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Water-soluble carotenoid proteins of cyanobacteria

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Abstract

In photosynthetic organisms, carotenoids function in light harvesting and in photoprotection. In cyanobacteria, there have been numerous reports of proteins that bind exclusively carotenoids. Perhaps the best characterized of these proteins are the 35 kDa water-soluble orange carotenoid proteins (OCPs). Structural, biochemical, and genomic data on the OCP and its paralogs are gradually revealing how these proteins function in photoprotection.

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Keywords: Carotenoid; Photoprotection; Photosynthesis; Pigment–protein; Protein structure

Photosynthetic organisms both need light and need to be protected from it. Excess excitation energy, beyond that which can be used in downstream photosynthetic processes such as carbon fixation, results in direct chemical damage to components of the photosynthetic apparatus and generates harmful reactive oxygen species. Stresses such as drought, salinity, and high temperature are all known to exacerbate the sensitivity of photosynthetic organisms to light-induced damage. For optimal photosynthetic function, photosynthetic organisms must respond to variation in light intensity and environmental stresses by shifting absorbed light energy between light harvesting and photoprotective functions. Carotenoids play a central role in this balance (reviewed in [1–3]).

In photosynthesis, carotenoids carry out their specialized functions as components of integral membrane, protein–pigment complexes such as the photosynthetic reaction center and the associated light-harvesting antennae. In contrast, the cyanobacterial carotenoid proteins considered here are water-soluble and do not bind chlorophyll or other pigments. Their abundance increases under high light treatment. These characteristics suggest that they function in photoprotection rather than in light harvesting.

Biochemical characterization and distribution of the OCPs

Orange carotenoid proteins (OCPs)¹ are readily detectable in crude, water-soluble extracts of cyanobacteria that have been isolated from natural blooms or laboratory cultures. OCPs, 35 kDa proteins that contain a non-covalently bound keto-carotenoid, 3'-hydroxyechinenone, were first identified in *Arthrospira* (*Spirulina*) *maxima*, *Microcystis aeruginosa*, and *Aphanizomenon flos-aquae* in 1981 [4]. Subsequently, a similar protein was purified from *Synechocystis* PCC 6803 [5]. The spectral features of the *Syn* 6803 OCP suggested it likewise contained 3'-hydroxyechinenone, however molecular weight estimation of the carotenoid by mass spectrometry indicates a mass of 819 Da, 254 Da larger than that expected for 3'-hydroxyechinenone, suggesting that this OCP contains a carotenoid glycoside [6].

The primary structure of the OCP was first elucidated by a combination of N-terminal amino acid sequencing, gene isolation, and the subsequent identification of the corresponding open reading frame in the *Syn* 6803 genome [5]. A survey of the genome databases reveals that highly conserved homologs of the OCP are found in all of the cyanobacteria for which genomic data are available with the exception of the *Prochlorococci* (Table 1).

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¹ Abbreviations used: OCP, orange carotenoid protein; RCP, red carotenoid protein; NTF-2, nuclear transport factor 2.

