Effects of high temperature on chlorophyll fluorescence induction and the kinetics of far red radiation-induced relaxation of apparent F₀ in maize leaves

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Abstract

Temperature dependence (25-50 °C) of chlorophyll (Chl) fluorescence induction, far-red radiation (FR)-induced relaxation of the post-irradiation transient increase in apparent F₀, and the trans-thylakoid proton gradients (ΔpH) was examined in maize leaves. Temperatures above 30 °C caused an elevation of F₀ level and an enhancement of F₀ quenching during actinic irradiation. Millisecond delayed light emission (ms-DLE), which reflects the magnitude of ΔpH, decreased strikingly above 35 °C, and almost disappeared at 50 °C. It indicates that the heat-enhanced quenching of F₀ under actinic irradiation could not be attributed mainly to the mechanism of ΔpH-dependent quenching. The relaxation of the post-irradiation transient increase in apparent F₀ upon FR irradiation could be decomposed into two exponential components (τ₁ = 0.7-1.8 s, τ₂ = 2.0-9.9 s). Decay times of both components increased with temperature increasing from 25 to 40-45 °C. The bi-phasic kinetics of FR-induced relaxation of the post-irradiation transient increase in apparent F₀ and its temperature dependence may be related to plastoquinone (PQ) compartmentation in the thylakoid membranes and its re-organisation at elevated temperature.

Additional keywords: heat stress; Zea mays L.

Introduction

The complicated syndrome of photosynthesis under heat stress can be probed by non-invasive methods such as chlorophyll (Chl) fluorescence measurements. Increased temperature causes an increase in non-photochemical quenching (qₑ) (Bilger et al. 1987, Georgieva and Yordanov 1994). Heat treatment is believed to limit ATP consumption in the Calvin cycle, which may lead to an accumulation of ΔpH gradient with an increase in high-energy dependent Chl fluorescence quenching (qₑ) during actinic irradiation (Bilger et al. 1987, Joshi et al. 1995). On the other hand, membrane permeability probably increases during heat stress, which may bring about a decrease in ΔpH across thylakoid membranes (Berry and Björkman 1980, Bukhov et al. 1999) and consequently a suppression of qₑ. In this study, the significance of heat-induced increase of qₑ was examined in intact maize leaves by comparing the Chl fluorescence induction curve with ms-DLE, an indicator of trans-thylakoid ΔpH (Evans and Crofts 1973, Xu and Shen 1984). The results suggest that heat-enhanced Chl fluorescence quenching under actinic irradiation could not be ascribed mainly to qₑ.

In cyanobacteria (Mi et al. 1997) and higher plants (Bukhov et al. 1992, Asada et al. 1993, Mano et al. 1995, Burrows et al. 1998, Feild et al. 1998, Shikanai et al. 1998, Jin et al. 2000, Yamane et al. 2000) the minimal Chl fluorescence yield (apparent F₀) transiently increases after a light-to-dark transition. This increase in apparent

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Abbreviations: AR – actinic radiation; apparent F₀ – dark level of chlorophyll fluorescence yield measured after light-to-dark transition; Chl – chlorophyll; F₀ – dark level of Chl fluorescence yield measured after dark-adaptation; F₀' – the dip of Chl fluorescence level after the turning off of actinic radiation; F₀'' – the fraction of the post-irradiation transient increase in apparent F₀ that can be quenched by far-red radiation; F₀''/F₀' – the steady state level of apparent F₀ during far-red irradiation; FR – far-red radiation; LHC – light-harvesting complex; ms-DLE – millisecond delayed light emission; PQ – plastoquinone; PS – photosystem; qₑ – trans-thylakoid-ΔpH-dependent Chl fluorescence quenching; qₑ – non-photochemical quenching of Chl fluorescence; ΔpH – proton gradient.

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F₀ can be quenched by FR (Asada et al. 1993, Burrows et al. 1998, Shikanai et al. 1998). It is inhibited by a specific inhibitor of ferredoxin-plastoquinone reductase (Mano et al. 1995) or by site mutation of the genes encoding the sub-units of NAD(P)H dehydrogenase complex (Burrows et al. 1998, Shikanai et al. 1998), and enhanced by specific inhibitor of cytochrome b₅f (Mano et al. 1995). The post-irradiation increase in apparent F₀ probably reflected the reduction by reducing equivalents of the intersystem electron transport chain that has been accumulated during actinic irradiation. FR preferentially drives photosystem (PS) 1, depletes electrons from the intersystem chain, and consequently brings about a decrease in Chl fluorescence level. Hence, the analysis of the relaxation of the post-irradiation transient increase in apparent F₀ upon FR irradiation can provide information about the efficiency of energy transfer in PS1 and the structural and functional change in photosynthetic membranes. In this work, the temperature dependence of the FR-induced relaxation of the post-irradiation transient increase in apparent F₀ in an intact maize leaf was investigated.

