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State transitions in the cyanobacterium *Synechococcus elongatus* 7942 involve reversible quenching of the photosystem II core

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ABSTRACT

Cyanobacteria use chlorophyll and phycobiliproteins to harvest light. The resulting excitation energy is delivered to reaction centers (RCs), where photochemistry starts. The relative amounts of excitation energy arriving at the RCs of photosystem I (PSI) and II (PSII) depend on the spectral composition of the light. To balance the excitations in both photosystems, cyanobacteria perform state transitions to equilibrate the excitation energy. They go to state I if PSI is preferentially excited, for example after illumination with blue light (light I), and to state II after illumination with green-orange light (light II) or after dark adaptation. In this study, we performed 77-K time-resolved fluorescence spectroscopy on wild-type *Synechococcus elongatus* 7942 cells to measure how state transitions affect excitation energy transfer to PSI and PSII in different light conditions and to test the various models that have been proposed in literature. The time-resolved spectra show that the PSII core is quenched in state II and that this is not due to a change in excitation energy transfer from PSII to PSI (spill-over), either direct or indirect via phycobilisomes.

1. Introduction

The first step of photosynthesis is light harvesting [1] and in cyanobacteria, the light is harvested by chlorophyll *a* (Chl *a*) molecules in photosystems I and II (PSI and PSII) and bilins in the phycobilisomes (PBSs). The excited-state energy is delivered to the reaction centers (RCs) of both photosystems, where charge separation takes place and the subsequent photochemistry reactions lead to the production of biomass. PSI and PSII are embedded in the thylakoid membrane and both photosystems work in series. In addition, PBSs are used as accessory light-harvesting antennae that are mainly associated with PSII on the cytoplasmic side [2]. Depending on the spectral composition of the light, the relative amounts of energy arriving at the RCs of PSI and PSII can vary. The excitation energy can be redistributed between PSI and PSII, due to a change in the interaction between PBS, PSI, and PSII under redox control of inter-photosystem electron carriers, a process called state transitions [3]. State I is induced when PSI is preferentially excited (illumination by blue or far-red light [3]) and hence the inter-photosystem electron carriers are more oxidized. State II is induced by preferential excitation of PSII (illumination by orange-green light) or by dark-adaptation [3], which results in more reduction of the inter-photosystem electron carriers. State I (state II) is characterized by a high (low) fluorescence yield of PSII at room temperature and a high (low) PSII/PSI emission ratio at 77 K [3]. While dark-adaptation induces state II and blue-light adaptation induces state I, other colors of light such as yellow-orange bring the cells to a state between state I and II [3,4].

Several mechanisms have been suggested for state transitions. One possible mechanism is the transfer of excitation energy from PSII to PSI, which is called spill-over. According to the spill-over model [5,6], excitation energy transfer (EET) takes place between Chl *a* molecules of PSI and PSII without any involvement of PBSs. Reversible migration of PBSs between PSI and PSII has also been used to explain state transitions and in this case only PBSs play a role [7]. A combination of spill-over and slight PBS movement has also been suggested to regulate the energy (re)distribution [8]. In this model PBSs feed excitation energy to both PSI and PSII. The isolation of a functional cyanobacterial mega-complex that includes PSI, PSII and PBS may support this idea [9]. In another study it was concluded that upon dark to light transitions a fraction of the PBSs decouples from PSI, but does not attach to PSII afterwards [10].

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In this study, we used time-resolved fluorescence measurements at 77 K to study state transitions in wild-type (WT) *Synechococcus elongatus* 7942 cells. Time-resolved fluorescence measurements have been used before to study light harvesting and its regulation in cyanobacteria [10–18]. This study attempts (1) to reveal the differences in EET in state I and II and to verify/falsify existing models for state transitions; and (2) to assess whether state I-light II transitions and state II-light II transitions involve the same processes (light I/II is the light with spectral composition that preferentially excites photosystem I/II).

2. Materials and methods

2.1. Strains and growth conditions

Wild-type *Synechococcus elongatus* 7942 and *Synechocystis* PCC 6803 cells were grown at 30 °C in a medium containing 20 ml/l BG-11, 0.85 g/l sodium bicarbonate, and 1.75 g/l sodium nitrate at pH 8.0. A white light source with an intensity of 50 μmol photons $m^{-2} s^{-1}$ was used to illuminate the cells. The spectrum of the growth light is presented in Fig. S1 in the SI. The cells were grown in 250 ml flasks with culture volume of 60 ml that were shaken at 100 rpm.

2.2. Sample preparation

The cells were grown in a fresh medium, starting at an optical density of 0.3 at 800 nm (OD_{800} scattering and 1 cm light path). For time-resolved spectroscopy measurements the cells were harvested at an OD_{800} of ~0.55–0.70. They were diluted with fresh medium to an OD_{630} of ~0.30, as measured with a spectrophotometer with integrating sphere.

