its corresponding protein sequence in the genus *Symbiodinium*. Previous attempts only included cDNA sequence [16,23], or a partial PCP protein sequence [19], or a very small portion of PCP protein sequence that corresponds to cDNA [8,13,15], except for the MFPCP and HSPCP from *A. carterae*, whose crystal structures have been determined [3,18,22]. Only three groups have reported the descriptions of the PCP gene organization in *Symbiodinium* [8,13,24], but none of them was able to determine the PCP sequence at both gene and protein level. The sequencing and molecular experiments show that the major form of PCP in *Symbiodinium* sp. CS-156 is monomeric, and intronless PCP genes are organized in tandem arrays. The arrangement of PCP genes in this organism is very similar to that of other dinoflagellates, although the DNA and protein sequences are different from those of others. The occurrence of heterogeneity in the PCP gene tandem repeats is also observed. The PCP gene heterogeneity was believed to be inconsistent with concerted evolution [24], which leads to the sequences of related genes to co-evolve over some period of time. However, although the variations can be detected at both mRNA [22,24] and protein level [1,2,6,13,14], the number of major isoforms in an individual organism seems to be very limited: an isoform with a single pI or isoforms with subtle pI differences always overwhelm other PCP species. This situation could also be applied to *Symbiodinium* sp. CS-156: we have found that there are some other isoforms expressed, but that the one characterized in this study is the main one (data not shown). It is noted that the hypothesis of the existence of major isoform(s) does not conflict with the fact that both monomeric and homodimeric forms were detected in *S. microadriaticum* [14], in which major isoforms have pIs of 7.2, 7.3, 7.6 and 7.7. Since each fraction with the same pI was able to separate into two PCP

species with distinct sizes but the same spectroscopic properties, the monomeric form could have arisen by duplication and fusion of gene(s) encoding the homodimeric form: the two PCPs of different lengths but the same pI could be the products of the same gene. In sum, there could be some posttranscriptional mechanism that leads to the mature apoPCP expression preference. At this point, the PCP expression pattern appears to be consistent with the concerted evolution model.

3.2. Absorption spectroscopy

The absorption spectrum of PCP measured at room temperature is shown in Fig. 4. The spectrum consists of a broad band between 400 and 550 nm, two shoulders at 420 nm and 520 nm, and three peaks at 438 nm, 476 nm and 670 nm. The peridinin S_0–S_2 transition results in the dominant broad band between 400 and 550 nm with the peridinin absorption maximum at 476 nm. The Chl *a* Soret band, which overlaps with the broad peridinin band, is at 438 nm. The Chl *a* Q_y band peaks at 670 nm, while the weak Q_x band is overlapped by the peridinin S_0–S_2 transition band. The absorption spectrum taken at 77 K (Fig. 5) has a similar shape, except that the Chl *a* Soret band is split into two peaks with maxima at 433 nm and 438 nm, indicating the different protein environments of two Chl *a* molecules. To locate the possible Chl *a* binding sites in the *Symbiodinium* PCP, the *Symbiodinium* PCP putative protein sequence (as listed in Fig. 3) was aligned with the *A. carterae* PCP (PDB ID: 1PPR), in which His66 interacts with Chl *a* through a water molecule in the first half of the mature protein [43], and Leu254 along with His229 are part of the environment of the COOH-terminal chlorophyll [3] in the second half of the mature protein. The two boxes in Fig. 3 are very likely to bind Chl *a* molecules in *Symbiodinium* PCP because these two fragments have the same amino acid sequences with the PCP (PDB ID: 1PPR) Chl *a* binding regions (the region around His66 and the region around Leu254 and His229). Region 1 (AAEAHHKAIGSISGPNGVTSRADWD) and region 2 (LKAAAEAHHKAIGSIDA) share a sequence of AAEAHHKAIGSI, but have the rest of the amino acid residues with distinct properties, resulting in different protein environments that can be detected in 77 K absorption spectrum.

In addition, at 77 K, the peridinin absorption maximum is red-shifted from 476 nm to 483 nm, while the Chl a Q_y band is blue-shifted from 670 nm to 666 nm. Compared with the RT absorption spectrum, the 77 K spectrum has more distinct shoulders at 420 nm and 520 nm and more resolved Q_y 0–1 vibronic and/or Q_x transition band between 600 nm and 650 nm [44]. This is because the lower temperature reduces the randomness of orientation of protein molecules in the system, thus providing more information.

