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Rapid report

Low and high temperature dependence of minimum F_0 and maximum F_M chlorophyll fluorescence in vivo

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Abstract

The aim of this paper is to give a global insight into the behaviour of F_0 and F_M in a wide temperature range from -100°C to 75°C. We show that the F_0 increases upon linear freezing, similarly to the widely published increase of the F_0 upon linear heating. In contrast to this the F_M decreases upon linear heating in the whole temperature range from -100°C to 75°C. A comparison of low and high temperature induced increase of the F_0 is presented. © 1998 Elsevier Science B.V.

Keywords: Fluorescence temperature curve; Fluorescence, minimum and maximum ; Fluorescence induction; Low temperature emission spectrum chlorophyll

Chlorophyll fluorescence measurements are widely used as an indicator of functional changes of photosynthetic apparatus under temperature stress [1,2]. The changes in the intensity of chlorophyll fluorescence measured upon linear heating have been extensively studied [1,3,4] and were also designated as fluorescence temperature curve (FTC) [5,6]. The FTC is usually measured in temperature range of 25–75°C. Upon so called F_0 conditions, the measured F_0 increases slightly from 32°C to 42°C and then sharply from 42°C to about 50°C [7,8], whereas the F_M decreases in the whole temperature range of 25–75°C [9]. The heat-induced enhancement of the F_0 seems to be a result of the blocking of RCII, probably of the inhibition of Q_A–Q_B electron transport [10,11]. The temperature at which the F_0 [1,12] or F_s (steady state fluorescence) [13,14] starts to rise sharply is routinely used as an indicator of heat stability of PSII. As far as we know, the behaviour of fluorescence at temperatures below 0°C upon linear freezing has not been yet investigated because the fluorescence measurement upon freezing is influenced by changes in the optical properties of the leaf. To minimize the creation of the ice on the surface of leaf we realized the low temperature measurements in a special cryostat in presence of vacuum (20 kPa) (short-term presence of vacuum during measurements had no effect on photosynthetic activity) and the leaf segment was infiltrated before measurement with glycerine (it is

Abbreviations: F_0 and F_M, minimum and maximum fluorescence; $F_V = F_M - F_0$, variable fluorescence; FTC, fluorescence temperature curve; PSII and PSI, photosystem II and I; LHCII and LHCI, light-harvesting complex of photosystem II and I; RCII, reaction centre of PSII; OEC, oxygen evolving complex; Q_A and Q_B, the primary and secondary quinone electron acceptors of RCII; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea

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used as an additive to make an amorphous glass with limited alternation of the photosynthetic activity).

Green plants of spring barley (*Hordeum vulgare* L. cv. Akcent) were grown from seedlings cultivated 7 days after sowing in a growth chamber on artificial soil composed of perlit and Knopp solution, under low irradiance ($90\ \mu mol\ m^{-2}\ s^{-1}$) in a light regime of 16 h light/8 h dark, at 25°C and relative air humidity 85%. The 1 cm long tip of the primary leaf blade was detached and following 2 cm long segment was used for measurements. To reach the F_M level the segment was infiltrated with 1 mM DCMU for 60 min at lowered pressure of 60 kPa. Fluorescence was measured with PAM 2000 fluorimetr (H. Walz, Effeltrich, Germany). The leaf segment was excited by weak red light ($1\ \mu mol\ m^{-2}\ s^{-1}$, maximum emission at 655 nm) and fluorescence was detected at wavelengths above 700 nm. The leaf segment fixed on the metal holder was linearly cooled or heated with the rate of 30°C/min in a home-made vacuum optical cryostat. Cooling of the leaf segment was performed by filling up the internal space of the cryostat with liquid nitrogen, whereas heating was realized by heaters connected directly to the sample holder. The temperature was detected by a copper/constantant thermocouple placed on the abaxial side of the leaf. For measurement of fluorescence induction kinetics the leaf segment was linearly heated (up to 50°C) or linearly cooled (up to −50°C) with the rate of 30°C/min in darkness under vacuum. After reaching the desired temperature the leaf segment was immediately transported in darkness into the leaf clip (Hansatech) and measured under normal air pressure. The fluorescence induction kinetics was detected with Plant Efficiency Analyzer PEA (Hansatech Ltd., King's Lynn, Norfolk, England), with $3400\ \mu mol\ m^{-2}\ s^{-1}$ of actinic light intensity (measured by quantum radiometer LI-1989, LI-COR, USA). The ratio F_V/F_M was measured with PAM 2000 fluorimetr (H. Walz, Effeltrich, Germany) using standard procedure with analytic light and saturating pulse [15]. Fluorescence emission spectra of leaf segments were measured after heating of segments or freezing in temperature range from 25°C to 50°C and 0°C to −50°C, respectively. After reaching the desired temperature the leaf segment was rapidly frozen to −196°C in liquid nitrogen bath. Then fluorescence emission spectra were recorded with fluorescence spectrophotometer Hitachi, model F-4500 (Hitachi Instruments, Japan) with sample immersed in liquid nitrogen in a Dewar-type optical cryostat. The excitation wavelength was 436 nm, the emission was detected in the wavelength range from 600 to 800 nm. The spectral halfwidths were 5 and 2.5 nm for excitation and emission monochromator, respectively.

