# The Photophysics of the Orange Carotenoid Protein, a Light-Powered Molecular Switch

Rudi Berera,\*<sup>,†</sup> Ivo H. M. van Stokkum,<sup>†</sup> Michal Gwizdala,<sup>‡,§</sup> Adjélé Wilson,<sup>‡,§</sup> Diana Kirilovsky,<sup>‡,§</sup> and Rienk van Grondelle<sup>\*,†</sup>

<sup>†</sup>Division of Physics and Astronomy, Department of Biophysics, VU University Amsterdam, The Netherlands <sup>‡</sup>Commissariat à l'Energie Atomique, Institute de Biologie et Technologie de Saclay, 91191 Gif sur Yvette, France <sup>§</sup>Centre National de la Recherche Scientifique, 91191 Gif sur Yvette, France

**Supporting Information** 

**ABSTRACT:** To cope with the deleterious effects of excess illumination, photosynthetic organisms have developed photoprotective mechanisms that dissipate the absorbed excess energy as heat from the antenna system. In cyanobacteria, a crucial step in the process is the activation, by blue-green light, of a soluble protein, known as orange carotenoid protein (OCP), which binds the carotenoid 3'-hydroxyechinenone as its only pigment. While the spectroscopic properties of the inactive form of OCP have been described, the nature of the excited states in the active form still awaits elucidation. We applied transient absorption spectroscopy to the dark and the light activated forms of OCP to study and compare the excited state dynamics of both forms. We show that excitation of the photoactivated OCP leads to the population of new carotenoid excited states. One of these states populated shortly after excitation is characterized by a very pronounced charge transfer character and a



lifetime of about 0.6 ps. When the illuminated sample is exposed to a dark relaxation period, it responds to excitation as the original dark sample, showing that photoactivation and decay of the photoactivated state are fully reversible. Thus OCP functions as a light-powered molecular switch that modulates its spectroscopic properties as a response to specific changes in light environment. We discuss the importance of this switch in cyanobacteria photoprotection and propose a mechanism wherein the red state of OCP echinenone acts as an energy dissipator via its charge transfer state.

## INTRODUCTION

While photosynthesis relies on the efficient collection of light, too much of it can be lethal. In order to cope with the deleterious effects of excess light, photosynthetic organisms have developed a sophisticated machinery that can be finetuned to either provide efficient light harvesting (photosynthesis) or efficient energy dissipation (photoprotection). The process, generally known as nonphotochemical quenching (NPQ), has been extensively investigated in plants.<sup>1-3</sup> In this process, a crucial role is played by carotenoids, a class of pigments ubiquitous in photosynthetic systems and more generally in living organisms.<sup>4,5</sup> In photosynthetic organisms, carotenoids are active in energy transfer, structure stabilization, and photoprotection,<sup>5,6</sup> both by quenching cholorophyll triplets and by quenching chlorophyll singlet states. It has recently been shown that carotenoids can also act as light sensors.<sup>7</sup> For artificial photosynthetic complexes it has been demonstrated that carotenoids can dissipate the excited state energy from a neighboring tetrapyrrole, via electron and/or energy transfer.<sup>8–10</sup> Evidence of those mechanisms being active in nature has been reported in the literature.<sup>11–15</sup> Cyanobacteria, related to the ancestor of eukaryotic algae and higher plants, possess at least two mechanisms of energy dissipation.<sup>16</sup> One of these mechanisms consists of energy dissipation in the IsiA complex, a stress-inducible protein that forms strongly quenched aggregates in the cell. This mechanism is triggered by a number of stress factors, among them high light, iron deficiency, and salt stress,<sup>17,18</sup> and is irreversible, i.e., cells containing IsiA are in a permanently quenched state. Another dissipative mechanism is induced by high intensity blue-green light illumination, which triggers energy dissipation within or from the phycobilisome, the main light harvesting antenna of the organism.<sup>19-24</sup> It was shown that the action spectrum for the activation of this process matches with the spectral shape of a carotenoid molecule<sup>21</sup> subsequently identified as 3'hydroxyechinenone,<sup>22</sup> which is noncovalently bound to a soluble protein, the orange carotenoid protein (OCP).<sup>25</sup> This molecule is a crucial player in the energy dissipation process since mutants lacking OCP cannot provide fluorescence quenching.<sup>22</sup> OCP binds one pigment only, the carotenoid 3'-