Table 1
Orange carotenoid proteins found in cyanobacterial genomes

A. max	MPFTIDTARSIFPETLAADVVPATIAARFKQLSAEDQLALIWFAYLEMGKTIITIAAPGAANMQFAENTLQETIRQMTPLQQT
Syn 6803	MPFTIDSARGIFPNTLAADVVPATIAARFSQLNAEDQLALIWFAYLEMGKTLTIAAPGAASMQLAENALKEIQAMGPLQQT
Nos 7120	MAITIDSARRIFPNTLQADAVPALTARFNQLSAEDQLAWTWFALFMGKTIITVAAPGAASMQFAEGLKQIKEMTFEEQT
N. punc 1	MSFTIKSAQSIIFPGTLVADVPTVVESFSQLNAEDQLALLWFAYTEMGRSITVAAPGAANMVLAQGLLEQIKQMPFEAQ
Syn 8102	M-FTLDKARQIFPDTLSAAAVPAITARFKLLSAEDQLALIWFAYLEMGRTITVAAPGAARMALAQPTLDEIQAMSFDEQT
G. viol	MAFTLESAQAIFAETGKPSIPGILADFNRLSLEDRLALLWYAYTETGRITITRAALGAASMALVEGMLDQIKQMPPEAQ
N. punc 2	MNYTIESARNIFSSSTQVADAVPATTAMFAELNIDDQLAFLLWYAYAEELGRTITPAAPGKANLQLMEGIFNEIKQMSHEEQ
A. max	QAMCDLANRDTTPICRTYASWSPNIKLGFWYELGRFMDQGLVAPIPEGYKLSANANAILVTIQGIDPGQITVLRNCVVD
Syn 6803	QAMCDLANRADTTPICRTYASWSPNIKLGFWYRLGELMEQGFVAPIPAGYQLSANANAVLATIQGLESQQITVLRNAVVD
Nos 7120	QVMCDLANHTDTPICRTYATWSPNIKLGFWNLGGEWMEQGAVAPIPAGYQLSANANAVLETLKSLEDQGGQITVLRSSVVD
N. punc 1	RVMYDLANRADTTPICRSYASFTVNIKLGFYQWLGEMAQGIVAPIPEGYKLSPKAADVLEAIRNADSGQQITILRNTVVS
Syn 8102	KVMCDLAGKINSPIASARYAYWSVNVKLCFWYELGEMRQGVAPIPQGYRLSANANSVLEAVKKVEQQQITLLRNFVVD
G. viol	RVMFDLARRADTTPISRSYGYFSVNTKLGFYQLAEWMAQGTVAPIPANYQMSDQAQLLFESIKNLDGGQQIQVLRDIVLN
N. punc 2	QLMRDLASNADTTPISRSYAYFGVNAKLGFWWQLGEMWKEGIVAPMPVGYQMSDQVKAVLEAVQKIDQSQQITVLRNTVVD
A. max	MGFDTSKLGSYQVAEPV-VPPQEMSQRKVKQIEGVTNSTVLQYMDNLNANDFDNLISLFAEDGALQPPFQKPIVKGENA
Syn 6803	MGFTAGKDG--KRIAEPV-VPPQDTASRTKVSIEGVTNATVLNYMDNLNANDFDTLIELFTSDGALQPPFQRPVIGKENV
Nos 7120	MGFDAAKLDGYTRVAEPL-VAPKDISQRVQVTIEGINNSTVLNYMNNLNANDFDELIKLFVEDGALQPPFQRPVIGKDAI
N. punc 1	MGFDPNAPGSYKVKVSEVPAPTAPAFRTKVSIEGINNPTVLGYINNMNANDFDDAVALFTSEGGLOPPFERPIVQDAIR
Syn 8102	MGYDPDVEDD-SQVVTEPI-VAPTVDQREEILIPGVLNQITLSYMQLLNANDFDQLIDLFLNDGALQPPFQRPVIGRDAI
G. viol	MGFDPSVVPDEAPEAEDQFERTEPASTERIDVKGVDPTPLRYFEAMNSDNFEAAVALFEPEGALQPPFQKPIVGREAI
N. punc 2	MGFDPSLADKKQAQ-INFKFRPTS--LSPQFTIEGVTETVLKYIEAMNADNFEAAVALFANNALQPPFQKPIVGREAI
A. max	LRFFREECQNLKLI PERGVSEPTEDGYTQIKVTGKQVTPWFGNVGMNIAWRFLNPNKVVFFVAIDLLASPKELNL--
Syn 6803	LRFFREECQNLKLI PERGVTEPAEDGFTQIKVTGKQVTPWFGNVGMNIAWRFLNPEGKIFFVAIDLLASPKELNLFAR
Nos 7120	LRFFREECQNLNLLPERGVAEPAEDGYTQVKVTGKQVTPWFGAAVGMNMAWRFLNPPQKIFFVAIDLLASPKELNLVR
N. punc 1	AYMREECQGLKMI PERGISEPVEDGYTQVKVTGKQVTPWFGASVGMNIAWRFLDPPQKIFFVAIDLLASPKELNLVR
Syn 8102	LKFFKRDCQNLKLMPOGGYGEPTEGGFNQIKVTGKQVTPWFGREVMNVAVWRFLDENDKIYFVAIDLLASPAELLLKGG
G. viol	AAYMREEAQGLTMKPIEGITEVLDPGSKKLKVTGKQVTPWFGVNVAMNIAWRFLNPEGKIFYVVIDLLASPELNLNLR
N. punc 2	TAYLRDEGQGLVMKPTKGVSETIEDGYTQHKITGTVETPWFGNVGMNIAWRFLNPPQGIYFVAIDLLASPKELNLTR

Abbreviations: *A. max*, *Anthrospira maxima* [11]; Syn 6803, *Synechocystis* PCC 6803 [Kazusa Institute, www.kazusa.org.jp]; Nos. 7120, *Nostoc* PCC 7120 (Anabaena) [Kazusa]; Syn 8102, *Synechococcus* WH 8102 [DOE Joint Genome Institute, www.jgi.doe.gov]; *G. viol*, *Gloeobacter violaceus* [Kazusa].

