

Examination of chlorophyll fluorescence decay kinetics in sulfur deprived algae *Chlamydomonas reinhardtii*

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Received 29 September 2006; received in revised form 17 April 2007; accepted 24 April 2007

Available online 3 May 2007

Abstract

Chlorophyll fluorescence decay kinetics was measured in sulfur deprived cells of green alga *Chlamydomonas reinhardtii* with a home made picosecond fluorescence laser spectrometer. The measurements were carried out on samples either shortly adapted to the dark ('Fo conditions') or treated to reduce Qa ('Fm conditions'). Bi-exponential fitting of decay kinetics was applied to distinguish two components one of them related to energy trapping (fast component) and the other to charge stabilization and recombination in PS 2 reaction centers (slow component). It was found that the slow component yield increased by 2.0 and 1.2 times when measured under 'Fo' and 'Fm conditions', respectively, in sulfur deprived cells as compared to control ones. An additional rapid rise of the slow component yield was observed when incubation was carried out in a sealed bioreactor and cell culture turned to anaerobic conditions. The obtained results strongly indicate the existence of the redox control of PS 2 activity during multiphase adaptation of *C. reinhardtii* to sulfur deficiency stress. Probable mechanisms responsible for the observed increased recombinant fluorescence yield in starved cells are discussed.

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Keywords: Chlorophyll fluorescence; Laser spectrometry; Photosystem 2; Sulfur deprivation; *Chlamydomonas reinhardtii*

1. Introduction

Sulfur deprivation (SD) is a biotechnological procedure providing photoinduced production of molecular hydrogen by green alga *Chlamydomonas reinhardtii* cultivated in bioreactor [1]. As known, SD causes dramatic alterations of cell physiology, including cessation of cell division, accumulation of starch, elevation of intracellular 'reducing pressure' and a respiration rates, and decline in photosynthesis [2,3]. The latter occurs due to the significant degradation of D1 protein of photosystem (PS) 2 and Rubisco [3]. The elevated respiratory and reduced PS 2 activity lead to a progressive decrease in oxygen concentration when cultivation is carried out in a sealed bioreactor. The establishment of anaerobic conditions is followed by the complete

reversible down regulation of PS 2 activity and expression of O₂-sensitive hydrogenase which generates H₂ to decrease the excess of the 'reducing power' in a cell [1]. Examination of the PS 2 functional state is of great importance to understand the mechanisms regulating cell adaptation to SD stress, including H₂ production.

As known, limitation of higher plants and algae of macronutrients, such as nitrogen or sulfur, causes gradual irreversible inactivation of PS 2 accompanied by an increase in chlororespiration [3,4]. PS 2 photoinhibition during the limitation of macronutrients can be related to the accumulation of long-lived reduced forms of Qa, which may leave the binding place in D1-protein, thus inducing disassembling and irreversible damage of PS 2 [5]. The reversible mechanism of PS 2 activity regulation via the Qa redox state was also proposed to occur during the anaerobic phase of *C. reinhardtii* incubation in sulfur-depleted medium in a sealed bioreactor [6]. However, there is no detailed evidence on the interrelationship between PS 2 activity and the redox state of Qa during multiphase adaptation of *C. reinhardtii* to sulfur-deficiency stress. A pronounced

Abbreviations: Chl, chlorophyll; PS, photosystem; PQ, plastoquinone; SD, sulfur deprivation or sulfur deprived; Qa, primary quinone acceptor; OEC, oxygen-evolving complex; DCMU, -3-(3,4-dichlorophenyl)-1,1-dimethylurea

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increase in chlorophyll (Chl) fluorescence emission, including maximal yield (Fm), was demonstrated in SD *C. reinhardtii* during aerobic incubation [7], indicating the modified processes of energy deactivation in closed PS 2 centers.