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hydroxyechinenone.<sup>25,26</sup> The OCP is a photoactive protein: absorption of blue-green light induces conformational changes in the carotenoid and the protein, converting the stable orange dark OCP form (OCP°) into a metastable red active form (OCP<sup>r</sup>).<sup>7</sup> Only OCP<sup>r</sup> is able to bind to the phycobilisomes and to induce fluorescence quenching.<sup>27,28</sup> The presence of a ketocarotenoid is essential for photoactivity: OCP binding hydroechinenone or echinenone shows the same kinetics of photoactivity and fluorescence quenching induction.<sup>29</sup> OCP is also able to bind zeaxanthin, which lacks the carbonyl group of ketocarotenoids, but zea-OCP is photoinactive, remaining yellow under strong illumination.<sup>29</sup> In cyanobacteria cells, under high irradiance, OCPr induces a decrease of phycobilisome fluorescence by increasing thermal energy dissipation in/ from the phycobilisome and, consequently, a decrease in the amount of excitations arriving at the reaction centers.<sup>22</sup> In the dark, the isolated OPC<sup>r</sup> spontaneously reverses to OCP<sup>o.30</sup> This reaction is strongly dependent on the temperature: the higher the temperature, the faster the recovery; in fact, the  $t_{1/2}$ ranges from a few seconds at 32 °C to more than 1 h at 15 °C.<sup>30</sup> By contrast, the light-induced OCP°-to-OCP<sup>r</sup> conversion shows a small temperature dependence in this range of temperatures. Thus, in vitro, the steady state concentration of OCP<sup>r</sup> during continuous illumination decreases with increasing temperature.<sup>30</sup> In vivo, OCP<sup>r</sup> is stabilized by its binding to the phycobilisomes.<sup>27</sup> The phycobilisome fluorescence remains quenched as long as the cells are under strong illumination; when they are transferred to the dark or to low light intensities, total fluorescence recovery occurs in only 10-15 min at 32 °C. By binding one pigment only, OCP represents the ideal and minimal system if one wants to study how the protein environment can modulate the excited state properties of a bound pigment. Echinenone (and hydroxyechinenone) belong to the class of keto-carotenoids, which is characterized by the presence of a carbonyl group in their structure. Carbonyl carotenoids are among the most abundant carotenoids on Earth; they are the main carotenoid species in dinoflagellates<sup>31</sup> where peridinins play a pivotal role in the light harvesting properties of the peridinin-chlorophyll protein (PCP).<sup>32,33</sup> The presence of a carbonyl group in the carotenoid structure leads to very specific changes in the photophysical properties of the molecule. The electron-withdrawing nature of the carbonyl group makes keto-carotenoids very sensitive to the polarity of the environment, a property exploited by nature, for instance, in PCP to fine-tune the spectroscopic properties of peridinin to allow very efficient light-harvesting.34 In particular, a polar environment leads to the stabilization of an intramolecular charge transfer (ICT) state.<sup>35,36</sup> In general, carbonyl carotenoids have a smaller  $S_1-S_2$  energy gap compared to their noncarbonyl counterparts, a property exploited by marine organisms, where the S<sub>2</sub> state can efficiently absorb green light and at the same time the  $S_1/ICT$  state is still high enough in energy to absorb orange/red light in combination with very efficient car to chl energy transfer.<sup>37</sup>

It was also shown that the  $S_1/ICT$  state of a carbonyl carotenoid can not only be tuned to optimize the light harvesting function of the molecule, but also to efficiently dissipate the excited state energy of a neighboring chlorophyll mimic.<sup>9</sup>

In this paper we present results from transient absorption spectroscopy upon excitation of the  $S_0 \rightarrow S_2$  transition of echinenone bound to OCP from the cyanobacterium *Synechocystis* PCC 6803. We studied OCP both in its dark

and in its photoactivated form. The aim of the experiments is to determine the excited state properties of the photoactivated OCP with the perspective to unravel the mechanism by which the phycobilisome fluorescence gets quenched under high light conditions.

