Trimeric Photosystem I facilitates energy transfer from phycobilisomes in *Synechocystis* PCC 6803

Parveen Akhtar¹, Avratanu Biswas^{1,2}, Fanny Balog-Vig¹, Ildikó Domonkos¹, László Kovács¹, Petar H. Lambrev¹

¹Biological Research Centre, Szeged, Institute of Plant Biology, Temesvári krt. 62, Szeged 6726, Hungary

²Doctoral School of Biology, University of Szeged, Közép Fasor 52, Szeged 6726, Hungary

Short Title

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Author for contact

Petar H. Lambrev, lambrev.petar@brc.hu

Author Contributions

P.A. and P.H.L. conceived the project and designed the experiments. P.A., A.B., and F.B.-V. prepared the biological samples and conducted most of the spectroscopic measurements. L.K. and P.A. performed P₇₀₀ oxidation measurements; I.D. and F.B.-V. did protein gel electrophoresis. P.A., A.B. and P.H.L. performed data analysis. P.A., A.B., and P.H.L. wrote the paper with contributions from all authors. P.H.L. is responsible for communication.

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One-sentence summary

Cyanobacterial mutants with monomeric PSI have higher abundance of smaller phycobilisomes transferring more energy to PSII than to PSI, thus trimeric PSI is important for balanced energy flow.

ABSTRACT

In cyanobacteria, phycobilisomes serve as peripheral light-harvesting complexes of the two photosystems, extending their antenna size and the wavelength range of photons available for photosynthesis. The abundance of phycobilisomes, the number of phycobiliproteins they contain, and their light-harvesting function are dynamically adjusted in response to the physiological conditions. Phycobilisomes are also thought to be involved in state transitions that maintain the excitation balance between the two photosystems. Unlike its eukaryotic counterpart, PSI is trimeric in many cyanobacterial

species and the physiological significance of this is not well understood. Here we compared the composition and light-harvesting function of phycobilisomes in cells of *Synechocystis* PCC 6803, which has primarily trimeric PSI, and the *ApsaL* mutant unable to form trimers. We also investigated a mutant additionally lacking the PsaJ and PsaF subunits of PSI, as PsaF has been proposed to facilitate interaction with phycobilisomes. Both strains with monomeric PSI accumulated significantly less phycocyanin (which constitutes the phycobilisome rods) per chlorophyll, while the allophycocyanin content was unchanged compared to WT. These data show that cells with monomeric PSI have higher abundance of smaller phycobilisomes. Steady-state and time-resolved fluorescence spectroscopy at room temperature and 77 K revealed that PSII receives more energy from the phycobilisomes at the expense of PSI in cells with monomeric PSI, regardless of the presence of PsaF. Taken together, these results show that the trimeric organization of PSI is advantageous for efficient and balanced excitation energy transfer from phycobilisomes in *Synechocystis*.

INTRODUCTION

Although the photosynthetic apparatus of cyanobacteria is largely similar to that of eukaryotic algae and plants, cyanobacteria are distinct in several key aspects. Cyanobacteria lack the membrane-intrinsic antenna proteins of the LHC family but utilize membrane-peripheral phycobilisomes (PBSs) to increase the absorption cross-section of the two photosystems, PSI and PSII. The organization and function of the cyanobacterial thylakoid membranes is defined by the large PBSs attached to them and the coexistence of the photosynthetic and respiratory electron-transport chains sharing electron carriers. Apart from their peripheral antenna systems, cyanobacterial and plant PSI differ by their quaternary structure and subunit composition. Four small hydrophobic protein subunits - PsaF, PsaJ, PsaK and PsaX (only present in thermophilic species) cover the membrane-exposed surface of cyanobacterial PSI (Fromme et al., 2003). The functional role of PsaF in cyanobacterial PSI, apart from structural stabilization, is not established. It has been suggested that PsaF may be involved in the docking of the PBSs (Hippler et al., 1999) and, under iron deficiency, to mediate the interaction of the PSI core with the peripheral antenna IsiA (Fromme et al., 2003; Akita et al., 2020). Whereas eukaryotic PSI is monomeric, in cyanobacteria it is found in the form of trimers and in some species tetramers. The PsaL subunit is crucial for trimer formation and mutants lacking PsaL only accumulate PSI monomers (Chitnis and Chitnis, 1993). The physiological advantages of PSI oligomerization in cyanobacteria are not clear. PSI trimers have higher far-red absorption thanks to the presence of long-wavelength chlorophylls (Chls) and it may facilitate quenching of excess excitation energy by the oxidized reaction center (RC) and help protect against photoinhibition and ROS generation (Karapetyan et al., 1999; Kłodawska et al., 2020). It has also been proposed that the trimeric state could facilitate excitation energy transfer (EET) from the PBS to PSI (Şener et al., 2004).

