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$P700^+$- and $^3P700$-induced quenching of the fluorescence at 760 nm in trimeric Photosystem I complexes from the cyanobacterium *Arthrospira platensis*

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Abstract

The 5 K absorption spectrum of Photosystem I (PS I) trimers from *Arthrospira platensis* (old name: *Spirulina platensis*) exhibits long-wavelength antenna (exciton) states absorbing at 707 nm (called C707) and at 740 nm (called C740). The lowest energy state (C740) fluoresces around 760 nm (F760) at low temperature. The analysis of the spectral properties (peak position and line width) of the lowest energy transition (C740) as a function of temperature within the linear electron–phonon approximation indicates a large optical reorganization energy of ~110 cm^{-1} and a broad inhomogeneous site distribution characterized by a line width of ~115 cm^{-1}. Linear dichroism (LD) measurements indicate that the transition dipole moment of the red-most state is virtually parallel to the membrane plane.

The relative fluorescence yield at 760 nm of PS I with P700 oxidized increases only slightly when the temperature is lowered to 77 K, whereas in the presence of reduced P700 the fluorescence yield increases nearly 40-fold at 77 K as compared to that at room temperature (RT). A fluorescence induction effect could not be resolved at RT. At 77 K the fluorescence yield of PS I trimers frozen in the dark in the presence of sodium ascorbate decreases during illumination by about a factor of 5 due to the irreversible formation of $P700^+F_{A/B}^-$ in about 60% of the centers and the reversible accumulation of the longer-lived state $P700^+F_X^-$.

The quenching efficiency of different functionally relevant intermediate states of the photochemistry in PS I has been studied. The redox state of the acceptors beyond A_0 does not affect F760. Direct kinetic evidence is presented that the fluorescence at 760 nm is strongly quenched not only by $P700^+$ but also by $^3P700$. Similar kinetics were observed for flash-induced absorbance changes attributed to the decay of $^3P700$ or $P700^+$, respectively, and flash-induced fluorescence changes at 760 nm measured under identical conditions.

A nonlinear relationship between the variable fluorescence around 760 nm and the $[P700_{red}]/[P700_{total}]$ ratio was derived from titration curves of the absorbance change at 826 nm and the variable fluorescence at 760 nm as a function of the redox potential imposed on the

Abbreviations: A_0, primary electron acceptor in PS I; A_1, secondary electron acceptor in PS I (a phylloquinone); Chl *a*, chlorophyll *a*; Chl *a*′, C-13 epimer of Chl *a*; C708 (C740), Chl absorbing at 708 (740) nm; CAPS, 3-(cyclohexylaminol-1-propanesulfonic acid); β-DM, *n*-dodecyl-β-D-maltoside; DPIP, 2,6-dichlorophenolindophenol; E_m, midpoint potential; FeS, iron–sulfur cluster; F_X, F_A and F_B, three [4Fe–4S] clusters in PS I; F760, fluorescence with an emission maximum at 760 nm; LHC I, light-harvesting complex of Photosystem I; P700 ($P700^+$), primary electron donor of PS I in the reduced (oxidized) state; $^3P700$, P700 in the excited triplet state; P_A and P_B, the two chlorophylls constituting P700 coordinated by subunit PsaA and PsaB, respectively; PMS, phenazine methosulfate; PS I (PS II), Photosystem I (Photosystem II); RT, room temperature; $t_{1/2}$, half-life; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine dihydrochloride; T−S, triplet-minus-singlet

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sample solution at room temperature before freezing. The result indicates that the energy exchange between the antennae of different monomers within a PS I trimer stimulates quenching of F760 by $P700^+$.

Keywords: Photosystem I; P700; Energy transfer; Fluorescence quenching; Transient absorbance spectroscopy; Long-wavelength antenna chlorophyll; *Arthrospira platensis*

1. Introduction

Photosystem I (PS I) is a pigment–protein complex located in the thylakoid membranes of cyanobacteria, algae and plants that mediate light-induced electron transfer from plastocyanin or cytochrome c_6 on the lumenal side to ferredoxin on the stromal side (for a review see Refs. [1,2] and references therein). PS I of higher plants and algae (named PS I-200) consists of the PS I core complex and the peripheral light-harvesting complex (LHC I). Cyanobacteria lacking LHC I contain only the PS I core. The PS I core complex coordinates all of the redox cofactors and the core antenna of ~100 chlorophyll *a* (Chl *a*) and ~20 β-carotene molecules. The PS I core complexes in cyanobacteria are organized preferentially as trimers [3–6], whereas PS I in higher plants and algae is present only as a monomer [7]. Structural characterization of green plant PS I by electron microscopy [8], as well as the recent X-ray structure of plant PS I at 4.4 Å resolution [9] indicate that LHC I (Lhca1–4) is only attached in a half-moon shape to the core complex at the side of the subunits PsaF/PsaJ of the core and thus does not cause structural hindrances for trimerization.

