Thermally-induced Delayed Fluorescence of Photosystem I and II Chlorophyll in Thermophilic Cyanobacterium *Synechococcus elongatus*

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Abstract. Stationary delayed fluorescence (DF) of chlorophyll in isolated membrane preparations from thermophilic cyanobacterium *Synechococcus elongatus* was investigated as a function of temperature. Two peaks at different temperatures were observed. The low-temperature peak (54–60 °C) coincided with the main maximum of the thermally-induced delayed fluorescence of chlorophyll in intact cells and PSII-particles with active oxygen-evolving system. The high-temperature peak (78 °C) coincided with the minor band of delayed light emitted by intact cells. It was also observed in the delayed fluorescence emission from a PSI-enriched fraction preparation. The intensities of the DF peaks were dependent on the presence of inhibitors, donors and acceptors that cause specific effects on electron transport of the two photosystems. The low-temperature and high-temperature peaks were related to PSII and PSI, respectively. The manifestation of delayed fluorescence from PSI and PSII at different temperatures seems to be a specific property of thermophilic cyanobacteria. The reason for this may be a high thermal stability of the photosystems and the lack of the PSII antenna complex in isolated membranes. Consequently, the relative yield of delayed fluorescence from PSI markedly increases. Thermally-induced fluorescence seen in membranes of cyanobacteria showed a high sensitivity to structural and functional membrane alterations induced by pH changes, different electron transport stabilizing agents or different concentrations of $MgCl_2$.

Key words: Delayed fluorescence — Photosystem I — Photosystem II — Thermophilic cyanobacteria

Introduction

Delayed fluorescence of chlorophyll is a universal phenomenon, characteristic of both eucaryotic and procaryotic photosynthesizing organisms (Lavorell 1975; Malkin 1977; Ono and Murata 1977; Fork et al. 1985). It arises from charge recombination, chiefly in photosystem II (Lavorell 1975; Malkin 1977). The delayed fluorescence from chlorophyll of photosystem I constitutes as little as 1% of total delayed fluorescence emitted by green plant chloroplasts (Shuvalov et al. 1976). One approach to the study of delayed fluorescence is the investigation of thermally-induced chlorophyll fluorescence (Fork et al. 1985; Veselovskij and Veselova 1983; Havaux and Lannoye 1983). Delayed fluorescence is best studied in higher plants. In procaryotic organisms, particularly in cyanobacteria, thermally-induced delayed fluorescence has been much less studied, in particular in isolated membrane systems. DF has specific features in cyanobacteria, due to specific structural arrangement of the photosynthetic membranes of these organisms.

For example, in contrast to other algae, some parameters of delayed fluorescence from cyanobacteria show specific changes within the temperature range at which membrane lipids undergo thermophilic phase transitions (Fork et al. 1985). The photosynthetic apparatus of cyanobacteria, and especially that of thermophilic species, is highly stable. Owing to this, it is possible to investigate the temperature effects on delayed fluorescence of chlorophyll over a wide range of positive temperatures, both in intact cells and in isolated photosynthetic membranes and membrane complexes.

In the present work we investigated thermally-induced delayed fluorescence of chlorophyll in isolated membranes of the thermophilic cyanobacterium *Synechococcus elongatus*.

Abbreviations:

PS — photosystem; DF — delayed fluorescence; LDAO — lauryldimethylamine-N-oxide; DCIP — 2,6-dichlorophenolindophenol; DCMU — 3-(3,4-dichlorophenyl)-1,1-dimethylurea; HEPES — 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

Materials and Methods

Synechococcus elongatus (No. 120, Museum of the Institute of Plant Physiology, Russian Academy of Sciences) was grown in a Kratz-Myers medium (Kratz and Myers 1955) at 55 °C. The incubation medium was bubbled with air containing 0.2% CO_2. Luminescent lamps were used for illumination. The light intensity used was 1500 lux at the beginning and 10,000 lux at the final stage of growing. The photosynthetic membrane isolation procedure used has been described elsewhere (Kaurov et al. 1986). *S. elongatus* cells, collected at the end of the logarithmic growth phase, were treated with lysozyme (0.25% solution, 3 h) in a medium, containing 5 mmol/l K^+-Na^+ phosphate buffer, 10 mmol/l