The PBS is composed of phycobiliproteins (PBPs) and linker proteins organized as rods radiating from a membrane-attached core (MacColl, 1998; Arteni et al., 2009; Zheng et al., 2021). In the cyanobacterium *Synechocystis* PCC 6803 (hereafter called *Synechocystis*), six PBS rods connect three hexameric phycocyanin (PC) discs each and the core consists of three cylinders, each with two stacked allophycocyanin (APC) hexamers (Arteni et al., 2009). The ApcD and ApcE (L_{CM}) polypeptides of the core are crucial for the interaction with the photosystems and contain the longest-wavelength (680 nm) ‘terminal emitter’ pigments of the PBS that transfer energy to Chls in both photosystems (Ashby and Mullineaux, 1999; Rakhimberdieva et al., 2001; Liu and Blankenship, 2019). In situ cryoelectron tomography has revealed the ordered arrays of PBS-PSII supercomplexes, where energy can presumably migrate also laterally between PBS making for a very efficient light-harvesting system (Rast et al., 2019; Li et al., 2021). Plausible routes for energy migration from PBS to PSI can be directly via interaction between them (Mullineaux, 1994; Liu et al., 2013) or indirectly via “spillover” from PSII to PSI (McConnell et al., 2002; Ueno et al., 2017). ApcE is responsible for EET to PSII, whereas ApcD is proposed to serve as an energy donor primarily for PSI (Ashby and Mullineaux, 1999; Dong et al., 2009; Liu and Blankenship, 2019).

The relative excitation of PSI and PSII can be rapidly regulated by the mechanism of state transitions, which is triggered by the redox state of the PQ pool (for reviews, see Mullineaux and Emlyn-Jones, 2005; Calzadilla and Kirilovsky, 2020). Several mutations in the PBS core are known to block or reduced the ability to perform state transitions, at least in some species (Ashby and Mullineaux, 1999; Dong et al., 2009; Calzadilla et al., 2019; Zlenko et al., 2019), highlighting the key role of the PBS in the process. However, the exact mechanism of cyanobacterial state transitions is under debate and alternative models are proposed, including a mobile PBS shuttling between PSI and PSII, regulated spillover (PSII–PSI energy transfer), and PSII quenching.

Cyanobacterial cells can modify the characteristics and abundance of PBSs in response to changes in the environmental conditions. Shortening of the PC rods under high growth light has been reported in several species, whereas low-intensity light elevates the PC content (Raps et al., 1985; Samson et al., 1994; Nomsawai et al., 1999). Macronutrient limitation results in extensive PBS breakdown (Salomon et al., 2013) that could provide the cell with amino acids – thereby, PBPs have obtained a secondary role as intracellular storage compounds.

Changes in the PBS can in turn affect the relative abundance of the membrane pigment-protein complexes. Genetically manipulated strains with shorter PBS rods or devoid of PBS accumulate more PSII relative to PSI (Nagarajan et al., 2014; Liberton et al., 2017). The PSI:PSII ratio in cyanobacteria is typically 2–4 and varies depending on the light intensity and quality that the organism is cultured in (Murakami and Fujita, 1991). Low growth irradiance increases the abundance of PSI and vice versa. The sensing mechanisms controlling the PBPs and photosystem abundance are not well elucidated (Stadnichuk et al., 2015). Different growth conditions, such as light, temperature and available nutrients

may change the PSI oligomeric state and the ratio of monomers to trimers (Ivanov et al., 2006; Salomon and Keren, 2011; Kłodawska et al., 2015). Furthermore, mutants unable to form PSI trimers have shown changes in the relative fluorescence emission of PBS components suggesting that the oligomeric state influences the PBS composition (Kłodawska et al., 2020).

In this work, we take a look into the relationship between the oligomeric state of PSI, the PBS composition and abundance, and the EET from PBS to the photosystems, by comparing *Synechocystis* (which has predominantly trimeric PSI) and the $\Delta psal$ mutant (unable to form trimers). In addition, we investigated a mutant lacking also the F and J subunits ($\Delta FIJL$) to test if the PsaF and PsaJ subunits have specific role in EET from the PBS to PSI. We compare the PSI and PSII ratios as well as the PBP (PC and APC) content of the mutant cells with WT and will show that mutants with monomeric PSI have an altered composition and abundance of PBS. We also employed steady-state and time-resolved fluorescence spectroscopy at ambient and cryogenic temperature to evaluate the EET from PBS to the photosystems. By low-temperature time-resolved fluorescence spectroscopy we could separate PSII and PSI emission components and as well as populations of free, weakly, and strongly coupled PBPs. We show that the changes in the PBS composition affect the dynamics of EET in the cells and the excitation distribution between PSII and PSI, supporting the idea that PBS transfer energy more efficiently to trimeric than to monomeric PSI.

MATERIALS AND METHODS

Cyanobacterial cultures and preparations

Cyanobacterial cultures (*Synechocystis* sp. PCC 6803 wildtype (WT), $\Delta psal$, and $\Delta FIJL$), thylakoid membranes, and isolated PSI complexes were prepared as described in Akhtar et al. (2021).

