Journal of Photochemistry and Photobiology B: Biology 133 (2014) 153–160

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Effect of APCD and APCF subunits depletion on phycobilisome fluorescence of the cyanobacterium *Synechocystis* PCC 6803

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ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form 12 March 2014
Accepted 18 March 2014
Available online 27 March 2014

Keywords:
Cyanobacteria
Energy dissipation
Fluorescence
Non-photochemical quenching
Orange carotenoid-binding protein
Phycobilisome

ABSTRACT

Long-wavelength allophycocyanin (APC) subunits in cyanobacteria (APCD, APCE, and APCF) are required for phycobilisome (PBS) assembly, stability, and energy transfer to photosystems. Here we studied fluorescence properties of PBS *in vivo*, using *Synechocystis* PCC 6803 mutant cells deficient in both photosystems and/or long-wavelength APC subunits. At room temperature, an absence of APCD and APCF subunits resulted in ~2-fold decrease of long-wavelength APC (APC680) fluorescence. In 77 K fluorescence spectra, we observed only a slight shift of long-wavelength emission. However, 77 K fluorescence of a PSI/PSII/APCF-less mutant was also characterized by increased emission from short-wavelength APC, which suggested the importance of this subunit in energy transfer from APC660 to APC680. Under blue-green actinic light, all mutants showed significant non-photochemical fluorescence quenching of up to 80% of the initial dark fluorescence level. Based on the mutants' quenching spectra, we determined quenching to originate from the pool of short-wavelength APC, while the spectral data alone was not sufficient to make unambiguous conclusion on the involvement of long-wavelength APC in non-photochemical quenching. Using a model of quenching center formation, we determined interaction rates between PBS and orange carotenoid protein (OCP) *in vivo*. Absence of APCD or APCF subunits had no effect on the rates of quenching center formation confirming the data obtained for isolated OCP–PBS complexes. Thus, although APCD and APCF subunits were required for energy transfer in PBS *in vivo*, their absence did not affect rates of OCP–PBS binding.

1. Introduction

Phycobilisome (PBS) is a major light harvesting antenna complex of cyanobacteria and red algae [14,1]. In the cyanobacterium *Synechocystis* PCC 6803 (hereafter *Synechocystis*), PBS is a fan - like structure that consists of phycocyanin rods radiating outward from an allophycocyanin core (Fig. 1A) [29,1]. The PBS core of *Synechocystis* is tri-cylindrical (two bottom cylinders attach to the thylakoid membrane surface) and contains four types of allophycocyanin (APC) trimers that are stacks of disk-type structures (hereafter disks) forming each cylinder [1,5,29,30,14,27,28] (Fig. 1B). The upper cylinder of a tri-cylindrical cyanobacterial core consists of 4 disks (2 $\alpha_3\beta_3$ and 2 $\alpha_3\beta_3L_C$ trimers) of only short-wavelength APC [3,5,16]. Two asymmetrically arranged bottom cylinders have four different disks ($\alpha_3\beta_3$, $\alpha_3\beta_3$Lc, $\alpha^B\alpha_3\beta_3L_C$, $\alpha_2\beta_2\beta^{18}L_{CM}$). Here α, α^B (APCB protein encoded by the *apcD* gene, hereafter APCD), β, and β^{18} (APCF protein encoded by the *apcF* gene, hereafter APCF) are different APC subunits; L_C – core linker polypeptide (contains no chromophore but shifts fluorescence towards the longer wavelength) [16]; L_{CM} (APCE protein encoded by the *apcE* gene, hereafter APCE) is a core-membrane linker polypeptide (one of its domains is structurally similar to APC α subunit and contains the long-wavelength emitting chromophore) [27,28]. The named APC trimers' emission is peaked at: ~660 nm (for $\alpha_3\beta_3$ trimer), ~662 nm ($\alpha_3\beta_3L_C$), ~680 nm ($\alpha^B\alpha_2\beta_3L_C$), and ~680 nm ($\alpha_2\beta_2\beta^{18}L_{CM}$). Overall PBS core contains 5 groups of chromophores: APC (represented by α and β subunits), $APCL_C$ (represented by α and β subunits in the presence of L_C), APCD, APCF, and APCE. APCD and APCE subunits are known to be final acceptors (terminal emitters) of PBS excitation [6,11,31,14,15]. The exact role of the APCF subunit is not clear; however it was proposed to be an intermediate in energy transfer from short- wavelength APC to terminal emitters [15,6,31]. Although much effort has been put into studies of energy transfer

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http://dx.doi.org/10.1016/j.jphotobiol.2014.03.012
1011-1344/

pathways in the PBS core, we still lack a complete understanding of this process.