The separate, but adjacent, single copy genes for the N- and C-terminal domains of the OCP in the genome of *Thermosynechococcus elongatus* (Table 2) suggest that the 35 kDa OCP is the product of a gene fusion event.

There are scattered reports of other cyanobacterial carotenoid proteins in the literature. A carotenoid protein was isolated from the cytoplasmic membrane fraction of *Synechocystis* PCC 6714 [7]. The apparent molecular mass of this protein, determined by SDS-PAGE, was estimated to be either 35 or 45 kDa depending on the solubilization temperature. This protein cross-reacted with antibodies prepared against a 46 kDa carotenoid protein isolated from *Anacystis nidulans* R2 [8]. The thylakoid membrane of *A. nidulans* R2 also contains a carotenoid protein of 42 kDa [9] but it shows no sequence homology to the OCP [10]. Fractionation of *A. nidulans* yields a red water-soluble carotenoid protein; this carotenoprotein contains zeaxanthin and is

a homodimer of two 23 kDa polypeptides [11]. A water-soluble zeaxanthin-binding protein complex of 58 and 56 kDa proteins was isolated from the cell envelope of *Prochlorothrix hollandica* [12]. As a group, carotenoid-binding proteins vary with regard to cellular location, detergent versus water solubility, and the type of associated carotenoid. Furthermore, care must be taken in interpretation based on molecular mass estimations for the OCPs; molecular weights determined by SDS-PAGE and size exclusion chromatography data have been known to vary significantly from that determined by mass spectrometry (D.W. Krogmann pers. comm.). One common feature noted for several of these carotenoid-binding proteins is that they increase in abundance in response to high light [9,10,12]. In the present discussion, the use of "OCP" is restricted to carotenoid proteins that are verifiably orthologs of the 35 kDa protein that corresponds to the slr1963 gene product of *Syn* 6803 [5].

Table 2
Alignment of orange carotenoid protein domain orthologs

		1	18	54	124	165	317	residue length	e score	percent identity	
<i>A. max</i> OCP											
Nostoc punctiforme						4	contig 589.gene 11	139	143	2e-27	59
		15	contig 626.gene 18			154			163	3e-25	40
		1	contig 626.gene 15			149			149	2e-13	27
		24	contig 589.gene 11			162			176	4e-12	30
		12	contig 626.gene 18			130			149	1e-05	24
Nostoc PCC7120 (Anabena)						15	all4940	136	140	6e-27	44
		1	all4941			118			122	3e-27	49
		15	all1123			154			163	7e-23	39
		33	all3221			170			172	6e-14	28
		21	all4783			157			176	3e-12	29
Thermo synechococcus elongatus						10	tl1268	130	138	3e-18	34
		1	tl1269			159			162	1e-35	48
Gloeobacter violaceus						4	gll0258	131	138	0.16	21
		1	glr3935			267			274	1e-70	50
		60	gll0260			209			184	1e-14	32
		30	gll0259			181			138	5e-12	28

The three-dimensional structure of the OCP

The structure of the OCP of *A. maxima* has been determined to 2.1 Å resolution [13,14]. The asymmetric unit of the crystal contains an OCP dimer (Fig. 1) and the two molecules are essentially identical with few exceptions (noted below). Dimerization in the crystal buries 1411 Å² of surface area, suggesting that the asymmetric unit corresponds to the dimer observed in solution (D. Krogmann, pers. comm.). Homodimerization is a distinctive feature of the OCP relative to known structures of light-harvesting proteins. The relative disposition of the carotenoids in the OCP dimer is also unique; the 3'-hydroxyechinenone molecules are nearly parallel. In contrast, the carotenoids in peridinin-chlorophyll protein [15]—the only known light-harvesting protein structure with multiple closely interacting carotenoids—show no obvious preference for parallel alignment. The carotenoids of the ring-shaped bacterial light-harvesting complexes (composed of nine heterodimeric units) are nearly parallel, but very widely spaced [16–20].