PBSs were prepared according to the protocol described in Garnier et al. (1994) with some adjustments. Briefly, photoautotrophically grown cells were centrifuged to pellet at 6000 g at 25 °C. The pellet was washed twice with phosphate buffer (0.75 M phosphate buffer, 1 mM benzamidine hydrochloride hydrate, 1 mM EDTA, pH 7.0 and 1 mM of phenylmethylsulfonyl fluoride). The pellet was collected and treated with 0.2 % of lysozyme and incubated for 1 hour in dark at 37°C with continuous shaking at 200 rpm. After incubation cells were pelleted down by centrifugation at 6000 g for 7 minutes, 14 °C and washed twice in phosphate buffer to remove the remaining lysozyme. The cells were then broken with glass beads ($\leq 106 \mu\text{m}$ diameter) using a homogenizer (Precellys Evolution) equipped with dry ice cooling compartment. The remaining glass beads were removed by centrifugation at 3000 g for 5 min at 14 °C. The supernatant was then treated with 3% Triton-X100 with continuous stirring for 30 min at room temperature in dark and centrifuged at 21,000 g for 30 min to remove the unsolubilized material. The appropriate sample fraction was collected and loaded onto a sucrose density step gradient (0.25, 0.5, 0.75, 1 M) and centrifuged for 16 h at 104,000 g 14 °C for further purification. The gradient fraction containing PBSs was collected.

Pigment analysis

Chls were extracted from the cell suspensions in 90% methanol and the Chl contents were determined spectrophotometrically using molar absorption coefficients described in Lichtenthaler (1987).

The phycobiliprotein content was determined as described in Zavřel et al. (2018). For PBPs isolation, 100 ml of the cells from each type were pelleted by centrifugation at 6000 g for 5 min and resuspended in phosphate buffer (50 mM, pH 6.5) to total vol of 5 ml. The cells were broken using a Precellys Evolution homogenizer with dry-ice-cooled chamber (10 cycles of braking, 30 s vortex, 5500 rpm and two min cooling). The homogenate was then sonicated intermittently (five sec sonication with interval of 10 sec rest, four times) by ultrasonicator at ice water temperature. Unbroken cells and cell debris were removed by low-speed centrifugation. Cell homogenates were then ultracentrifuged at 104 000g for 60 min. Absorption spectra of the transparent supernatant in the range of 220–750 nm were recorded to determine the soluble PBP content of the cells.

Quantification of PSI and PSII

The PSI and PSII concentrations were determined spectrophotometrically using the protocol described by Fujita and Murakami (1987). For P_{700} measurements the samples were suspended to 20 $\mu\text{g/ml}$ (or optical density of 2 at 680 nm) and for cytochrome (Cyt) to 60 $\mu\text{g/ml}$ (or optical density of 6 at 680 nm) in a buffer containing 20 mM MES/NaOH, pH 6.4, 10 mM MgCl_2 , 10 mM CaCl_2 . The control absorption spectra in the range of 350–750 nm were recorded from each sample. To estimate the concentration of P_{700} and Cyts, absorption spectra were recorded in the range 650–750 and 500–600 nm, respectively, with bandwidth of 1 nm. The P_{700} was first oxidized with 1 mM potassium ferricyanide and then reduced with sodium ascorbate. The difference spectra (690–720 nm) between oxidized and reduced P_{700} were identical to P_{700} and the difference at 700 nm was taken as the signal of P_{700} . P_{700} abundance was estimated from the absorption difference with a molar extinct coefficient $\Delta\epsilon_{\text{ox-red}} = 64 \text{ mM}^{-1} \text{ cm}^{-1}$ at 700 nm. For PSII determination, first all Cyts were oxidized with 1 mM of potassium ferricyanide. Then few grains of hydroquinone were added, followed by addition of sodium ascorbate and sodium dithionite. The difference spectra (520–580 nm) between hydroquinone-reduced and ascorbate-reduced had peak at 559 nm – Cyt b_{559} .

Redox kinetics of P_{700}

The functional antenna size of PSI was estimated by the rate of light induced oxidation of P_{700} RC under light limiting conditions. The oxidation kinetics of P_{700} upon illumination was followed by the measurement of absorbance change at 830 nm using Dual-PAM 100 Chl a fluorometer (Walz, Germany). Prior to measurement samples were dark-adapted for 3 minutes in the presence of 100 μM methylviologen (MV) and 20 μM DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) then cells equivalent to 20 μg Chl were filtered onto a 25 mm diameter glass fibre syringe filter disc (Whatman GF/C). The filter discs, placed between two microscopy slides with a spacer, were inserted between the

fibre optics of the emitter-detector unit. Samples were illuminated with 5-s long 635-nm pulses at various intensities (6, 31, 140, 251, and 805 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$) consecutively and the oxidation kinetics were recorded at a millisecond sampling rate.

Steady-state absorption and fluorescence spectroscopy

Absorption spectra in the range of 350–750 nm were recorded at room temperature with a Thermo Evolution 500 dual-beam spectrophotometer. The measurements were performed in a standard glass cell of 1-cm optical path length with 1 nm spectral bandwidth.

