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Dramatic Domain Rearrangements of the Cyanobacterial Orange Carotenoid Protein upon Photoactivation

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Abstract

Photosynthetic cyanobacteria are an important contributor to global carbon and nitrogen budgets. A protein, known as the Orange Carotenoid Protein (OCP) protects the photosynthetic apparatus from damage by dissipating excess energy absorbed by the phycobilisome, the major light-harvesting complex in many cyanobacteria. OCP binds one carotenoid pigment, but the color of this pigment depends on conditions. It is orange in the dark and red when exposed to light. We modified the orange and red forms of OCP by using isotopically coded cross-linking agents and then analyzed the structural features by using LC-MS/MS. Unequivocal cross-linking pairs uniquely detected in red OCP indicate that, upon photoactivation, the OCP N-terminal domain (NTD) and C-terminal domain (CTD) reorient relative to each other. Our data also indicate that the intrinsically unstructured loop connecting NTD and CTD is not only involved in the interaction between the two domains in orange OCP but also, together with the N-terminal extension, provides a structural buffer system facilitating an intramolecular breathing motion of the OCP, thus helping conversion back and forth from orange to red form during the OCP photocycle. These results have important implications for understanding the molecular mechanism of action of cyanobacterial photoprotection.

Graphical Abstract

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Keywords

Cyanobacteria; Orange Carotenoid Protein; Mass Spectrometry; Protein Cross-linking; Isotopic Cross-linker; Photoprotection; Photosynthesis

Introduction

Photosynthesis converts solar energy to chemical energy in plants, algae, cyanobacteria, and other phototrophic bacteria ¹. In this process, light energy initially absorbed by light-harvesting antenna complexes (LHC) is delivered to the reaction centers (RCs) where photochemistry takes place ^{2, 3}. Energy currency (ATP) and reducing power (NADPH) generated from light-driven electron transport are then used for carbon fixation in a light-independent manner. Solar energy conversion rates in photosynthetic systems, however, are not constant. Instead, cellular energy requirements vary depending on cellular developmental stage, growth condition, carbon-fixation capacity, and others. If solar energy absorbed by LHC's exceeds the photochemical conversion capacity and cellular energy requirements, the excess energy has to be safely dissipated as heat in a process called Non-Photochemical Quenching (NPQ) ⁴. NPQ is one of multiple strategies that cells have evolved to regulate the energy arriving at RCs and to reduce the production of reactive oxygen species that are damaging or lethal if not properly managed. In plants and algae, the thermal dissipation of excitation energy occurs at the membrane-bound chlorophyll antenna of Photosystem II ^{5, 6}; an equivalent photoprotective mechanism in cyanobacteria takes place, however, in the cytosol where the major LHC, known as the phycobilisome (PBS), is located ^{7, 8}.

The orange carotenoid protein (OCP) is a pigment-protein complex with molecular weight (MW) of 35 kDa^{9–11} and is involved in NPQ in many cyanobacteria¹². The OCP has two structural domains, with an N-terminal domain (NTD) consisting of mainly α -helix secondary structure and a C-terminal domain (CTD) consisting of two discontinuous four-helix bundles and a mixed α/β structure. A 4-keto carotenoid (3'-hydroxyechinone) spans both domains and is almost entirely buried by the protein matrix. In addition to this connection, the NTD and CTD interact through two other distinct regions. The first 19 amino acids of the NTD, which are comprised of a short α helix (α A) and an unstructured loop, extend away from the N-terminal α -helix bundle and interact with the solvent-exposed surface of the C-terminal domain β -sheet. The second interaction is at the interface of NTD and CTD, which buries 1,722 Å² of surface area¹³. Upon light illumination, OCP (orange OCP, OCP^o) undergoes conversion to a red form (OCP^r) and consequently becomes able to bind to the PBS, enabling the excitation energy dissipation of the latter via energy transfer to the carotenoid molecule of OCP, and thereby preventing oxidative damage under high light conditions.