HEPES-NaOH pH 7.5, 10 mmol/l $MgCl_2$, at 37 °C. All subsequent procedures were performed at 4 °C. Spheroplasts obtained were disrupted by glass beads in a mechanical homogenizer (7000 r.p.m, 3 min). Membranes were obtained by differential centrifugation at 3500 × *g* for 5 min followed by centrifugation at 38,000 × *g* for 15 min. The membrane sediment was suspended in a buffer supplemented with 25% glycerol.

PSII particles with active oxygen-evolving system and membrane fragments enriched in PSI were obtained by selective fragmentation; the membranes prepared as above were treated with lauryldimethylamine-N-oxide (LDAO) (Kaurov et al. 1986; Stewart and Bendall 1979). The detergent (2.4-2.7 mg LDAO/mg chlorophyll) was added to the membrane suspension (chlorophyll concentration 1 mg Chl/ml) and incubated for 50 min, with subsequent centrifugation at 150,000 × *g* for 90 min. The supernatant containing PSII particles was placed on a Sepharose-6B column. The particles, purified from the detergent and free phycobilins, were concentrated by centrifugation at 200,000 × *g* for 90 min. The PSI-enriched sediment, obtained by centrifugation at 150,000 × *g* for 90 min, was resuspended in a buffer containing glycerol. To remove the detergent, the 150,000 × *g* fraction was resuspended twice by centrifugation at 150,000 × *g* for 60 min. The preparations obtained were stored on liquid nitrogen until used.

The properties of the PSII particles were described in our earlier paper (Kaurov et al. 1986). The PSII particles contained 1 mol P700 per 1000 mol chlorophyll. Oxygen was evolved in the presence of *p*-benzoquinone (1 mmol/l) at a rate of 600 μmol O_2/mg Chl per hour. The low-temperature fluorescence emitted by PSII particles (λ_{exc} = 435 nm) exhibited a distinct band with a peak at 685 nm and an arm at 695 nm. The band is typical of PSII chlorophyll.

The PSI-enriched fragments showed no oxygen-evolving activity. The oxygen uptake rate in the presence of reduced dichlorophenolindophenol (2×10^{-5} mol/l DCIP, 2 mmol/l Na ascorbate) and methylviologen (0.1 mmol/l) was 65 μmol O_2/mg Chl per hour. P700 content was 12 mol per 1000 mol of chlorophyll in the PSI preparation, and 7 mol per 1000 mol of chlorophyll in initial membranes. The low-temperature fluorescence spectra of chlorophyll in the PSI-enriched fragments preparation (λ_{exc} = 435 nm) have a long wavelength band with a peak at 730 nm; this band is typical of PSI chlorophyll.

The chlorophyll content was determined by the method of Arnon (Arnon et al. 1974). Oxygen evolution and uptake were measured at 25 °C with a Clark electrode. A MF-2 Hitachi fluorometer (Japan) was used to measure low-temperature fluorescence spectra.

Delayed fluorescence measurements were carried out using a home-made fluorometer, based on phosphoroscope (Rubin 1974). The time lag between excitation and measurement was 1.2 ms; the excitation time and the measurement time were both 1.9 ms. Chlorophyll fluorescence was excited with a red light 250 W Halogen lamp (KC-15 red light filter, $\lambda > 650$ nm, 5 mm thick), with the sample being heated at a rate of 4 °C/min. Incident irradiance was 30 mW/cm^2. The temperature of the sample in the temperature-controlled cuvette was measured with a chromal-capel thermocouple with an accuracy of ±1 °C. The initial intensity of the long-lived component of delayed fluorescence was monitored 2 s after the exciting light was switched off.

Commercial preparations of lysozyme and HEPES-NaOH used in the experiments were obtained from Serva (BRD), and LDAO from Onyx Chemical Co. (USA). Reagents purchased from home manufacturers were at least of analytic quality.