Fluorescence emission spectra in the visible range were measured from the same samples at room temperature and 77K on a FP-8500 (Jasco, Japan) spectrofluorometer. The sample were diluted to absorbance of 0.1 per cm at the red maximum. Emission spectra in the range of 620–780 nm were recorded with excitation wavelength of 440 nm and 580 nm and excitation/emission bandwidth of 3 nm. The measurements were performed with 1 nm increment and 1 s integration time. For 77K measurements samples were cooled in an optical cryostat (Optistat DN, Oxford Instruments, UK).

Time-resolved fluorescence spectroscopy

Picosecond time-resolved fluorescence measurements were performed with a time-correlated single-photon counting instrument (FluoTime 200/PicoHarp 300 spectrometer, PicoQuant). Excitation was provided by Fianium WhiteLase Micro (NKT Photonics, UK) supercontinuum laser, generating white-light pulses with a repetition rate of 20 MHz. Excitation wavelengths of 440 and 580 nm were used to excite selectively Chls and PBSs. The fluorescence decays were recorded at wavelengths of 600–744 nm with 8 nm steps, at room temperature, and 605–760 nm with 5 nm steps at 77 K. All the samples were diluted to an absorbance of 0.03 at excitation wavelength. For the room temperature measurements, the suspension (whole cells or isolated complexes) was placed in 1 mm flow cell and circulated at a flow rate of 4 ml/min. For 77 K measurements, the suspension was placed in a 1 mm demountable cryogenic quartz cell and cooled in an optical cryostat (Optistat DN, Oxford Instruments, UK). The total instrument response (IRF) measured using 1% Ludox as scattering solution has width of 40 ps. Global multiexponential lifetime analysis with IRF deconvolution was performed using MATLAB.

RESULTS

Changes in the pigment stoichiometry

We cultured *Synechocystis* (WT), which contains PSI trimers and the two mutants with monomeric PSI, ΔpsaL and ΔFIJL , under the exact same conditions, to examine the phenotype effects of the mutations. Both mutants appeared more greenish in colour suggesting a change in the pigment composition of the cells. Accordingly, absorption spectra of the supernatant obtained after sedimenting the broken cell debris (Supplementary Fig. S1) show a distinct shoulder around 650 nm in both mutants – ΔpsaL and ΔFIJL – suggesting increased APC content. We estimated the PC and APC composition of the cell

cultures from absorption spectra of the supernatant (Table 1). The Chl content was measured from methanol extracts of either the cell debris or whole cell sediment, yielding approximately equal results. We found that the ratio of PC to Chl was unchanged between the WT and mutant cultures. However, the monomeric PSI mutants contained significantly higher amount of APC (up to 80% more than in the WT), hence lower PC:APC and Chl:APC ratios. If we assume that all, or almost all, PC in the WT is found in the PBS rods, the lower PC:APC ratio means that the PBS in the mutants has either fewer or shorter PC rods. On the other hand, the more abundant APC indicates a higher number of PBS cores in the cells. Assuming a basic tricylindrical APC core architecture of the PBS, wherein each PBS contains 36 APC $\alpha\beta$ subunits, we can estimate the Chl:PBS ratio (Table 1). From these data it follows that both the composition and the number of the PBSs are altered in the mutants with monomeric PSI – they contain more PBSs as a whole (on Chl basis) but the PBSs are smaller, binding fewer PC units.

It must be noted that the PC:APC ratios in the WT are higher than expected. PBSs are typically found to contain six rods with three PC hexamers per tricylindrical core (Arteni et al., 2009; Rast et al., 2019), which amounts to a PC:APC ratio of 3:1. Although one could merely attribute the discrepancy to a systematic error in the PC:APC estimation, it is interesting to point out that we obtained different results from isolated PBS using the same measurement methodology (Supplementary Table S1). The PC:APC ratio of isolated PBS was found to be around 4:1 – similar to previous reports. More importantly, the ratios were the same in PBSs of the WT and mutants, as evident from their nearly identical absorption spectra (Supplementary Fig. S1). It must be concluded that WT cells under our growth conditions contain extra PC, which is either not connected to the PBS in vivo or (more likely) is weakly connected such that is dissociated during isolation of PBS.

Table 1. Pigment content of cell cultures

Type	PC ($\mu\text{mol/L}$)	APC ($\mu\text{mol/L}$)	Chl ($\mu\text{mol/L}$)	PC:APC	Chl:PC	Chl:APC	Chl:PBS
WT	$2.0 \pm 0.4^*$	0.28 ± 0.04	10 ± 1	7.2 ± 0.6	5.1 ± 0.3	36 ± 2	1305
ΔpsaL	2.2 ± 0.3	0.50 ± 0.07	11 ± 1	4.4 ± 0.2	4.9 ± 0.3	19 ± 2	767
ΔFIJL	1.8 ± 0.1	0.37 ± 0.14	8 ± 2	5.1 ± 0.3	4.9 ± 0.5	24 ± 4	915

* values are represented as the mean $\pm$ standard error of four biological replicates