The NTD and CTD likely have discrete functions during the OCP photocycle. Mass spectrometry-based carboxyl group footprinting indicates that the N-terminal extension (NTE), containing α A (the first α -helix from N-terminus of OCP), plays a key role in the structural rearrangements of OCP during photoactivation^{14, 15}. Although the NTE is located on and associated with the outside of the β -sheet core in the CTD of OCP under dark conditions, light-induced changes in the pigment molecule disrupt this interaction, allowing global tertiary structural changes and favoring complete domain dissociation of NTD and CTD¹³. It was suggested that the NTD's newly exposed surface, which is normally buried in the interface of NTD and CTD in OCP^o, is involved in the association with PBS¹⁶. The restoration of light harvesting by PBS to the RCs represents the reverse process. Fluorescence recovery is facilitated by the Fluorescence Recovery Protein (FRP)¹⁷, which is thought to interact with the OCP CTD through a domain that is not exposed until OCP is fully activated^{14, 18}. Even though structural and functional models of the OCP have been proposed^{15, 16, 19–24}, many questions regarding the details of the mechanism and the associated protein dynamics in the OCP photocycle remain unanswered.

In the present study, we used isotopically coded chemical cross-linking reactions and FPLC separation and mass spectrometry to investigate the reorientation of NTD and CTD upon OCP photoactivation. We report here that chemical cross-linking can lock in and thereby track structural features of OCP^r. The results indicate that NTD and CTD are juxtaposed in a way that the NTE, Loop J-K (the linker domain between CTD and NTD), and the surface of the β -sheet core of CTD are in structural proximity, presenting a fully exposed interface of NTD for PBS binding and excitation energy quenching. The distance constraints are used for generating a molecular model of OCP^r.

2. Materials and Methods

2.1 Growth of *Synechocystis* sp. PCC 6803 and OCP isolation

The cell growth and the OCP isolation were described elsewhere^{21, 25}.

2.2 Chemical Cross-linking

OCP was resuspended at 0.1 mg/mL in 20 mM MOPS buffer (pH 7.0). NHS-ester based chemical cross-linking (BS³, DSS, Thermo Scientific, Rockford, IL) was performed according to the manufacturer's protocol followed by desalting by using ZebaTM spin columns (Thermo Scientific). Isotopically coded cross-linkers (BS³-H₁₂/D₁₂ and DSS-H₁₂/D₁₂) were purchased from Creative Molecules, Inc (<http://www.creativemolecules.com/>).

2.3 Protein Analysis and FPLC isolation of monomeric, cross-linked OCP

Protein electrophoresis was performed as described previously²⁶ unless otherwise indicated. A SuperdexTM 75 10/300GL column was used for separating the cross-linked products of OCP⁰ and OCP^r.

2.4 Sample Digestion, LC-MS/MS and Data Processing

Cross-linked OCP samples were precipitated by acetone precipitation and then digested by LysC and trypsin by following the previously published method²⁷. In brief, protein pellets were dissolved in 8 M urea solution (20 μ L) followed by incubation with *tris*(2-carboxyethyl)phosphine (2.5 mM) at 37 °C for 30 min and Iodoacetamide (5 mM) for 30 min at room temperature. LysC stock solution (0.5 μ g/ μ L, 2 μ L for each sample) was added to initiate the digestion at 37 °C. After two hours of LysC digestion, the protein solution (8 M urea) was diluted (1 M urea) by adding Tris buffer (100 mM). The protein solution was further incubated with trypsin overnight at 37 °C. The digestion was quenched by adding 0.1% formic acid.

