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Excited-state properties of the 16 kDa red carotenoid protein from *Arthrospira maxima*

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ABSTRACT

We have studied spectroscopic properties of the 16 kDa red carotenoid protein (RCP), which is closely related to the orange carotenoid protein (OCP) from cyanobacteria. Both proteins bind the same chromophore, the carotenoid 3′-hydroxyechinenone (hECN), and the major difference between the two proteins is lack of the C-terminal domain in the RCP; this results in exposure of part of the carotenoid. The excited-state lifetime of hECN in the RCP is 5.5 ps, which is markedly longer than in OCP (3.3 ps) but close to 6 ps obtained for hECN in organic solvent. This confirms that the binding of hECN to the C-terminal domain in the OCP changes conformation of hECN, thereby altering its excited-state properties. Hydrogen bonds between the C-terminal domain and the carotenoid are also absent in the RCP. This allows the conformation of hECN in the RCP to be similar to that in solution, which results in comparable excited-state properties of hECN in solution. The red-shift of the RCP absorption spectrum is most likely due to aggregation of RCP induced by hydrophobic nature of hECN that, when exposed to buffer, stimulates formation of assemblies minimizing contact of hECN with water. We suggest that the loss of the C-terminal domain renders the protein amphipathic, containing both hydrophobic (the exposed part of hECN) and hydrophilic (N-terminal domain) regions, and may help the RCP to interact with lipid membranes; exposed hECN can penetrate into the hydrophobic environment of the lipid membrane, possibly to provide additional photoprotection.

1. Introduction

Photoprotective mechanisms involving carotenoids are the key processes protecting photosynthetic organisms from excess light [1,2]. These processes range from direct quenching of (bacterio) chlorophyll triplet states and quenching singlet oxygen to complex reactions involving specialized proteins and cofactors. In plants for example, photoprotection involves chlorophyll-binding antenna proteins and is triggered by a pH change in thylakoid membrane that, with a help of a specialized protein called PsbS, activates enzymes that perform mutual interconversion of two carotenoids, violaxanthin and zeaxanthin [3,4]. This complicated process, known as non-photochemical quenching (NPQ), eventually results in thermal dissipation of absorbed energy that is manifested by a decrease of chlorophyll fluorescence.

Until recently the NPQ has been associated only with plants, but a number of studies in the past decade showed that NPQ exists also in cyanobacteria to decrease the amount of energy transferred from phycobilisomes to PSII [5–8]. In contrast to plants, however, a 35 kDa water-soluble carotenoid-binding protein known as the orange carotenoid protein (OCP) plays an essential role [7–9]. This protein, discovered nearly twenty years ago [10] and whose structure is known [11,12], binds a single carotenoid, 3′-hydroxyechinenone (hECN). The OCP is composed of two domains, an all-helical N-terminal domain (Pfam09150; http://pfam.sanger.ac.uk/) which has not been observed in any other protein structures determined to-date, and a C-terminal domain which is a member of the nuclear transport factor 2 (NTF2) superfamily (Pfam02136; http://pfam.sanger.ac.uk/) of α/β proteins. The carotenoid has a conjugated chain consisting of 11 conjugated C C bonds spanning two protein domains. The conjugated chain is terminated by a carbonyl group that is nestled in the binding pocket of the C-terminal domain (Fig. 1A), forming hydrogen bonds between the keto oxygen and absolutely conserved tyrosine and tryptophan residues [11–13]. Binding to the protein forces the terminal ring of hECN to the s-trans conformation, while s-cis orientation occurs in solution (Fig. 1). This change is associated with changes of hECN excited-state properties [14].

