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Short Term Acclimation of Spinach to High Temperatures

EFFECT ON CHLOROPHYLL FLUORESCENCE AT 293 AND 77 KELVIN IN INTACT LEAVES

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ABSTRACT

Using intact leaves of *Spinacia oleracea* (L.), reversible temperature-induced changes in chlorophyll fluorescence emitted at room temperature and at 77K were studied. Interpretation of fluorescence at 77K was largely facilitated by developing a new method to minimize reabsorption artifacts ('diluted leaf-powder'). Leaves of plants grown at 15 to 20°C were exposed for several hours to different temperatures. Upon incubation at 35°C in the dark or in the light, the following changes in 77K fluorescence occurred with a half-time of less than 1 hour: (a) the initial fluorescence (F_0) of photosystem I increased by 15%, while that one of photosystem II somewhat decreased; (b) although variable fluorescence declined in both photosystems, the decrease in photosystem II (40%) was more severe; (c) the changes were less significant after 480-nanometer excitation light was replaced by 430-nanometer light. The data were interpreted in terms of a reversible, temperature-induced change in thylakoid structure and related change in the distribution of the absorbed energy in favor of photosystem I, at the expense of photosystem II excitation, probably accompanied by an increase in the rate of thermal deactivation of excited states. The considerable decrease in the variable part of room temperature fluorescence gives rise to the suggestion that this transition has lowered the reduction level of plastoquinone, *i.e.* has increased electron flow through photosystem I, relative to photosystem II. Possible physiological and mechanistic analogies between this temperature-induced state transition and the light-dependent state 1-state 2 regulation has been discussed.

In a large number of investigations, photosynthetic acclimation of higher plants to high temperatures is regarded predominantly as a long term response of leaves to changes in temperature regime in the time range of days or weeks, often accompanied by alterations in the molecular composition of pigment systems (*e.g.* Ref. 2) and in the physical state of matrix lipids (11; for a review, see 5). However, considerable fluctuations in temperature also occur in the time range of several hours during a day and less is known about mechanisms of photosynthetic acclimation to such fast variations in temperature. Since temperature coefficients of various types of photosynthetic reactions such as primary photochemical events, electron transport, and enzymic catalysis differ from each other, fluctuations in temperature will affect the balance between these reactions. In the field, temperature normally co-varies with light. During variations in light conditions, the balance between carbon cycle reactions, energy charge, and electron transport is assumed to be maintained by reversible changes in the distribution of the absorbed energy between the two photosystems, which in turn is related to the lateral distribution of the different pigment systems within the thylakoid membrane (state 1-state 2 regulation, *e.g.* Ref. 1). A similar mechanism might exist for optimizing photosynthesis during fast fluctuations in leaf temperature.

The distribution of excited states between the two photosystems is reflected by characteristics of Chl fluorescence emitted from the pigment systems (for a review, see Refs. 6 and 12). First experimental evidence for reversible temperature-induced alterations in fluorescence yield and the conformational state of the PSII complex came from experiments with spinach, which showed that exposing plants to high but not damaging temperatures for a few hours gives rise to an increase in thermal stability (14, 22) and an accompanying decrease in the fluorescence of PSII (22). It has been concluded that changes in the aqueous phase of the stroma rather than alterations in the molecular composition of the thylakoid membrane affects its physical state (14). For example, the thermal stability of pigment structures can be considerably modulated by changing the electrolytic conditions of the aqueous phase around the membrane (20, 21).

The intention of the present study is to gain further insight into mechanism and possible function of reversible changes in the state of photosynthetic pigment systems that take place after spinach leaves are exposed for a few hours to high (not injurious) temperatures. The distribution of excited states between the three pigment structures, PSI, PSII, and LHC[1] within leaves will be characterized by means of 77K fluorescence, whereby a new method developed to minimize reabsorption artifacts will be presented. Room temperature fluorescence is used to demonstrate the redox state of the electron transport chain between the two photosystems. A reversible increase in the rate of PSI excitation, relative to PSII, induced by exposure of spinach leaves to high temperature, is regarded as an adaptive response of leaves to high temperatures and is discussed in relation to the light-dependent state 1-state 2 regulation.