Results

Fig. 1 (curve 1a) shows the temperature dependence of light-induced stationary delayed fluorescence in samples of isolated photosynthetic membranes of *Synechococcus elongatus*. The curve has two pronounced peaks at 54°C and 78°C. The low-temperature peak, noted peak I, has a similar location as the main peak of delayed fluorescence of PSII chlorophyll in intact cells (Fig. 1, curve 2a). A high-temperature peak (II) was observed mainly in isolated membranes (Fig. 1, curve 1a). Cells exhibit a similar band but only as a minor component. Its maximum intensity is approx. 50 times smaller than that of peak I (Fig. 1, curve 2a). PSII-particles isolated from membranes show only one peak of DF at 48°C(Fig. 1, curve 3). On the other hand, the membrane fraction enriched in PSI shows a high-temperature peak in the delayed fluorescence emission (Fig. 1, curve 4a, 4b). In the absence of electron-donating substances the peak is expressed weakly (curve 4a). The addition of DCIP and Na ascorbate, which donate electrons to P700 (Izawa 1980), caused a significant rise of peak II (curve 4c). The addition of $DCIPH_2$ increases the intensity of peak II also in intact cells (Fig. 1, curve 2b).

In all the preparations used, delayed fluorescence of chlorophyll was found to be sensitive to the presence of electron acceptors. For instance, upon addition of ferricyanide to isolated membranes (ferricyanide undergoes reduction by both PSI and PSII (Izawa 1980)) quenching of delayed light occurred at all temperatures tested (Fig. 1, curve 5). Methylviologen, an acceptor of electron flow from PSI, appeared to quench only peak II (Fig. 1, curve 6).

Inhibition of electron flow to PSII in isolated membranes indicated that different species are responsible for peaks I and II. Treatment with Tris buffer (0.8 mol/l), which inactivates the water-splitting system (Izawa 1980), caused quenching of peak I (Fig. 1, curve 7). Inhibition with DCMU of PSII-driven electron flow at the level of plastoquinone increased the DF yield (Fig. 1, curve 8). In both cases the amplitude and position of peak II remained virtually unchanged.

In *S. elongatus* peak I of delayed fluorescence is composed of components with different lifetimes similar to those characteristic for intact photosynthesizing organisms (Malkin 1977). The major contribution to the maximum of the band comes from the millisecond components. The second component, whose lifetime is 7 s, has an initial intensity, which makes up approx. 10% of total delayed fluorescence. Peak II of delayed fluorescence of chlorophyll contains only a millisecond component.

The position, intensity and shape of the DF peaks depend on the characteristics of the medium which grants the membrane its functional persistence. In particular, this is true for the DF peak I. Presented in Fig. 2 are thermograms of chlorophyll delayed fluorescence for membranes suspended in media with different $MgCl_2$ contents; this compound at neutral pH keeps the PSII-driven electron trans-