An aliquot (5 μ L, 1 pmole protein digest) was loaded onto a trap column (180 μ m $\times$ 2 cm, C18 Symmetry, 5 μ m, 100 Å, Waters, Milford, MA) using solvent A (Water with 0.1% formic acid). Peptides were eluted from a reverse phase C18 column (BEH 130, 75 μ m $\times$ 150 mm, Waters, Milford, MA) by increasing the fraction of solvent B (80 % Acetonitrile, 20 % Water, 0.1% formic acid). The gradient was supplied by an Dionex UltiMate 3000 (Thermo Scientific, Inc., Sunnyvale, CA) and was run from 2% solvent B to 40% solvent B over 90 min, then to 95% solvent B for 10 min at 250 nL/min followed by a 5 min re-equilibration step with 98% solvent A. The flow was directed by a Nanospray Flex source into a Q Exactive Plus mass spectrometer (Thermo-Scientific, San Jose, CA) with a spray voltage of 1.8 kV and a capillary temperature 300 °C. The Q Exactive Plus was operated in standard data-dependent acquisition mode controlled by Xcalibur 3.0.63 software. Peptide mass spectra (m/z range 380–1500) were acquired at high mass resolving power (70,000 for ions of m/z 200) with the Fourier transform (FT) mass spectrometer. The Q Exactive plus was externally calibrated using a standard mixture of caffeine, MRFA, and Ultramark 1621. The mass calibration was checked and repeated before the LC-MS experiments to optimize mass measurement accuracy. In the data-dependent mode, the ten most abundant multiply charged ions with a minimum intensity of 5e4 counts were subjected to high-energy collision-induced dissociation (HCD). Precursor activation in HCD was performed with an isolation width of 1.5 m/z , and normalized collision energy of 27%.

The raw data were directly loaded into PEAKS (v 7.0, Bioinformatics Solution Inc. Waterloo, ON, Canada) for protein identification. The raw data were converted to .mgf file by ProteoWizard^{28, 29}. The compact data files (raw data and .mgf files) were submitted to ProteinProspector^{30, 31}. The same data were searched twice with H₁₂ and D₁₂ cross-linker separately. The score cut-off was 40 in the identification of cross-linked peptides.

2.5 Protein Modeling

After collection of cross-linking data, a feasible model for photoactivated OCP^r was sought by loading the OCP^o crystal structure into PyMol³² (Schrödinger, Ltd.). An *x-y* plane that bisects, and is perpendicular to, the long-axis of the carotenoid cofactor was defined. This *x-y* plane, therefore, occupies the boundary between NTD and CTD. Eight models were then generated in which the CTD is allowed to flip over, as if on a hinge, at 45° increments along the plane around the diameter of the protein, so that the interface of the NTD, which usually interacts with the CTD in OCP^o, was totally exposed. Cross-linking pairs observed in our experiments were then used as structural constraints to eliminate unfavorable orientations of the CTD relative to the NTD. Minimizing the spatial conflicts between each cross-linking pair from our experiments led to a single acceptable model.

After locking in the position of the NTD and the new position of the CTD, the linker domain (Loop J–K, residues 161–196) was left as an unstructured, disoriented loop region. The Modeller v.9.15 environment (<https://salilab.org/modeller/>)^{33, 34} was utilized to generate possible structural models for this region. Modeller is generally used for comparative protein modeling with spatial restrains for applications such as homology modeling, but it contains functions for the refinement of existing protein structures. A Loop Refinement Python script was generated using Modeller's "loopmodel" class, which generates low-energy models for a loop region of an existing protein structure without comparative modeling. An example script for this process can be found in the online manual (<https://salilab.org/modeller/manual/>). In this script, the loopmodel.select_loop_atoms routine was redefined so that refinement of the loop in question did not require a comparative model. The script was written such that the NTD and CTD domains would remain spatially static, with loop refinement performed on just residues 161–196. One hundred models were generated, and the five models with the lowest Discrete Optimized Protein Energy (DOPE) scores were assessed³³. Of the five, the single model that best satisfied the spatial restraints imposed by chemical cross-linking results was retained. This final model, incorporating the new CTD position and refined Loop J–K was chosen as our preferred model for OCP^r.

Results and Discussion

Chemical cross-linking in combination with mass spectrometry has become a widely used tool in structural proteomics to study protein-protein interactions and determine low-resolution structures and topology^{35, 36}. This method can additionally provide important supplementary information concerning the structural organization and identification of conformational changes occurring during enzymatic reactions. Modification of OCP^o and OCP^r by a chemical cross-linker (BS³, DSS [non-isotopically coded]) resulted in different cross-linked species (Fig. 1A). In the absence of a cross-linker, OCP migrated as a single