MATERIALS AND METHODS

Plant Material. Plants of *Spinacia oleracea* L. were grown either in a growth chamber (10-h light at 20°C/14-h dark at 15°C; light intensity, 30 $w \cdot m^{-2}$; 75% RH) or in a glasshouse (October to December) equipped with supplemental light (10-h photoperiod) at a temperature adjusted to 17 to 20°C (day) and 14 to 17°C (night).

Temperature Treatments. Either intact plants or freshly cut leaves were placed into water vapor-saturated chambers which then were adjusted (1 to 1.5°C/min) to the indicated temperatures (±1°C). If indicated, the lucid chambers were illuminated with blue-green light (Corning filter 9782). After the indicated incubation times, the leaves were taken from the chamber and rapidly prepared at room temperature in the dark for fluorescence meas-

[1] Abbreviations: LHC, light-harvesting complex; PQ, plastoquinone, F_I (F_{II}, F_{LHC}), fluorescence emitted from PSI (PSII, LHC); F_0, initial fluorescence with open PSII traps; F_m, fluorescence with closed PSII traps; F_v, F_m minus F_0; Q, primary electron acceptor of PSII.

urements.

For testing the thermal stability of PSII, preincubated leaves were plunged into a 45°C water bath (6 min) and then measured for fluorescence.

Fluorescence Measurements. Room temperature (18 to 20°C) fluorescence was measured from the upper surface of leaf segments (20-mm diameter). The excitation beam was passed through glass filters (Corning 5030, 8782; Schott KG 4), a heat-reflecting filter (Balzers Calflex c), and a 20-mm water layer; the lamp was equipped with a photoshutter (Prontor Electronic-m-1). Fluorescence was measured through a 685-nm interference filter (Balzers) and a RG 665 cut-off filter (Schott) and recorded with a photomultiplier (EMI 9558B).

For measuring fluorescence in the presence of DCMU after removing the lower epidermis, preincubated leaves were bathed for 5 min under slightly reduced pressure in a solution of 300 mM sorbitol in the presence or absence of 50 μM DCMU. Control experiments confirmed that leaves infiltrated with sorbitol (minus DCMU) showed essentially the same fluorescence characteristics as non-infiltrated leaves, suggesting that the infiltration procedure had no significant effect on electron transport.

Fluorescence at 77K was measured from the upper surface of leaf discs (20-mm diameter) which were punched from intact leaves immediately prior to measuring. The leaf discs were lightly appressed against the lucid bottom of a Dewar vessel and then kept for 3 min at room temperature in darkness to allow complete oxidation of PSII reaction centers (which was confirmed in trial experiments). Liquid N_2 was added in the dark and fluorescence induction was measured after at least 1-min equilibration at 77K. The excitation beam (0.5 $w \cdot m^{-2}$) was passed through a glass filter (Corning 9782), a 477-nm interference filter (Balzers), and heat-absorbing filters. Fluorescence emission was measured through a RG 715 cut-off filter (Schott) and a 740-nm, 7-nm half-bandwidth interference filter from Balzers (PSI) or through RG 665 (Schott) and a 694-nm, 7-nm half-bandwidth interference filter (PSII).

The fluorescence spectra were recorded with a spectrofluorimeter (Farrand Mark 1) and were corrected for fluorimeter sensitivity; 480-nm light (20-nm bandwidth, 8 $w \cdot m^{-2}$) was used for excitation. Emission was analyzed with 1-nm bandwidth.