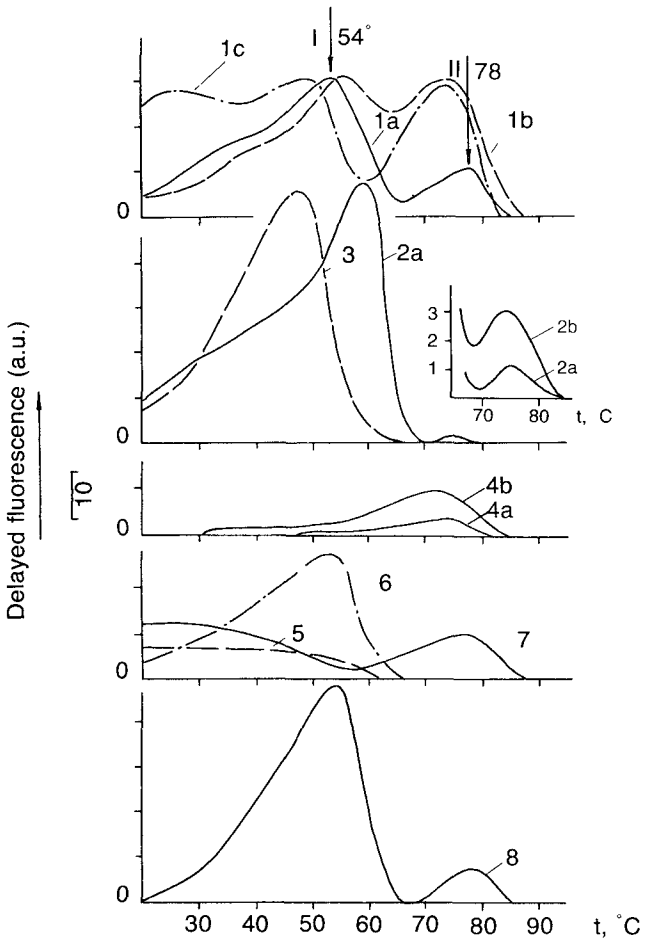

Figure 1. Delayed fluorescence of chlorophyll in isolated membranes, membrane fragments enriched in PSI and PSII, and intact cells from the thermophilic cyanobacterium *Synechococcus elongatus* as a function of temperature. 1*a*, *b*, *c* - isolated membranes (*a*, *b* - in the absence and presence of DCIP (20 μmol/l) and sodium ascorbate (2 mmol/l), respectively; *c* - pH 6.0* in the absence of donors); 2*a*,*b* - intact cells (*a*, *b* - in the absence and presence of DCIP (20 μmol/l) and sodium ascorbate (2 mmol/l), respectively); 3 - PSII particles with active water-splitting system; 4*a*,*b* - PSI-enriched fragments (a,b-in the absence and presence of DCIP (20 μmol/l) and sodium ascorbate (2 mmol/l), respectively); 5,6,7,8 - isolated membranes (5- in the presence of potassium ferricyanide (10 mmol/l); 6 - in the presence of methylviologen (0.1 mmol/l); 7 - Tris-treated membranes (0.8 mol/l, pH 8.0); 8 - in the presence of DCMU (10^{-5} mol/l)). Chlorophyll concentration: 13 μg/ml in isolated membrane preparations and membrane fragments, 1.5 μg/ml in intact cells. *Buffer contained 126 mmol/l Na_2HPO_4; 37 mmol/l citric acide and 10 mmol/l $MgCl_2$.

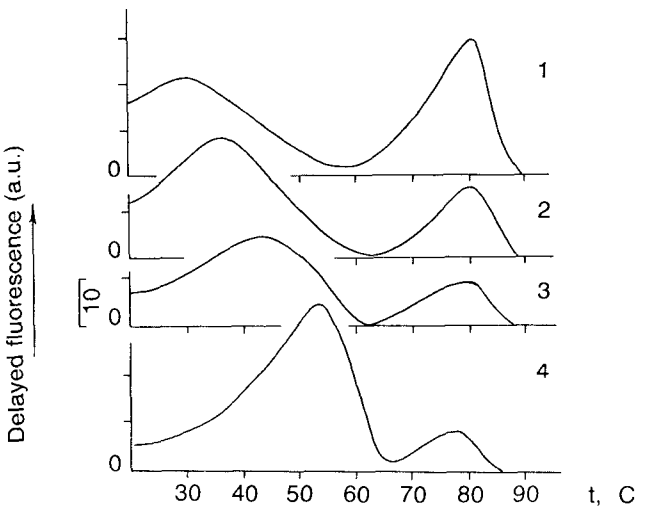

Figure 2. Effect of $MgCl_2$ on the temperature dependence of chlorophyll delayed fluorescence for an isolated membrane preparation from *Synechococcus elongatus*. 1, 2, 3, 4 - isolated membranes in the absence (1) and presence of 1 mmol/l (2), 2.5 mmol/l (3), 10 mmol/l (4) $MgCl_2$. For chlorophyll content see legend to Fig. 1.