The striking change in the PBS content and composition found in the monomeric PSI mutants compared to WT *Synechocystis* prompted to test whether there was a corresponding change in the photosystem stoichiometry. To this end, we estimated the number of PSI and PSII RCs in the cells by the absorption difference of the oxidized and reduced forms of P₇₀₀ and Cyt *b*₅₅₉, respectively (Table 2 and Supplementary Fig. S2). We found P₇₀₀:Cyt *b*₅₅₉ ratios of 1.5–1.7 that were not significantly different between mutants and WT. These values are similar to the ones reported by Murakami and Fujita (1991). However, in this paper it was assumed that there are two Cyt *b*₅₅₉ per PSII RC, hence the reported

PSI:PSII ratios were twice as high. A RC ratio of 1.5 means that there are equimolar ratios of PSI trimers (PSI₃) and PSII dimers (PSII₂) in WT or three monomeric PSI per PSII₂ in the mutants. From these ratios and the number of Chls in PSI and PSII, we can estimate 3–4 PSI and the same number of PSII complexes per PBS in the WT. In the monomeric mutants, although the number of PBS is apparently increased, there are still more (monomeric) PSI complexes per PBS (6–7) but fewer PSII (2–3). Thus, we could potentially interpret the increased number of PBS in the mutants as an adaptive response that compensates for the number of PSI per PBS.

Table 2. Stoichiometric ratios of PSI, PSII and PBS

Type	P ₇₀₀ :Cyt <i>b</i> ₅₅₉	PSI ₃₍₁₎ :PSII ₂	Chl:PSI ₃₍₁₎	P ₇₀₀ :PBS*	Cyt <i>b</i> ₅₅₉ :PBS	PSI ₃₍₁₎ :PBS	PSII ₂ :PBS
WT (PSI ₃)	1.6 ± 0.2	1.0	285	11.4	7.2	3.8	3.6
<i>ΔpsaL</i> (PSI ₁)	1.5 ± 0.2	3.0	92	6.3	4.0	6.3	2.0
<i>ΔFIJL</i> (PSI ₁)	1.7 ± 0.1	3.1	88	7.8	5.0	7.8	2.5

* assuming all PBS have a tricylindrical core with two APC hexamers per cylinder

Steady-state fluorescence emission spectra

As a further confirmation of the pigment stoichiometry changes, we recorded fluorescence emission spectra of intact WT, *ΔpsaL*, and *ΔFIJL* cells at 77 K (Figure 1). The spectra recorded with 580 nm excitation (primarily absorbed by PC) have well-defined peaks at 650, 660, 687, and 720 nm. The peaks at 650 and 660 nm correspond to PC and APC and the ones at 687 and 720 nm – to PSII and PSI, respectively. In accordance with the higher amount of PC determined in the WT, the spectra showed more intense emission at 650 nm. The ratio of fluorescence emitted at 650 nm to 660 nm decreased in the monomeric PSI types in line with the decreased PC:APC ratio. Increased PC emission is also detectable in the room-temperature fluorescence spectra of the WT cells, albeit not as pronounced (Supplementary Fig. S3). No significant differences in the fluorescence spectra were found between the two monomeric PSI types, *ΔpsaL* and *ΔFIJL*.

Figure 1. Fluorescence emission spectra of intact cells of *Synechocystis* WT, $\Delta psaL$ and $\Delta FIJL$ recorded at 77 K. (A) Excitation wavelength 580 nm; (B) Excitation wavelength 440 nm. The spectra are normalized at 720 nm.

The photon energy absorbed by the PBSs is ultimately distributed between both PSI and PSII as evident from their corresponding emission peaks. The relative amplitudes of the 687 and 720 nm peaks suggest that in $\Delta psaL$ and $\Delta FIJL$ cells more energy is transferred to PSII compared to WT. In contrast, the fluorescence spectra recorded with 440 nm excitation (almost exclusively absorbed by the photosystems) show no significant difference in the ratio of the PSII and PSI peaks. This is expected because the PSI: PSII stoichiometric ratio (on monomer basis) is unchanged. Thus, the stronger PSII emission upon 580 nm indicates that the distribution of excitation energy from PBSs to PSII/PSI is altered in the monomeric PSI types.

P₇₀₀ oxidation kinetics

To compare the effective antenna size of PSI, we recorded the oxidation kinetics of P₇₀₀ (absorption transients at 830 nm) in intact cells and isolated PSI complexes upon illumination in the presence of DCMU and MV. DCMU prevents re-reduction of P₇₀₀⁺ by electrons from PSII and MV accepts electrons from PSI keeping the acceptor side of PSI in oxidized state and minimizing cyclic electron flow. The oxidation curves at different light intensities are shown in Supplementary Fig. S4 and the oxidation rates obtained by fitting logistic or exponential kinetics to the curves are in Figure 2. At all light intensities in the range 6–805 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the oxidation rates in WT *Synechocystis* cells were higher than either of the monomeric PSI types – up to 60% at 140 $\mu\text{mol m}^{-2} \text{s}^{-1}$. These data indicate that the effective antenna

size of monomeric PSI in vivo is smaller compared to trimeric PSI. The oxidation kinetics were measured with 635 nm light, predominantly absorbed by the PBS. Hence, a possible explanation for the different rates could be that PBS transfer energy more effectively to trimeric than to monomeric PSI. This hypothesis is supported by the fact that the P_{700} oxidation rates were similar in monomeric and trimeric isolated PSI (Figure 2B) as well as in thylakoid membranes, which lack PBSs (Supplementary Fig. S5).

