

Activated OCP unlocks nonphotochemical quenching in cyanobacteria

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Most aerobic photosynthetic organisms have enough light-harvesting capacity so that on bright sunny days their photosynthesis is saturated for the major part of the day (1, 2). Early on in such sunny days the capacity of the reaction centers to process the absorbed solar energy is exceeded. The excess absorbed photons are potentially very dangerous. For example, if the chlorophyll-excited singlet states created in the antenna complexes cannot be used productively by the reaction centers, then these singlet states can potentially last long enough to undergo intersystem crossing to produce triplets. Excited chlorophyll triplet states can interact with molecular oxygen to produce very dangerous products,

such as singlet oxygen (3). Singlet oxygen is an extremely powerful oxidizing molecule that can irreversibly damage lipids and, indeed, most major biological polymers. Cells exposed to singlet oxygen are rapidly killed (4). Oxygenic phototrophs have evolved a process, called nonphotochemical quenching (NPQ), to mitigate this problem of overexcitation (5). These phototrophs are able to control the efficiency of their light-harvesting systems. Under nonsaturating incident light intensities, the antenna complexes work as efficient light-harvesters, transferring absorbed solar energy effectively to the reaction centers. As incident light intensities become saturating, the antenna complexes

switch to a quenched state, where the lifetime of their excited singlet states are strongly reduced and the efficiency of energy transfer to the reaction centers is much lower. The excess absorbed light-energy is effectively detoxified and converted harmlessly into heat.

The process of NPQ has been extensively studied in plants and algae, where the major light-harvesting complexes are integral membrane chlorophyll-containing pigment-protein complexes (6). Cyanobacteria also show NPQ, but their major light-harvesting proteins are the water-soluble phycobiliproteins, where the light absorbing pigments are bilins (linear tetrapyrroles) rather than chlorophylls (7). So how does NPQ work in this very different type of light-harvesting system? The answer involves a protein called the orange carotenoid protein (OCP) (8). Under nonsaturating incident light intensities, OCP^O remains orange and the phycobilisome functions as an efficient antenna system. When incident light becomes saturating, the excess light switches OCP^O into its active red form, called OCP^R. OCP^R interacts with the allophycocyanin molecules at the base of the phycobilisome to strongly accelerate the decay of the excited singlet states of the allophycobillin's bilin pigments, thereby shutting down the efficiency of energy transfer from the phycobilisome to the photosynthetic reaction centers in the membrane (Fig. 1B) (6). It is as if OCP^R can close a "tap" at the end of the light-harvesting funnel to reduce the flow of energy through the funnel to the reaction centers in the photosynthetic membrane. The important question addressed in the paper by Gupta et al. (9) is: What are the structural changes occurring in OCP that underlie this form of NPQ?

Previously, the groups of Kerfeld and Kirilovsky have collaborated to determine the X-ray crystal structures of both OCP^O (10) and OCP^R (11). However, they could only determine the structure of part of OCP^R because the complete protein, in this form, has up until now been resistant to attempts to crystallize it.