port chain of thermophilic cyanobacteria stable (Stewart and Bendall 1980; 1981). In the absence of $MgCl_2$, the DF yield decreases and peak I is shifted towards lower temperatures (30 °C, curve 1), compared with the controls (curve 4). Upon elevating the content of $MgCl_2$ a recovery of the functional activity of the membrane, and an increase of the intensity of peak I and its temperature occur (curves 2,3,4). The effect of $MgCl_2$ on peak II is opposite to that on PSII. In contrast to $MgCl_2$, high concentrations of Na citrate (0.75 mol/l) produce a pronounced stabilizing effect on macromolecules (Hippel and Schleich 1973) and photosynthetic membranes (Kato and Gantt 1979; Stewart 1982; Kaurov et al. 1988b), with identical changes in bands of delayed fluorescence. In the presence of this compound (Fig. 3), the DF intensity increases and both peaks are shifted by 5–6 °C towards higher temperatures.

A specific feature of the temperature dependence of delayed fluorescence of chlorophyll in isolated membranes (curve 1a,b) is the presence of an arm within 25–45 °C. It is clearly seen in particular at low pH values (see Fig. 1, curve 1c).

For membranes of *S. elongatus* the general pattern of the thermograms of delayed fluorescence showed little dependence on the rate of heating. For example, increasing the rate of heating from 4 °C/min to 15 °C/min caused only a shift of the peaks by 2–4 °C towards higher temperatures. The decay of DF, observed in

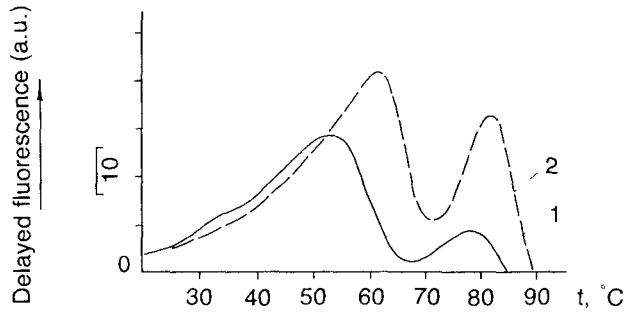

Figure 3. Effect of sodium citrate on the temperature dependence of delayed fluorescence of chlorophyll for an isolated membrane preparation from *S. elongatus*. 1,2 - isolated membranes in the absence and presence of 0.75 mol/l Na-citrate, respectively. For chlorophyll content see legend to Fig. 1.

all experiments (see Figs. 1 to 3) at temperatures of 85 to 90 °C, was completely irreversible. A partial recovery (to 10% of the initial amplitude) was seen only for peak I upon cooling a membrane sample previously heated to 65 °C.

It is worth of noting that thermally-induced delayed fluorescence is not associated with fluorescing products that may be formed as a result of the oxidative photodynamic destruction of the photosynthetic apparatus (Takahama and Nishimura 1975). In control experiments, Fe^{2+} ions ($FeSO_4$) and α-tocopherol, which have a specific influence on lipid peroxidation involving free radicals, were seen to cause no change in the DF yield in isolated membranes (results not shown).

Discussion

The above data for isolated membranes of *S. elongatus* provide evidence that peaks I and II on the temperature curve of DF relate to fluorescence of chlorophyll of PSII and PSI, respectively. Peak I (PSII) was observed in PSII particles and appeared to be highly sensitive to DCMU, ferricyanide and to high concentrations of Tris-HCl. Peak II (PSI) was lacking in PSII particles but it was observed in PSI-enriched particles. Its intensity did not change in the presence of agents known for their specific action on PSII, but was seen to drop markedly under the effect of ferricyanide and methylviologen. Moreover, the amplitude of peak II increased sharply in the presence of reduced DCIP.

It should be noted that some difference in the thermally-induced peaks of DF for PSII particles and the PSI preparation and those for intact membranes (displacement towards lower temperatures of the PSII peak, low intensity of the

PSI peak) are presumably caused by a deorganizing effect of the detergent used for fragmentation. The temperatures at which the peaks of DF occur with intact membranes (54 and 78 °C) are nearly the ultimate temperatures at which the photosystems retain their stability. The temperature at which electron transport is inactivated by 50% in *S. elongatus* is 59 and 76 °C, respectively, for PSII and PSI (Kaurov et al. 1988a; Koike et al. 1982; Hirano et al. 1981).