Figure 2. P_{700} oxidation rates. (A) intact cells of *Synechocystis* WT, ΔpsaL and ΔFIJL; (B) isolated PSI. The rates are calculated from the differential absorption at 830 nm induced by pulses of different light intensity, applying logistic or exponential fit (for intensities under and above 100 μmol m⁻² s⁻¹, respectively). Error bars indicate standard errors from four independent experiments. Note the logarithmic vertical scale.

Fluorescence kinetics of cells at room temperature

We employed picosecond time-resolve fluorescence spectroscopy to gain better understanding of the excitation energy partitioning in the intact cells of *Synechocystis* with trimeric (WT), monomeric (ΔpsaL) and subunit-depleted (ΔFIJL) PSI. Time-resolved fluorescence enables better separation of emission components, for example emission of PBSs that are energetically coupled to the photosystems from free PBPs and can detect changes in the architecture and supramolecular organisation of the photosynthetic complexes that affect the EET. We applied global five-exponential analysis of the fluorescence decays recorded in the wavelength range 600–720 nm after excitation at 580 nm. Figure 3 compares the lifetimes and decay-associated emission spectra (DAES) of WT, ΔpsaL, and ΔFIJL cells.

The lifetimes and spectra are very similar between genotypes and are comparable to previously published results on cyanobacterial cells (Mullineaux and Holzwarth, 1991; Tian et al., 2011; Akhtar et al., 2020). The first two components (Figure 3A,B), with respective lifetimes around 30 ps and 90–100 ps, represent EET within the PBS (Akhtar et al., 2020). The DAES have characteristic positive and negative peaks around 650, 660, and 680 nm, signifying decay and rise of the emission from PC, APC and red-shifted APC (APC₆₈₀), respectively. The third component with a lifetime about 170 ps represents mainly decay of APC excitations in PBS coupled to photosystems and the fourth, around 500–600 is associated with trapping in PSII. A longer-lived component (1.4–1.6 ns, not plotted) indicated the presence of a negligible amount (2–3%) of uncoupled PBP.

Figure 3. DAES of *Synechocystis* cells obtained by global lifetime analysis of the fluorescence decays recorded at room temperature with 580 nm excitation. Panels A-D compare individual DAES for WT (black circles), $\Delta psaL$ (red squares), and $\Delta FIJL$ (gold triangles) cells. The spectra are scaled to the total number of photons counted. The longest-lived (1.3-1.6 ns) component is omitted.

As seen in Figure 3, there is little difference between the genotypes as concerning the shape of the DAES. On close inspection, the DAES of WT cells display relatively larger amplitudes at 640–650 nm (Figure 3B,C), consistent with their higher PC:APC ratio. There was also a small but significant difference in the PBS-associated decay lifetimes – in WT we obtained 35 ± 1 , 97 ± 2 , and 172 ± 3 ps, compared to 31 ± 1 , 90 ± 2 , and 162 ± 2 ps in $\Delta psaL$ (each value an average of four culture batches). This result is consistent with longer PC rods in the PBSs of WT cells, which slows down the rod-core equilibration dynamics (Sandström et al., 1988; Zhang et al., 1997).

The most notable kinetic difference between WT cells and those with monomeric PSI is in the PSII decay component (Figure 3D). In the WT, the peak amplitude of this component was $15 \pm 2\%$, whereas in either of the monomeric PSI types it was $20 \pm 2\%$. This result confirms the finding that a larger proportion of excitation energy is transferred to PSII in cells with monomeric PSI, compared to cells with trimeric PSI. Moreover, as we do not observe significant amount of uncoupled PBSs in any of the *Synechocystis* cells, we can make the reverse conclusion – namely, that PBSs transfer more energy to PSI when it is trimeric rather than monomeric.

Fluorescence kinetics of cells at 77 K

We further examined the fluorescence decay kinetics of intact cells at 77 K for better resolution of the different pigment groups. Most importantly, the emission from the long-wavelength “red” Chls in PSI is well pronounced allowing us direct comparison of the energy flow to the two photosystems. Global analysis of the fluorescence decays resulted in six decay lifetimes and DAES. Individual DAES for the three *Synechocystis* genotypes are compared in Figure 4. We will first briefly describe the kinetic components in WT cells. The fastest component (13 ps, Figure 4A) shows decay of PC emission at 640 nm and concomitant rise of APC emission at 660 nm. In addition, EET from bulk to red Chls in PSI occurs on this timescale (690 to 720 nm). The second component (56 ps, Figure 4B) shows decay of both PC and APC and rise of the red-shifted APC₆₈₀. The 145-ps component (Figure 4C) has positive peaks corresponding to all three PBP groups (PC, APC and APC₆₈₀) that evidently decay as energy is transferred to Chls. Also notable are the negative peaks at 690 nm (PSII) and 720 nm (PSI). The PSII and PSI emission components decay mainly with lifetimes of 373 ps and 982 ps (Figure 4D,E) while the final component (3.8 ns, Figure 4F) is of negligible amplitude. Remarkably, PC fluorescence detected at 640–650 nm spans the entire range of decay timescales (in WT). A sizeable fraction of long-lived PC is less efficient in transferring excitation energy downstream (note the 650 nm peaks in Figure 4C–E). It is also of note that a considerable proportion of photon energy absorbed by the PBS is delivered to PSI, judging by the height of the DAES peaks around 720 nm.