In this work we investigated processes of energy transduction in PS 2 of SD *C. reinhardtii* by means of a fluorescence laser spectrometer, which provides data on Chl fluorescence decay kinetics in a picosecond time range [8]. Bi-exponential fitting of decay kinetics was applied to distinguish two components one of them related to energy trapping (fast component) and the other to charge stabilization and recombination in PS 2 reaction centers (slow component). Changes of the slow component of the decay kinetics during culture incubation in sulfur depleted medium correlated fairly well with PS activity, estimated by the $\Delta F/F_m'$ ratio. Sulfur deficiency increased the yield of the slow component in closed PS 2 centers where Qa was reduced shortly before measurements. The results suggest: (a) redox control of the PS 2 activity during aerobic and anaerobic phases of *C. reinhardtii* adaptation to SD, and (b) primary photochemical events are modified under SD conditions.

2. Materials and methods

2.1. Culture conditions and sulfur depletion

C. reinhardtii strain Dang 137+ was grown photoheterotrophically in tris-acetate-phosphate medium, pH 7.0, in Erlenmeyer flasks at 25 °C under continuous illumination ($100 \mu\text{E m}^{-2} \text{s}^{-1}$) accompanied by constant shaking (60 rpm). Late-log cells ($4\text{--}6 \times 10^6$ cells/ml) were pelleted three times upon centrifugation and resuspended in the same medium or in S-depleted medium to a concentration of 4×10^6 cells/ml, followed by 72 h aerobic incubation in conic vials (250 ml) or aerobic/anaerobic incubation in a sealed reactor vessel (1 l) under the same light and temperature conditions, and constant shaking/stirring.

2.2. Measurements and analysis of fluorescence decay kinetics in a picosecond time range

Fluorescence kinetics was registered in cell suspensions of *C. reinhardtii* (Chl ($a+b$) = $20 \mu\text{g/ml}$, OD_{532} = 0.6) with a home-made pulse fluorometer [8]. A sample in the cuvette with optical length 2 mm was excited by ps-pulses of light ($\lambda = 532 \text{ nm}$, $\text{fwhm} = 30 \text{ ps}$, $E = 10^{-5} \text{ J}$) from a frequency-doubled Nd:YAG laser at a repetition rate of 1 Hz. Laser energy was $1.5 \mu\text{J}$ per pulse, and the laser beam at the sample had the diameter 2 mm. Optical density of the sample at $\lambda = 530 \text{ nm}$ was 0.15. These conditions provide the absorbed intensity of single pulse not more than 4×10^{13} photon/ cm^2 at $\lambda = 530 \text{ nm}$. The time-course of pulse-induced fluorescence kinetics was obtained by means of a streak-camera (Agat SF3, Russia) connected to the multichannel CCD matrix detector (C7041, Hamamatsu). To separate fluorescence signal from excitation, the long-pass filter KS-11 (transparency $\lambda > 600 \text{ nm}$) was applied. During experiments the sample was continuously stirred. To improve the signal-to-noise ratio 40 measurements of kinetics with repetition rate of 1 Hz were performed and averaged.

Total fluorescence intensity φ was calculated as the area under fluorescence decay kinetic normalized to Chl *a* concentration. We suggest that $\varphi = \varphi_0$ ('Fo conditions') when fluorescence kinetics was registered after 5 min of dark adaptation of samples. Fluorescence kinetics was estimated also in samples containing closed PS 2 centers with a fully reduced primary quinone acceptor Qa. In this case fluorescence yield is maximal and $\varphi = \varphi_m$ ('Fm conditions'). To transfer PS 2 into the closed state, electron transport between Qa and Qb quinones was blocked by the addition of $10 \mu\text{M}$ DCMU followed by 5 min continuous illumination by the He–Ne laser.

Kinetic traces were analyzed using the model developed by Shatz et al. [9]. A fitting was performed by a bi-exponential theoretical curves $\varphi(t) = A_1 e^{-t/\tau_1} +$

$A_2 e^{-t/\tau_2}$ convoluted with the apparatus function of a pulse fluorometer where $\tau_{1,2}$ and $A_{1,2}$ are the duration and amplitude of the fast (1) and slow (2) component in fluorescence decay kinetics ($A_1 + A_2 = 1$). The yields of fast and slow components were calculated as $\varphi_1 = A_1 \times \tau_1$ and $\varphi_2 = A_2 \times \tau_2$, respectively, where $\varphi = \varphi_1 + \varphi_2$. The fast component is related to the energy trapping in reaction center by primary charge separation, whereas the slow one is assigned to the electron transfer from Pheo to the Qa in the open PS 2 center, and to the charge recombination in a radical pair $\text{P}_{680}^+ \text{Pheo}^-$ when PS 2 is closed [9,10]. The rate constants k_1 , k_{-1} , and k_2 were calculated on the basis of the photoreaction scheme for PS 2 in the open and closed states as described in [11] (Fig. 1). The rate constant k_3 was assigned for core complex preparations as 0.24 ns^{-1} as suggested in [12].