## MATERIALS AND METHODS

Synechocystis Culture Conditions and OCP Purification. The OCP utilized in this work was isolated from the overexpressing His-tagged OCP- $\Delta$ CrtR mutant<sup>38</sup> of the mesophilic freshwater cyanobacterium Synechocystis PCC 6803. This OCP contains only echinenone. The cells were grown photoautotrophically in a modified BG11 medium,<sup>39</sup> containing double amount of sodium nitrate, in 3 L Erlenmeyers in a rotary shaker (120 rpm) under a CO<sub>2</sub>enriched atmosphere, at 30 °C with a light intensity of 70–90  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>. The cells were harvested at OD<sub>800</sub> = 1.

Purification of the OCP was performed as previously described:<sup>30</sup> cells in 0.1 M Tris–HCl pH 8.0 (1 mg chl/ml) were broken in mild light using a French Press. The membranes were pelleted, and the supernatant was loaded onto a column of Ni-ProBond resin. The OCP was further purified by loading it onto a Whatman DE-52 cellulose column.

**Time-Resolved Measurements.** The measurements were carried out on a Coherent Legend-USP setup described in detail in ref 40; the repetition rate was 1kHz, the energy per pulse was in the range 15–100 nJ with a spot size diameter of ~160  $\mu$ m, and the polarization was set at magic angle. The instrument response function was described by a Gaussian of ~120 fs (full width at half-maximum). The recorded time-resolved spectra were globally analyzed.<sup>41</sup> In global analysis, the evolution of time-resolved spectra is distilled into a relatively small number of components, which sequentially interconvert into one another by exponential decay. Each component is characterized by an evolution associated difference spectrum (EADS) and a corresponding lifetime.<sup>41</sup>

#### RESULTS

**OCP Photoactivation.** Figure 1 shows the steady state absorption spectrum of OCP in its inactive orange and



**Figure 1.** Steady state absorption spectrum of OCP in its inactive (black) and active (red) forms. The vertical lines correspond to the excitation wavelengths used for the time-resolved experiments: 480, 495, 540, and 550 nm respectively.

photoactivated red forms. The vertical lines correspond to the excitation wavelengths used for the time-resolved experiments: 480, 495, 540, and 550 nm. For all 4 excitation wavelengths, we obtained very similar results. However, redder excitation, which is more selective for the red versus the orange form, allowed us to more clearly determine the excited state manifold and spectral evolution of the photoactivated form. Each timeresolved experiment consists of a series of three measurements: (1) a measurement of OCP in the dark; (2) a measurement of OCP after blue-green light illumination; (3) a measurement of OCP in the dark after relaxation of the photoactivated state. The aim of the first and third measurement is to show that any change to OCP induced by the external light source is completely reversible, meaning that the new spectroscopic phenomena observed in the photoactivated state are intrinsic properties of the photoactivated OCP. Besides measurements 1 and 3, steady state absorption spectra recorded before and after the experiment (Figure SI.1 of the electronic Supporting Information (ESI)) confirmed sample stability.

In order to photoactivate OCP, the sample was illuminated for 15 min with a 466 nm light-emitting diode (LED) with an average photon flux of ~350  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>. The orange-to-red photoconversion was confirmed by monitoring the transient absorption spectrum at 200 fs delay; at the onset of illumination, the transient spectrum starts changing in shape due to the increase in concentration of the red, photoactivated OCP, until it reaches equilibrium and its spectral shape remains constant. In order to slow down the red-to-orange reconversion, the cuvette was put in touch with an ice bath, which lowers the sample temperature to about 13.5  $^{\circ}C_{r}^{7}$  both during photoactivation and during the measurement. The timeresolved measurement of the photoactivated OCP was performed with the light source (LED) on. After the measurement, the sample was placed in the dark for  $\sim$ 30 min after which measurement 3 was performed.