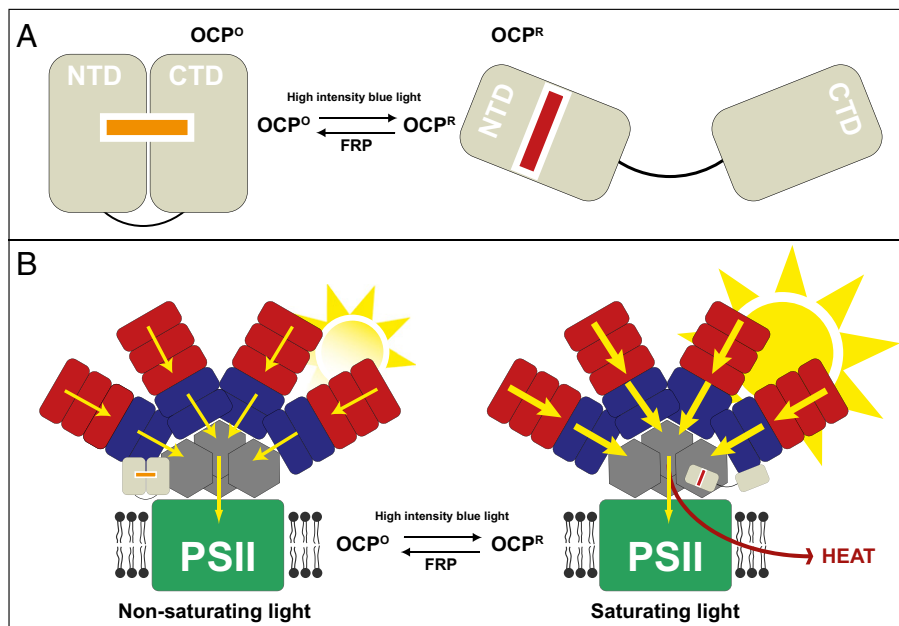


Fig. 1. (A) Schematic representation of the reversible light-induced conversion of OCP from the inactive OCP^O globular form to the active OCP^R elongated form by intense blue light. OCP^O contains two domains, the NTD and the CTD, which are bolted together by the carotenoid molecule. Upon conversion to OCP^R, the carotenoid moves into the NTD and the CTD dissociates. (B) A schematic model for how OCP induces NPQ in cyanobacterial phycobilisomes. Under nonsaturating conditions, light energy (yellow arrows) is harvested by (red) phycoerythrin and (blue) phycocyanin pigment-protein molecules and transferred to photosystem II (PSII) via (gray) allophycocyanin. The OCP^O protein is inactive and in a more globular form. When too much light is present OCP^O is converted to OCP^R. The NTD domain of OCP^R can now bind to allophycocyanin and induce NPQ. Now the excess light energy is dissipated as heat, reducing the flow of energy to PSII, thereby preventing damage to the system. OCP^R is converted back to OCP^O by the fluorescence recovery protein (FRP).

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The result of comparison between these two OCP crystal structures shows that upon conversion of OCP^O to OCP^R the carotenoid moves by about 12 Å. In OCP^O the two main structural domains, the N-terminal domain (NTD) and the C-terminal domain (CTD), are “bolted” together by the carotenoid, whose binding site extends into both domains (Fig. 1A). On conversion to OCP^R the carotenoid “bolt” is withdrawn into the NTD. This then releases the CTD and a short N-terminal helix. This transition is illustrated in Fig. 1A. The absorption spectra of carotenoids are very sensitive to the polarizability of the solvent (12). The shift in color from orange to red just reflects the change in the polarizability of the carotenoid’s binding site during the conversion to OCP^O to OCP^R. Although it is clear that light absorbed by the carotenoid is responsible for triggering the conversion of OCP^O to OCP^R, the photochemical mechanism that results in the large-scale reorganization of the carotenoid remains unclear, especially because this light-driven reaction has a low quantum yield. As shown in Fig. 1A, the back reaction that converts OCP^R into OCP^O is catalyzed by a protein called the fluorescence recovery protein (13). The model proposed to account for how OCP is activated by intense blue light to produce OCP^R was based on a comparison of the two crystal structures. However, because the structure of OCP^R was only a structure of part of the molecule, there was still some uncertainty that all of the structural changes had been fully described. The results presented in the Gupta et al. report (9) address these uncertainties.

Gupta et al. (9) show very clearly, from small angle X-ray scattering data, that using

the full-length OCP^O, light-activation to produce OCP^R is accompanied by structural changes that convert a compact globular protein into an elongated tubular conformation.

The Gupta et al. report now provides a clear structural basis for the detailed molecular mechanism of how cyanobacterial NPQ is determined.

This structural change is consistent with the crystallographic data and reflects the dissociation of the NTD and the CTD, as shown in Fig. 1A. Gupta et al. also used two separate techniques (X-ray radiolytic labeling with mass spectrometry and hydrogen–deuterium exchange mass spectrometry) to show how this gross structural transition is accompanied

by local changes in the organization of water molecules at the surface of OCP. The authors suggest that part of the way in which the result of “unbolting” the carotenoid is transmitted to the surface of the NTD is because of changes in chains of bound water molecules. Water often plays key roles in protein–protein interactions and it is intriguing to speculate that changes in the bound water molecules might play a major part in the subsequent interaction between the NTD and the phycobilisome. The Gupta et al. (9) report now provides a clear structural basis for the detailed molecular mechanism of how cyanobacterial NPQ is determined. One can even dream of seeing a structure of OCP^R bound to the phycobilisome, where structural changes in the phycobilisomes could be correlated with precise measurements of fluorescence lifetimes, thereby fully allowing the process of NPQ to be visualized. The Gupta et al. report now makes this a real possibility.

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