Estimations of chlorophyll fluorescence parameter $\Delta F/F_m'$, which is a measure of photochemical activity of PS 2 under ambient light [13], and oxygen concentration in algae suspension were performed, as described in [6].

3. Results

3.1. Chl fluorescence decay kinetics during aerobic incubation

Typical fluorescence decay kinetics, measured in *C. reinhardtii* cells during 72 h incubation in complete (control) (A and B) and sulfur-depleted medium (C and D) are presented in Fig. 2. Measurements were performed following 5 min of dark adaptation ('Fo conditions', A and C) or after reduction of the primary quinone acceptor Qa by short exposure of a sample to the illumination in the presence of DCMU, as described in Materials and methods ('Fm conditions', B and D). In this paper we also use the term 'closed' PS 2 center in relation to the photosystem with reduced Qa. The analysis of fluorescence decay kinetics showed that the best fitting is achieved by a sum of two exponential components convoluted with the apparatus response function with the lifetimes varying in the time range of 70–100 ps for the fast component (τ_1) and 300–800 ps for the slow one (τ_2). Thus, the fluorescence decay kinetics measured in control cells under 'Fo conditions' have the following characteristic time values for the fast and slow components: $\tau_1 = 70 \text{ ps}$ and $\tau_2 = 400 \text{ ps}$,

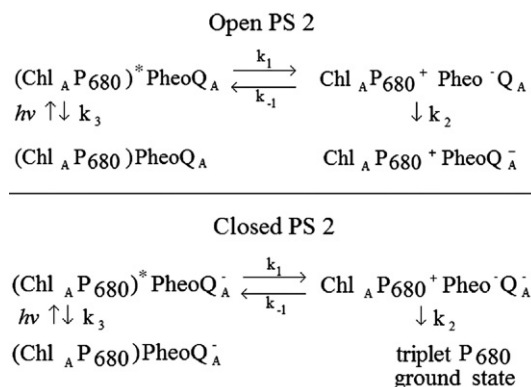


Fig. 1. First-order reversible photoreaction scheme for PS 2 in open and closed state with singly reduced Qa (adapted from [11]). $(\text{Chl}_A \text{P}_{680})^*$ designates the excited state equilibrated between antenna Chls and P_{680} ; k_1 is the rate constant of primary charges separation; k_{-1} is the rate constant of primary charges recombination accompanied by generation of singlet P_{680} state; k_2 is either the rate constant for the electron transfer from Pheo^- to Q_A in open PS 2 or the rate constant of primary charges recombination with generation of triplet excited or ground P_{680} states; k_3 is the rate constant of radiative deactivation in PS 2 antenna.

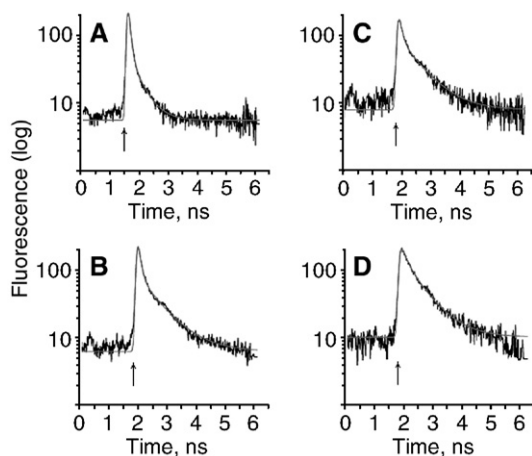


Fig. 2. Typical kinetics of Chl fluorescence decay measured in cell suspensions of *C. reinhardtii*. Cells were incubated during 72 h in complete media (A, B) or in S-depleted media (C, D) under aerobic conditions. Before measurements cells were set in the dark for 5 min (A, C) or treated to reduce Qa (see Materials and methods) (B, D). Arrows show an application of 30 ps exciting light pulse. The fluorescence is normalized to the maximal value.