Materials and methods

Plants of maize (Zea mays L.) were grown in Hoagland solution under natural light at temperature range of 13-24 °C. The third fully unfolded leaves were used for measurements.

Chl fluorescence was measured using PAM101 (Walz, Effeltrich, Germany) integrated with a microcomputer. The modulated measuring beam (1-μs duration, 1.6 kHz, peak emission at 650 nm) was 0.05 μmol(phonon) m⁻² s⁻¹. Actinic irradiation (620 μmol m⁻² s⁻¹) was provided by an halogen lamp. Far-red radiation (>720 nm, 7.3 μmol m⁻² s⁻¹) was from a halogen lamp filtered by a GR720 filter (Schott, Mainz, Germany). For the measurement of Fₘ, 1-s saturating pulse [655 nm, 1 200 μmol(phonon) m⁻² s⁻¹] was provided by a LED-array cone (High-Power-LED-Lamp, Walz). The abaxial side of an intact attached leaf was pressed on a thermostatted glass platform covered by a piece of black cotton cloth, and the adaxial side of the leaf was placed facing the detector encircled by a piece of plastic foam, as described in Jin et al. (2001). The temperature was increased from 25 to 50 °C by 5 °C steps. The dark period between two measurements was 20 min, and the temperature was increased thermostatically within 3 min at the beginning of each dark period.

Results and discussion

Temperature dependence of the profile of Chl fluorescence induction, the transient post-irradiation increase in apparent F₀, and its relaxation upon FR irradiation measured in an intact attached maize leaf are depicted in Fig. 1. The post-irradiation transient increase in apparent F₀ was quenched by FR applied 20 s after the turning off of actinic radiation. The values of parameters used to characterise the Chl fluorescence traces in Fig. 1, i.e. the dip upon light-to-dark transition (F₀'), the fraction of the post-irradiation increase of apparent F₀ that could be quenched by FR (F₀'/F₀), the steady state level of apparent F₀ during FR irradiation (Fₜ'), and the F₀ level before actinic irradiation are shown in Fig. 2. Elevated temperature caused not only a rise of F₀ but also an acceleration of Chl fluorescence quenching under actinic irradiation, and a decline of the Chl fluorescence below the F₀ level at temperatures above 35 °C.

The heat-induced rise of F₀ has been well documented and attributed mainly to the disconnection of light-harvesting complex (LHC) from PS2 (Schreiber and Armond 1978, Armond et al. 1980, Yamane et al. 1997) and/or to the accumulation of reduced QA (Bukhov et al. 1990, Cao and Govindjee 1990, Goltsvev et al. 1994, Havaux 1996, Szanov et al. 1998, Yamane et al. 2000). In the present experiment, F₀ could not be quenched by FR (values not shown), indicating that QA reduction mechanism was not involved in the heat-induced rise of the original F₀ in maize leaves. Comparative study among various higher plants made by Yamane et al. (2000) revealed that the functioning of QA reduction mechanism in

Measurement of ms-DLE was carried out using a home-equipped apparatus (Xu and Shen 1984, Li and Shen 1995, Wei et al. 1998). The outputs were fed to an analogue-digital converter attached to a microcomputer. Intact attached maize leaves treated in the same way as that used for Chl fluorescence measurement were cut into 4.5 cm long sections and inserted into a flat simple cell made of plexiglass, and the ms-DLE was measured immediately at room temperature (22 °C).

Data analysis: Processing of all the digitised records was made using a series of home-designed software. The relaxation of the post-irradiation transient increase in apparent F₀ upon FR irradiation was described by a sum of exponential decaying components with amplitude aᵢ and decay time τᵢ,

\[ F(t) = F₀ + \sum aᵢ \exp(-t/τᵢ) \]  

(1)

where t represents the time since the turning on of FR, F(t) the level of apparent F₀ as a function of t, and F₀ the estimated asymptotic level of apparent F₀ under FR irradiation. Experimental points were fitted to Eq. 1 by software based on non-linear Levenberg-Marquardt algorithm as described by Jin et al. (2001).
the heat-induced enhancement of \( F_0 \) was species-dependent. The quenched \( F_0 \) could not return to the original level instantly upon the turning off of actinic radiation, as indicated by the observation that the steady state level of apparent \( F_0 \) under FR irradiation (\( F'_0 \)) dropped below \( F_0 \) at temperatures above 35 °C. This was caused by heat-induced quenching of \( F_0 \) under actinic irradiation (Bilger et al. 1987). \( F_0 \) quenching suggests a limitation of energy transfer from LHCII to PSII (Bilger and Schreiber 1986), which takes place upon LHCII phosphorylation (Krause and Behrend 1983, Hodges and Barber 1986).