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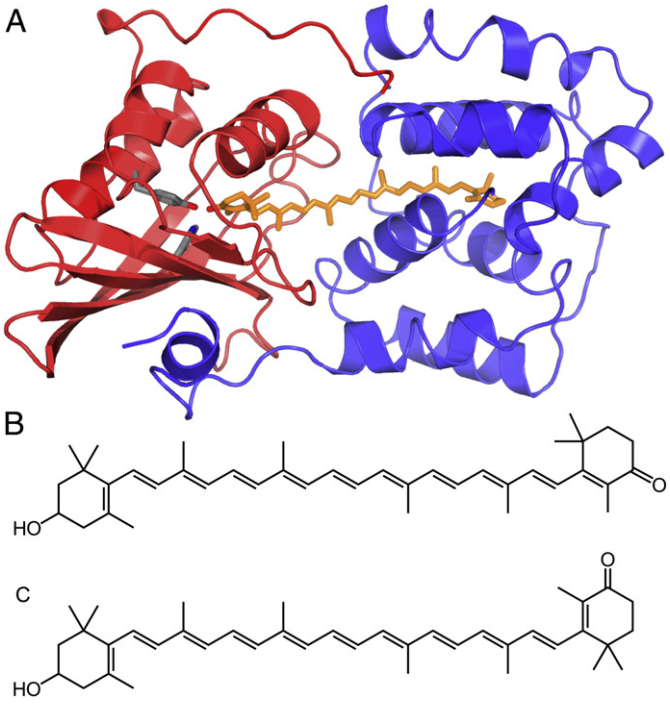

Fig. 1. (A) Structure of *A. maxima* OCP showing the two helical bundles of the N-terminal domain (blue), and the C-terminal domain (red). The hECN (orange) and the absolutely conserved tyrosine and tryptophan residues that hydrogen bonds to the carotenoid are shown in sticks. Figure was prepared with PyMOL (http://www.pymol.org/). Molecular structure of hECN when bound to OCP (B), and in solution (C).

Although increasing transcript levels for the OCP during exposure to intense light suggested its possible involvement in photoprotection, the mechanism of its action was not known until recently.

It was shown that action spectrum of NPQ in *Synechocystis* [15] matches the absorption spectrum of OCP [14,16,17]. Moreover, the absence of the OCP in cyanobacteria prevents fluorescence quenching induced by blue light [7]. Thus, the OCP was identified as photoreceptor triggering the NPQ in cyanobacteria. Later, the same group demonstrated that OCP itself is photoactive. Upon illumination with blue-green light, the OCP converts to a red form, which is proposed to be the active form of the protein responsible for induction of NPQ [9]. Using mutants accumulating different carotenoids in OCP, Punginelli et al. [18] proved that the conjugated carbonyl group of hECN is the key structural feature leading to the photoactivity of OCP.

A red form of the OCP can be also produced by acidification of the protein resulting in a red-shifted absorption spectrum. The origin of the red-shift is unknown, as well as the origin of the red-shift of the active form of OCP. While these red forms of OCP have all molecular weights of 32 kDa, there is another form of this protein, a 16 kDa red carotenoid protein (RCP) that co-purifies with the OCP in certain cyanobacteria [13]. RCP also has a red-shifted absorption spectrum and lacks the C-terminal domain, presumably resulting in exposure of hECN to solvent (Fig. 1A). Interestingly, the accumulating genomic sequence data indicates that many genomes also contain, in addition to a full length OCP gene, one or more open reading frames encoding homologs to the N-terminal domain while containing a single copy of the C-terminal domains (with the exception of the draft genome of *Microcoleus chthonplastes* which does not contain a gene encoding C-terminal domain) [19]. The primary structures of these fragments are highly conserved. When they were first noted [11], it was suggested that they could combine in modular fashion to produce OCP variants. More recently evidence for their expression has been reported [20]. For example, in *Nostoc punctiforme*, proteomic analysis showed that several of the N-terminal paralogs are known to be expressed [21]. Here we apply transient absorption spectroscopy to study excited-state properties of the 16 kDa RCP protein from *Arthrospira maxima* and compare the results with hECN in solution and in the OCP.

2. Materials and methods

A. maxima OCP and hECN were prepared as described previously [14]. In many organisms, including *A. maxima* the RCP co-purifies with the OCP and is subsequently separated from the purified OCP by gel filtration chromatography [22]. The purified OCP and RCP samples were stored in dark at 4 °C and prior to experiments diluted in a buffer (5 mM Tris pH 8, 0.5 M EDTA, and 80 mM NaCl) to yield optical density of 0.1/mm at absorption maximum. hECN was dissolved in methanol or acetone (Sigma Aldrich, spectroscopic grade) to give optical density 0.3/mm at the excitation wavelength. Absorption spectra were measured on UV-300 spectrophotometer (Spectronic Unicam), and CD spectra were recorded on J-715 spectropolarimeter (Jasco).