band at 35 kDa in SDS-PAGE. Treatment with NHS-ester type cross-linker (i.e., BS³ or DSS of OCP⁰), resulted in the formation of a major product with MW of approximately 78 kDa as well as a major band with MW of 35 kDa (Fig. 1A, lane 2, 3). According to these values and using unmodified OCP as a reference (Fig. 1A, lane 1), we suggest that they correspond to cross-linked, dimerized OCP and cross-linked OCP monomer, respectively. In contrast, treatment of OCP^r resulted in a more complex protein gel pattern (Fig. 1A, lane 4): two bands appeared corresponding to proteins of MW approximately 76 kDa (band a) and 70 kDa (band b), respectively. A dense band for a 35 kDa monomer and a diffused band corresponding to a 28 kDa band (band c) were also significant. We suggest that the 76 and 70 kDa bands represent the cross-linked OCP dimer with gel migration behavior altered by the chemical modification introduced by chemical cross-linking (e.g., altered MW compared to the unmodified protein, altered overall polypeptide shape that affects the gel migration mobility in the electrical field). The 28 kDa band may represent a cross-linked, monomeric OCP, similar to the major 35 kDa band (cross-linking-induced gel migration variations are documented in previous publications^{37–39}).

In this study, we were particularly interested in the intramolecular structural changes of OCP upon photoactivation as could be revealed by comparing the cross-linked, monomeric OCP^r to OCP⁰ (Fig. 1). The oligomerization state of OCP is controversial according to various analytical perspectives^{11, 13, 21, 40}. It seems that dimerization occurs at higher protein concentration¹³. Whether OCP *in vivo* exists as a monomer or dimer, however, is not certain. We used size-exclusion chromatography (SEC) to purify cross-linked, monomeric OCP protein products after chemical cross-linking. A typical elution profile of OCP^r treated with DSS H₁₂/D₁₂ is shown (Fig. 1B). The two elution peaks in the chromatogram correspond to OCP dimer and monomer as assigned by comparing to protein standards. To determine if SEC is effective at separating OCP dimer and OCP monomer, different fractions were collected and subjected to further biochemical characterization. In brief, the resulting gel analysis (Fig. 1C) indicates that fraction 15 (F15) and F16 contain mostly cross-linked, dimeric OCP with significantly less 35 kDa OCP monomer (Fig. 1A, lane 4 and Fig. 1C, F15). Remarkably, both F17 and F18 show the major, monomeric 35 kDa and 28 kDa bands whereas dimeric OCP is not visible (Fig. 1C). It should be noted that chemically cross-linked, monomeric OCP^r becomes locked in its red form, failing to convert back to its orange form (data not shown) upon dark relaxation, indicating the red-to-orange conversion pathway is blocked by chemical modification. Similarly, cross-linked, monomeric OCP⁰ is unable to convert to OCP^r.

Chemical cross-linking covalently joins amino acid pairs found in close proximity to each other in a protein or a protein complex^{41–43}. After more than a decade of method development, multiple mature platforms of cross-linked species identification have become available^{42, 44, 45}. To determine the locations of intramolecular cross-links of OCP⁰ and OCP^r, purified monomeric OCP⁰ and OCP^r samples were subjected to direct trypsin digestion in solution and the resulting peptides were submitted to extraction and LC-MS/MS analysis (Fig. 2A shows the intramolecular cross-links in OCP⁰ and OCP^r, respectively).

A consensus has been reached that local and global structural changes take place in OCP upon photoactivation^{11, 13, 14, 21, 46}. Compared to its red form, OCP⁰ is more compact. In

practice, the outcome of protein cross-linking reactions is affected by solvent accessibility and distance between two functional groups. In our experiments, we detected many mono-links that account for peptides that are modified once by the cross-linker but are not linked to a second peptide because a second functional group to complete the cross-link reaction is not readily available. We detected one cross-linked pair in OCP^o (i.e., K²⁴⁹–K²⁷², Fig. 2B and 2C). K²⁴⁹ and K²⁷² are located in β 2 and β 3, respectively, of the OCP crystal structure¹¹. This result is consistent with the arm span of 12 Å, which determines the cross-linking capability of DSS. The measured Euclidean Ca–Ca distance in the crystal structure of OCP^o between K²⁴⁹–K²⁷² is 8.6 Å (Fig. 2B, PDB ID: 4XB5), the distance of two primary amines in this pair is 13.0 Å. Also listed is the SAS (solvent accessible surface) distance, which is 11.3 Å in the case of K²⁴⁹–K²⁷². This distance corresponds to the shortest path length between two amino acids, a path that leads through solvent occupied space without penetrating the protein surface. The K²⁴⁹–K²⁷² cross-link was also detected in OCP^r, suggesting that the orientation of β 2 and β 3 upon OCP photoactivation is relatively unaltered (or, at least the spatial distance between this pair doesn't increase to prevent the cross-linking reaction occurring). This result is consistent with published data¹³.