The light intensity used in the experiments shown in Figures 2 and 3 is relatively low for typical OCP in vitro experiments.<sup>7</sup> While this on one hand does not allow for a complete orange-to-red conversion (we estimate about 50% conversion, see below), it does allow long measuring times (typically several hours of continuous illumination without appreciable sample degradation) and for a full recovery of the photoactive state when the sample is exposed to a dark period, as demonstrated in Figure 3.

Figure 2 shows selected kinetic traces for a measurement upon 550 nm excitation (a more comprehensive set of kinetic traces is shown in the ESI (Figure SI.2). When compared to the dark measurements (1 and 3, black and blue curves, respectively), the light measurement (2, shown by the red curves), clearly exhibits several specific changes. While the overall difference in amplitude is mainly due to the fact that the orange and red forms have a different extinction coefficient at the excitation wavelength (Figure 1), the underlying kinetics are remarkably different. As mentioned above, all measurements contain both orange and red OCP contributions but in different proportion depending on the light conditions (dark vs LED illumination). The 565 nm trace is taken as representative of the ground state bleach (GSB) region of the activated form (Figure 1): the red trace shows an increased bleach signal meaning that LED illumination leads to a carotenoid bleach signal that is red-shifted compared to the one in the dark form (Figure 2A). The 598 nm trace, representative of the carotenoid  $S_1 \rightarrow S_n$  transition, clearly shows a different evolution



**Figure 2.** Selected kinetic traces. The black, red, and blue lines correspond to the three measurements upon 550 nm excitation: in the dark (1), after illumination (2), and in the dark after recovery (3), respectively. The data are shown as solid lines, while the dashed lines show the corresponding fit. The units in the *y* axis are in mOD. The red and blue traces are divided by 0.73 and 0.86, respectively (scaling factors). Further explanation in the text.

for the light (red trace) and dark (black and blue traces) measurements (Figure 2B). In fact, the red trace features a rise taking place in about 0.6 ps, indicative of a decay of GSB and possibly an increase of excited state absorption (ESA) on this time scale; this feature is absent in the black and blue traces; the 0.6 ps rise is nearly absent in the 635 nm red trace trace (Figure 2C). Figure 2D shows the trace recorded at 748 nm, corresponding to the maximum absorption of the carotenoid ICT state (vide infra). Interestingly, the increase in amplitude of the red curve when compared to the dark traces is more pronounced at this wavelength when compared to the traces in Figure 2A–C, suggesting the presence of a pronounced ICT<sup>36,42</sup> state in the red form. The 748 nm trace also features a decay in ~0.6 ps. If one compares the black and blue traces for all four wavelengths, they are virtually identical,



**Figure 3.** (A) The kinetic model used for the simultaneous target analysis. With OCP in the dark, the orange branch receives a 0.5 input, and the red branch receives a 0.07 input. With OCP after illumination, both branches receive a 0.5 input. (B) The estimated spectra for the orange, inactive form of OCP, OCP<sup>o</sup>. (C) The estimated spectra of the red, active form, OCP<sup>r</sup>. Note that the spectra are less accurate around the excitation wavelength (550 nm).

demonstrating that the new spectroscopic features of the red form are completely reversible.