The femtosecond spectrometer used for collecting transient absorption spectra is based on an amplified 1 kHz Ti:sapphire laser system (Integra-i, Quantronix) producing 795 nm pulses of ~130 fs duration with energy of 2.1 mJ/pulse. The amplified pulses were divided into two paths. The first generates excitation pulses in optical parametric amplifier whose output was set to desired excitation wavelength and focused to sample to a spot of 400 μm in diameter. The energy of excitation was attenuated by neutral density filters to 200 nJ/pulse resulting in excitation density of $\sim 4\times 10^{14}$ photons $\text{pulse}^{-1}\,\text{cm}^{-2}$ at all excitation wavelengths. The second part of the amplifier output was used to produce white-light continuum probe pulses in a 2-mm sapphire plate. In order to minimize dispersion, the white-light continuum was collimated by parabolic mirror and divided into the probe beam, which overlapped with the pump beam at the sample, and a reference beam that passes the sample outside the excited area. Probe and reference beams were focused to the sample by a pair of 30 cm spherical mirrors. After passing the sample, both beams were dispersed in spectrograph onto a double photodiode array with 1024 elements allowing measurements of transient spectra in a spectral window of ~240 nm. The instrument response function was ~130 fs as determined by fitting the instantaneous rise of bleaching signal in a laser dye. Mutual polarization of pump and probe beams was set to the magic angle (54.7°) by placing a polarization rotator in the pump beam. All measurements were carried out in a rotating cuvette consisting of two 1 mm quartz windows separated by a 1 mm Teflon spacer.

Transient spectra collected by the diode-array detection system were fitted globally using DAFit software (Pascher Instruments) to a sum of exponentials, including numerical deconvolution of the response function, and a fourth degree polynomial describing the chirp. To visualize the excited-state dynamics, we assume that the excited system evolves according to a sequential, irreversible scheme A→B, B→C, C→D… The arrows represent increasingly slower monoexponential processes and the time constants of these processes correspond to lifetimes of the species A, B, C, D… The spectral profiles of the species are called evolution-associated difference spectra (EADS), and provide information about the time evolution of the whole system [23].

3. Results

The absorption spectrum of the 16 kDa RCP is compared to the absorption spectra of hECN in methanol and in the OCP is shown in Fig. 2. The 0–0 peak of the S_2–S_0 transition of hECN, located in methanol at 476 nm, shifts to 496 nm when hECN is bound to the OCP. In addition, resolution of vibrational bands is enhanced in the OCP. This is explained by prolongation of the effective conjugation length

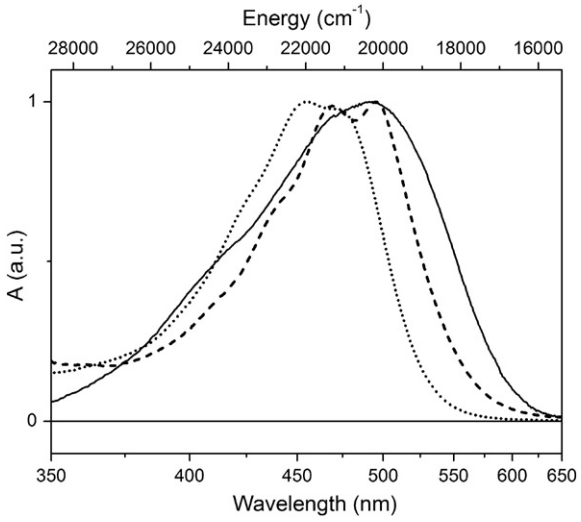

Fig. 2. Absorption spectra of OCP (dashed) and RCP (solid) compared with absorption spectra of hECN in methanol (dotted). All spectra are normalized.

induced by binding to the OCP [14]. In the RCP, however, the resolution of vibrational bands is nearly lost and, although the absorption maximum is at about the same wavelength as in the OCP, the absorption spectrum has a red shoulder that extends up to 600 nm. Furthermore, the RCP absorption spectrum exhibits an additional shoulder at 410 nm that is not present in either hECN in solution or in the OCP. Clearly, this shoulder cannot be a higher vibrational band, because the energy gap between the blue shoulder at 410 nm and the absorption maximum is too large to match the known vibrational spacing commonly observed in carotenoid absorption spectra [24].