Preparation of 'Diluted Leaf Powder.' In order to avoid artifacts due to the reabsorption of short wavelength fluorescence in leaves, 77K emission spectra were obtained from a diluted leaf powder prepared as follows. Leaf discs containing about 20 μg Chl were frozen in liquid N_2 and carefully ground under subzero temperatures in a cold mortar. Afterwards, the resulting fine leaf powder was mixed with ground ice crystals (equivalent to 4 ml water). For fluorescence measurements, a small portion of this diluted leaf powder was placed into a Dewar vessel containing liquid N_2. The emission spectra obtained from those samples did not show significant artifacts by reabsorption of PSII fluorescence: the shape of the spectra remained unaffected by Chl concentration up to 30 μg Chl/sample. At Chl concentrations exceeding 30 μg/sample, the F_I/F_{II} ratio gradually increased and the F_{LHC} peak at 685 nm disappeared, indicating the beginning of reabsorption of short wavelength fluorescence.

RESULTS

77K Fluorescence. Emission bands of 77K Chl fluorescence at 735, 695, and 685 nm has been attributed to emission by PSI, PSII, and LHC (15). The analysis of fluorescence emitted from intact leaves is complicated by the phenomenon that a large fraction of F_{II} and F_{LHC} is reabsorbed due to multiple scattering between tight packed chloroplasts, whereas F_I emission is less affected. Especially in the case of exposure to different environmental conditions, it cannot be excluded that changes in scattering properties could change the fraction of F_{II} (F_{LHC}) to become reabsorbed. In order to minimize this artifact, a diluted leaf powder has been prepared as described above. Using this procedure, F_I/F_{II} ratios of leaves preincubated in the dark at room temperature varied from 1.3 to 1.7 between different batches of plants (Fig. 1). Similar values were obtained from a diluted suspension of isolated intact chloroplasts (conditions as in Ref. 10): F_I/F_{II} ratio from a preparation of diluted leaf powder and from intact chloroplasts isolated from the same batch of leaves did not differ from each other by more than 10% (data not shown). Unfortunately, the absolute fluorescence yield of the diluted leaf powder varied from sample to sample due to inevitable small variations in the Chl content of different preparations. Therefore, F_I emitted at 740 nm from the surface of intact frozen leaf discs was taken as a standard, because these values were reproducible from leaf to leaf and were expected to be less affected by reabsorption artifacts. The far-red peaks of the emission spectra of diluted leaf powder were then normalized on these F_I standards.

Figure 1 demonstrates that incubation of leaves for 3 h at 35°C results in a large decrease in F_{II} (30 to 40%) and a concomitant small increase in F_I (3 to 6%). As a consequence, the F_I/F_{II} ratio increased from about 1.5 (20°C) to 2.3 (35°C). Essentially the same effect was observed with leaves from *Valerianella locusta* (not shown). Figure 2 shows the temperature profile of F_I/F_{II} from 5 to 35°C (5- to 7-h incubation). The ratio increased with increasing temperature over the range studied. However, the increase was most pronounced at temperatures exceeding 20°C. For a first approach, this increase in the F_I/F_{II} ratio induced by high temperatures may be taken as a relative measure for a

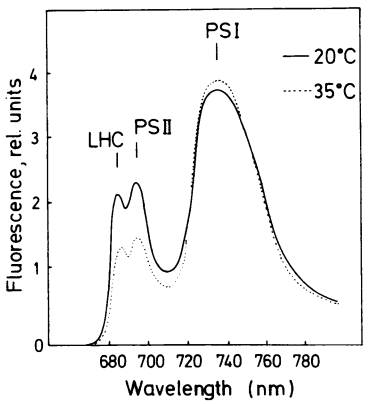

FIG. 1. Fluorescence emission spectra at 77K of diluted leaf powder prepared from leaves which were preincubated for 3 h in the dark at 20°C (——) or at 35°C (- - -). The far-red peaks were normalized to $F_{m(I)}$ obtained from separate measurements with intact frozen leaf discs. The spectra were recorded in the F_m state (closed traps). Actinic light: 480 nm.

