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Crystals of the Carotenoid Protein from *Arthrospira maxima* Containing Uniformly Oriented Pigment Molecules

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Abstract

Crystals of a carotenoid protein from the cyanobacterium *Arthrospira maxima* have been grown in space group *C*2 with unit-cell dimensions $a = 219.6$, $b = 40.3$, $c = 75.5$ Å and $\beta = 95.5°$. The crystals diffract X-rays to 2.3 Å resolution and display unusual optical properties in polarized light that suggest that all of the carotenoid molecules in the crystals are oriented similarly. A slight increase in the concentration of a crystallization additive in the mother liquor induces macroscopic twinning, which is also visible when the crystals are illuminated with polarized light.

1. Introduction

The carotenoids are a diverse family of isoprenoid-based pigments with a variety of functions in plant and animal systems. The essential role of carotenoids in photosynthesis is underscored by their presence in all naturally occurring photosynthetic organisms. As accessory pigments in light harvesting, carotenoids are noncovalently associated with both soluble or transmembrane proteins or protein complexes. The proteins provide hydrophobic binding environments for the apolar pigments and influence their absorption properties. Most often, carotenoids are present in protein complexes with (bacterio)chlorophyll, where they increase light-harvesting efficiency by capturing blue–green light (470–550 nm), which is poorly absorbed by (bacterio)chlorophyll. The captured light energy is then transferred with high efficiency to the photosynthetic reaction center *via* the chlorophylls. Carotenoids also presumably play a role in photoprotection by quenching chlorophyll triplets and singlet oxygen.

Structural studies of light-harvesting proteins have provided details of the diverse strategies used by photosynthetic organisms for organizing pigments for optimal function in different ecological niches. Three-dimensional structures of the integral membrane light-harvesting protein complexes (LHC's) from higher plants (Kuhlbrandt, Wang & Fujiyoshi, 1994) and photosynthetic bacteria (McDermott *et al.*, 1995; Koepke, Hu, Muenke, Schulten & Michel, 1996) reveal how the coordination of carotenoid within the hydrophobic interior of the protein and in van der Waals contact with the (bacterio)chlorophyll facilitates light-harvesting, energy transfer and photoprotective roles. Moreover, the coordination of the carotenoids between transmembrane helices of the protein subunits in these complexes suggests they also function in maintaining the structural integrity of pigment–protein complexes. A similar positioning between transmembrane helices of the single carotenoid molecule was observed in the structures of bacterial photosynthetic reaction centers (Yeates *et al.*, 1988; Arnoux *et al.*, 1989; Deisenhofer, Epp, Sinning & Michel, 1995).

Additional structural information is known about three soluble LHC's: phycocybiliprotein (Schirmer, Bode, Huber, Sidler & Zuber, 1985), the bacteriochlorophyll a protein (Matthews, Fenna, Bolognesi & Schmid, 1979) and the peridinin-chlorophyll protein (Hofmann *et al.*, 1996). Despite the evolutionary distance between the organisms from which these proteins are derived and their lack of similarity in protein fold and pigment composition, each of these LHC's is organized as a trimer in which the pigments are tightly packed. Of these soluble LHC's, only the recently described chlorophyll-peridinin protein contains carotenoids [eight, in addition to two chlorophyll and two lipids (Hofmann *et al.*, 1996)].

To date the only report of crystallization of a protein that binds exclusively carotenoid pigments is that of β-crustacyanin isolated from lobster carapace. Structural studies have been hampered by the length of time required to achieve crystallization (3–4 months) and the radiation sensitivity of the crystals (Chayen, Gordon, Phillips, Saridakis & Zagalsky, 1996).

Several carotenoid proteins have been identified in cyanobacteria (reviewed by Hirschberg & Chamovitz, 1994). The level of carotenoid proteins varies with growth conditions, increasing during exposure to intense light (summarized by Hirschberg & Chamovitz, 1994). This suggests a photoprotective role for these proteins that bind exclusively carotenoid; this is also consistent with the absence of chlorophyll (or other tetrapyrrole)

pigments, which function in light harvesting but would be unnecessary in a photoprotective protein.