The transient absorption spectrum of the RCP measured at 1 ps after excitation at 500 nm shows a single excited-state absorption band peaking at 615 nm (Fig. 3). This is due to the S_1–S_n transition that appears after the fast S_2–S_1 internal conversion, which in hECN occurs within 100–200 fs depending on solvent [14]. With the exception of the large red-shift, the shape of the RCP transient absorption spectrum is comparable to that of hECN in methanol, which also exhibits a single, broad S_1–S_n band that peaks at 580 nm. On the other hand, the transient absorption spectrum of the OCP is qualitatively different. It has two excited-state absorption bands

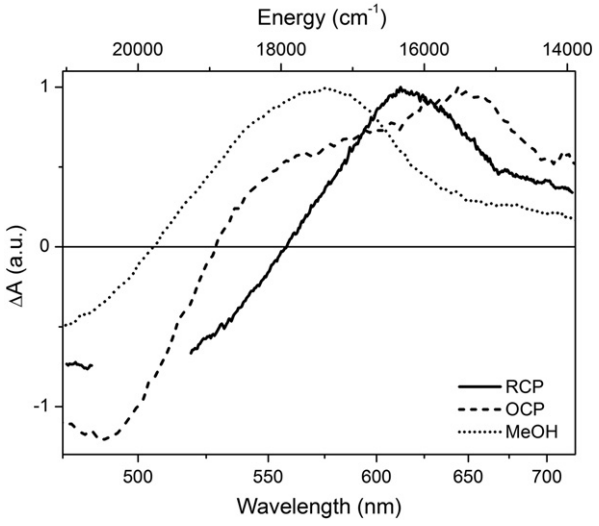

Fig. 3. Transient absorption spectra of hECN in methanol (dotted), OCP (dashed) and RCP (solid) recorded at 1 ps after excitation at 485 nm (hECN in methanol), 495 nm (OCP) and 500 nm (RCP). For RCP, the part of the spectrum close to the excitation wavelength is removed due to scattering. All spectra are normalized at their maxima.

located at 565 and 650 nm. While the 565 nm band is due to the S_1–S_n transition, the other band is indicator of an intramolecular charge transfer (ICT) state; the 650 nm band is due to the ICT–S_N transition [25,26]. The stabilization of the ICT state in OCP is most likely caused by the s-trans conformation of hECN in OCP caused by binding of hECN to the C-terminal domain that leads to a specific interaction of the conjugated carbonyl group with amino acid residues nearby [14]. The absence of the ICT band in RCP again points to a release of the protein lock caused by lacking the C-terminal domain, resulting in the s-cis conformation of the terminal ring with conjugated carbonyl group of hECN in RCP.

Fitting the spectro-temporal dataset obtained for the RCP globally allowed for extraction of time constants associated with the excited-state processes of hECN bound in the RCP. The results are shown in Fig. 4. The first EADS corresponding to the S_2 spectrum of hECN decays in 130 fs to produce the second EADS that is reminiscent of the S_1–S_n transition. However, the second EADS decays in 0.8 ps and the third EADS (dotted line in Fig. 4) generated via the 0.8 ps process has essentially the same shape. It should be noted that although the 0.8 ps time constant is in the range expected for vibrational relaxation in the S_1 state [27,28], the shape of the second EADS prevents assignment to the hot S_1 state. Even though it has larger magnitude in the red part of the spectrum, a feature usually associated with the hot S_1 state [28,29], there is also a concomitant loss of bleaching indicating that the 0.8 ps process repopulates the ground state. The third EADS generated by the 0.8 ps process decays within 5.5 ps and it is clearly due to the S_1 lifetime. Yet another EADS is necessary to obtain good fits in the 550–650 nm spectral region. The final spectrum has a lifetime of 20 ps and its possible origin will be discussed later.