In order to gain more insight into the spectroscopic features of the photoactivated OCP, a simultaneous target analysis was applied to the three measurements. Figure 3A shows the kinetic model, which consists of two independent parallel and sequential decay channels corresponding to the excited state relaxation of the orange and red forms respectively. For the sake of simplicity, we call the states  $S_{20}(r)$ , ICTo(r), and  $S_1o(r)$ , even though the ICT and  $S_1$  states contain both  $S_1$  and ICT character (the name thus reflects the dominant contribution to the spectrum). The excitation beam populates the carotenoid S<sub>2</sub> state, and in each measurement there is a fraction of both orange and red forms; the LED illumination shifts the equilibrium toward the active form, i.e., increases the population of the red form relative to the population of the orange form. In the dark measurements, the model allows for a small percentage (~12%) of photoconverted (red) OCP; this photoconversion is induced by the excitation beam and represents a "baseline" value. Since the applied kinetic model still contains features of global analysis (sequential decay of possibly more complicated kinetics), the resulting spectra will be referred to as EADS rather than SADS.

The EADS of the dark and light (activated) forms estimated from the simultaneous target analysis of the 550 nm excitation experiment are shown in Figure 3B,C, respectively. The magenta spectrum in Figure 3B corresponds to the carotenoid  $S_2$  state with GSB and stimulated emission (SE) below ~650 nm and ESA above this wavelength. The  $S_2$  state decays in ~60 fs to the green EADS; this state features GSB below 540 nm and a broad region of ESA in the 540 to 800 nm region; it decays in 0.9 ps to the blue EADS characterized by a blueshifted bleach and a broad ESA region. The blue EADS also features a loss of car bleach, and this was ascribed to the presence of two or more OCP subpopulations, each characterized by a different excited state decay.43 This blue EADS decays to the ground state in  $\sim$ 3.8 ps. The shape of the ESA region in the green and blue spectra is rather broad compared to the conventional  $S_1 \rightarrow S_n$  transition of noncarbonyl carotenoids, and this feature is ascribed to an S1 state coupled to an ICT state originating from the interaction of the keto group with the protein environment. The spectra match very well with those previously published for orange OCP.<sup>7,43</sup> The carotenoid spectra created upon OCP exposure to LED illumination are shown in Figure 3C. The  $S_2$  state (cyan line) shows a more pronounced and red-shifted GSB/SE in the 560 to 620 nm region when compared to the  $S_2$  state of the orange form, consistent with the fact that in the activated OCP, echinenone has an extended conjugation length. The cyan spectrum relaxes in  $\sim 60$  fs to the black EADS. The shape of this spectrum is very different when compared to the green EADS in Figure 3B. The ICT contribution to this spectrum, reflected by the broad band in the 670 to 800 nm region, is greatly enhanced with respect to the  $S_1 \rightarrow S_n$  band (600 to 670 nm region), showing that this spectrum is characterized by a very strong ICT character. It must be noted that the ICT band in the 700-800 nm region is already partly present in the first EADS, suggesting that the ICT state is populated extremely fast after excitation, possibly on a time scale shorter than 60 fs (the lifetime of the cyan spectrum).

The black EADS decays in ~0.6 ps to the red spectrum. The ICT contribution to the ESA region is greatly decreased when compared to the  $S_1 \rightarrow S_n$  transition. The evolution also shows a decrease in the GSB; as for the inactive form, this may be due to the presence of two or more OCP subpopulations (see Discussion section). The remaining  $S_1$  state decays to the ground state in 3.2 ps. Its lifetime is shorter when compared to

the lifetime of the blue spectrum in panel (b) and those published in refs 7 and 43 for the  $S_1$  state, consistent with the fact that the carotenoid in the red form has an increased effective conjugation length.<sup>7</sup> Figure 4 shows the relative



Figure 4. The relative concentrations of the various species from Figure 3A showing the effect of illumination: OCP in the dark (A) and OCP after illumination (B). Note that in B, the  $S_2$  concentration of the OCP<sup>o</sup> and OCP<sup>r</sup> forms overlap.

concentrations of the orange and red form obtained from the target analysis of the 550 nm excitation experiment and demonstrates the effect of the LED illumination; the cyan, black, and red curves, which correspond to the red form, show a 4-fold increase upon illumination, corresponding to an increase in the concentration of the photoactivated OCP from 12% to 50% upon LED illumination.