In general, the steady-state absorption spectra of *Symbiodinium* PCP at both temperatures are very similar to those of *A. carterae* PCP in the aspects of shape and locations of peaks and shoulders except for some minor differences (e.g. the ratios of peridinin absorption maximum to Chl *a* Q_y peak at both temperature are slightly different). The protein sequence analysis revealed the conservative binding sites for peridinin and Chl *a* molecules in *Symbiodinium* PCP (Fig. 3, two boxes and two dotted-underlined sequences are the possible binding pockets for Chl *a* and peridinin molecules, respectively) [3,43,45]. *Symbiodinium* PCP and *A. carterae* PCP share AAEAHHKAIGSISGPNGVTSRADWD and LKAAAEAHHKAIGSIDA for Chl *a* binding, as well as VNAALGRV and VNAALGR for peridinin binding, leading to the almost identical absorption spectra, which are dependent on the protein environments of the pigments.

3.3. Fluorescence spectroscopy

The fluorescence emission spectra of PCP were taken at room temperature and 77 K (Fig. 6). The two spectra exhibit a similar shape, with a peak maximum at 675 nm and 674 nm,

respectively, and a broad vibronic band between 700 and 770 nm, centered around 735 nm. The 77 K spectrum is narrower than the RT spectrum: the former has a full width at half-maximum (fwhm) value of 10 nm, while the latter has a value of 15 nm.

Symbiodinium PCP fluorescence emission spectra at both temperatures are very similar to *A. carterae* MFPCP and HSPCP spectra, as well as the spectra of Chl *a* in 2-MTHF [20] (Fig. 6). All the spectra are typical of monomeric Chl species [20]. However, the fluorescence emission maxima of 3 PCPs are different (Table 2). The *Symbiodinium* PCP spectrum maximum only blue-shifts by 1 nm upon cooling to 77 K, as opposed to 4 nm of the MFPCP spectrum maximum. Although the HSPCP spectrum red-shifts by 1 nm, the spectral maxima are closest to those of *Symbiodinium* PCP.

The fluorescence excitation spectrum taken at 77 K was overlaid with the corresponding 1-T spectrum, as shown in Fig. 7. The fluorescence excitation spectrum was normalized with the 77 K 1-T spectrum Q_y band. The height difference between the peridinin absorption peaks of both spectra indicates the peridinin-to-Chl *a* energy transfer loss. The calculated energy transfer efficiency is 95%, which is very close to the reported peridinin-to-Chl *a* energy transfer efficiencies [20].

3.4. Stoichiometry of pigments in PCP

The molar ratio of peridinin to Chl *a* was determined by measuring the steady-state absorbance of the PCP methanol extract. The UV–vis absorption spectra of peridinin and Chl *a* fractions eluted from HLPC were confirmed by comparison with literature values [31,46] (data not shown). The spectra of the PCP methanol extract and Chl a in methanol were normalized at the Chl *a* Q_y band. The contribution of Chl *a* absorbance at 469 nm was deducted from the PCP methanol extract absorbance at the same wavelength (Fig. 8). The calculated value of the molar ratio is 4.07, which is very close to 4. This result along with the spectroscopic properties indicates that *Symbiodinium* PCP is very likely to share the same structure of *A. carterae* MFPCP, although the sequence identity is ~83% based on the alignment of the *Symbiodinium* PCP sequence (listed in Fig. 3) with the *A. carterae* PCP (PDB ID: 1PPR). The pigment stoichiometry and the conservative pigment binding sites suggest that there is consistently high energy transfer efficiency in dinoflagellate PCPs.

4. Conclusion

In this study, we identified and characterized PCP and its gene organization in *Symbiodinium* sp. CS-156. We found that this strain possesses the monomeric form of PCP of 32.7 kDa, encoded by the PCP gene cassette composed of 1095-bp coding regions and spacers in between. Despite the occurrence of the PCP gene heterogeneity, a single form of PCP was identified and sequenced, indicating the possible existence of a PCP major form. The resulting sequence matches the deduced sequence of PCP gene clone 7. The detection of PCP protein and its corresponding gene is first reported in the dinoflagellate *Symbiodinium* and may provide an important hint for future evolutionary studies. The protein sequence analysis, along with the spectroscopic studies, revealed the conservative pigment binding sites, which may be one of the key factors that determine the consistently high peridinin-to-Chl *a* energy transfer efficiency in dinoflagellate PCPs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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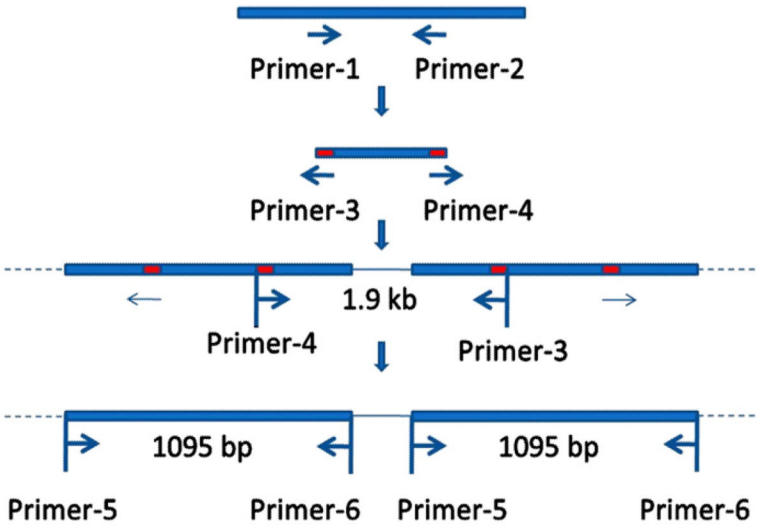