Fig. 1. The *A. maxima* OCP dimer. The N-terminal, all-helical domain is shown in dark gray, the C-terminal domain in light gray. The carotenoid is shown in white, in space-filling representation. Chloride ions are shown in black, and the sucrose molecule observed in one of the molecules in the asymmetric unit is shown in sticks. This figure and Figs. 3, 5, and 6 are prepared with Pymol [52].

The structure of the OCP consists of two domains (Fig. 1). The all- α -helical N-terminal domain resembles two four-helix bundles, however the helices forming each bundle are made up of discontinuous segments of the primary structure of the protein (Fig. 1, and Table 1) suggesting that the eight helices form an intact domain. The C-terminal domain resembles a NTF-2 (nuclear transport factor 2) domain as predicted by P-FAM analysis [21] of the OCP primary structure. NTF-2 domains are observed in a wide range of functionally distinct proteins including enzymes and transport proteins [22–26]. The NTF-2 fold is a mixed α/β fold, characterized by the formation of a hydrophobic pocket. In the OCP, the keto group of the 3'-hydroxyechinenone molecule is nestled into the core of the NTF-2 domain. The N- and C-terminal domains are joined by a long extended loop (residues 161–185). In one of the molecules of the dimer, a sucrose molecule (a component of the crystallization mother liquor) is nestled into a cavity formed by a cluster of highly conserved residues [14] in this linker region (Figs. 1 and 2A). The fructose moiety is in van der Waals distance of sidechain of Trp 279 adjacent to the 3'-hydroxyechinenone molecule (Fig. 3).

It is well known that the protein environment plays an important role in tuning the spectral characteristics of the pigment in pigment–protein complexes. This is dramatically demonstrated in the OCP (Fig. 4). The isolated carotenoid, 3'-hydroxyechinenone, appears yellow in organic solvents ($\lambda_{\max} = 450$ nm) whereas it appears orange ($\lambda_{\max} = 465$ and 495 nm) in the OCP. In the OCP, the 3'-hydroxyechinenone molecule has an all-*trans* configuration with an average deviation from 180° of 16° and a radius of curvature of approximately 28 \AA . The 3'-hydroxyechinenone molecule is approximately 26 \AA in length and spans both domains of the OCP (Fig. 1).

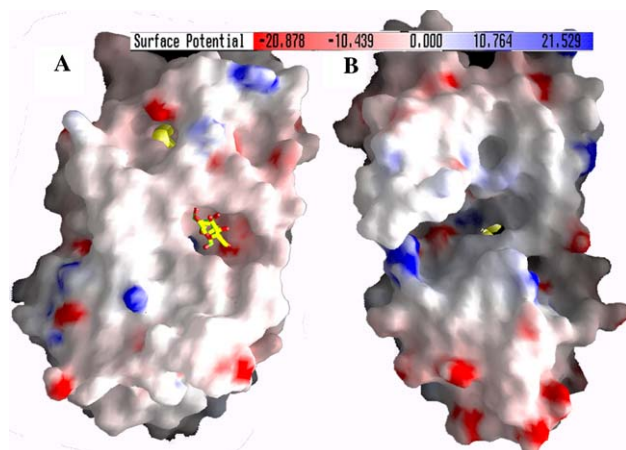


Fig. 2. Surface and charge representation of the OCP. (A) The carotenoid molecule is yellow and shown in space filling representation. The sucrose molecule is yellow and rendered in sticks. The view in (B) is oriented 180° relative to the view shown in (A). Figure prepared with GRASP [53].