## DISCUSSION

Activation of OCP is a necessary step that triggers photoprotection in cyanobacteria.<sup>22</sup> Absorption of light by echinenone induces conformational changes of the carotenoid and the protein;<sup>7</sup> these conformational changes are indispensable for the binding of the OCP to phycobilisomes, and only the red activated form binds to the phycobilisome.<sup>27</sup> In this study, we have demonstrated that OCP is a light-powered molecular switch capable of modulating the excited state properties of its bound pigment upon changes in light environment. With our work we elucidated the excited state properties of the activated form of OCP, the form that is, either directly or indirectly, involved in the quenching of excitations from the phycobilisome. The most remarkable feature of the spectra corresponding to the excited activated OCP is the presence of a very pronounced carotenoid ICT state. Interestingly, in a proteolytic fragment of OCP lacking the C terminal domain, known as red carotenoid protein (RCP) and binding the same carotenoid, no evidence of an ICT state was found.<sup>44</sup> This suggests that hydrogen bonds between the echinenone carbonyl group and the protein play a crucial role in modulating and stabilizing the charge transfer character of the carotenoid excited state(s). Carotenoid ICT states possess remarkable biological significance; they were shown to act as a mediator in the very efficient energy transfer process between the carotenoid peridinin and chlorophyll a.<sup>33,34</sup> We have shown that in a minimal system for energy dissipation, a biomimetic carotenoid-phthalocyanine dyad, a carotenoid ICT state acts as a mediator in the energy dissipation process.<sup>9</sup>

The evolution of the ICT state (second EADS in Figure 3B,C) is associated with a decrease of GSB. This phenomenon has been explained for the inactive form of OCP in terms of heterogeneity in the OCP population, where at least two populations, characterized by different hydrogen bonding of their keto group, are present in the sample.<sup>43</sup> If we look at the spectra of the photoactivated form of OCP, the second EADS in Figure 3C shows predominantly ICT features, while the third EADS is reminiscent of an almost pure  $S_1$  state. If we suppose that these two states correspond to two subpopulations of OCP, possibly the same two subpopulations suggested to be present in the inactive form, this would imply that OCP activation would give rise to a stabilization of hydrogen bonding in one population and almost complete destabilization in the other (black and red EADS in Figure 3C, respectively), which is, in our opinion, unlikely. The kinetic traces in the 580 to 595 nm in the light measurement (Figure SI.1 of the ESI) show a rise on a  $\sim 0.6$  ps time scale suggesting a decay of GSB and possibly an internal conversion and/or relaxation process leading to the population of the S<sub>1</sub> state. We suggest that the spectra in Figure 3C and possibly those in Figure 3B may be associated with the same population of OCP, and that the loss of carotenoid bleach can be explained in terms of a complete or partial decay of the second EADS (ICT state) to the ground state. The same state may also partly decay to the S<sub>1</sub> state, in which case the ICT state would act as an intermediate in the S<sub>2</sub> to S<sub>1</sub> relaxation pathway. Further work on OCP mutants with altered stability of the active form and a higher orange to red photoconversion is needed, however, to better understand this internal conversion process. To further support the absence of subpopulations of orange and red OCP with different hydrogen bonding strength within the orange and red OCP populations, crystal structures of OCP independently refined showed the same degree of hydrogen bonding between OCP and the carotenoid;<sup>25</sup> resonance Raman spectroscopy, a technique very sensitive to slight changes in the carotenoid conformation/ configuration, showed a high degree of homogeneity in both the inactive and active form.

