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Characterization of wave phenomena in the relaxation of flash-induced chlorophyll fluorescence yield in cyanobacteria ☆

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ABSTRACT

Fluorescence yield relaxation following a light pulse was studied in various cyanobacteria under aerobic and microaerobic conditions. In *Synechocystis* PCC 6803 fluorescence yield decays in a monotonous fashion under aerobic conditions. However, under microaerobic conditions the decay exhibits a wave feature showing a dip at 30–50 ms after the flash followed by a transient rise, reaching maximum at ~1 s, before decaying back to the initial level. The wave phenomenon can also be observed under aerobic conditions in cells preilluminated with continuous light. Illumination preconditions cells for the wave phenomenon transiently: for few seconds in *Synechocystis* PCC 6803, but up to one hour in *Thermosynechocystis elongatus* BP-1. The wave is eliminated by inhibition of plastoquinone binding either to the Q_B site of Photosystem-II or the Q_o site of cytochrome b_6f complex by 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea or 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone, respectively. The wave is also absent in mutants, which lack either Photosystem-I or the NAD(P)H-quinone oxidoreductase (NDH-1) complex. Monitoring the redox state of the plastoquinone pool revealed that the dip of the fluorescence wave corresponds to transient oxidation, whereas the following rise to re-reduction of the plastoquinone pool. It is concluded that the unusual wave feature of fluorescence yield relaxation reflects transient oxidation of highly reduced plastoquinone pool by Photosystem-I followed by its re-reduction from stromal components via the NDH-1 complex, which is transmitted back to the fluorescence yield modulator primary quinone electron acceptor via charge equilibria. Potential applications of the wave phenomenon in studying photosynthetic and respiratory electron transport are discussed. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.

1. Introduction

Measurement of flash-induced chlorophyll (Chl) fluorescence relaxation has been proven to be a highly informative method to study the kinetics of photosynthetic electron transport processes, which lead to changes in the redox state of the Q_A primary quinone electron acceptor of Photosystem II (see Refs. [1–7]). The underlying phenomenon behind the method is the modulation of the yield of variable Chl fluorescence by the reduction state of Q_A, which leads to high fluorescence yield when Q_A is in the reduced state, and to low fluorescence yield when Q_A is in the oxidized state (see [8–10]). As a consequence, all of the electron transport processes that lead to changes in the redox state of Q_A can be monitored in a technically straightforward way by measuring Chl fluorescence yield.

Single turnover flash illumination transfers an electron from the water oxidizing complex to the first quinone electron acceptor, Q_A, which results in a prompt increase of fluorescence yield due to formation of Q_A^-. In PSII centers with functional donor and acceptor side electron transfer the flash-induced increase of fluorescence yield usually relaxes in a monotonously decreasing fashion, consisting of three main phases (see [1–6]). These phases are assigned to different pathways of Q_A^- reoxidation as discussed in [11–13]: (i) by Q_B (or Q_B^-) which is bound to the Q_B site at the time of the flash (fast phase, T_1 ~ 300–500 μs), (ii) by plastoquinone (PQ) which binds to the Q_B site after the flash (middle phase, T_2 ~ 5–15 ms), and (iii) by recombination of the electron on $Q_AQ_B^-$, via the $Q_A^-Q_B \leftrightarrow Q_AQ_B^-$ charge equilibrium, with the oxidized S_2 (or S_3) state of the water-oxidizing complex (slow phase, T_3 ~ 10–20 s). In the presence of electron transport inhibitors, which block the Q_B site, such as DCMU, the relaxation is dominated by a slow component (1–2 s), that reflects the reoxidation of Q_A^- via charge recombination with the S_2 state. When Q_B site inhibition occurs in PSII centers in which electron transfer is partly, or completely inhibited between the water oxidizing complex and Tyr-Z, that serves

Abbreviations: Chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DMBQ, 2,5-dimethyl-p-benzoquinone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; NDH-1, NAD(P)H-quinone oxidoreductase; PQ and PQH_2, plastoquinone and plastoquinol, respectively

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as intermediate electron donor to P_{680}^{+}, Tyr-Z^{+} will be stabilized and will act as a recombination partner of Q_A^- instead of the S_2 (S_3) state leading to a fast decaying (few ms) fluorescence relaxation component [2,14].

Analysis of the fluorescence decay phases gives useful information on the kinetics of the above described electron transport processes, and makes it possible to calculate the rate of forward electron transport between Q_A and Q_B (Q_B^-), the binding rate of PQ to the Q_B site, as well as the rates of charge recombination between the S_2 state and the reduced Q_A and Q_B acceptors. In addition, it is possible to estimate the free energy (redox potential) differences between various acceptor ($Q_A \leftrightarrow Q_B$) and donor (Tyr-Z $\leftrightarrow$ S_2) components (see [1–5]). Since the method can be applied both in isolated thylakoid membrane preparations, and in intact cells it has become a widely used approach to study modifications of PSII electron transfer caused either by environmental stress effects, or by various mutations occurring naturally or produced by targeted research efforts (see Refs [1–7,15–20]). Although the dominating amount of literature data shows monotonic decay of the flash-induced fluorescence yield we have previously observed unusual wave features in the fluorescence relaxation in intact cells of the cyanobacterium *Thermosynechococcus elongatus* BP-1 (denoted as *Thermosynechococcus*) [21]. A biphasic decay of fluorescence showing a shoulder was also observed in *Synechocystis* sp. PCC 6803 (which will be denoted as *Synechocystis* hereafter) [18]. This wave phenomenon was investigated here in more detail in various cyanobacteria. The results demonstrate that wave features in the decay of flash-induced fluorescence yield represent a general phenomenon that reflects transient changes in the reduction level of the PQ pool due to imbalance between the different electron transport pathways of the thylakoid membrane.