In addition to this pair, there are seven cross-links uniquely detected in monomeric OCP^r (Fig. 2B). Among them, three cross-linked pairs have a median Euclidean distance less than 30 Å: K²⁴⁹–K²⁶⁸ (20.1 Å), P²–K²⁶⁸ (22.0 Å), P²–K¹⁶⁷ (Fig. 2B, 2D). The SAS distance of K²⁴⁹–K²⁶⁸ is about 42 Å, and this value decreases to 34.9 Å (Fig. 2B, 2E, pink, sphere) if the C-terminal loop moves away from the β -sheet core as α A does in OCP^r^{13, 14}. It is possible that photoactivation of OCP leads to the dissociation of the C-terminal loop away from the β -sheet core. Increased GEE modification extent of E³¹¹ on peptide 311–317 was observed in our mass spectrometry-based protein footprinting analysis¹⁴. It is more likely, however, that this increase results from α A detachment (blue sphere in Fig. 2E) from the β -sheet core rather than the direct detachment of the C-terminal loop. This is consistent with recent HDX exchange results showing no increased solvent accessibility of P³⁰⁹ and K³¹⁰¹³. Therefore, the observed cross-link of K²⁴⁹–K²⁶⁸ must result from conformational change between β 2 and β 3 where K²⁴⁹ and K²⁶⁸ are located, respectively.

Although carboxyl footprinting-based MS¹⁴ and HDX MS¹³ have reached a consensus that the NTE dissociates from the β -sheet core of the CTD upon OCP photoactivation, the orientation of this NTE fragment in OCP^r remains unclear. It is likely that the NTE becomes very flexible. The cross-links of P²–K²⁶⁸ and P²–K¹⁶⁷ may result from the NTE's increased conformational freedom and, thus, provide direct evidence to support such a hypothesis. The OCP^o structure has been resolved to atomic level^{11, 40} in contrast to that of OCP^r, which thus far has not been crystallized. Loop J–K¹¹, the NTE, and the C-terminal domain in OCP^r may be highly disordered, making crystallization difficult.

We further mapped the remaining four cross-links with the Euclidean distance longer than 30 Å onto the OCP^o structure (Fig. 2B). The SAS distances of some cross-link pairs are abnormally longer than the cross-linking chemistry could afford. For example, although we found that K¹⁷⁰ is cross-linked to K²⁴⁹ by isotopically coded DSS H₁₂/D₁₂ (Fig. 3), the SAS is 45 Å, almost four times longer than the accepted arm span of DSS. The mass spectrum of the cross-linked peptides NAVVDMGFTAGK¹⁶⁷DGK¹⁷⁰R and EECQNLK²⁴⁹LIPER (Fig.

3A) shows that the use of isotopically coded cross-linker enables unequivocal detection of the cross-linked species. The reaction products of DSS- H_{12}/D_{12} are obvious in the mass spectra and manifest themselves as characteristic doublets of peaks of equal intensity corresponding to light (H_{12}) and heavy (D_{12}) forms of the reagent separated by 12.07573 Da divided by charge state⁴⁷. In this pair of peptides, K^{170} is clearly cross-linked to K^{249} and a mono-link is found on K^{167} . Owing to the double modification of lysine residues K^{170} – K^{249} (cross-link) and K^{167} (mono-link) by two isotopically coded cross-linkers respectively, a characteristic triplet with ratio of 1:2:1 separated by m/z 4.025 (charge = 3) is seen as major peaks of the mass spectrum. The accurate modification sites are confirmed by production (MS/MS) spectra (Fig. 3B, 3C, 3D).