![Fig. 1. Effects of elevated temperatures on Chl fluorescence induction, the post-irradiation transient increase in apparent \( F_0 \), and its relaxation upon FR irradiation in an intact attached maize leaf. After 50-min dark-adaptation, the leaf was exposed sequentially to a series of step-wise increased temperatures. The measurement was performed after 20-min leaf adaptation to the treated temperatures. Arrows indicate switching on (↑) or off (↓) of actinic or FR irradiation.](image)

Heat-induced change in ΔpH across the thylakoid membranes in maize leaves was followed with ms-DLE measurement (Fig. 3). First discovered by Strehler and Arnold (1951) and having been used to probe a variety of parameters which change during adjustment to steady-state photosynthesis (Malkin 1977), ms-DLE generally consists of a fast phase and a subsequent slow increase. The slow phase depends on trans-thylakoid ΔpH proton gradient (Evans and Crofts 1973) and the fast phase is related to a rapid establishment of the thylakoid membrane potential (Wraight and Crofts 1971). Furthermore, Xu and Shen (1984) showed that adding NH₄Cl or nigericin plus K could diminish the intensity of the fast phase; suggesting also a ΔpH-dependence. As shown in Fig. 3, the magnitude of ms-DLE changed slightly with temperature in the range 25-35 °C, strikingly decreased above 35 °C, and almost vanished at 50 °C. Although heat-induced inactivation of PSII may lead to the inhibition of linear electron transport and consequently a decrease in ΔpH, the temperature-dependence pattern of \( F_0/F_m \) (Fig. 3, inset) indicates that this effect was not significant at temperatures below 45 °C. Thus, the dimi-

![Fig. 2. Quantitative representation of the temperature-dependent Chl fluorescence changes in Fig. 1. \( F_0 \) quenching under actinic irradiation was indicated by \( F'_0 \), which dropped below \( F_0 \) at temperatures above 30 °C. Magnitude of the post-irradiation transient increase in apparent \( F_0 \) was estimated by the fraction that could be quenched by FR (\( F'_0 \)).](image)

![Fig. 3. Effects of elevated temperatures on ms-DLE in maize leaves. The leaves had gone through the same heating and darkening regimes as those used for Chl fluorescence measurement at corresponding temperatures. For details, see Materials and methods. The oscillations were due to instrumental noise. The inset demonstrates temperature-dependence of \( F_0/F_m \).](image)
nishing of ms-DLE above 35 °C arose mainly from heat-induced increase in membrane permeability and the resultant abolishment of trans-thylakoid proton gradient, as suggested by Berry and Björkman (1980) and Bukhov et al. (1999). The discrepancy between thermal response patterns of ms-DLE and Chl fluorescence induction curve suggests that qE was not the main mechanism causing the heat-induced quenching of Chl fluorescence during actinic irradiation, especially at temperatures above 35 °C.

Heat stress causes the detachment of LHC2 from PS2 and the subsequent lateral migration of PS2. A portion of LHC tightly bound to it goes to the non-appressed regions, leaving behind the free LHC2 in the appressed membranes (Sundby and Andersson 1985, Sundby et al. 1986, Ruban and Trach 1991), which might be transferred to PS1 located in the grana margins (Ruban and Trach 1991), but not necessarily so (Carpentier 1999). Conceivably, it was the quenching related to the heat-induced state transition that accounted for the main part of the enhanced quenching of Chl fluorescence induction curve at elevated temperature (Figs. 1 and 2).

When the actinic irradiation was turned off, non-photochemical reduction of the intersystem chain brought about a transient increase in apparent F0 (Fig. 1). At temperatures above 30 °C, a substantial fraction of the post-irradiation increase in Chl fluorescence arose from the nullification of light-induced F0 quenching (Fig. 2). The FR-quenchable portion of the post-irradiation transient increase in apparent F0, as represented by F0p in Fig. 2, went through a maximum around 35 °C. This form of the temperature-dependent pattern might be related to the change in size of the pool of electrons available for the reduction of the intersystem electron transport chain (Jin et al. 2000).

As shown in Fig. 4, the kinetics of the FR-induced relaxation in apparent F0 can be decomposed into two exponentially decaying components. Although FR-irradiation could also induce ΔpH-dependent Chl fluorescence quenching, the effect would be too small under the FR irradiance employed in this experiment (Feild et al. 1998). Bi-phasic kinetics of the FR-induced relaxation of apparent F0 indicates the structural and functional heterogeneity of the thylakoid membranes.