Compared to WT, the DAES of both monomeric PSI types showed notable differences. Remarkable are the kinetic differences in the 640–680 nm wavelength range reflecting PBPs emission. In $\Delta psal$ and $\Delta FIJL$ cells, PC emission decays faster than in WT – the peaks at 650 nm in the long-lived DAES all but vanish. Conversely, the amplitudes of the APC peaks at 660 and 680 nm are larger than in WT. Especially obvious is the larger negative peak at 680 nm in the 58-ps DAES (Figure 4B) and corresponding positive peak in the 136-ps DAES (Figure 4C). These results show unequivocally that the altered PBP stoichiometry results in changes in the excitation dynamics of the intact and energetically coupled PBSs.

The fluorescence kinetics at 77 K also unambiguously confirm that a larger proportion of PBS excitations end up on PSII when PSI is monomeric (compare the amplitudes of the 1-ns DAES at 695 nm relative to 725 nm in Figure 4E).

Figure 4. DAES of *Synechocystis* cells obtained by global lifetime analysis of the fluorescence decays recorded at 77 K with 580 nm excitation. Panels A-F compare individual DAES for WT (black circles), $\Delta psal$ (red squares), and $\Delta FIJL$ (gold triangles) cells. The spectra are scaled to the total number of photons counted.

As a control, we analysed the fluorescence kinetics at 77 K upon 440 nm excitation, which excludes the PBS contribution to the dynamics. In this case, the lifetimes and DAES of intact cells were virtually identical to the ones reported earlier for isolated thylakoid membranes (Supplementary Fig. S6). The fluorescence decays reflect mainly the dynamics in PSI, as it has 4–5 times larger absorption cross section than PSII. The most significant difference in the fluorescence kinetics is the reduced amplitude of the DAES emitting at 710–715 nm, attributed to the loss of red Chls at the trimerization region (Akhtar et al., 2021).

Additionally, we compared the fluorescence kinetics of isolated PBSs of WT, *ΔpsaL*, and *ΔFIJL* at room temperature and 77 K (Supplementary Figs. S7, S8). Interestingly, the clear differences in the PC dynamics, that were observed in whole cells, could not be detected in the isolated PBS. This result is in line with the fact that changes in the PC:APC ratio were detected in whole cells but not in isolated PBS.

DISCUSSION

A key finding of this study is that the oligomeric state of PSI exerts control over the abundance of PBPs in *Synechocystis* and, likely, the architecture of the PBSs. The *ΔpsaL* and *ΔFIJL* genotypes with monomeric PSI contained more PBSs on Chl basis compared to the WT genotype with trimeric PSI but, at the same time, fewer PCs per PBS (Tables 1 and 2). In agreement with the spectroscopic quantification, semiquantitative Tricine-SDS-PAGE analysis showed lower PC:APC ratios in cells of the monomeric PSI mutants (Supplementary Fig. S9). Our results are in line with the changes in fluorescence spectra of the *ΔpsaL* mutant of *Synechocystis*, reported by Kłodawska et al. (2020) and attributed to differences in the PBS composition.

By comparing the data from cells and isolated PBSs we can conclude that under the present cultivation conditions WT *Synechocystis* cells contain weakly bound PCs that are dissociated upon PBS isolation. We cannot conclusively determine whether the additional PC makes up for longer rods radiating from the PBS core (the PC:APC ratio suggests six PC hexamers per rod) or more (laterally bound) rods. It is evident, however, that the higher PC content slows down energy migration in the PBS and consequently from the PBS to the photosystems. It has been demonstrated that the rod-core equilibration time increases with the length of the PC rods (Sandström et al., 1988; Zhang et al., 1997). Here, we have found that a fraction of the PC is exceedingly long-lived, suggesting weak energetic coupling that favours parallel or unspecific binding of PC rods. These “semi-free” rods may be easily detached during PBS isolation.

Remarkably, both genotypes with monomeric PSI, grown under the same conditions, contain less PC per PBS. We detected no long-lived PC population in the fluorescence kinetics and there is no evidence for loss of PC during biochemical isolation, which suggests that the PBSs have shorter strongly-coupled PC rods. The modulation of the size and number of PBSs can be understood as an adaptive response. Cyanobacteria are capable of acclimatory regulation of the PBS in response to the physiological conditions. Growth under high irradiance changes the abundance of PBS, reducing the cell PC content (Raps et al., 1985) and shortening of the PC rods has been reported in *Synechococcus* (Samson et al., 1994), *S. maxima* (Garnier et al., 1994), and *S. platensis* (Nomsawai et al., 1999). The rod length seems to be regulated by specific linker proteins (Nomsawai et al., 1999). We could not detect any differences in the PBPs or linker protein content of the PBSs (Supplementary Fig. S9), except for a reduced content of FNR in PBS of *ΔFIJL*. Interestingly, FNR was also missing from a mutant with truncated rods (Leganes et al., 2014). PBPs are also reservoirs for nutrients, degraded when nutrients, e.g. nitrogen, are

scarce (Salomon et al., 2013), so it is plausible that downstream metabolism changes will also alter the PBP content.