Fig. 1 shows FTC (F_0) (curve a) and FTC (F_M) (curve b) of barley leaves in temperature range from −100°C to 75°C. The curve (a) is composed of two parts: part (a_1) was obtained by cooling a segment from 25°C to −100°C, whereas the other part (a_2) was obtained by heating of another segment from 25°C to 75°C. The rise of temperature above 32°C leads to slight non-linear increase of the F_0, whereas a sharp linear increase of the F_0 was detected above 42°C (insert 2). At 50°C the F_0 reaches the F_M level. Similarly to the effect induced by increasing temperature, the decrease of temperature below 0°C leads to a two-step rise of the F_0: the F_0 increases slightly non-linearly and then sharply linearly below −10°C and −25°C, respectively (insert 1). At about −50°C the F_0 reaches the F_M level. Above 50°C and below −50°C the F_0 follows the course of the F_M. The F_M decreases in the whole temperature range of −100°C to 75°C.

Results on the effect of temperature on the ratio F_V/F_M, reflecting potential quantum yield of photochemical reaction of RCII [16], are presented in insert 3 of Fig. 1 (open circles). The F_V/F_M ratio is used as parameter of the physiological state of photosynthetic apparatus in intact plant leaves. Under optimal physiological conditions this parameter was found to have the value of 0.832 [17]. The environmental stresses affect PSII efficiency and lower the F_V/F_M value. Our results show that at temperatures from −10°C to 32°C the ratio F_V/F_M does not change considerably and keeps the value ∼0.8. However, in parallel with the F_0 increase both at high and low temperature, the F_V/F_M sharply decreases reaching the 0 value at −50°C and 50°C. Below −50°C and above 50°C the F_V/F_M was found constant with 0 value.

The chlorophyll fluorescence induction kinetics is used as a monitor of the electron transport processes in the RCII. At room temperature, the chlorophyll fluorescence transient follows an O-J-I-P pattern from the minimum fluorescence F_0 to the maximum fluo-