Since the global fitting of hECN in methanol and in the OCP was discussed in detail earlier [9,14], we compare only kinetics at the key wavelengths to demonstrate differences among the three samples. Fig. 5 compares kinetic traces taken at the maxima of the respective S_1–S_n bands whose decays monitor the S_1 lifetime. hECN in methanol has S_1 lifetime of 6.2 ps that is shortened to 3.3 ps in the OCP. The shorter S_1 lifetime in the OCP reflects the change of hECN structure (see Fig. 1) induced by the OCP's binding pocket, which makes the effective conjugation length longer, and consequently the S_1 lifetime shorter. In the RCP, hECN exhibits excited-state dynamics similar to that in solution. The S_1 lifetime extracted from global fitting (Fig. 4) is 5.5 ps. However, contrary to hECN in methanol the kinetic trace measured for hECN in the RCP at the S_1–S_n maximum does not decay to zero within the first 25 ps. Instead, additional decay component of 20 ps (see global fitting described previously) is necessary to fit the kinetic at long times as shown in the inset of Fig. 5, which shows the kinetic trace extending to longer delays.

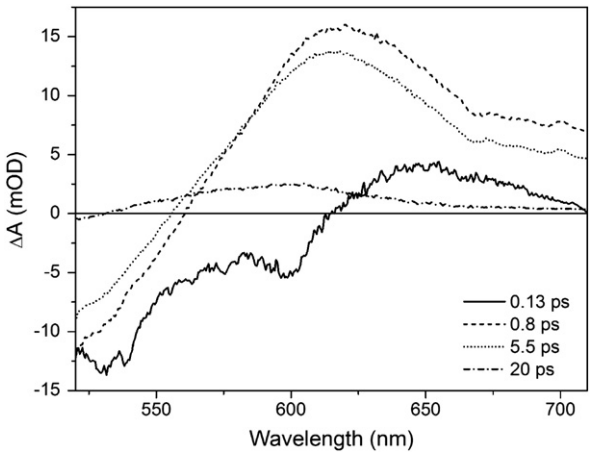

Fig. 4. EADS obtained from global fitting of data measured for RCP after excitation at 500 nm. See text for details.

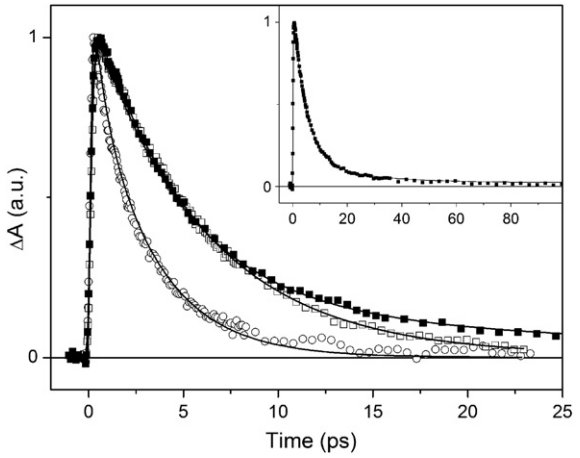

Fig. 5. Kinetic traces measured at maxima of the S_1–S_n transitions for hECN in methanol (585 nm, open squares) and RCP (615 nm, full squares), and the ICT–S_N transition of hECN in OCP (650 nm, open circles). Solid lines are fits obtained from global fitting analysis. Inset shows the decay of the S_1–S_n signal in RCP at longer delays.

4. Discussion

The main structural difference between the OCP and the RCP is the absence of the C-terminal domain in RCP (Fig. 1). Thus, while in the OCP the hECN is completely buried in protein [11,12], the lack of the C-terminal domain in the RCP apparently exposes part of the carotenoid. Moreover, the C-terminal domain of the OCP provides the specific binding cleft and hydrogen bonding between the conjugated carbonyl group of the hECN and the protein. Thus, while in the OCP the terminal ring with the conjugated carbonyl group is locked in s-trans orientation (Fig. 1), the lack of the C-terminal domain in RCP should release the protein lock, resulting in the s-cis conformation of the terminal ring of hECN. In addition, since the s-trans conformation of hECN in the OCP enables hydrogen bonding of the carbonyl oxygen to the tyrosine and tryptophan nearby [11,12], and since this hydrogen bond is supposed to be the key factor in stabilizing the ICT state of hECN in OCP [14], the spectroscopic features characteristic of the s-trans conformation and ICT state are expected to disappear in RCP.