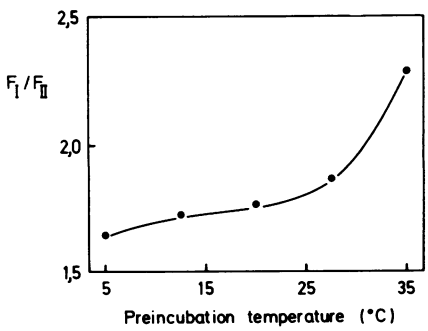

FIG. 2. The ratio F_I/F_{II} as a function of the temperature of a 5 to 7 h preincubation in the dark. The F_I/F_{II} ratios were calculated from emission spectra as shown in Figure 1.

change in the overall distribution of excitation energy in favor of PSI, at the expense of PSII excitation.

Changes in the distribution of excitation energy in favor of PSI has been shown to be initiated by illumination with light preferentially absorbed by PSII/LHC (state 1-state 2 transition; for a review, *cf.* Ref. 12). Table I shows F_I/F_{II} ratios from leaves incubated either in the dark or in blue-green light to establish a state 2 (*cf.* Ref. 8) at the respective temperature. At moderate temperatures, only a small light-dependent increase in F_I/F_{II} was observed and at 35°C, light had no effect. Essentially the same results were obtained by incubating plants in white light (30 w·m^{-2}) instead of blue-green light (not shown). The data demonstrate that the F_I/F_{II} ratios in dark-adapted leaves essentially reflect a state 2, in agreement with data of Satoh and Fork (16). This indicates that the temperature-induced increase in F_I/F_{II} was not equivalent to a light-induced state 1-state 2 transition. Rather, adaptation of leaves to high temperature enhances the maximum F_I/F_{II} ratio that can be established in state 2.

For more detailed analysis, induction of 77K fluorescence has been measured at 740 and 694 nm from leaves preincubated at different temperatures and then frozen in the dark (Fig. 3). At 77K, all photosynthetic reactions other than primary photochemistry are inhibited. Basing the analysis of 77K fluorescence on the model developed by Kitajiama and Butler (6, 9) $F_{0(II)}$ represents the weak fluorescence emitted from PSII when all traps are open: $F_{v(II)}$, *i.e.* the increase from $F_{0(II)}$ to the maximum level, $F_{m(II)}$, is related to the accumulation of closed traps, whereby the reoxidation of the reduced acceptor Q is almost completely prevented. $F_{0(I)}$ reflects the fraction of excitation energy initially delivered to PSI, whereas $F_{v(I)}$ mainly results from energy spilled over from closed PSII centers to PSI, *i.e.* changes in the ratio $F_{v(I)}/F_{v(II)}$ may indicate changes in the rate of spill-over of excitation energy from PSII to PSI. Table II shows a complete pattern of 77K fluorescence parameters obtained from whole leaves preincubated in the dark at two extreme temperatures (5 and 35°C). $F_{m(I)}$ values have been taken as a standard for correcting the short wavelength fluorescence: F_{II} values were normalized on $F_{m(I)}$ on the basis of F_I/F_{II} ratios obtained from preparations of diluted leaf powder. The most important results are: (a) after incubation at 35°C $F_{0(I)}$ increased by about 15% (compared to 5°C leaves), whereas $F_{0(II)}$ remained unchanged or even somewhat decreased; (b) $F_{v(I)}$ and $F_{v(II)}$ both decreased after incubation at 35°C; however, the decrease in $F_{v(II)}$ was more severe, and as a consequence, the ratio $F_{v(I)}/F_{v(II)}$ increased from 0.65 (5°C) to 0.88 (35°C). In most experiments, the values obtained from leaves incubated at 5 and 20°C did not significantly differ from each other (*cf.* also Fig. 2). The data suggest that incubation at high temperature gives rise to a change in the initial distribution of excitation energy as well as in the spill-over from PSII to PSI in favor of PSI. However, in spite of a considerable increase in $F_{v(I)}/F_{v(II)}$ (indicating increased spill-over), the overall decrease in the variable fluorescence ($F_{v(I)}$ and $F_{v(II)}$) may suggest that the temperature-induced increase in the rate of spillover is superimposed on an increase in the rate of thermal deactivation. The yield of variable fluorescence depends in a competitive manner on the rate of thermal (nonradiative) deactivation of excited states at the PSII centers (*cf.* Ref. 6).