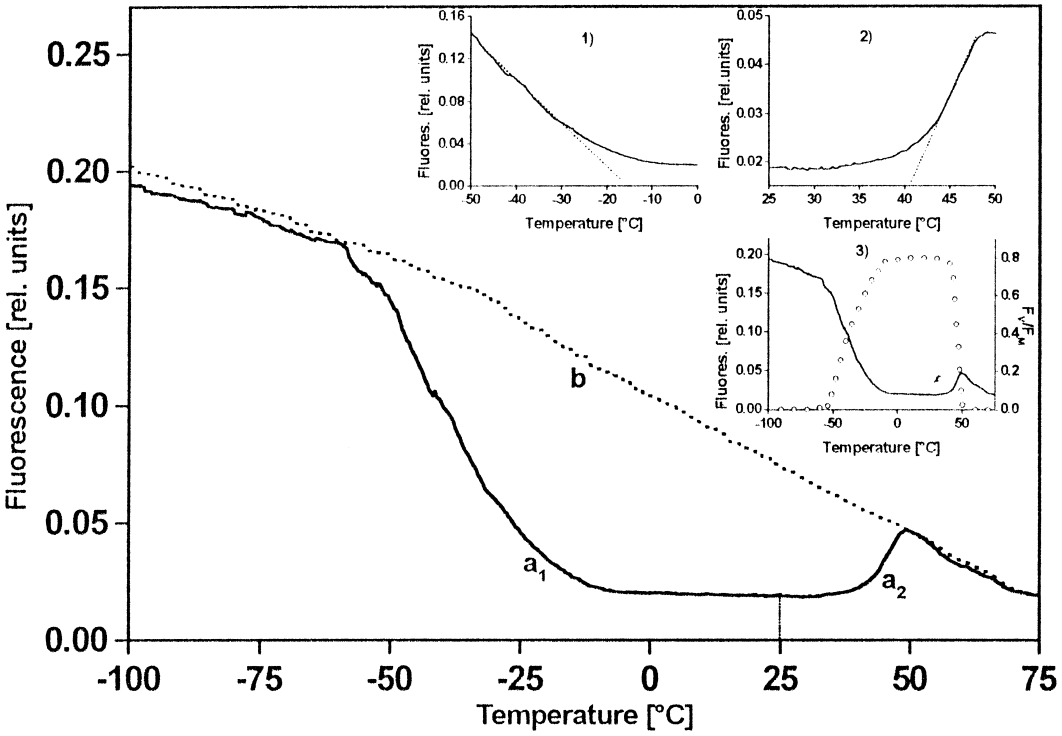

Fig. 1. FTC (F_0) (curve a) and (F_M) (curve b) of barley leaf in temperature range from −100°C to 75°C. Leaf segment infiltrated with glycerine was linearly cooled from 25°C to −100°C with cooling rate 30°C/min (trace a_1), another leaf segment was linearly heated from 25°C to 75°C with heating rate 30°C/min (trace a_2). The intensity of fluorescence (at 25°C) of (a_1) and (a_2) curves was normalized to the same value. Leaf segment infiltrate with DCMU and glycerine (curve b) was linearly heated from −100°C to 75°C. The intensities of fluorescence at 50°C of (a_2) and (b) curves were normalized to the same value. The fluorescence changes at 0°C (slight increase, about 5%) connected with the creation of ice on the surface of leaf was corrected. The samples were excited by weak red light ($1\,\mu mol\,m^{-2}\,s^{-1}$, maximum emission at 655 nm) and fluorescence was detected above 700 nm. Inserts 1 and 2 show two parts of FTC (F_0) from −50°C to 0°C and 25°C to 50°C, respectively, demonstrating the non-linear and linear parts of the F_0 rise. Insert 3 shows comparison of temperature dependence of F_0 (full line) and ratio F_V/F_M (open circles) in temperature range from −100°C to 75°C.

rescence F_P or F_{max} [18] (Fig. 2, trace 25°C). The chlorophyll fluorescence induction kinetics was measured at 50°C and −50°C (that is at temperatures, where the F_0 reaches the F_M) (Fig. 2). As is evident from this figure both the freezing and heat treatment changes drastically the shape of the chlorophyll fluorescence induction kinetics. A sharp increase of fluorescence in the induction curve is followed by a nearly constant part.

Low temperature fluorescence emission spectra are used to provide information on the distribution of excitation energy between PSII and PSI [19]. The emission spectrum of leaves (at −196°C) shows three major bands with maxima at around 685, 695 and 735 nm (Fig. 3, trace a). It is generally accepted that the bands at 685 nm (F685) and 695 nm (F695) originate from PSII core antenna, CP43 and CP47, respectively [20], whereas the emission around 735 nm (F735) is predicated to LHCI [21]. The conditions which affect the structural organisation of photosynthetic apparatus alter the normal pattern of excitation energy distribution between the photosystems. To compare the excitation energy distribution between PSII and PSI at low and high temperatures, the chlorophyll fluorescence emission spectra (at −196°C) were measured after linear heating from 25°C to 50°C and linear freezing from 0°C to −50°C (Fig. 3, trace b and c, respectively). As is evident from Fig. 3, both linear freezing and heating lead to a rise of the emission band at 735 nm relative to that at 685 nm. The heating causes the rise of F735/F685 ratio from 2.5 at room temperature to about 5 at 50°C. Similarly freezing leads to a rise of F735/F685 from 2.5 at 0°C to about 5 at −50°C. Inserts 1 and 2