Indeed, the excited-state dynamics of hECN observed here supports this scenario. The transient absorption spectrum of the RCP lacks the splitting of the S_1–S_n transition into two bands that is a typical marker of the involvement of the ICT state in excited-state dynamics [25,26]. With the exception of the red-shift, the transient absorption spectrum of hECN in the RCP is similar to that in solution, supporting the notion that the orientation of the C=O group in respect to the main conjugation is the same for hECN in both RCP and in solution. This hypothesis is further strengthened by the 5.5 ps S_1 lifetime of hECN in RCP. Even though the S_1 lifetime in solution is somewhat longer, yielding values that slightly depend on solvent (6.2 ps in methanol, 6.4 ps in n-hexane, and 6.8 ps in CS_2 [14]), it is clear that the effective conjugation of hECN in the RCP is significantly shorter than in the OCP where the S_1 lifetime of hECN is shortened to 3.3 ps. The slight difference between the S_1 lifetime of hECN in solution and in the RCP may be caused by a π–π stacking interaction. The structure of the OCP revealed such an interaction between tryptophan in the N-terminal domain and the terminal ring of hECN that does not contain the carbonyl group. Since the π–π interaction may shorten the S_1 lifetime of carotenoids with terminal rings [30], it could be the origin of the slightly shorter S_1 lifetime of hECN in RCP.

It is important to note that changes in the S_2 lifetime further support the idea of similar spectroscopic properties of hECN in solution and RCP. While in solution the S_2 lifetime varies between 135 fs (CS_2) and 230 fs (hexane), the S_2 lifetime in the OCP is markedly shorter, yielding a sub-100 fs value [14]. The 130-fs S_2 lifetime measured here for hECN in the RCP matches those measured in CS_2. Moreover, the solvent-dependence of the S_2 lifetime of hECN follows the energy gap law. The larger polarizability of CS_2 pushes the S_2 state down, while the energy of the S_1 state remains unaffected due to its negligible dipole moment. Consequently, the S_2–S_1 energy gap is narrow in CS_2, making the S_2 lifetime shorter than in hexane or methanol. Because the 0–0 band of the S_2 state, and consequently also the S_2–S_1 energy gap, of hECN in the RCP are close to that in CS_2 [14], the 130 fs S_2 lifetime of hECN in the RCP matches nicely the S_2 lifetime of hECN in CS_2, again strengthening the observation that the spectroscopic properties of hECN in the RCP are very similar to those in solution.

Thus, both the shape of transient absorption spectra and the S_1 and S_2 lifetimes of hECN in the RCP are similar to those in solution, likely because the s-cis conformation of the exposed terminal group. Yet, because the s-cis conformation makes the conjugation length effectively shorter than for the s-trans conformation in OCP [14], the absorption spectrum of the RCP should be similar to that of hECN in solution and thus blue-shifted as compared with the OCP. Obviously, this is not the case. It is true that absorption spectrum of hECN may be significantly red-shifted even in solution, but this is achieved only in the highly-polarizable CS_2 [14]. For the RCP in buffer, however, the hECN is likely exposed to water, which has a polarizability comparable to hexane or methanol. Therefore dispersive interactions cannot be the reason for the absorption spectrum of the RCP extended to nearly 600 nm (Fig. 2).

What is then the origin of the red-shifted absorption band in the RCP? It is worth noting that absorption spectrum of the 16 kDa protein shown in Fig. 2 is (except the 410 nm shoulder in the 16 kDa RCP) remarkably similar to the absorption spectrum of the active form of the OCP [9,18]. The origin of the red-shift of the active form of the OCP remains unknown, but since the carbonyl oxygen is crucial for photoconversion of the OCP into the active form [18], and since the 16 kDa RCP exposes the part of hECN containing carbonyl oxygen, it is tempting to suggest that the interactions, resulting in the red-shifted absorption spectrum, may be of the same origin. However, since the carbonyl oxygen in the RCP is not buried in protein but exposed to buffer, it is likely the hydophobicity of hECN that is responsible for the red-shift of the RCP absorption spectrum.

