FLUORESCENCE DECAY AND ENERGY TRANSFER CHARACTERISTICS OF THE CP43-PREASSEMBLY COMPLEX OF PHOTOSYSTEM II IN *SYNECHOCYSTIS* 6803

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INTRODUCTION

- Nonphotochemical quenching (NPQ) in cyanobacteria is attributed to orange carotenoid protein and phycobilisome antenna with no contribution from chlorophyll-containing PSII assembly intermediates.
- Photosystem II assembly intermediates (primarily, CP43- and CP47-preassembly complexes) have both chlorophyll *a* and β-carotenes bound and thus they are capable of light absorption.
- Our previous work[1] suggested that energy transfer from CP43 to reaction center is much faster than from the CP47 antenna protein.
- Cells normally accumulate ~10% Psb27 containing CP43-preasembly complexes of total PSII complexes[2]. The contribution of the CP43-preassembly complex to overall NPQ thus could not be overlooked.
- With negligible efforts made towards spectroscopic assessment of the CP43-preassembly complex, it becomes difficult to comment on its contribution to the overall NPQ.
- We constructed a CP47-deletion (ΔCP47) mutant in the CP43-6×His (CP43H) background of *Synechocystis* 6803 to isolate and evaluate the CP43 preassembly complex spectroscopically.

CONCLUSIONS

Global analysis of TA after excitation of β-carotene clearly shows that S_1 excited state of the carotenoid does not contribute to energy transfer (its lifetime is identical with one measured in organic solvents) and all excitation transfer is exclusively associated with transfer from the S_2 state.

Chlorophyll triplet is efficiently formed, and estimated triplet formation yield is 21%. Chl *a* triplet is not efficiently quenched by carotenoid.

Effective triplet lifetime of Chl *a* was estimated at 31 ns, which is also the time constant of the carotenoid-triplet rise curve; therefore, even under aerobic environment, Chl-triplets could be quenched by carotenoids and by molecular oxygen.

Chlorophyll triplet quenching seems to be complete. The remaining signal that has triplet-like characteristic decays with the same time constant as carotenoid triplet (~2.5 μs); therefore, is associated with the so-called interaction spectrum due to the response to the neighboring carotenoid triplet.

RESULTS

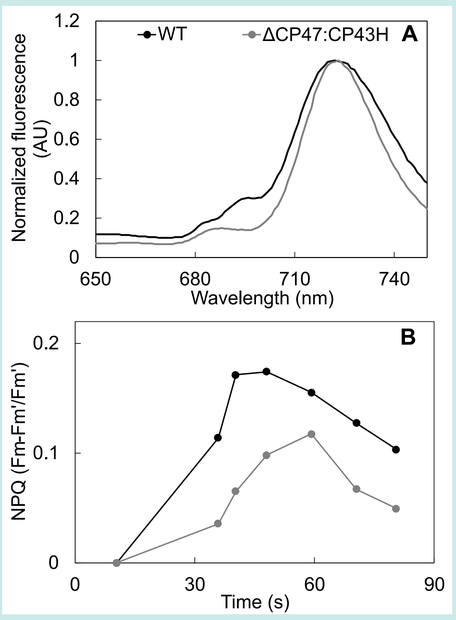

Figure 1: Physiological assessment of the ΔCP47:CP43H strain.

A) 77 K fluorescence emission spectra on excitation at 435 nm. The ΔCP47:CP43H strain is compared with wild type (WT) to highlight the changes in emission when PSII assembly is blocked.

B) Nonphotochemical quenching in the whole cells assessed by exposing dark-adapted cells to intense blue light (preferential excitation of PSII chlorophyll *a*) between 30 to 60 s.

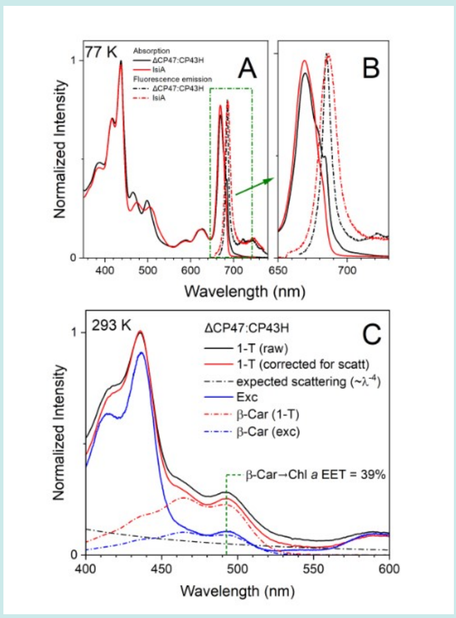

Figure 2: Basic spectroscopic characterization of ΔCP47:CP43H pre-assembly complex.

(A) Steady-state absorption and fluorescence spectra taken at 77 K in glycerol/buffer medium (60/40 v/v). For comparison, the complementary spectra of the IsiA protein (CP43′) are added.