2. Materials and methods

2.1. Culture conditions

Synechocystis and *Synechococcus* sp. PCC 7942 (denoted as *Synechococcus* hereafter) cells were grown in BG-11 medium on a rotary shaker under continuous illumination of 40 μmol photons m^{-2} s^{-1} intensity white light at constant temperature of 30 °C, supplied by 3% CO_2. The M55 (ΔndhB) mutant of *Synechocystis* [22] was a kind gift of Dr. Teruo Ogawa, and was grown under the same conditions as the WT in BG-11 medium supplemented with kanamycin (20 μg mL^{-1}). The PSI-less (Δ*psaAB*) mutant obtained from the laboratory of Prof. Vermaas [23] was grown under 5 μmol photons m^{-2} s^{-1} light intensity in BG-11 medium supplemented with glucose (10 mM) and chloramphenicol (25 μg mL^{-1}). High NDH-1 activity of the WT cells was achieved by growing the cultures under higher light intensity of 60–80 μmol photons m^{-2} s^{-1}, without extra CO_2 supply. Unless stated otherwise, cells used for measurements were grown under white light illumination of 40 μmol photons m^{-2} s^{-1} and the growing chamber was supplied by 3% CO_2.

Thermosynechococcus elongatus BP-1 cells were routinely grown in BG-11 medium in a rotary shaker at 40 °C under 0.1% CO_2 atmosphere. The intensity of the illumination was 40 μmol photons m^{-2} s^{-1}. For freeze–thaw treatment cells were kept at −80 °C for 2 h, then thawed on ice and kept at room temperature for 2 h in the dark. Thylakoids from *Thermosynechococcus* were isolated as in [15], and were a kind gift from the laboratory of Prof. James Barber. Chl concentration of the thylakoids was 10 μg mL^{-1}.

Acaryochloris marina MBIC11017 (denoted as *Acaryochloris*) cells were obtained from the laboratory of Dr. Min Chen and were grown in artificial sea water plus iron (4 mg L^{-1}) at 6–10 μmol photons m^{-2} s^{-1}, at 27 °C in conical flasks during continuous shaking. For measurements cells were harvested in their late exponential growth phase. After centrifugation (4000 ×*g* for 10 min) cells were resuspended in fresh culture medium to achieve final Chl concentration of 5 μg mL^{-1}.

2.2. Microaerobic conditions

Microaerobic conditions were achieved by the addition of glucose, glucose oxidase (Sigma) and catalase (Sigma) at final concentration of 10 mM, 7 U mL^{-1} and 60 U mL^{-1}, respectively. Under our experimental conditions oxygen content of the sample dropped below 1 μmol L^{-1} within 3 min as monitored with an immersible fiber-optic oxygen meter (Fibox 3, Presens).

2.3. Flash-induced increase and subsequent decay of chlorophyll fluorescence yield

Flash-induced increase and subsequent decay of chlorophyll fluorescence yield was measured using a double-modulation fluorometer (FL-3000, Photon System Instruments, Brno) [24]. For measurements cells were harvested in their late exponential growth phase. After centrifugation (4000 ×*g* for 10 min) cells were resuspended in fresh culture medium to achieve final Chl concentration of 5 μg mL^{-1}. The instruments LED system provides both single turnover saturating actinic flashes (20 μs, 639 nm) and measuring flashes (8 μs, 620 nm). Fluorescence decay was recorded in the 150 μs to 100 s time range on a logarithmic time scale. Flash-induced fluorescence relaxation curves were analyzed as described by [2]. Multicomponent deconvolution of the monotonically decreasing curves was done by using a fitting function with two exponential components (fast and middle phase) and one hyperbolic component (slow phase):

$$F_{v,corr} = A_1 \exp(-t/T_1) + A_2 \exp(-t/T_2) + A_3/(1 + t/T_3) + A_0$$

where $F_v = F(t) - Fo$, F(t) is the fluorescence yield at time t, Fo is the basic fluorescence level before the flash, A_1–A_3 are the amplitudes, and T_1–T_3 are the time constants. Non decaying fluorescence component in the time span of the measurement is described by a constant A_0 amplitude. $F_{v,corr}$ is the variable fluorescence yield corrected for non-linearity, taking into consideration the nonlinear correlation between fluorescence yield and the redox state of Q_A using the Joliot model [25], with a value of 0.5 for the energy-transfer parameters between PSII units.

2.4. Post-illumination rise of the fluorescence yield

Post-illumination rise of the fluorescence yield [26] was measured by a double-modulation fluorometer (FL-3000, Photon System Instruments, Brno). Continuous red (639 nm) actinic illumination was applied for 30 s corresponding to the growth light intensity of the examined cells followed by a dark period. Fluorescence was detected by weak measuring flashes (8 μs, 620 nm) at every 1 s in the last 5 s of the illumination period, then every 50 ms in the first 2 s of the dark period, followed by 0.5 s repetition rate of measuring flashes in the remaining time of dark adaptation.

2.5. Simulation of fluorescence traces

Simulation of fluorescence traces was performed by using a mathematical model of electron transport processes on the basis of an electron transport network, which is shown in Scheme 1. The model includes 52 electron transport processes, which are listed in Supplementary Table 1. These processes are described by a set of connected linear differential equations according to the topology shown in Scheme 1, and solved numerically using the Matlab software package. The rate constants of the forward and backward electron transport processes (shown in Supplementary Table 2) were taken from the literature, or obtained from our own measurements. When data were not available for the backward rates we used the reasonable estimation that the reverse processes proceed with 5–10% rate of the forward processes.