There is growing documentation on the structural and functional heterogeneity of photosynthetic membranes of higher plants. Apart from the heterogeneous distribution of photosystems and other integral protein complexes in thylakoid membranes (Albertsson 2001), PQ compartmentation indicated by difference in rate of photoreduction has also been seen in chloroplasts (Joliot et al. 1992, Kirchhoff et al. 2000). As proposed by Kirchhoff et al. (2000), PQ molecules in thylakoid membranes are segregated into micro-domains by large aggregates formed by a hierarchy of specific PS2-LHC2 and LHC2-LHC2 interactions. At least 70 % of PS2 may be located in small domains, with an average number of only 1 to 2 PS2 centres in each domain, while a small fraction of PS2 is located in large domains, with more than 10 PS2 centres in a domain. Such micro-organisation allows rapid shuttling of electrons between PS2 and cytochrome b6/f by PQ diffusion within each micro-domain, but redox equilibration throughout the membrane is slow. The structural and functional difference between the large and the small micro-domains of PQ in thylakoid membranes may result in a variation in the decay-time of FR-induced depletion of electrons accumulated in these domains, as reflected by the bi-phasic kinetics of FR-induced relaxation of the post-irradiation transient increase in apparent F0.

Fig. 4. Relaxation kinetics of the post-irradiation transient increase in apparent F0 upon FR irradiation in the intact maize leaf. The figure illustrates the fitting procedure and the decomposition with the best fit. A, B: Residual plots of the fitting of experimental data to mono- and bi-exponential model, respectively. C: Decomposition of the fitted bi-exponential curve, showing the individual contributions of the two kinetic components. The treating and measuring temperature was 35 °C.

The temperature-dependence of the kinetic parameters is shown in Fig. 5. As the temperature increased sequentially from 25 to 50 °C, the fast phase was slowed down and its decay-time increased with temperature up to 45 °C, with a slight decrease above 45 °C. The slow phase, with a decay-time increasing monotonously with temperature in the range from 25 to 40 °C, degenerated at 45 °C, and could not be resolved at 50 °C.

Heat-treatment caused not only the detachment of LHC2 from PS2 but also the randomisation and dissociation of other integral protein complexes in the thylakoid membranes, leading to a re-organisation of PQ compartmentation. Meanwhile, heat-treatment also enhanced random motion of PQ, which in turn muddled PQ diffusion within micro-domains. All these effects would limit the FR-induced oxidation of the PQ pool in the thylakoid.
membranes, and thus slow down the relaxation of apparent F₀, or even change the pattern of decay. This interpretation seems contradictory to the observations that the dark re-reduction of P700 was accelerated by heat treatment (Havaux 1996, Bukhov et al. 1999, Jin et al. 2001). As an accommodation, heat-induced de-stacking of the thylakoid membranes (Carpentier 1999) may be made easy for reduced ferredoxin molecules to enter into the narrow, crowded space between two neighbouring apressed membranes to reach the cytochrome b₅f complex in the grana, bringing about a promotion of P700 reduction. Although the direct reaction between ferredoxin and cytochrome b has been questioned (Bendall and Manasse 1995), there might be other pathways through which electrons can be transferred from stromal reductants to cytochrome b₅f without the participation of the PQ pool. In Chlorella sorokiniana, a redox-active component loosely associated with the stromal surface of cytochrome b₅f complex has been identified, which undergoes redox equilibration with the haem b₅ of cytochrome b-563 (Lavergne 1983, Joliot and Joliot 1988). Recently, Zhang et al. (2001) found the activity of ferredoxin:NADP⁺ oxidoreductase in spinach chloroplast cytochrome b₅f complex. Since the PQ pool is not involved in all these pathways, the limitation arising from heat-induced randomisation of PQ can be avoided.

Alternatively, we cannot exclude the possibility that the retardation of FR-induced relaxation of the post-irradiation transient increase in apparent F₀ at high temperatures was caused by the detachment of LHCl from PS I. We observed that the FR-induced relaxation of the post-irradiation transient increase in apparent F₀ in intact maize leaves was slowed down under lower irradiance by FR (data not shown), indicating that the absorption of radiation may become the rate-controlling step. Although we have not found evidence in the literature, it is conceivable that LHCl may dissociate from PS I under severe heat stress, leading to a decrease in functional cross section of the light-harvesting system.

In summary, the present study provides evidence that the ΔpH-dependent quenching is not the dominating mechanism of heat-enhanced quenching of Chl fluorescence induction. As to the kinetic analysis of FR-induced relaxation of the post-irradiation transient increase in apparent F₀, our interpretation is tentative. More investigations are needed before it can be employed as a probe to explore conformational changes in photosynthetic apparatus.

References


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