As part of their photoacclimation strategy, cyanobacteria have evolved a special type of non-photochemical quenching (NPQ). Illumination of cyanobacteria with an intense blue-green light results in significant quenching of PBS fluorescence in the 660–680 nm region [13,34,39]. The identity of the action spectrum of blue-green light induced quenching [34] with the absorption spectrum of 3′-hydroxyechinenone, or hECN [32] – a carotenoid present in a water-soluble orange carotenoid-binding protein (OCP) of cyanobacteria – suggests a key role of OCP in the process of NPQ [44]. Blue-green light induced NPQ is not observed in *Synechocystis* mutant cells lacking PBS or OCP [9,44] or in the absence of the linker polypeptide L_{CM}, responsible for the assembly of the PBS core [35,44]. Reversible quenching is observed in *in vitro* experiments when OCP is added to a solution of isolated PBS upon blue-green light illumination [18]. Thus both PBS and OCP are essential for carotenoid-triggered NPQ in cyanobacteria [7,20–24].

Formation of the quenching state in the course of NPQ development has been extensively studied and shown to correlate with the light-induced transformation of the OCP molecule [17,26,36]. In dark or low light conditions, OCP is present in its orange form (OCP^o, named after the color of purified protein solution) and does not interact with PBS. However, when activated by blue-green actinic illumination, OCP converts to its red form (OCP^r, with a ~20 nm red shift of the absorption maximum [45] and the purified protein solution appears red). OCP^r is able to interact with PBS to quench its fluorescence [22,24,45]. Interaction of PBS with OCP^r could result in alteration of PBS core structure, leading to quenching of PBS fluorescence. However, it is more likely that binding of activated OCP^r to PBS leads to direct exciton energy transfer from PBS chromophores to carotenoid hECN in OCP [37,45]. Light activated hECN has a lower S_1 state [33] thus making OCP^r a likely energy acceptor of PBS excitation [8,33,37]. Abrupt cessation of actinic illumination leads to transition of OCP from the active red form (OCP^r) to the inactive orange form (OCP^o) and its subsequent decoupling from PBS, which is accompanied by a rise in PBS fluorescence [17,34,44]. The latter process accelerates by protein involved in blue-green light induced NPQ – fluorescence recovery protein, or FRP [10,40]. FRP interacts with OCP^r and enhances its deactivation to OCP^o [10]. The kinetics of NPQ development in cyanobacteria and light-induced transformations in OCP appear to follow these same patterns. However, it is not yet clear how OCP alters the energy transfer pathways within the light-harvesting antenna of cyanobacteria.

Several approaches have been employed to study the exact site of OCP binding [19,26,37,41,42]. It has been established that OCP alters exciton transfer to photosystems at some place within the PBS core [37,41]. In a PSI/PSII-less mutant that lacks chlorophyll, peaks in fluorescence emission at 660 and 680 nm belonging to PBS core are quenched upon NPQ induction [26,36]. Due to effective energy transfer and spectral overlap of absorption and emission spectra for short- and long-wavelength APC it is unclear which chromophore is the primary subject of quenching *in vivo*. There are two major possibilities: quenching of APC emitting at 660 nm [19,39,41] or quenching of both 660 and 680 nm emitting chromophores [26,36].