A high-resolution X-ray structure is available for trimeric PS I core complexes from the cyanobacterium *Thermosynechococcus elongatus* [10]. The structure exhibits 12 protein subunits, 96 Chls, 22 carotenoids (Car), 2 phylloquinones, 3 iron–sulfur [4Fe–4S] clusters, 4 lipids, about 200 water molecules and a metal ion (presumably Ca^{2+}) for each PS I monomer.

The two large subunits, PsaA and PsaB, each consisting of 11 transmembrane helices, coordinate most of the antenna pigments and the following redox cofactors involved in the electron-transfer process: the primary electron donor P700 (a heterodimer of chlorophyll *a* (P_B) and *a*′ (P_A), the primary acceptor A_0 (a Chl *a* monomer), the secondary acceptor A_1 (a phylloquinone), and F_X (a [4Fe–4S] iron–sulfur–cluster). The terminal electron acceptors F_A and F_B (two [4Fe–4S] iron–sulfur clusters) are both coordinated by subunit PsaC, one of the three extrinsic subunits located on the stromal side.

After absorption of light by an antenna pigment, the excitation energy is efficiently trapped via charge separation in the reaction center. P700 in the lowest excited singlet state donates an electron to the primary acceptor A_0. Charge stabilization is achieved by subsequent electron transfer to the secondary acceptor A_1, then further to F_X and finally to the terminal electron acceptors F_A and F_B. An interesting aspect of the electron transfer in PS I is the reported heterogeneity at low temperature. In one fraction of the PS I complexes, an irreversible charge separation due to the stable formation of $P700^+F_A^-$ and $P700^+F_B^-$ takes place, whereas in the other fraction, forward electron transfer to the terminal iron–sulfur clusters is completely blocked at cryogenic temperatures [11,12]. In this fraction, the charge separation is reversible at low temperature and can be attributed to the formation and decay of $P700^+A_1^-$ and $P700^+F_X^-$. The charge recombination of $P700^+A_1^-$ occurs with $t_{1/2}$ ~200 μs. This half-life was found to be almost temperature independent [12]. The charge recombination between F_X^- and $P700^+$ takes place in the millisecond time range at low temperature.

When the forward electron transfer to A_1 is prevented by prereduction or removal of A_1, the primary radical pair, $P700^+A_0^-$, can decay by three pathways: (i) by charge recombination to the singlet ground state, (ii) by an activated back reaction to the excited singlet state of P, and (iii) after singlet–triplet mixing in the radical pair, by charge recombination to $^3P700A_o$. The lowest excited triplet state of P700, ^{3}P700, is formed at 5 K with high yield and decays in the millisecond time range ($t_{1/2} \approx 0.7$ ms (65%) and 7 ms (35%)).

The function of the antenna is to harvest solar energy and to transfer this energy to the reaction center, where the excitation energy is converted into a charge separated state. One striking feature of the light-harvesting antenna of PS I is the presence of long-wavelength (red, low-energy) Chls that absorb at energies lower than that of the primary electron donor P700 (for reviews see Refs. [13–16]). Plants and some algae contain their most-red Chls in the LHC I, which give rise to the emission around 735 nm at low temperature, whereas long-wavelength Chls in the PS I core of plants and algae absorb around 705 nm and emit at 720 nm. The existence of red Chls in the core antenna absorbing even further to the red is unique to cyanobacteria. The content and the spectral characteristics of long-wavelength Chl *a* antenna states are species-dependent. PS I trimers usually contain more red Chls than monomers. The 5 K absorption spectrum of PS I trimers of *T. elongatus* exhibits Chl *a* antenna states absorbing at 708 and 720 nm [17,18], whereas PS I trimers of *Synechocystis* sp. PCC 6803 contain red Chls peaking at 708 and 714 nm [19–22]. The most-red Chl *a* antenna state absorbing at 740 nm (C740) and emitting at 760 nm (F760) at low temperature is present in PS I trimers of *Arthrospira platensis* [5,23,24]. The intensity of the fluorescence at 760 nm (F760) in PS I trimers of *A. platensis* is highly dependent on the redox state