respectively (Fig. 2A). Kinetic curves registered in control samples under ‘Fm conditions’ demonstrated insignificant rise of τ_1 from 70 to 80 ps, whereas τ_2 increased almost twice from 400 to 750 ps. Our data agree fairly well with results reported in [11] demonstrating that lifetimes of the fast and slow components of fluorescence decay kinetics measured in spinach photosynthetic membranes increased their lifetimes from 120 to 160 ps and from 370 to 1500 ps, respectively, in samples with reduced Qa. The shorter lifetime values of τ_1 and τ_2 obtained in our experiments as compared to [11] can be due to different intact states of preparations (whole cells vs. photosynthetic membranes). In fact, intact preparations usually give shorter lifetimes, e.g. in the case of a radical pair $P_{680}^+ \text{Pheo}^-$ [14]. The considerable increase in τ_2 in PS 2 centers with reduced Qa (‘Fm conditions’) can be interpreted as a shift of the equilibrium between the population of charge separated states and excited states toward the latter [9] provided by the electrostatic effect of Qa^- on the primary charge recombination [15]. In closed PS 2 the increased slow component reflects the contribution of the recombinant (variable) fluorescence. The fast component can be interpreted as reflecting the overall energy migration/charge separation process in PS 2.

The fluorescence decay kinetics measured under ‘Fo conditions’ in *C. reinhardtii* incubated during 72 h in sulfur-depleted medium demonstrated a slightly increased τ_1 value from 70 to 82 ps and a substantial rise of τ_2 from 400 ps to 675 ps as compared to the control samples (Fig. 2A, C). The effect of SD on the fast and slow components of the fluorescence decay kinetics was very similar to those observed in the samples with reduced Qa (compare Fig. 2A, B, C), indicating the accumulation of closed PS 2 centers. The lifetime of the slow component of decay kinetics measured under ‘Fm conditions’ in SD cells (Fig. 2D) reached 850 ps exceeding τ_2 values measured in control cells under ‘Fm conditions’ (Fig. 2B).

The overall fluorescence yields ϕ_o and ϕ_m were calculated as the area under the fluorescence decay curve measured in *C. reinhardtii* cells under ‘Fo’ and ‘Fm conditions’, respectively.

Changes of the ϕ_o and ϕ_m values during 72 h incubation in sulfur-depleted medium are shown in Fig. 3. As seen in the figure ϕ_o increased by a factor of 1.5, whereas ϕ_m increased by 1.2 times at 72 h of starvation. The most pronounced changes in ϕ_o and ϕ_m were observed during the first 24 h period of incubation, when ϕ_o and ϕ_m increased by about 50 and 70%, respectively. These changes are in accordance with the increase in Fo and Fm parameters observed in SD *C. reinhardtii* [7]. However, the yields of Fo and Fm were affected to a greater extent increasing by a factor 2.25 and 1.7, respectively, that can be explained by a peculiar characteristics of the used picosecond technique, which does not allow to estimate the absolute value of fluorescence amplitude with sufficient accuracy. However, the latter may change during SD, as the antenna proteins structure is essentially modified [16].

The relative values of amplitudes and absolute values of lifetimes of the fast and slow components resulting from a fit of Chl fluorescence decay kinetics under ‘Fo’ and ‘Fm conditions’ in sulfur-depleted medium are presented in Table 1. As seen from the table, the amplitude and lifetime of the fast component (A_1 and τ_1) measured in control cells changed when cells were transferred from ‘Fo’ to ‘Fm conditions’. Thus, A_1 decreased from 80% to 75% whereas τ_1 increased from 83 ps to 95 ps in control cells with reduced Qa. This result is in a good agreement with data reported in [11] where slight reduction of A_1 and an increase in τ_1 in photosynthetic membranes from spinach with reduced Qa were shown. The increase in τ_1 value seems to be due to the reduced energy trapping (primary charge separation) in closed PS 2 centers. Very similar changes of A_1 and τ_1 were also observed when measurements were carried out under ‘Fo conditions’ in samples incubated during 24 h in sulfur-depleted medium. Thus, A_1 decreased from 80 to 75%, while τ_1 increased from 83 to 91 ps, which points to the reduced state of Qa in SD cells.