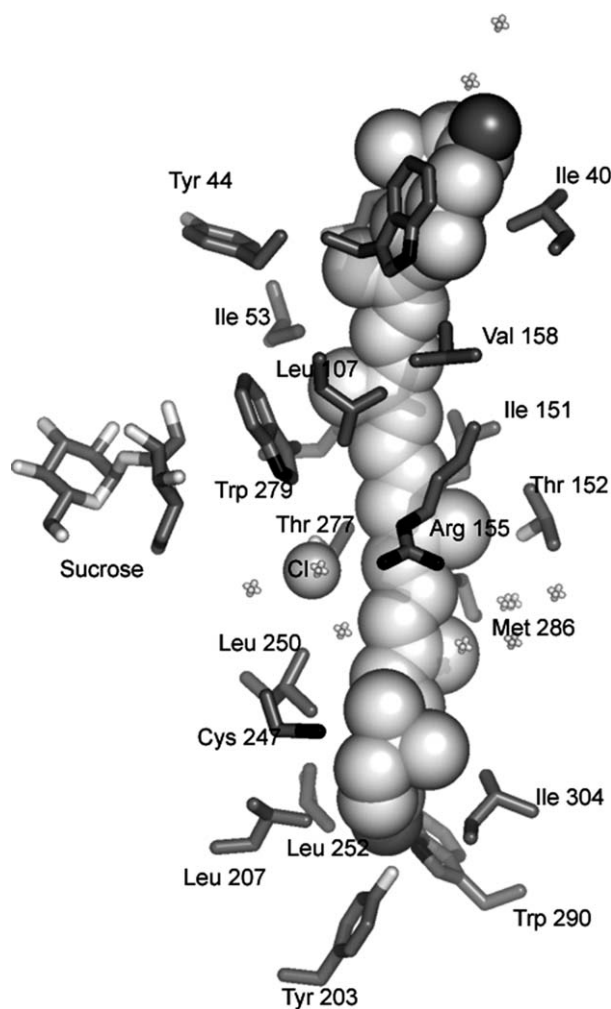


Fig. 3. Amino acid sidechains within 3.7 \AA of the 3'-hydroxyechinenone molecule in the OCP. The sucrose molecule is shown in sticks. The chloride ion and nine water molecules (space-filling crosses) found in the carotenoid-binding cleft are also shown.

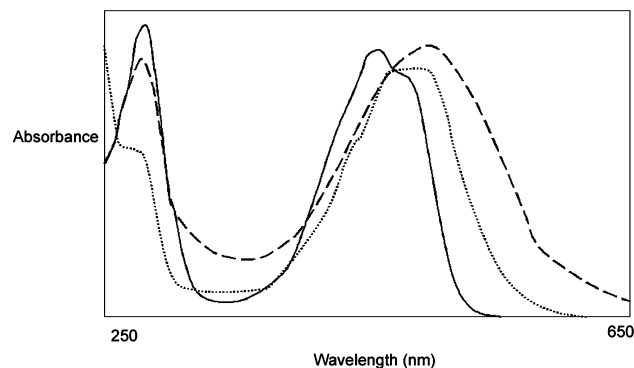


Fig. 4. Absorption spectra of the OCP (dotted line), the RCP (dashed line), and 3'-hydroxyechinenone in hexane (solid line) isolated from *A. maxima*.

The carotenoid binding cleft of the *A. maxima* OCP is lined with residues conserved in the primary structures of OCPs (Table 1) including Trp 290 and Tyr 203 which

hydrogen bond to the keto oxygen atom of the 3'-hydroxyechinenone molecule. All residues within 3.7 Å of the carotenoid molecule are shown in Fig. 3. There are also eight water molecules surrounding the center of the carotenoid molecule at the interface between the two domains. The carotenoid binding cleft also contains a chloride ion hydrogen bonded to Thr 277 (Figs. 1 and 3), a conserved residue in the primary structure of the OCP.

A notable feature of the OCP structure is the presence of six Met residues (five absolutely conserved among the known primary structures of OCPs, Table 1), positioned so that their thioether groups extend toward the carotenoid molecule [14]. The importance of a Met residue in chromoprotein integrity has been demonstrated in bacteriophytochrome [27]. The unusual distribution of methionine residues in the OCP may indicate that these residues play an important yet undetermined role in its function. The flexibility of the Met sidechain as well as the polarizability of the sulfur atom could be important in binding the highly conjugated carotenoid molecule in the OCP. Furthermore, the oxidation of Met residues has been characterized in numerous biological systems and could be expected to occur in the context of the light reactions of photosynthesis. Conversion of the thioether moiety into Met sulfoxide or Met sulfone could influence the interaction of these sidechains with pigment. It is also possible that oxidation could affect the stability or alter the structure of the protein [28]. Methionine sidechains also have singlet oxygen quenching activity [29] which may contribute to the OCP's putative photoprotective function.

In the OCP, the carotenoid is almost entirely buried; only 3.4% of the surface of the 3'-hydroxyechinenone molecule is exposed to solvent. Solvent accessibility is restricted to two regions on the surface of the pigment molecule. The first is near the hydroxyl terminus of the carotenoid, which is wedged between the helical bundles (Fig. 1). The carotenoid is also slightly solvent accessible through the large elongated depression (Fig. 2B) formed by residues from both domains. The total volume of this cavity is 895 Å³, large enough for a substantial interaction with another protein. The cavity is continuous with the portion of the carotenoid binding pocket formed by the NTF-2 domain.