Apart from a few exceptions [31], carotenoids are highly hydrophobic molecules that usually aggregate when exposed to water [32,33]. It is therefore likely that the RCP, with apparently nearly half of the hECN molecule exposed to buffer, will self-organize into aggregates in which the hydrophilic protein part is exposed to water while the carotenoid is protected from contact with buffer. The changes in absorption spectra of aggregated carotenoids depend on organization of molecules within the aggregate [33–35], and indication of the aggregation-induced changes can be found also in absorption spectrum of the RCP. The shoulder at 410 nm is reminiscent of the H-band of carotenoid aggregates resulting from tight packing of the aggregated molecules [33–35]. This band could be for example due to an RCP dimer, in which the hECN molecules are in close contact. On the other hand, red-shifted absorption band is usually indicator of weakly-coupled carotenoid aggregates. Thus, the red-shift of the RCP absorption spectrum may be due to formation of micelles consisting of RCP proteins.

To further test the hypothesis that the RCP forms aggregates, we have measured circular dichroism (CD) spectra of the OCP, RCP and hECN in solution (Fig. 6). As for other carotenoids, hECN has virtually no optical activity in acetone in the 450–550 nm spectral region. Binding to the OCP induces chirality of hECN, a phenomenon known also for other carotenoids. For example, optical activity was observed for the otherwise nonchiral salinixanthin when bound to protein xanthorhodopsin [36], strong carotenoid CD bands were also produced upon binding of carotenoids to light-harvesting complexes of purple bacteria [37]. The CD bands in such cases originate from

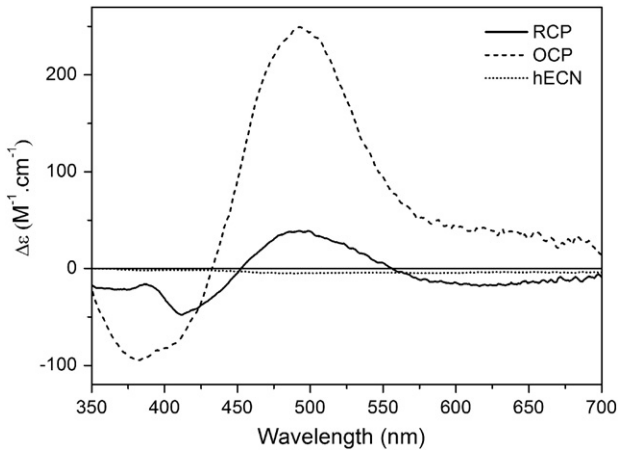

Fig. 6. CD spectra of hECN in acetone (dotted), OCP (dashed), and RCP (solid).

interaction of the carotenoid with the protein in an asymmetric environment or from an enforced asymmetric conformation of the carotenoid chain. Thus, in the OCP the non-conservative CD bands results from the asymmetric binding site, in which hECN is bowed, exhibiting an average deviation of 16° from all-trans conformation (Fig. 1). In the RCP, the missing C-terminal domain releases the protein lock, resulting in decrease of intensity of the protein binding-induced CD bands, but the binding cleft in the N-terminal domain still generates CD spectrum whose overall shape is comparable to that of the OCP except for two features characteristic of the RCP. First, there is a clear Cotton effect at ~400 nm consisting of negative signal at 412 nm, and approximately equal positive lobe at 390 nm. This conservative signal, superimposed on the non-conservative CD background generated by interaction with protein, suggests a presence of excitonic interaction between hECN molecules typical for carotenoid aggregates [34]. Similarly, the weak negative CD signal above 550 nm observed exclusively in the RCP could be due to red-shifted weakly interacting carotenoid aggregates [34]. Thus, CD spectra further supports our hypothesis that the red-shift of the RCP absorption spectrum is likely caused by aggregation of RCP, which is induced by exposition of the highly hydrophobic hECN to buffer.