The amplitude and lifetime of the slow component (A_2 and τ_2) also demonstrated very similar changes if we compare

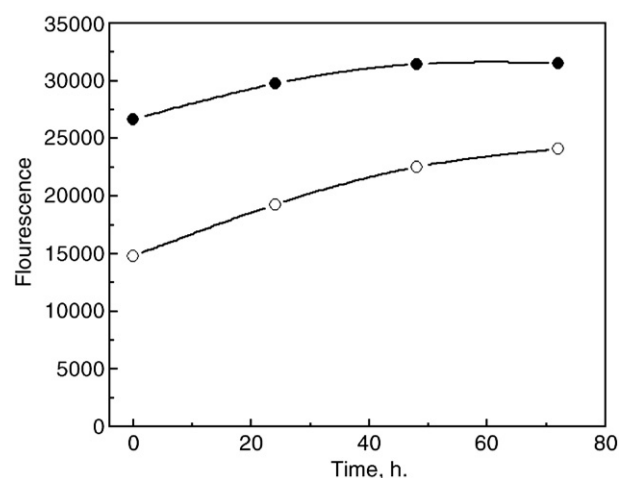


Fig. 3. Time course of integral fluorescence intensity during incubation of *C. reinhardtii* cells in S-depleted media under aerobic conditions. Integral fluorescence intensity was calculated as the area under fluorescence decay kinetic measured in dark adapted cells (ϕ_o , open symbols) and cells with reduced Qa (ϕ_m , filled symbols) (see Materials and methods).

Table 1
Change of amplitude and duration of fast (A_1 , τ_1) and slow (A_2 , τ_2) components of fluorescence decay kinetics measured in *C. reinhardtii* during cell growth in S deficient medium

Time (h)	A_1 (%)		τ_1 (ps)		A_2 (%)		τ_2 (ps)	
	DA	Qa ⁻	DA	Qa ⁻	DA	Qa ⁻	DA	Qa ⁻
0	80	75	83	95	20	25	393	781
24	75	73	91	94	25	27	504	844
48	74	70	89	96	26	30	611	836
72	72	71	91	98	28	29	628	840

Fluorescence was measured in dark adapted cells (DA, 'Fo conditions') and cells with reduced Qa centers (Qa⁻, 'Fm conditions') (see Materials and methods). The standard deviation has not exceeded 5% for all measurements.

the sensitivity to SD with that of the reduction of Qa (Table 1). Thus, the contribution of A_2 increased from 20% in control cells with oxidized Qa to 25% both in control cells with reduced Qa and in SD cells at the end of the 24 h period of incubation. The value of τ_2 increased from 393 in control with oxidized Qa to 781 ps in control with reduced Qa, while the gradual rise of τ_2

from 393 to 628 ps was observed following 72 h of SD. However, an additional increase in both A_2 (from 25 to 29%) and τ_2 (from 781 to 840 ps) was induced in the course of 72 h of sulfur starvation when Qa was reduced before picosecond fluorescence measurements.

The relative yields of the fast and slow components of Chl fluorescence decay kinetics measured in *C. reinhardtii* were calculated according to the formulas: $\phi_{1,2o} = A_{1,2o} \times \tau_{1,2o}$ for 'Fo conditions', and $\phi_{1,2m} = A_{1,2m} \times \tau_{1,2m}$ for 'Fm conditions'. The values of amplitudes and lifetimes were taken from Table 1. The dynamics of the relative yields of the fast and slow components are shown in Fig. 4A and B, respectively. The yield of the fast component was practically invariable during starvation measured under 'Fo' and 'Fm conditions' (Fig. 4A). On the contrary, the yield of the slow component changed significantly during SD. Thus, the slow component yield estimated under 'Fo conditions' increased by 2.0 times during 72 h of sulfur starvation attaining about 90% of the yield observed in samples with completely reduced Qa at 0 h of deprivation (Fig. 4B). This result can be understood provided that the portion of closed PS 2 centers in SD cells reaches about 90% after 72 h of incubation. However, the contribution of long-lived slow components (several nanoseconds), originated from detached antenna or centers lacking Qa cannot be also excluded. This result is in agreement with a decrease in PS 2 activity measured as $\Delta F/F_m'$ ratio by about 80% during the same period of incubation (data is not shown). Under 'Fm conditions' the yield of the slow component demonstrated a slower rise by only about 20% during 72 h of starvation, implying the increased emission of recombinant fluorescence in SD cells.