The red carotenoid protein

In the course of purification of OCPs from several cyanobacterial species, a red carotenoid protein (RCP) with distinctive optical properties (Fig. 4) was isolated [5,6]. N-terminal sequencing and mass spectrometry analysis indicates that this is a 16 kDa proteolytic fragment of the OCP [5]. This fragment is the result of removal of the first 15 and approximately the last 160

amino acid residues of the OCP. The proteolysis removes the entire C-terminal domain. Without concomitant structural changes (that are likely to occur in solution), this would result in the exposure of nearly half of the carotenoid to solvent (Fig. 5). The proteolysis of the OCP into an RCP also occurs under certain storage conditions. The 16 kDa RCP elutes as a monomer in size-exclusion chromatography and has an isoelectric point (5.0) similar to that of the OCP (4.7) [6]. Aggregates of the 16 kDa RCP induced by concentration or successive cycles of freeze–thawing appear orange and are retained by a YM 30 membrane [30], suggesting that native OCP spectral properties may be achieved through oligomerization of the RCP.

The observation of shorter OCP homologs in some cyanobacterial genomes (Table 2) resembling the RCP suggests that there may be smaller OCP-like proteins with altered spectral and oxygen quenching properties in these organisms. For example, in *Gloeobacter violaceus* and in both of the *Nostoc* genomes for which sequence data are available, in addition to full-length OCP genes, there are four paralogs that correspond to RCP-like proteins. These genomes also contain an open reading frame encoding a protein similar to the C-terminal, NTF2-like domain of the OCP. It is possible that different N-terminal domains may combine with the C-

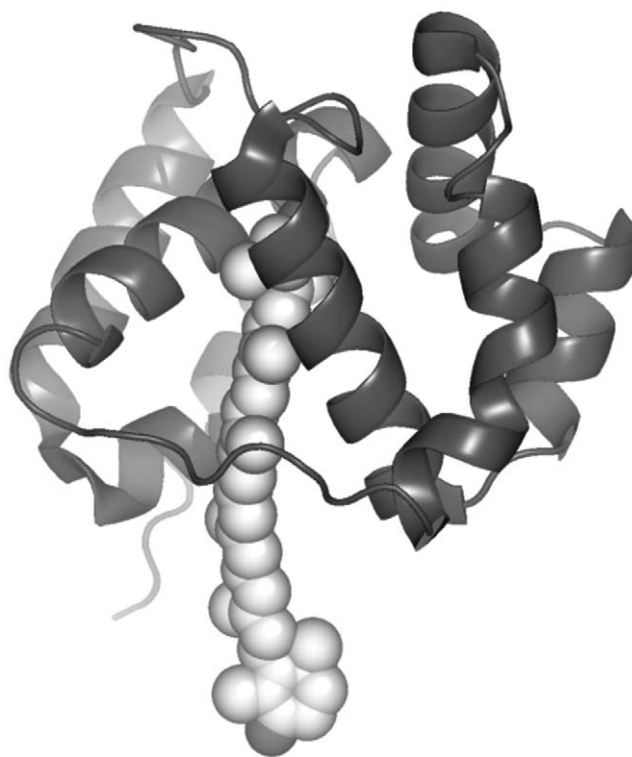


Fig. 5. Hypothetical model of the 16 kDa RCP based on the known proteolysis sites and the structure of the OCP. The carotenoid is shown in the conformation it holds in the OCP form. The protein and pigment conformational changes that are likely to occur with proteolysis to the RCP are not yet known.

terminal domain and carotenoid to create spectrally distinct OCP-like proteins in these organisms. A paradigm for a modular assembly of carotenoid-containing subunits into distinct holoproteins occurs in crustacyanin, isolated from lobster carapace. Various combinations of five β -crustacyanin subunits (each ~ 20 kDa) combine to form dimers in which two carotenoids span the interface [31].