Our present cross-linking data observed for OCP^f add credibility to the hypothesis that OCP undergoes significant conformational changes upon photoactivation, as reached by outcomes from site-directed mutagenesis^{11, 15, 46}, protein footprinting^{13, 14}, small angle X-ray scattering (SAXS)¹³, and other biophysical techniques²⁴. Now the question arises, how do the NTD and CTD re-orient relative to each other in OCP^f? Even without a crystal structure for OCP^f, we can posit a meaningful model using our cross-linking data as spatial constraints. In the crystal structure of OCP^o, the NTD and CTD are stabilized in two ways: via the major interface between NTD and CTD and via the minor interface of the NTE and the CTD^{11, 40}. Additionally, the carotenoid cofactor should also play an important role stabilizing the NTD and CTD. Another factor that could contribute to the OCP photocycle, in our opinion, is the Loop J–K linker region (Fig. 4A). This ~25 (depending on species) amino acid residue fragment starts from a short α helix (α_J) in the NTD and winds its way onto the surface of the CTD and ends at α_K of the CTD (Fig. 4B). In other words, Loop J–K extends like an arm from the interface between NTD and CTD to the bottom of the latter, holding the CTD from the opposite side of NTE. It is conceivable that the process of OCP photoconversion from orange to red (or vice versa) relates to how the CTD exits from and re-enters the “cradle” formed by Loop J–K and the NTE, accompanied by the carotenoid shuttling back and forth into the interior of the NTD.

Our ultimate aim is to generate a model using our cross-linking data as structural constraints⁴⁷. We began by generating eight models in which the CTD of the OCP^o crystal structure is allowed to flip, as if on a hinge, at 45° increments around an x – y plane that bisects normal to the long-axis of the carotenoid cofactor. As a result of the CTD flipping, the interface of the NTD that usually interacts with its counterpart of the CTD in OCP^o is totally exposed¹⁶. Cross-linking pairs that we observed were then used as structural constraints to judge the best orientation of the CTD relative to the NTD. By minimizing the spatial conflicts between each cross-linking pair, we found that the model generated by flipping the CTD outward along axis 3 (Fig. 4C) has the fewest spatial conflicts. Then, we used the computer program Modeller to refine and produce a feasible model for Loop J–K, which becomes unstructured when reorienting the CTD relative to the NTD. In our final model of OCP^f (which includes the translocated CTD and refined Loop J–K; Figs. 4D, 4E, and 4F), all of the amino acids implicated in cross-linking are in close proximity, within 30 Å. We must assume that for the flexible NTE, the meaningful structural constraints come from the K^{167} – K^{249} and K^{170} – K^{249} cross-links, the distances of which are 12.7 and 18.7 Å, respectively. We admit that this modeling treatment is preliminary, and that the refined

structure of Loop J–K is entirely dependent on our proposed reorientation of the CTD relative to the NTD. Nevertheless, we consider this a useful first step for future structural refinements. Our model doesn't necessarily exclude other models that the 12Å cross-linker fails to conjugate. It is highly possible that Loop J–K could allow multiple orientations of NTD and CTD in solution, while *in vivo* the PBS binding pocket could preferably select those OCP^r that are structurally available for interaction. The flexible NTE, with length of 30.6 Å from P2 to D19, also presents a problem. If unfolding of αA is considered, the arm span of NTE could be even longer. At any rate, our experiment establishes the cross-linking network in OCP^r, allowing for structural refinement of OCP^r by other methods. This model assumes that the separation of NTD and CTD as well as that CTD does not impose any structural effect upon the binding of the NTD and carotenoid cofactor to PBS.

In summary, we successfully modified OCP^o and OCP^r and locked them in their characteristic states using isotopically-coded cross-linkers. The spatial conflicts of cross-linking pairs uniquely detected in OCP^r compared to OCP^o support that OCP undergoes dramatic conformational changes from the perspective of NTD and CTD orientation. Based on previous and our current results, we propose an updated intramolecular signal propagation pathway for OCP activation in conjugation with the carotenoid translation. Upon photon absorption, the pigment's effective conjugation length is increased, contributing to the breaking of the H-bonding network within OCP^r. The interactions of the major interface between NTD and CTD and the minor interface between NTE and CTD decrease and collapse upon translocation of the pigment towards the NTD. The NTD and CTD are completely detached and are then guided by an intrinsic structural cue from Loop J–K so that the PBS and FRP binding interfaces on OCP^r are exposed for PBS energy quenching and fluorescence recovery.