The observation of thermally-induced delayed fluorescence of PSI and PSII at different temperatures is probably a specific feature of cyanobacteria. In higher plants, PSI DF may reliably be measured only in mutants devoid of PSII activity or in preparations enriched in pigment-protein complexes of PSI (Vasiliev et al. 1984). A possibility to differentiate between delayed fluorescence from PSI and PSII in intact chloroplasts has recently been reported. This possibility is provided by a kinetic analysis of fluorescence decay induced by applied electric field (Symons et al. 1987; Vos and Van Gorkom 1988). Similarly as in cyanobacteria, the PSI and PSII of higher plants have different resistivities to high temperatures. This is evidenced by quenching of PSII DF in chloroplasts occurring at a temperature lower by 15–20 °C than in isolated PSI complexes (Venediktov and Krivosheyeva 1984; Vasiliev et al. 1984). However, for intact chloroplasts attempts to separate delayed fluorescence of chlorophyll relating to PSI and PSII using temperature effects have failed. Probably, this is due to the low intensity of PSI DF in chloroplasts. Estimates show that PSI contributes as little as 1% to the overall delayed fluorescence from chloroplasts (Shuvalov et al. 1976). In intact cells of *S. elongatus*, PSI DF has nearly a similar low level (2% of the maximum intensity of PSII DF). However, in isolated membranes of *S. elongatus* the relative yield of thermally-induced delayed fluorescence of PSI is much higher (35–100%, depending on experimental conditions). This is primarily due to the sharp drop of PSII DF isolated membranes, as compared with intact cells. The amplitude of the PSI DF peak is the same for isolated membranes and intact cells (after relating the values to chlorophyll content). However, the amplitude of the PSII DF peak for membranes is 25 times smaller than that for intact cells. The membranes of cyanobacteria are known to easily lose, during the isolation procedure, the light-harvesting complexes associated with PSII. A result of this is a sharp degradation of the efficiency of light energy utilization. Probably, it is this characteristic that accounts for the comparable levels of PSI and PSII DF in isolated membranes of cyanobacteria. The relatively high content of chlorophyll associated with PSI in cyanobacteria may also add to this fact. An important point to note is that phenomena responsible for the extreme pattern of the temperature dependence are essentially different for PSI and PSII. As far as PSII is concerned, the rise of chlorophyll delayed fluorescence occurs at physiological temperatures for thermophils and correlates with the monotonic increase of the rate of electron flow from water to the primary acceptors (Kaurov et al. 1988a; Hirano et al. 1981). The delayed fluorescence is emitted by PSI at tem-

peratures at which irreversible degradation of the photosynthetic apparatus occurs. Under these conditions PSI DF is probably in general the result of deterioration of the mechanism of inactivation of chlorophyll P700 excited states. One may assume that the rise of PSI DF is due to the acceleration of the radiative recombination of charges in the $P700^+P430^-$ pair. These transitions are known to make a major contribution to PSI DF in higher plants with a lifetime of more than 1 ms (Vos and Van Gorkom 1988).

A fact that deserved attention is inhomogeneity of the bands of PSII DF which is well seen at low pH values. A similar temperature dependence of DF has earlier been observed for intact *Synechococcus lividus* cells (Fork et al. 1985). The two arms on the DF curve were ascribed to the temperature-induced phase transitions of the lipids. Presumably, the low-temperature inflexion of the DF curve for isolated membranes may be explained in a similar way. However, this assumption needs verification by additional experiments.

In contrast to chloroplasts, the general behavioral pattern of the temperature dependence of DF characteristic of intact cells is also observed in isolated membranes of *S. elongatus*. As follows from the results, the thermograms appear to be highly sensitive to different effects such as redox potential, contents of ions, pH of the medium, the presence of agents that produce a stabilizing effect, degree of fragmentation. This suggests that thermally-induced delayed fluorescence may be used to monitor the stability of the photosynthetic apparatus. It can also be used as a mean to study electron transitions in PSI and PSII of thermophilic cyanobacteria.

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