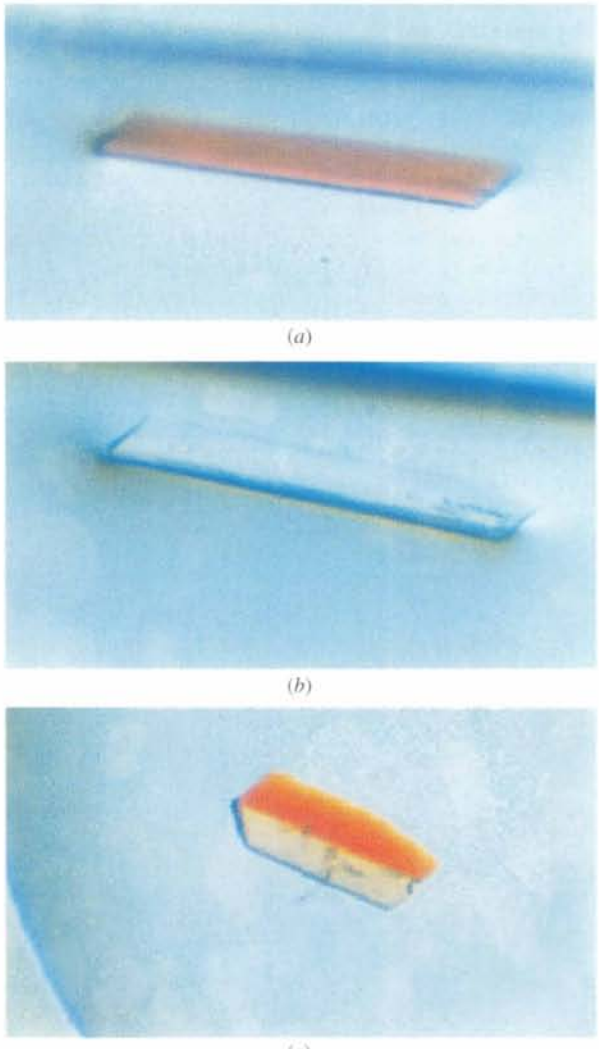
Estimates of the size of carotenoid proteins isolated and characterized from cyanobacteria have varied between 16 and 48 kDa; several appear to be dimers (Hirschberg & Chamovitz, 1994). They have several intracellular locations: loosely attached to either the photosynthetic or plasma membrane or to the outer surface of cell walls (Reddy, Bullerjhan & Sherman, 1993) or free in the cytosol. Water-soluble carotenoid proteins have been identified in several species of cyanobacteria (Holt & Krogmann, 1981; Diverse-Pierluissi & Krogmann, 1989; Engle, Burkhart, Sherman & Bullerjhan, 1991). The orange carotenoid protein (OCP) from the cyanobacterium *Arthrospira maxima* contains the keto-carotenoid 3-hydroxyechinenone (Cheng, 1996) and is similar to OCP's isolated previously from other cyanobacterial species (Holt & Krogmann, 1981). Although early estimates of the number of carotenoid molecules per apoprotein ranged from 10 to 12, more recent estimates indicate there are two carotenoid molecules per protein chain (Krogmann, unpublished work). The protein has absorbance maxima of 465 and 495 nm and a monomeric molecular weight, determined by mass spectroscopy, of 34 695 Da. N-terminal sequencing of the *A. maxima* OCP (Cheng, 1996) indicates that it is highly homologous (83% similarity) to the *Synecchococcus* 6803 slr1963 gene product (OCP/slr1963; Kazusa Institute, 1996). The deduced molecular mass of OCP/slr1963 is 34 658 Da, similar to that of *A. maxima* OCP. The primary structure of OCP/slr1963 shows no significant sequence similarity to any other proteins in the sequence database and structure-prediction searches have not detected a strong similarity to any other protein tertiary structure. However, slr1963 contains an ATP/GTP-binding motif and four protein kinase C phosphorylation sites. One of the protein kinase C phosphorylation sites is present in the N-terminal sequenced fragment of *A. maxima* OCP. The other three and the ATP/GTP binding motif are downstream of the sequenced region (Cheng, 1996).

Here we report the crystallization and preliminary X-ray analysis of the *A. maxima* carotenoid protein. The crystals have exceptional spectral properties in polarized light that suggest that all of the pigment molecules in the crystal are oriented similarly.

2. Experimental procedures

The *A. maxima* carotenoid protein was purified essentially as described by Holt & Krogmann (1981) and concentrated and dialyzed in a Centricon 10 (Amicon, Beverly, MA). The protein (~2.4 mg ml^{-1} in 0.05 *M* Tris pH 7.8) was crystallized by hanging-drop vapor diffusion at 295 K over a 1 ml reservoir containing 0.1 *M* Tris pH 7.7, 0.2 *M* NaCl, 30% PEG 3350 (all chemicals were purchased from Sigma Chemical Co., St Louis, MO). The hanging drop (2–6 µl) contained a 1:1 mixture of protein and reservoir solution. The size and radiation tolerance of the crystals were improved by the addition of 1–5% of either sucrose, glycerol or 1,2-methylpentanediol (MPD) to the reservoir. All crystals reached maximum size within one week.