Fig. 1.
The strategy to identify PCP genes in *Symbiodinium* sp. CS-156-b1 [24].

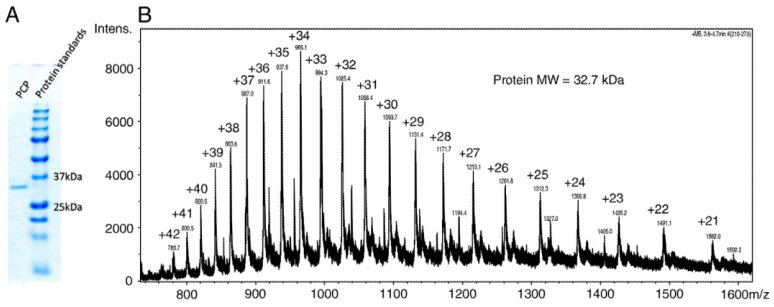

Fig. 2.

The identification of PCP by SDS-PAGE (A) and LC/MS (B). SDS-PAGE revealed a single band at a mass of about 33 kDa, which was determined to be 32.7 kDa by LC/MS.

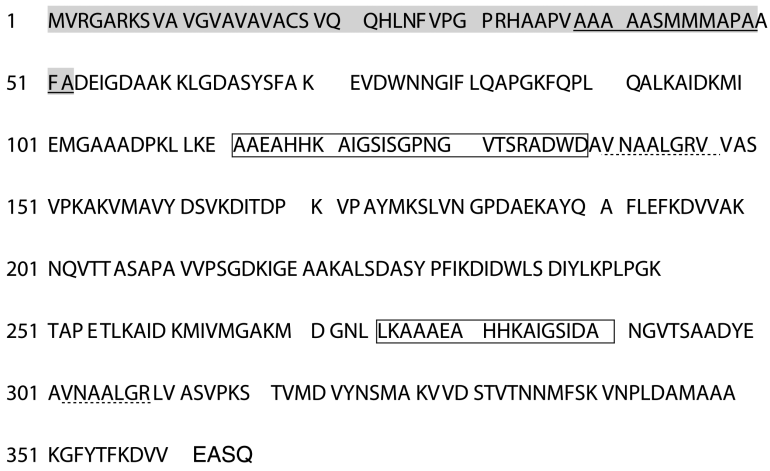

Fig. 3.

PCP protein sequence identification. The listed sequence is the predicted amino acid sequence of a PCP gene clone (Clone 7, JQ395030). LC/MSMS experiment by UPLC-QTOF (Waters, Synapt G2) identified multiple peptides, assembly of all of which matches the predicted sequence of clone 7. The transit peptide is in green; the solid-underlined sequence in green is the possible thylakoid-targeting domain; the two boxes indicate the possible Chl *a* molecules binding sites; the dotted-underlined sequences are the possible peridinin binding sites.