Spectral changes mimicking those in the 16 kDa RCP can be induced by acidification of the OCP. Mass spectrometry confirms that the acid-induced RCP has not been proteolyzed. The acid-induced RCP migrates similar to OCP under native PAGE conditions (Kerfeld, unpub.), suggesting no change in oligomerization in the conversion of the OCP to the acid-induced form of RCP. However, circular dichroism measurements indicate that it has altered secondary structure relative to the OCP. The acid-induced RCP is stable indefinitely at neutral pH; however, prolonged exposure in crystallization conditions similar to those used to crystallize the OCP (pH 8.5) results in orange crystals (Kerfeld, unpub.). There are precedents for reversible, low pH-induced spectral changes in photosensitive proteins. For example, green fluorescent protein undergoes a spectral shift in response to decreasing pH [32]. A pH-induced carotenoid spectral change in the context of photoprotective processes also occurs in the xanthophyll cycle of higher plants (reviewed in [33,34]), which is involved in the thermal dissipation of excess light energy in both Photosystem I and Photosystem II.

Putative functions of the OCP

The OCP is unusual in that its structural characterization precedes a precise understanding of its function. A photoprotective function for the OCP is supported by microarray data indicating that OCP transcript levels increase more than 600% upon transfer to high light [35]. Since their initial characterization, several specific photoprotective roles have been proposed for the OCP and other water-soluble carotenoid proteins; they include singlet oxygen quenching, carotenoid transport or light attenuation. Below, the putative functions of the OCP are considered in the context of the structural data.

Quenching singlet oxygen and triplet chlorophyll

Data from our laboratory show that the OCP is an effective quencher of singlet oxygen, with an activity comparable to that of copper superoxide dismutase [14]. This is a critical function in oxygen-evolving photosynthetic organisms which generate oxidizing molecules under a broad range of light intensities. Singlet oxygen quenching studies of the 16 kDa RCP show that it quenches singlet oxygen at a significantly faster rate

than the OCP [14], consistent with greater carotenoid accessibility. The methionine sidechains noted above may also play a significant role in the OCP and the RCP's efficacy as quenchers of singlet oxygen. However, the limited exposure of the carotenoid and the lack of evidence that the OCP localizes to the luminal surface of the thylakoid membrane where these reactive oxygen species would be most concentrated make a physiological role in quenching singlet oxygen less likely.

Carotenoid-mediated photoprotective mechanisms also include quenching triplet chlorophyll and dissipating excited singlet chlorophyll in excess of the capacity of the electron transport chain (non-photochemical quenching). To carry out these quenching functions, the OCP would necessarily need to be able to dock to the photosynthetic apparatus. The numerous protuberances and cavities on the OCP surface could facilitate these interactions (Fig. 2).

Carotenoid transport

The OCP has also been suggested to be a carotenoid transport protein, perhaps involved in (re-)assembly of the photosynthetic apparatus. For example, Photosystem II components are also known to undergo rapid turnover in response to high light intensity. Levels of the transcripts for the Photosystem II D1 polypeptide (known to be vulnerable to UV-induced damage) and the OCP show a similar pattern of regulation in response to high light treatment [35]. Recently, phenotypic characterization of an OCP deletion mutant of *Syn 6803* indicates that this strain is more sensitive to high light treatment (Diana Kirilovsky, pers. comm.) and to UV-B radiation (Imre Vass, pers. comm.).

Functioning as a transport protein, the OCP could shuttle carotenoids from their site of synthesis in the thylakoid membrane to other cellular destinations. In cyanobacteria carotenoids such as zeaxanthin, echinone and carotenoid glycosides such as myxoxanthophyll are found in the outer membrane [36–38] and the cell wall [39–44]. High light or other environmental stresses results in an increase in the types and quantities of cyanobacterial carotenoids in these locations. Likewise, carotenoids are found in the cytoplasmic as well as the thylakoid membrane [45,46] where they have been implicated in regulating membrane fluidity. The possibility that OCP is a general carotenoid transport protein is supported by observations in our laboratory and that of our collaborator Roberto Bassi that the *A. maxima* apo-OCP can be reconstituted with a variety of non-native carotenoids (Bassi, unpub.; Kerfeld, unpub.).

A role in transport may explain the conflicting results of early efforts to determine the cellular location of various carotenoid proteins, as well as the diversity of carotenoids associated with them [6–12]. Although speculative, several structural features of the OCP are

consistent with a transport function. In a carotenoid transport function, the methionine residues near the carotenoid could be important for pigment binding and release. The sucrose molecule binds at a highly conserved region that structurally resembles an allosteric site. The binding site is between the two domains, an interface that presumably would need to open to release the carotenoid. In addition, circular dichroism measurements show that apo-OCP has significant secondary structure in solution, perhaps indicating that the protein adopts a stable folded form prior to binding or after release of pigment (Kerfeld, unpub.).