The rate constants k_1 , k_{-1} and k_2 (see Fig. 1) were calculated from the decay kinetics parameters presented in Table 1 as described in [11]. The rate constant k_3 was assumed to be 0.24 ns^{-1} as proposed in [12]. The results of calculations, presented in Table 2, demonstrated the decrease in k_1 and k_2 from 9.9 to 8.1 ns^{-1} and from 2.8 to 1.8 ns^{-1} , respectively, during 72 h of *C. reinhardtii* incubation in sulfur-depleted medium, whereas k_{-1} increased from 1.5 to 2.2 ns^{-1} for the same period of SD, as measured in dark adapted cells. These results are in consistency with the proposed reduced state of Qa in SD cells, causing the repulsive electrostatic effect on electrons participating in charge separation/recombination [15]. When measurements were

Table 2
Change of the rate constants k_1 , k_{-1} , and k_2 during *C. reinhardtii* incubation in S deficient medium

Time (h)	k_1 (ns^{-1})		k_2 (ns^{-1})		k_{-1} (ns^{-1})	
	DA	Qa ⁻	DA	Qa ⁻	DA	Qa ⁻
0	9.9	8.0	1.5	2.0	2.8	1.3
24	8.5	7.7	1.8	2.2	2.2	1.2
48	8.5	7.4	2.1	2.4	1.8	1.3
72	8.1	7.4	2.2	2.3	1.8	1.2

The rate constants were calculated from decay kinetics parameters presented in Table 1 as described in [11]. The rate constant k_3 was assumed to be 0.24 ns^{-1} as suggested, e.g. in [12]. Fluorescence was measured in dark adapted cells (DA, 'Fo conditions') and cells with reduced Qa centers (Qa⁻, 'Fm conditions') (see Materials and methods).

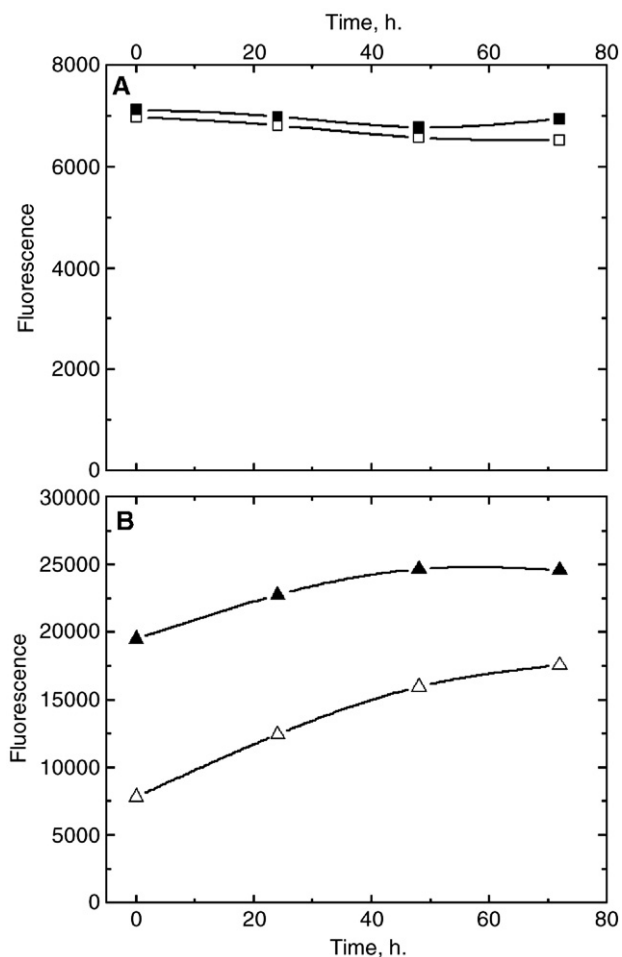


Fig. 4. Time courses of the fast (A) and slow (B) fluorescence component yields measured in *C. reinhardtii* cells during S-starvation under aerobic conditions. The yields of the fast and slow components of fluorescence decay were calculated according to formulas $\phi_1 = A_1 \times \tau_1$ and $\phi_2 = A_2 \times \tau_2$, respectively. Fluorescence was measured under 'Fo' (open symbols) and 'Fm' (filled symbols) conditions (see Materials and methods).