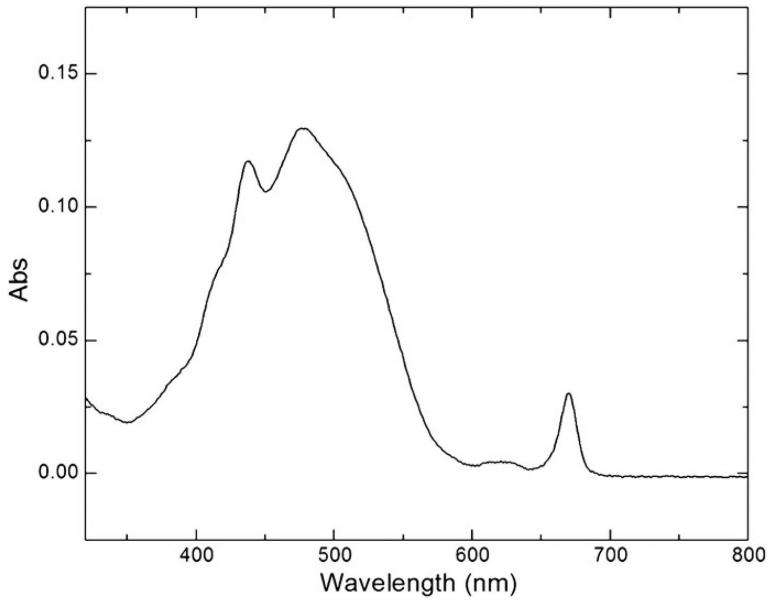

Fig. 4.
The absorption spectrum of PCP at room temperature.

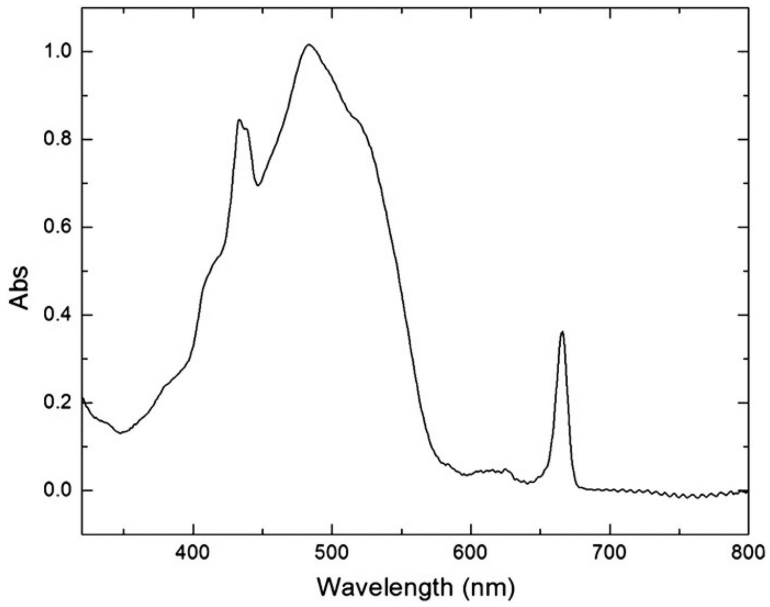

Fig. 5.
The absorption spectrum of PCP at 77 K.

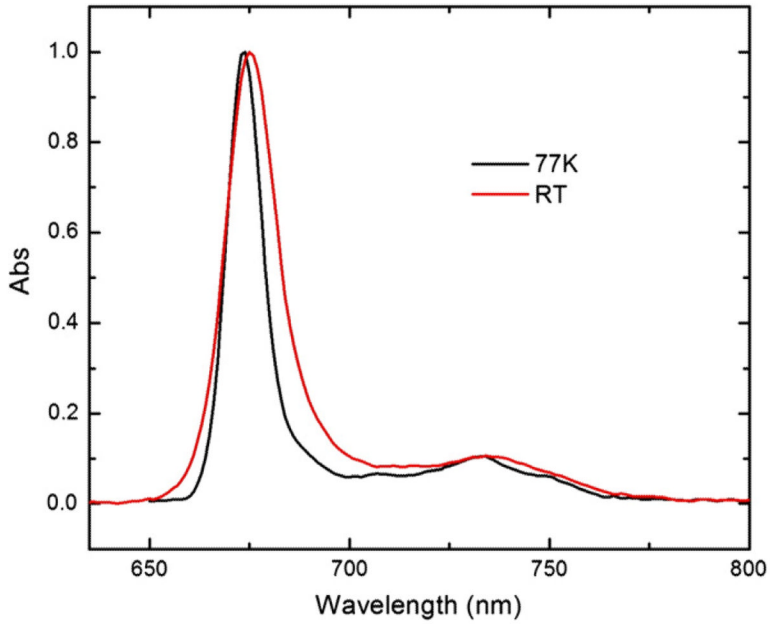

Fig. 6.
The fluorescence emission spectra of PCP at room temperature (red) and 77 K (black).