Diffraction data were collected on an R-AXIS II image-plate detector with a Rigaku RU-300 X-ray generator. Diffraction data were processed using *DENZO* and *SCALEPACK* (Otwinowski, 1993). The self-rotation function was calculated using *AMoRe* (Navaza, 1994).



(*a*)

(*b*)

(*c*)

Fig. 1. (*a*) Carotenoid protein crystal viewed under a single polarizer and (*b*) after 90° rotation of the polarizer. (*c*) A twinned crystal grown with 2.5% MPD as additive.

Crystals were examined with a Zeiss SV 8 stereomicroscope with a Series III linear-polarization filter over the transmitted-light unit (MAX ERB Instrument Co., Burbank, CA).

3. Results and discussion

The *A. maxima* OCP is water soluble only at low protein concentrations; at concentrations exceeding 3.0 mg ml^{-1}, the protein forms stringy aggregates that require detergent treatment to dissociate. Initial crystals were obtained from a protein preparation that contained 0.025% dodecyl maltoside by vapor diffusion against 0.1 *M* HEPES pH 7.5, 0.5 *M* ammonium sulfate, 30% MPD. These crystals were strongly birefringent but thin and were not characterized by X-ray diffraction analysis. After removal of the detergent by dialysis and concentration, a second crystal form was grown. Inclusion of 1–2% of glycerol or MPD or sucrose as a crystallization additive increased the size of the crystals. Analysis of X-ray diffraction data indicated the space group *C*2 with cell dimensions $a = 219.6$, $b = 40.3$, $c = 75.5$ Å and $\beta = 95.5°$. The choice of the additive does not appreciably affect the cell constants, but does affect tolerance to radiation, in the following order of decreasing protection: glycerol > sucrose > MPD > no additive.

The crystals display a striking optical property (Fig. 1). They grow as elongated prisms that lie horizontally in the crystallization drop. When illuminated through a single polarizing filter, each crystal is orange or perfectly clear, depending on the orientation of the crystal relative to the direction of polarization. The maximum carotenoid absorbance (*i.e.* the darkest orange color) occurs when the polarizer is approximately aligned with the long edge of the crystals, which corresponds to the crystallographic *a* axis. Since the carotenoid absorbance transition is along the long axis of the pigment molecule, we conclude that all of the carotenoid molecules in the crystal are aligned nearly parallel to the *a* axis. This is remarkable considering the expectation that each of the protein chains binds at least two pigment molecules, and that the protein molecules occur in multiple orientations in the crystal.

The Matthews (1968) parameter for the carotenoid protein crystals is 2.4 $Å^3$ Da^{-1}, assuming two molecules in the asymmetric unit. A self-rotation function calculated with the native diffraction data indicates that the putative dimer is oriented in the crystal with its local axis of symmetry nearly parallel to the crystallographic *a* axis (Fig. 2). This conclusion is consistent with the tendency of the crystals to grow as macroscopic twins when the concentration of glycerol or MPD is increased (Fig. 1*c*). The twinning is non-merohedral or 'epitaxial', with a twinning axis along the *a* axis.

Other pigment proteins for which structures are known function in light harvesting, while OCP may be photoprotective and therefore unique in structure. Moreover, the dimeric organization of OCP is unusual; all of the soluble LHC's that have been structurally characterized are trimeric. The functional significance (if any) of the oligomeric state is unknown. The chromophore organization in OCP is also unusual. The present crystallization study and *in vivo* linear-dichroism experiments (Jurgens & Mantele, 1991) suggest that carotenoids are uniformly oriented. The three-dimensional structure of *A. maxima* OCP should shed new light on the principles of energy transfer and photoprotection.

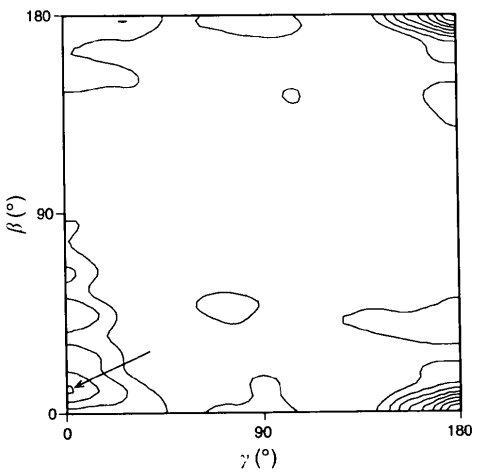


Fig. 2. Self-rotation function of the orange carotenoid protein (OCP) in the $\alpha = 180°$ section of rotation space. Data were included between 8 and 3.4 Å resolution. The large peaks at $\gamma = 180°$ are origin peaks. The arrow indicates the operation presumed to relate the two protein monomers in the asymmetric unit. The map is contoured in increments of one standard deviation.

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