(B) Qy spectral range zoomed to highlight apparent differences in Qy absorption band and different fluorescence band widths for both complexes.

(C) Determination of carotenoid (β-carotene)-to-Chl excitation energy transfer (EET) based on comparison of fluorescence excitation (Exc) and absorptance (1-T, T-transmittance) spectra. Detailed analysis demonstrates that EET=39%.

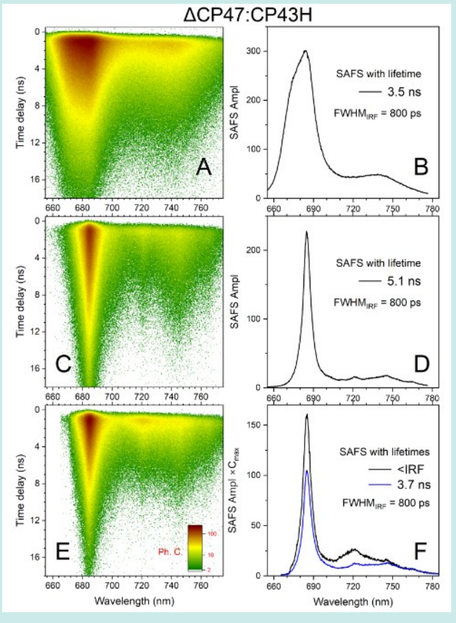

Figure 3: Time-resolved fluorescence imaging of ΔCP47:CP43H in various buffers and temperatures.

Pseudo-color 2D maps of fluorescence decay after excitation at 410 nm (Soret band) and global fitting (A, B) at 293 K (room temperature) (C, D) at 77 K in plain sample buffer and (E, F) at 77 K in glycerol/buffer (60/40 v/v).

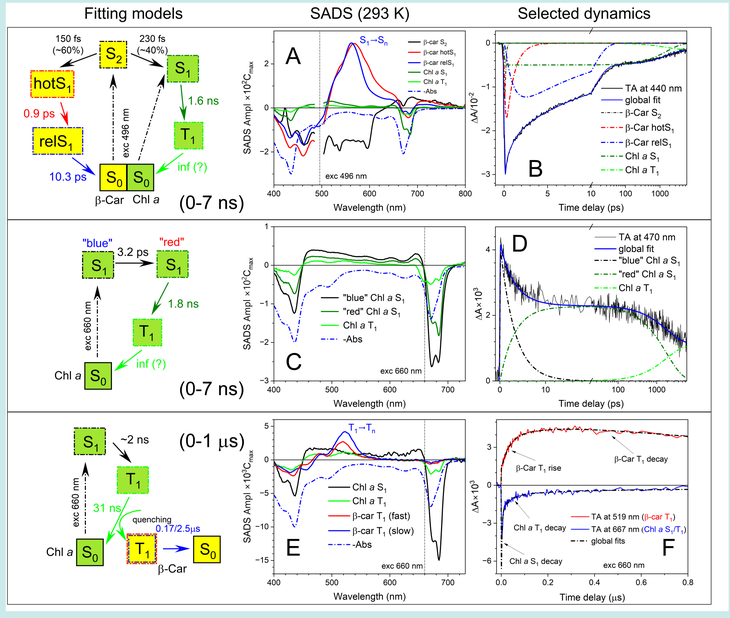

Figure 4: Transient absorption (TA) of ΔCP47:CP43H.

(A) Global analysis of TA (SADS) after excitation to S_2 state of β-carotene recorded in 7 ns delay time window and (B) selected TA trace reconstructed to contributions of individual transient species. (C) Global analysis of TA (SADS) after excitation to Qy band of Chl *a* recorded in 7 ns delay time window and (D) selected TA trace reconstructed to contributions of individual transient species. (E) Global analysis results of TA (SADS) after excitation to Qy band of Chl *a* recorded in 1 μs delay time window and (F) selected TA traces highlighting coupling of Chl *a* triplet decay with rise of β-carotene triplet (sensitization of carotenoid triplet). Each SADS results are accompanied by steady-state absorption spectrum of ΔCP47:CP43H (reversed and scaled) for reference. Fitting models are depicted on left side. Excitation wavelengths are marked with vertical dash-dot line.

The fluorescence emission spectra of IsiA is substantially wider compared to the CP43-preassembly complex, suggesting that only a few closely energetically spaced chlorophylls (monomers) contribute to the emission in IsiA.

Abbreviations: SADS – species associated difference spectra, inf – infinite (too long to be correctly defined), exc – excitation at, (hot)relS$_1$ – vibrationally (not-)relaxed S$_1$ state of β-carotene, SAFS – species associated fluorescence spectra, IRF – instrument response function, <IRF – shorter than FWHMIRF, FWHM – full width at half maximum, Ph. C. – photon counts, SAFS Ampl – SAFS amplitude.

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References

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