The ICT and  $S_1$  states in the activated OCP clearly follow different kinetics; this behavior has been proposed<sup>42,45</sup> and predicted by theory,<sup>46</sup> but most experiments to date have challenged this interpretation.<sup>35</sup>

We have shown, by making use of an artificial lightharvesting dyad, that a carotenoid can accept singlet excitation energy from a neighboring tetrapyrrole, thus quenching its

fluorescence. The carotenoid ICT state acts a mediator in the energy transfer process, and the more pronounced (stronger) the ICT state, the more efficient the quenching. From this perspective, the very pronounced ICT state in the activated form of OCP would be a potential quencher in the OCPmediated energy dissipation mechanism; a very pronounced ICT character with a very short lifetime would make the carotenoid an extremely effective energy dissipator. Thus, we propose that echinenone in the activated OCP accepts energy from the excited bilin via its ICT state. The ICT state (partly) decays to the ground state and may partly relax to the S<sub>1</sub> state, which in turn would decay to the ground state. Thus the energy would harmlessly dissipate as heat. The lifetime of the ICT and  $S_1$  states, 0.6 and 3.2 ps, is ~3 orders of magnitude shorter than that of the excited bilin, making the carotenoid a very efficient quencher (Figure 5).



**Figure 5.** Proposed quenching model. The carotenoid in the activated OCP receives energy from an excited bilin of the phycobilisome core in a process mediated by its ICT state.

On the other hand, activation of OCP leading to an increased effective conjugation length of echinenone<sup>7</sup> lowers the first oxidation potential of the molecule.<sup>47,48</sup> Thus echinenone would be a better electron donor when OCP is in its activated form. This may trigger a charge transfer reaction as suggested to take place in plants,<sup>11,12</sup> which in turn would lead to energy dissipation. This could be an alternative energy quenching mechanism.<sup>24</sup>

Our results show that, upon light illumination, OCP changes the excited states properties of its bound carotenoid. Interestingly, the almost pure  $S_1$  state in the active form (Figure 3C) has a lifetime of 3.2 ps, comparable to the lifetime of an 11 double-bond carotenoid<sup>5</sup> and shorter than the lifetime of echinenone in solution.43 This indicates a specific configuration of echinenone associated with this spectrum that is remarkably different from the one in solution. Most likely, the C-C double bond in the carbonyl-containing end ring of echinenone must be in an s-trans orientation, as is the case for the inactive OCP form.<sup>43</sup> Thus the spectroscopic changes in the OCP excited state manifold most likely involve only a small change in the configuration of the bound carotenoid. A conformational change has been proposed to take place in the major light harvesting complex of plants, the light harvesting complex II,<sup>49</sup> and this would allow the complex to switch from a light-harvesting to an energy-dissipating state  $^{13,50,51}$  by specifically changing the lutein 1 binding domain.52 In this respect, OCP is the ideal minimal system to study the interaction between a carotenoid and its protein environment. Our results demonstrate that the protein environment can modulate the excited state properties of a

bound carotenoid as a response to an external (light) stimulus. This is, to our knowledge, the first report of such phenomenon, and may have broad implications to the understanding of how photosynthetic organisms are regulated at the molecular level. While in OCP the trigger is provided by blue-green light illumination, and in higher plants, in addition to high light, the synergy of other factors such as the xanthophylls cycle, a trans-thylakoid delta pH, and the PsbS protein is required,<sup>1,3</sup> the modulation of the carotenoid environment and of its excited state properties may be a general requirement for singlet–singlet energy dissipation in oxygenic photosynthesis.

## ASSOCIATED CONTENT

#### Supporting Information

Steady state absorption of OCP before and after the timeresolved experiment, and a more comprehensive plot of kinetic traces. This material is available free of charge via the Internet at http://pubs.acs.org.

#### AUTHOR INFORMATION

#### **Corresponding Author**

\*E-mail: r.berera@vu.nl (R.B.); r.van.grondelle@vu.nl (R.v.G.).

#### **Author Contributions**

The manuscript was written through contributions from all authors. All authors have given approval to the final version of the manuscript.

#### Notes

The authors declare no competing financial interest.

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#### ABBREVIATIONS

OCP, orange carotenoid protein; NPQ, nonphotochemical quenching; GSB, ground state bleach; ESA, excited state absorption; ICT, intramolecular charge transfer; EADS, evolution associated difference spectra; SADS, species associated difference spectra; RCP, red carotenoid protein; PCP, peridinin-chlorophyll protein

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