We reason that the absence of trimeric PSI in *Synechocystis* results in suboptimal or imbalanced EET, consequently electron transfer, which causes a change in the PBS content and composition. The most logical explanation reconciling all our data is that EET from the PBS to PSI is less efficient in the $\Delta psal$ and $\Delta FIJL$ mutants than it is in WT. Results in support of this are the higher PSII:PSI emission ratio upon PBS excitation (but not upon Chl excitation) and the diminished effective PSI antenna size in $\Delta psal$ and $\Delta FIJL$ cells (but not thylakoid membranes or isolated PSI). As to why trimeric PSI might be more suited for accepting energy from the PBS, there could be several possible reasons. PSI trimers might have higher affinity to the PBSs forming a more stable PBS-PSI supercomplex. This may be in contrast to structural modelling that suggests the existence of a PBS fraction directly attached to PSI monomers (Zlenko et al., 2016). We must also consider the possible role of state transitions (Mullineaux and Emlyn-Jones, 2005; Calzadilla and Kirilovsky, 2020). The $\Delta psal$ mutant of *Synechococcus* PCC 7002 was found to be capable of performing a state II–state I transition faster than the wild type (Schluchter et al., 1996) and Aspinwall et al. (2004) concluded that this was because of higher rates of PBS diffusion. Notably, if the PBSs in $\Delta psal$ are indeed smaller, this should in itself contribute to their higher mobility (Sarcina et al., 2001). However, there is no indication that the mutant is locked or preferentially found in state I. On the contrary, as state transitions are regulated by the redox state of the PQ pool (Mullineaux and Emlyn-Jones, 2005), it should be expected that any relative loss of PSI excitation will shift the balance toward state II (which would cause an opposite change in the fluorescence spectra than observed).

It has been suggested that PsaF facilitates the association of the PBS to PSI (Fromme et al., 2003) and this has been linked to an elevated APC content in mutants lacking the PsaF subunit, observed especially under low growth light. Here we found that APC is equally increased in $\Delta psal$ and in $\Delta FIJL$ (Table 1) and even to a greater extent in low-light-grown cells of both mutants (data not shown). Therefore, we could not discern a particular role of PsaF in the EET from PBS to PSI but the data support the notion that EET is impaired in monomeric PSI. There could be a simple reason for it – supercomplexes of PBSs with trimeric PSI can be more efficient by sharing the PBS among all three PSI RCs – increasing the effective antenna size. Then the increased number of PBSs (APC) in the monomeric PSI strains can be seen as compensatory response for the loss of effective absorption cross-section. However, PBSs can transfer energy to both photosystems (Ashby and Mullineaux, 1999). The PSII:PSI energy partitioning factors have been determined in the range of 50:50 (Tian et al., 2011; Acuña et al., 2018), 60:40 (Mullineaux, 1994) or even higher (Rakhimberdieva et al., 2001). From the Chl ratio in PSII and PSI (4:1) and the fluorescence ratios at 690 and 720 nm in the low-temperature spectra upon Chls and PBSs excitation (Figure 1), we can estimate that 50–60% of the energy from PBS is transferred to PSII in WT. Thus, if there is an imbalance of excitation energy between the two photosystems (because of PSI

monomerization), merely increasing the number of PBSs will exacerbate rather than alleviate it. However, it is known that strains devoid of PBSs or with truncated PBSs rods compensate for the reduced PSII antenna size by increasing the abundance of PSII (Ajilani et al., 1995; Nagarajan et al., 2014; Liberton et al., 2017). Therefore, increasing the number of PBSs while simultaneously reducing their size is a viable strategy to compensate for the effects of PSI monomerization. A similar phenomenon is observed in *Anabaena* mutants with truncated PBSs, which compensate by increasing the number of PBSs per photosystem (Leganes et al., 2014). Still, the energy distribution in the monomer PSI types is shifted to PSII but to a lesser extent than it would be without the compensatory change in the PBSs.

In conclusion, we have found that the trimeric organization of PSI is advantageous for efficient and balanced EET from PBSs in *Synechocystis*. It is possible that trimeric PSI better anchors the PBSs and facilitates EET. Alternatively, the reduced EET efficiency to PSI monomers could be because of entropic reasons or simply because monomers are unable to share the same PBS. Regardless of the actual mechanism, the imbalanced excitation is sensed by the cell, which responds by adjusting the PBSs to optimize energy and electron flow. These results add to the existing body of evidence that the PBSs are a remarkably responsive and tuneable light-harvesting antenna system but also provide a hint of the evolutionary advantage of oligomeric PSI in conjunction with PBSs.

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