Component of photoprotective structures

The cell wall and the outer membrane of cyanobacteria are rich in protein as well as carotenoids [36–44]. Some of these proteins may be the OCP or its paralogs. For example, a protein of 37 kDa was observed in the carotenoid-rich outer membrane and cell wall fractions from *Synechococcus* PCC6307 (see Fig. 2 in [42]) but not further characterized. There are also proteins similar in size to the OCP homologs in the carotenoid-rich fraction of the outer membrane of *Synechocystis* PCC6714 [38]. Dissociation of the *P. hollandica* zeaxanthin-binding protein complex accompanied by staining for sugars indicates that it was composed of several proteins with molecular masses between 22 and 31 kDa [12].

In vivo studies of outer membrane carotenoids in *Synechocystis* PCC6714 indicate that the carotenoids are found at the periphery of the cell in a uniformly oriented array [47], suggestive of a photoprotective function. This spatial organization is suggested to be the result of the association of carotenoids with outer membrane proteins [47,48]. Packing of the OCP molecules in the crystals [13,14] is intriguing in that it suggests one way in which an array of aligned pigment molecules might be produced (Fig. 6). OCPs from some species are prone to

become covalently attached to some cellulose-based chromatography matrices [5], perhaps through chemistry similar to that required for association with the cell wall.

Variants of OCP and photoprotection

The photosynthetic apparatus of cyanobacteria is known to be structurally dynamic; the organisms alter the composition of their phycobilisomes in response to differences in the spectral composition of incident light [49]. Although speculative, the potential for modular assembly of OCPs in some cyanobacteria species could provide a means for tuning the response of the photosynthetic/photoprotective proteins to varying environmental conditions.

The OCP as a model system for study of carotenoid–protein interactions

The importance of carotenoids in the xanthophyll cycle and in anti-oxidant processes has stimulated efforts to manipulate the carotenoid content of different organisms. It is becoming apparent that biological systems are more versatile at synthesizing carotenoids than previously expected. For example, the introduction of an algal carotenoid biosynthetic gene into tobacco resulted in the accumulation of 3'-hydroxyechinenone [50], the cyanobacterial carotenoid found in the OCP. Furthermore, the importance of carotenoids as biological anti-oxidants has led to intensive efforts to express them in heterologous systems such as *Escherichia coli* [51]. To fully utilize this technology, it must be complemented by an understanding of how to integrate carotenoids into protein carriers which, for many applications, must be water-soluble. Spectroscopic and functional characterization as well as structural studies of the RCPs, apo-

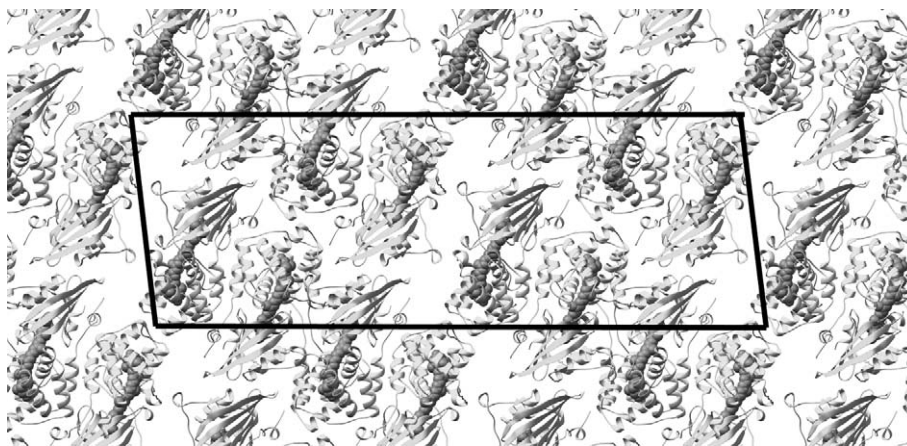


Fig. 6. Packing of the *A. maxima* OCP molecules within the crystals used for structure determination. The C2 cell (traced) dimensions were $a = 217 \text{ \AA}$, $b = 41 \text{ \AA}$, $c = 75 \text{ \AA}$ $\beta = 95.8^\circ$.

OCP, and site-directed mutants of the OCP will provide important information for manipulating carotenoid-protein content in other organisms.

Acknowledgments

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