Here we aimed at elucidating effects of APCD and APCF depletion on spectroscopic properties and OCP-triggered fluorescence quenching in *Synechocystis* PCC 6803 mutants deficient in PSI/PSII, PSI/PSII/APCD and PSI/PSII/APCF, using the previously developed kinetic model of NPQ in cyanobacteria [17,26] and deconvolution of the emission spectra into individual components. Although the role of long-wavelength APC in energy transfer and NPQ has been studied *in vitro* on isolated PBS [19], we wanted to test whether the native environment of PBS would affect these processes *in vivo*.

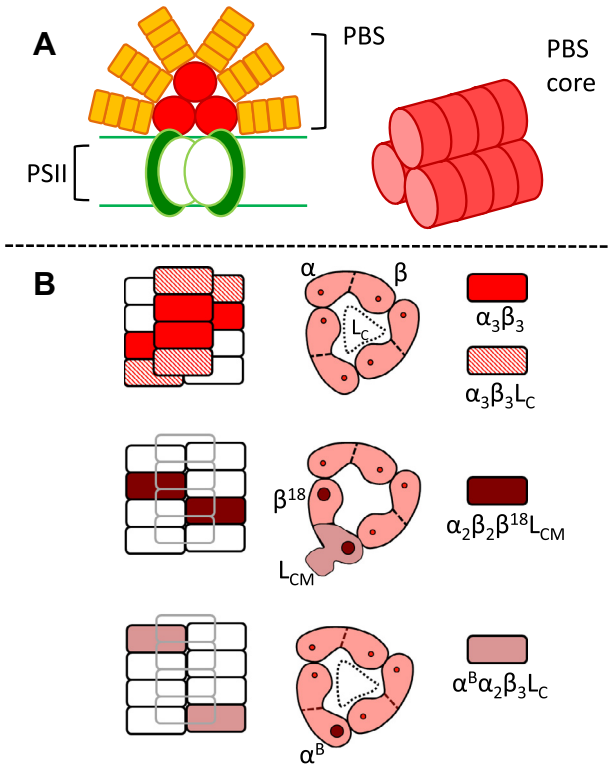

Fig. 1. A: Scheme of PBS attached to PSII of *Synechocystis* PCC 6803 (from Arteni 2009 and references therein). Note six phycocyanin rods and tri-cylindrical APC core (the latter shown separately). B: Top view of APC core with structure and position of different APC trimers (disks). Here α, α^B (APCD), β, and β^{18} (APCF) are different APC subunits; L_C – core linker polypeptide; L_{CM} (APCE) is a core-membrane linker polypeptide. See text for more details.

Mutants deficient in PSI/PSII are good model organisms for *in vivo* studies of exciton energy transfer since the absence of photosystem-related chlorophyll allows for a more accurate analysis of PBS fluorescence. In addition, PBS in these mutants, in contrast to isolated PBS, are structurally and functionally associated with the thylakoid membrane. PBS's in PSI/PSII-less mutants maintain their integrity; an absence of PSII/PSI does not affect the thylakoid membranes of mutant strains (thylakoid membrane sheets are present and number of membrane pairs per cell is similar to wild type) [43]. Our results support the existence of energy pathways from short-wavelength APC to terminal emitters (APCD and APCE) and the significant role of APCF in this process. We showed that an absence of APCD and APCF subunits does not affect *in vivo* rates of OCP–PBS binding.

2. Materials and methods

2.1. Strains and culture conditions

Synechocystis PSI/PSII-less mutants (Δ*psaAB*/Δ*psbDIC*/Δ*psbDII*) [12] were grown at 30 °C in BG-11 medium [38] supplemented with 10 mM glucose, 25 μg ml^{-1} spectinomycin, 20 μg ml^{-1} erythromycin, and 20 μg ml^{-1} chloramphenicol. The growth medium for PSI/PSII/APCD-less and the PSI/PSII/APCF-less mutants was additionally modified to contain double the amount of sodium nitrate and 80 μg ml^{-1} kanamycin. To create PSI/PSII/APCD-less and PSI/PSII/APCF-less mutants, *apcD* and *apcF* deletion plasmids were constructed by replacing parts of *apcD* and *apcF* genes with a kanamycin resistance cassette. The deletions covered the 68–109 bps region of *apcD* and the 86–330 bps region of *apcF*. Transformation