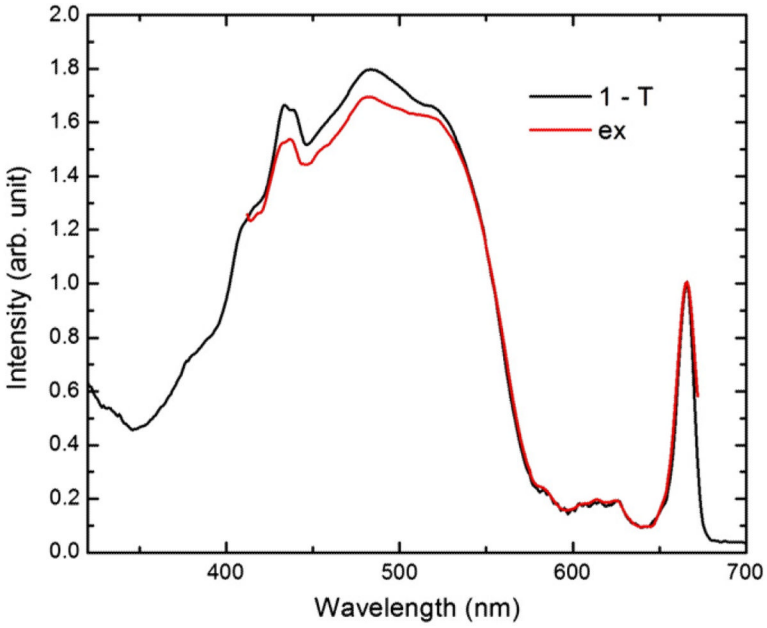

Fig. 7.

The overlay of fluorescence excitation (ex) spectrum at 77 K (red) with the corresponding 1-T spectrum (black). The fluorescence excitation spectrum was normalized at the 77 K 1-T spectrum Qy band. The calculated peridinin-to-Chl a energy transfer efficiency is 95%.

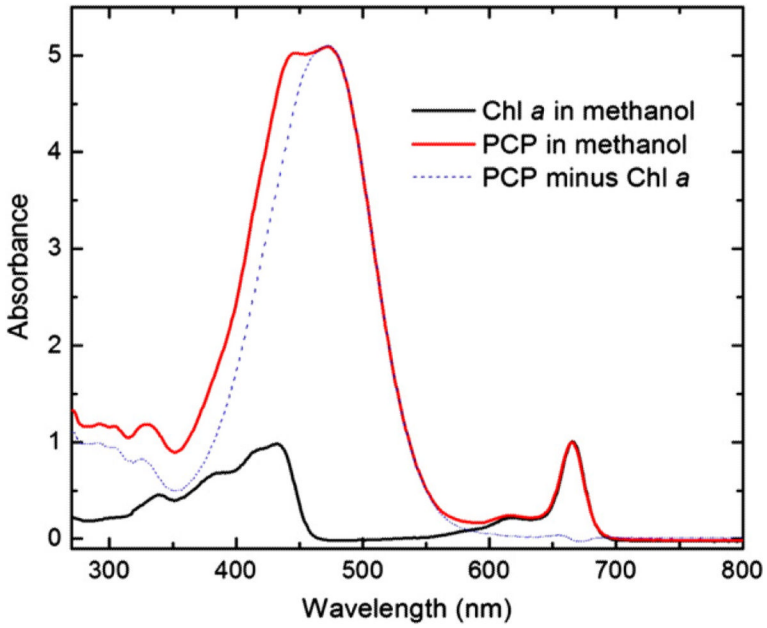

Fig. 8.

The spectra of the PCP methanol extract (solid red) and Chl a (in methanol) (solid black), normalized at Chl a Qy band. The difference between the solid red line and the solid black is indicated by the dotted blue line, which matches the shape of peridinin absorption spectrum. The contribution of Chl *a* absorbance at 469 nm was deducted from the PCP methanol extract absorbance at the same wavelength.

Table 1

Primers and the annealing conditions.

Primer name	Primer sequence (5′–3′)	Annealing
Primer-1	AAGAATTCGAAGGACGCAGCAGAAGC [24]	30 s at 52 °C
Primer-2	CAGAATTCOTCATGTACGCTGGCAC [24]	
Primer-3	TCGGTCCCCAAAGCAAAGGTCA [24]	30 s at 55 °C
Primer-4	CATTCACGGCATCCCAGTCAGC [24]	
Primer-5	TGGTGCGTGGAGCAAGGAAA	30 s at 55 °C
Primer-6	TTCACCTTCAGCGCTGGGAA	

Table 2

The comparison of *Symbiodinium* PCP, *Amphidinium carterae* MFPCP and HSPCP [20], and Chl *a* (in 2-MTHF) [20] fluorescence emission spectra at room temperature and 77 K.

	Peak maximum at RT	Peak maximum at 77 K
Symbiodinium PCP	675 nm	674 nm
Amphidinium carterae MFPCP	673 nm	669 nm
Amphidinium carterae HSPCP	674 nm	675 nm
Chl *a* in 2-MTHF	668 nm	671 nm