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ABBREVIATIONS

OCP	orange carotenoid protein
NTD	N-terminal domain
CTD	C-terminal domain
LHC	light harvesting complex
RC	reaction center

ATP	adenosine triphosphate
NADPH	nicotinamide adenine dinucleotide phosphate
NPQ	non-photochemical quenching
PBS	phycobilisome
MW	molecular weight
OCP^o	orange form of OCP
OCP^r	red form of OCP
NTE	N-terminal extension
FRP	fluorescence recovery protein
FPLC	fast protein liquid chromatography
LysC	Lys-C protease
HCD	high-energy collision-induced dissociation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis

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Fig. 1.

Chemical cross-linking of OCP. (A) SDS-PAGE analysis showing different cross-linking products of OCP^o and OCP^r after Coomassie Brilliant Blue (R-250) staining. Lane 1: OCP^o, control; lane 2 and 3 are OCP^o treated by BS³ and DSS respectively, *78 kDa; lane 4: OCP^r treated by DSS, band a (76 kDa), b (70 kDa), c (28 kDa). (B) Typical FPLC SEC elution profile (partial) of cross-linked species of OCP^r. Fractions (F) are labeled. (C) SDS-PAGE analysis of (B). The locations of OCP-M (monomer) and OCP-D (dimer) are indicated.

Fig. 2.

Identification of Intra-protein cross-links by using LC-MS/MS. (A) Cartoon representation of chemically cross-linked amino acid residues in OCP⁰ and OCP^r, respectively. (B) Xwalk analysis of cross-linking pairs detected using LC-MS/MS; listed are the Euclidean Ca-Ca distances between cross-linked lysines determined on x-ray structure of OCP (PDB ID: 4XB5), the side chain amine groups distance, and the SAS distance respectively (see text for explanation). (C) K²⁴⁹-K²⁷² cross-link in OCP⁰, NTE (marine), Canthaxanthin (orange, stick), C-terminal loop (purple), K²⁴⁹ and K²⁷² (red, sphere), PDB ID: 4XB5. (D) Amino acids residues involved in cross-linking in OCP^r. (E) K²⁴⁹-K²⁶⁸ cross-link in OCP^r, NTE (marine, sphere), C-terminal loop (purple, sphere), E311 (green, sphere).

Fig. 3. Mass spectra of cross-linked peptides. (A) Mass spectra of peptides NAVVDMGFTAGK¹⁶⁷DGK¹⁷⁰R and EECQNLK²⁴⁹LIPER. Unique triplet peak with ratio of 1:2:1 and 4.03 Da ($z = 3$) apart (results from isotopically coded cross-linker DSS-H₁₂/D₁₂ that modified two lysine residues, one mono-link at K¹⁶⁷ and one cross-link between K¹⁷⁰ and K²⁴⁹). (B) Production ion (MS/MS) spectra obtained for cross-linked peptides of (A). (C) and (D) are the theoretical (black) and identified ions (red) in (B).

Fig. 4.

Domain rearrangements of OCP upon light activation. (A) Side view of OCP showing Loop J–K (chocolate, joining α J and α K) and α A (marine) that form a clamp structure holding CTD (lime) in OCP⁰, NTD (wheat), C-terminal fragment (purple), carotenoid (yellow). (B) Bottom view from CTD, Loop J–K winding from α J located in NTD to α K which is located in the bottom of CTD, α A controls the exit of CTD from the opposite side of the Loop J–K. (C) View of NTD with CTD and Loop J–K removed, based on (B). (D) Modeled orientation of NTD and CTD in OCP^r, amino acid residues involved in cross-linking are labeled as sphere (red). (E) Side view (90° rotation of D). (F) Surface representation of OCP^r, circled carotenoid (red stick) region (lower) interacts with the Phycobilisome.