The cells were pre-conditioned in four different conditions: dark (state II), dark-blue (state I), dark-orange (state II-to-light II), and dark-blue-orange (state I-to-light II). Dark-blue-orange means that after the cells were dark adapted for at least ~15–20 minutes, they were then illuminated by blue light for ~20 minutes, and subsequently with only orange light for ~20 minutes. Each stage of adaptation took ~20 minutes and the maximum fluorescence upon illumination of a saturating pulse (closing the RCs) was monitored with the pulse-amplitude modulation (PAM) technique to make sure that cells reached an equilibrated state. A 440 nm (full with at half maximum (FWHM) of 40 nm) filter and a 605 nm (FWHM of 30 or 50 nm, both give the same result) filter were used for blue and orange light illumination. The intensity of either color of light was 50–65 μmol photons $m^{-2} s^{-1}$ for *Synechococcus elongatus* 7942 and 35–50 μmol photons $m^{-2} s^{-1}$ for *Synechocystis* PCC 6803. Samples were collected in a glass Pasteur pipette with a diameter of ~1 mm and were frozen in liquid nitrogen for the fluorescence measurements.

2.3. Steady-state absorption spectroscopy

A Cary 5E spectrophotometer, equipped with an integrating diffuse reflectance sphere (DRA-CA-50, Labsphere) was used to measure absorption spectra and correct for light scattering by the cells. To start growing the cells at OD_{800} of 0.3 and to harvest them at an OD_{800} of ~0.55–0.70, we did not use the integrating diffuse reflectance sphere.

2.4. Steady-state fluorescence spectroscopy

Steady-state fluorescence at 77 K was recorded with a Jobin Yvon Fluorolog FL3–22 spectrofluorimeter. The recorded spectrum was corrected for wavelength-dependent sensitivity of the detector and fluctuations in the lamp output. The excitation wavelengths were 577 nm and 430 nm with a bandwidth of 4.5 nm. The detection bandwidth was 2 nm. The spectra were averaged over several measurements.

2.5. Time-resolved fluorescence spectroscopy

Time-resolved fluorescence spectroscopy was performed using a picosecond streak-camera system as described before [10,16,19]. 800 nm pulsed light from a Ti:sapphire laser (Coherent, Mira) was fed into a FemtoWHITE 800 tube (NKT photonics, part of Thorlabs' Supercontinuum generation kit). From its broad spectral output (470–700 nm), light centered at 577 nm (FWHM of 10 nm) was selected with a bandpass filter. For 430 nm excitation the frequency-doubled output of the Ti:sapphire laser (860 nm) was used. The repetition rate of the laser was 3.8 MHz with a power of ~30 μW for 577 nm and ~100 μW for 430 nm excitation. A lens with focal length of ~7 cm was used to focus the light resulting in a spot size of ~0.1 mm in both cases.

2.6. Global analysis of time-resolved fluorescence data

The fluorescence decay was recorded with 2000-ps and 800-ps time windows. The 800-ps time window has a better time resolution and allows to resolve the faster fluorescence decay processes. The 2000-ps time window allows for a better estimation of longer lifetimes and it was used to verify if the ns fluorescence decay component recorded with 800-ps time window was estimated correctly, which was indeed the case. Each image was corrected for the wavelength dependency of the detector and then sliced into ~4 nm time traces.

Several measurements on dark-adapted cells (state II) were analyzed together so that all the measurements have the same lifetimes and decay-associated spectra (DAS); the DAS for different measurements on dark-adapted cells could only differ by a constant accounting for different levels of the fluorescence intensity. The same procedure was used for other measurements with the cells in the same condition, such as all measurements on blue-adapted cells (state I). The measurements performed on state I-to-light II and state II-to-light II were fitted with the same lifetimes while their amplitudes were fitted independent of each other. Global analysis, based on the singular-value decomposition (SVD) method, was performed as described in [10], using the Glotaran [20] and TIMP package [21] for R. The instrument response function (IRF) of the streak-camera setup was described as a Gaussian function with its FWHM as a free fitting parameter. Global analysis of the data led to a FWHM of ~11 ps and ~24 ps at time windows 800 ps and 2000 ps, respectively. In all cases a fit with five components was required according to the SVD method and no meaningful fits with six components were possible, despite the high signal-to-noise ratios. The quality of the fits is shown in the Supplementary material (Figs. S2–S4).

3. Results

3.1. Steady-state fluorescence spectroscopy

In Fig. 1, the steady-state fluorescence spectra of blue-light- and dark-adapted cells (state I and II, respectively) are shown. The spectra were recorded upon excitation with 577 nm (mainly PBS excitation) and 430 nm (mainly Chl *a* excitation) light, hereafter called 577 nm and 430 nm spectra. The 577 nm and 430 nm spectra were normalized to the peaks at ~655 nm and ~717 nm, respectively. In order to normalize the spectra in an independent way, we also added fluorescein for 430 nm excitation (77 K fluorescence peak at 500 nm) and rhodamine B for 550 nm excitation (77 K fluorescence peak at ~570 nm) to compare the samples under different light conditions. Normalizing the recorded spectra to the fluorescence peaks of the added dyes yielded the same results as those shown in Fig. 1 (see SI, Figs. S5–S6). Upon 430 nm and 577 nm excitation four peaks were resolved that belong to C-phycocyanin (C-PC) and allophycocyanin (APC) (655 nm) [22], CP43 in PSII and the terminal emitters of PBS (APC680) (683 nm) [22–24], CP47 and the RCs of PSII (695 nm) [22–24], and to PSI (717 nm) [25].

Steady-state spectra obtained both with 577 nm and 430 nm excitation light demonstrated that cells in state I showed a major increase