performed on PS 2 centers from SD cells but with additionally reduced Qa before measurements, the additional decrease in k_1 value from 8.0 ns^{-1} to 7.4 ns^{-1} , and increase in k_{-1} from 2.0 to 2.3 ns^{-1} was observed, while k_2 was virtually invariable. This result indicates a shift of the equilibrium between the population of charge separated states ($\text{P}_{680}^+ \text{Pheo}^-$) and singlet excited states ($\text{P}_{680}^* \text{Pheo}$) toward the latter in closed PS 2 centers of SD cells.

3.2. Chl fluorescence decay kinetics during anaerobic phase of incubation in a sealed bioreactor

As was demonstrated in [6], photochemical activity of PS 2, monitored by the $\Delta F/\text{Fm}'$ ratio in SD *C. reinhardtii* during cell incubation in a sealed bioreactor, decreases slowly during the aerobic period. However the additional sharp down regulation of PS 2 activity was observed exactly at the short transition period to anaerobiosis. Shortly thereafter, the PS 2 activity was partially restored, accompanied by the increase in H_2 production rate. The Chl fluorescence decay kinetics was measured under 'Fo conditions' in SD *C. reinhardtii* cells incubated in a sealed bioreactor, and the relative yields of the fast and slow components were calculated shortly before and after culture transition into anaerobic conditions. The dynamics of the fast and slow component yields as well as of the fluorescence parameter $\Delta F/\text{Fm}'$ are shown in Fig. 5. As seen from the figure, the increase in the slow component yield by 1.6 times was observed during 1 h period when anaerobic conditions were established. After that, the decrease in the slow component yield by 1.25 times occurred during anaerobic phase of H_2 evolution. The yield of the fast component demonstrated less significant change during the same period of incubation. The experimental conditions did not allow to perform measurements exactly at the

time when O_2 concentration in algae suspension dropped to zero or H_2 evolution just started. However, it is obvious, that the observed dynamics of the slow component yield changes during SD was similar to that of the ratio $\Delta F/\text{Fm}'$. This result shows that the PS 2 activity is determined by the redox state of Qa during anaerobic stage of sulfur starvation.

4. Discussion

As shown in the present work, the effects of sulfur deficiency on Chl fluorescence decay kinetic parameters measured in *C. reinhardtii* cells is very similar to that of when Qa is reduced (Table 1). As known, the redox state of Qa is the main factor determining the lifetime and the yield of the slow (DCMU-dependent) component of fluorescence decay kinetics [11]. The gradual increase in the yield of the slow component during aerobic incubation of SD *C. reinhardtii* is indicative of the appearance of closed PS 2 centers. The reduced state of Qa is related, apparently, to (a) the appearance of Qb-non-reducing centers [2], or/and (b) reduced state of the PQ pool due to the enhanced chlororespiration [3]. The closed state of PS 2 may lead to the loss of Qa, followed by the irreversible damage of PS 2 according to the acceptor-side mechanism [5]. The acceptor-side photoinhibition may also occur in the absence of oxygen, explaining PS 2 degradation at the anaerobic H_2 production phase during incubation in a sealed bioreactor.

As was shown in [6], PS 2 activity estimated as the $\Delta F/\text{Fm}'$ ratio during incubation of SD *C. reinhardtii* in a sealed bioreactor was completely but reversibly down regulated at the time when anaerobiosis was established, followed by a partial up regulation during the subsequent H_2 production phase. The yield of the slow component also demonstrated transitions comparable in their dynamics to those of $\Delta F/\text{Fm}'$ (Fig. 5), indicating that the reversible inactivation and subsequent reactivation of PS 2 are also related to the redox state of Qa.