The formation of aggregates also explains the presence of the additional kinetic components, 0.8 and 20 ps, in RCP. A component with a lifetime longer than the S_1 lifetime was observed in both blue- and red-shifted aggregates of the carotenoid zeaxanthin [33]. Although a decay component with a lifetime longer than the S_1 state with a spectrum that is blue-shifted in respect to the S_1–S_n transition could be also interpreted as due to the so-called S* state [38,39], this assignment is unlikely in the RCP. The S* lifetime measured in other carotenoids is always markedly shorter, 5–7 ps [40], than the 20 ps lifetime observed here. Also, the spectrum of the 20 ps component in the RCP is significantly broader that the characteristic spectrum of the S* state indicating different origin of this component. Thus, we assign the 20 ps decay component as due to excited-state lifetime of RCP aggregates. On the other hand, the lifetime of the shorter, 0.8 ps component is reminiscent of relaxation of the hot S_1 state [27,28], but its spectral profile, which is essentially identical to that of relaxed S_1 state (Fig. 4), suggests that it must be of different origin. In addition, it is clear from Fig. 5 that there is also a decrease of ground state bleaching during the 0.8 ps process, indicating that a fraction of the excited RCP returns to ground state with a time constant of 0.8 ps. Thus, the process characterized by the 0.8 ps component is another channel of depopulation of the S_1 state. Such process was identified in carotenoid aggregates and assigned to annihilation [33]. Consequently, we suggest that the 0.8 ps component corresponds to annihilation occurring within RCP aggregates.

Further support for RCP aggregation comes from experiments with adding detergent to the RCP buffer. Absorption spectra of RCP measured with different concentrations of dodecyl maltoside are shown in Fig. S1 (supporting information). It is clear that upon increasing concentration of dodecyl maltoside, RCP absorption spectrum exhibits a blue shift and the 410 nm band, assigned to aggregates disappears. However, the aggregation appears to be concentration-dependent. In gel filtration RCP elutes as a monomer, even in mobile phase lacking detergent (data not shown); the dilution of the protein sample during gel filtration may account for the lack of aggregation.

Thus, while spectroscopic data and addition of detergent to RCP buffer clearly support the notion of formation of RCP aggregates, gel filtration experiments suggest that no aggregates are present. This seemingly contradictory result may be explained by weakly-bound aggregates that dissociate during the gel filtration. Such situation is rather common in carotenoids, because carotenoid aggregates are unstable and very sensitive to changes in solvent parameters (e.g. pH, carotenoid concentration, water content in a hydrated solvent) [33,34]. The notion of disintegrating the aggregates during the gel filtration experiment is also supported by data shown in Fig. S2 (supporting information). We have measured absorption spectrum of RCP diluted in the buffer used for the gel filtration experiment. The absorption spectrum resulting from dilution of RCP to concentration comparable to that used in the gel filtration experiment exhibits comparable, though weaker effects as those observed after addition of dodecyl maltoside.

We conclude that the loss of C-terminal domain in the 16 kDa RCP from *A. maxima* makes the spectroscopic properties of hECN, which are markedly altered by binding to OCP [14], similar to those in organic solvents. The terminal group of hECN that contains the conjugated carbonyl group is in s-cis orientation in respect to the main conjugated chain. This orientation partially isolates the conjugated carbonyl, resulting in loss of ICT features in transient absorption spectra, which are readily observed in the OCP. The red-shift of the RCP absorption spectrum is most likely due to aggregation of the RCP proteins induced by hydrophobic nature of hECN that, when exposed to buffer, stimulates formation of assemblies minimizing contact of hECN with buffer.

It is thus likely that aggregation of the 16 kDa RCP is only a result of local environment and it is not directly related to RCP function that remains an open question. It is notable that the numerous cyanobacteria that contain open reading frames encoding N-terminal fragments of the OCP tend to inhabit environments that fluctuate between extremes [19]. It was demonstrated that exposure of hECN results in more efficient singlet oxygen scavenging, thus it is possible that while the OCP triggers the NPQ, the primary role of the 16 kDa RCP is scavenging singlet oxygen. The loss of the C-terminal domain splits the protein into two parts; one hydrophobic (the exposed part of hECN), the other hydrophilic (N-terminal domain). Such behavior may help the 16 kDa RCP to interact with lipid membranes as the exposed hECN can penetrate into the hydrophobic environment of the lipid membrane, possibly to provide additional photoprotection.

Supplementary materials related to this article can be found online at doi:10.1016/j.bbabio.2010.08.013.

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