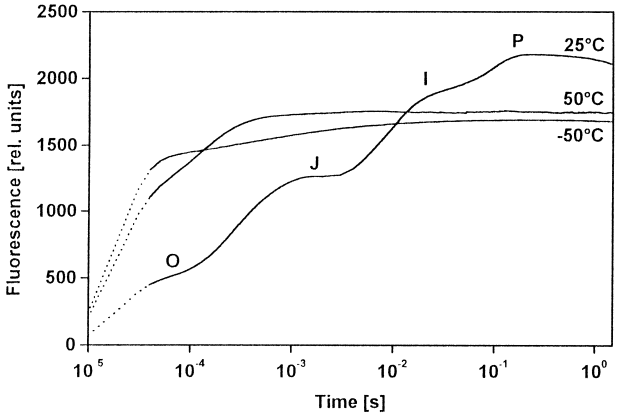

Fig. 2. Fluorescence induction kinetics recorded at 25°C and after cooling to −50°C or heating to 50°C. Fluorescence was recorded from 10^{-5} to 1.5 s. The F_0 is considered to be around 4×10^{-5} s (dotted curves correspond to LED switching). The graph is plotted on the logarithmic time scale.

of Fig. 3 show the temperature dependencies of F735/F685 (−196°C) on the temperature reached by heating or freezing, respectively. As is evident from the inserts, this ratio increases parallelly with a sharp increase of the F_0 at 42°C and −25°C.

The high temperature induced increase of the F_0 during linear heating occurs in two steps: a slight non-linear increase occurs in temperature range from 32°C to 42°C, whereas a sharp linear rise was observed from 42°C to 50°C. This increase has been described by Schreiber and Armond [9] and it seems to be connected with the blocking of electron transport in RCII. This interpretation seems to be correct taking into account the fact that the F_0 reaches the F_M level corresponding to the closed RCII. The RCII interpretation of the F_M increase is further supported by measurements of F_V/F_M and fluorescence induction kinetics. The ratio F_V/F_M reflecting quantum yield of PSII photochemistry is constant up to 32°C and then starts to decline parallelly with the F_0 rise. The shape of fluorescence induction kinetics after heating to 50°C with a sharp rise of fluorescence is similar to that measured in presence of DCMU. We show that also the temperature decrease leads to an