Therefore, both the photoinhibition and reversible regulation of PS 2 activity during different phases of *C. reinhardtii* adaptation to sulfur deficiency are regulated by the redox state of Qa, which is related to the redox state of PQ pool [17]. Consequently, the redox state of PQ pool plays an important regulatory role being an intermediate crossover link between chlororespiration, PS 2 activity and hydrogenase reaction in SD cells.

According to the results obtained in the present work, the slow component yield measured under 'Fm conditions' increased by 1.2 fold in SD cells (Fig. 4), indicating the rise of the recombinant fluorescence yield. Calculations of the rate constants for charge separation (k_1) and charge recombination accompanied by P_{680}^* generation (k_{-1}) in closed PS 2 centers (see scheme in Fig. 1 and Table 2) demonstrated the decrease in k_1 and increase in k_{-1} by about 10–15% in SD cells. At the same time k_2 which is the rate constant of charge recombination resulted in generation of triplet or ground P_{680} states did not change. Consequently, the increased yield of the recombinant fluorescence can be related to the shift of equilibrium between the population of charge separated states ($\text{P}_{680}^+ \text{Pheo}^-$) and singlet excited states ($\text{P}_{680}^* \text{Pheo}$) toward the latter in closed PS

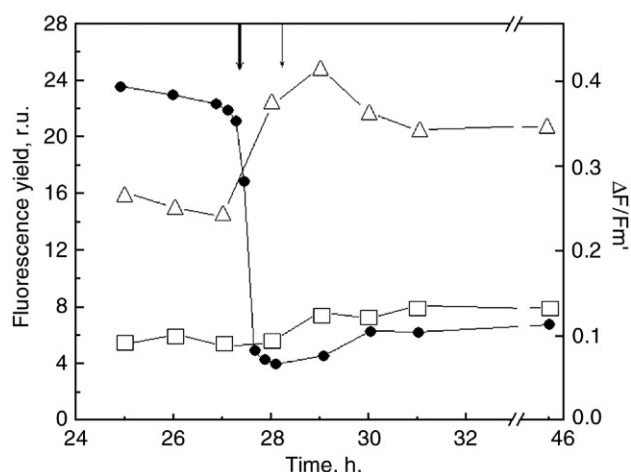


Fig. 5. Time courses of the fast (squares) and slow (triangles) fluorescence component, and $\Delta F/\text{Fm}'$ ratio measured in *C. reinhardtii* cells during S-deprivation in a sealed bioreactor and transition from aerobic to anaerobic conditions. Incubation in S-depleted medium started at 0 h. The onset of anaerobiosis and H_2 production are designated by closed and open arrows, respectively. The fluorescence decay kinetics was measured under 'Fo conditions', as described in Materials and methods. The yields of the fast and slow components of fluorescence decay were calculated according to formulas $\varphi_1 = A_1 \times \tau_1$ and $\varphi_2 = A_2 \times \tau_2$, respectively.

2 centers in SD cells, leading to the enhanced probability for the radiative deactivation of excitation. The observed changes in k_1 and k_2 values may reflect changes of intrinsic rate constants of charge separation/recombination in SD cells. According to the Marcus theory, these constants are proportional to the quantum mechanical matrix element and the reorganization energy [18]. The first depends on the van der Waals distance between the interacting redox centers, while the second is determined by the redox groups microenvironment (e.g., solvent polarization), respectively. The damage of PS 2 upon starvation may cause the modification of the primary photochemistry, including spatial and steric properties of the radical pair themselves and its local environment. The effect can be related, e.g. to an up-shift in the redox potential of Qa observed in PS 2 with inactive oxygen evolving complex [19,20], as the high-potential forms of Qa may have an altered electrostatic effect on electron of the radical pair. Indeed, our preliminary data demonstrates: (a) essential dysfunction of oxygen evolving complex in SD *C. reinhardtii*, and (b) the similar changes in the rate constants k_1 and k_{-1} in *C. reinhardtii* with impaired water splitting function, e.g. by hydroxylamine. The detailed mechanisms of these processes have to be studied in the future.

Acknowledgements

This work was supported by the Russian Foundation of Basic Research (07-04-00222; 05-04-48912), the U.S. Civilian Research and Development Foundation and The International Science (10841).

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