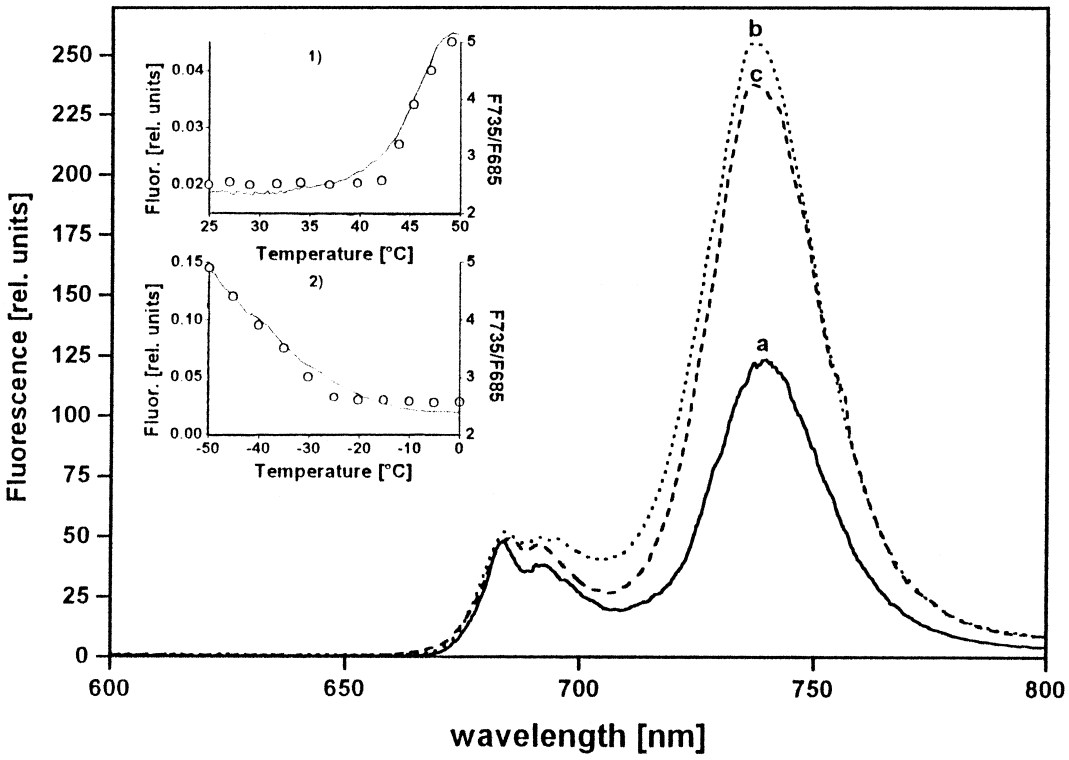

Fig. 3. Low temperature chlorophyll fluorescence emission spectra (−196°C) measured with control 25°C (a), after linear heating to 50°C (b) and linear freezing to −50°C (c). The spectra were normalized to F685. A comparison of the temperature dependence of F_0 (full line) and ratio F735/F685 (open circles) in temperature range from 25°C to 55°C (insert 1) and from 0°C to −50°C (insert 2).

F_0 increase. Like upon heating the low temperature induced increase of the F_0 occurs in two steps: a slight non-linear increase appears from -10 to -25°C whereas a sharp linear increase occurs from -25 to -50°C. We propose that the low temperature increase of the F_0 may be also caused by blocking of RCII because of the following reasons: (i) at -50°C the F_0 reaches the F_M level corresponding to the closed RCII; (ii) the F_V/F_M starts to decrease below -10°C parallelly with the F_0 rise; (iii) the shape of fluorescence induction kinetics after freezing to -50°C shows a sharp rise of fluorescence similarly to that measured in presence of DCMU.

Whether the blocking of RCII occurs on the donor or acceptor side of RCII is unclear. It was shown that inactivation of OEC occurs above 32°C [22] whereas the further heating above 42°C leads to an inhibition of Q_A–Q_B electron transport [10,11,22]. At low temperature the S-state advancement of OEC becomes inhibited step by step from the higher oxidation state to the lower upon lowering temperature. The half inhibition of S_3–S_0, S_2–S_3 and S_1–S_2 transition, deduced from thermoluminescence and EPR measurement, occurs at -7°C, -45°C and -100°C, respectively [23–26]. On the other hand the observations of Joliot [27] showed that the electron from Q_A to Q_B becomes very slow below -30°C.

The blocking of RCII is usually accompanied with a redistribution of excitation energy in favour of PSI. The changes in redistribution of excitation energy induced by heat stress has been published earlier [9]. The ratio F735/F685 reflecting the emission of antennae of PSI relative to that of PSII rises about twice. We show that similar changes in excitation energy redistribution occur upon freezing. This supports an idea that the freeze treatment may be connected with blocking of RCII as well as with concomitant redistribution of excitation energy in the antennae.

Freeze and heat treatment of leaves is accompanied by similar changes of F_0 – both upon freezing and heating the F_0 increases reaching the F_M value. The similar changes in fluorescence parameters F_V/F_M, F735/F685 and fluorescence induction kinetics indicate that not only heating but also freezing is connected with a blocking of RCII and redistribution of excitation energy.

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