Finally, it should be noted that at least part of the temperature-induced change in F_I/F_{II} ratio was retained after fast extraction of intact chloroplasts from 20 and 35°C leaves (data not shown).

Excitation of Chl *a* or Chl *b*. A change in the distribution of energy between the two photosystems is also supported by the observation that the increase in $F_{0(I)}$ and in F_I/F_{II}, observed after incubation of leaves at 35°C, is most significant if fluorescence is excited with light preferentially absorbed by Chl *b*, *i.e.* by the LHC, from where energy is distributed between the two photosystems. The effect of temperature was less marked if the fluorescence was excited with 430-nm light which is preferentially absorbed by Chl *a* (Table III). The Chl *a*/*b* ratio (determined after extraction of pigments from leaves) was not significantly affected from 5-h incubation at 35°C (data not shown).

Time Course of Temperature Effects. Figure 4 shows the time course of the change in F_I/F_{II} and in $F_{v(II)}$ following a rise in temperature from 14°C (night temperature in the glasshouse, from where the leaves were taken) to 35°C. The temperature has

Table I. *The Influence of Light on Temperature-Dependent Change in F_I/F_{II} (77K)*

The ratios were calculated from emission spectra (recorded in the F_m state) as shown in Figure 1. Diluted leaf powder was prepared from leaves which were preincubated for 2 to 4 h at the indicated temperatures either in the dark or in blue-green light. Illuminated leaves were taken from the light and rapidly frozen in liquid N_2 within 1 min. The ratios given in the table are mean values from four to six determinations.

	Dark	Blue-green (3 w·m^{-2})
°C		
18	1.61 ± 0.04	1.75 ± 0.05
28	1.81 ± 0.05	1.86 ± 0.07
35	2.33 ± 0.08	2.31 ± 0.09

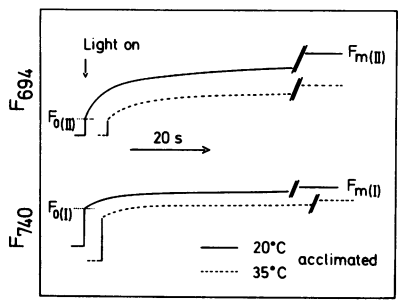

FIG. 3. Induction curves of 77K fluorescence emitted at 694 (PSII) and at 740 nm (PSI) from the upper surface of leaves which were preincubated for 3 h in the dark at 20 and at 35°C.

Table II. *Characteristics of 77°K Fluorescence*

The leaves were preincubated in the dark for 4 to 6 h either at 5 or at 35°C. Each value is a mean of 8 to 12 determinations from different leaves. Actinic light: 477 nm. If indicated, $F_{0(II)}$, $F_{v(II)}$, and $F_{m(II)}$ were corrected as follows: the original values obtained from measurements as shown in Figure 3 were normalized on $F_{m(I)}$, taking the ratio $F_{m(I)}/F_{m(II)}$ (obtained from a separate determination with diluted leaf powder) as a basis.

	5°C Leaves	35°C Leaves	Difference
	relative units		%
$F_{0(I)}$	3.36 ± 0.11	3.81 ± 0.09	+14
$F_{0(II)}$[a]	1.10 ± 0.04	1.15 ± 0.09	+4
$F_{0(II)}$[b]	0.76	0.72	−5
$F_{v(I)}$	1.67 ± 0.05	1.34 ± 0.05	−20
$F_{v(II)}$[a]	3.74 ± 0.22	2.42 ± 0.08	−35
$F_{v(II)}$[b]	2.58	1.52	−41
$F_{m(I)}$	5.01 ± 0.11	5.15 ± 0.09	+3
$F_{m(II)}$[a]	4.84 ± 0.17	3.58 ± 0.12	−26
$F_{m(II)}$[b]	3.35	2.23	−33
$F_{v(I)}/F_{v(II)}$[b]	0.65	0.88	+35
$F_{m(I)}/F_{m(II)}$[c]	1.03	1.44	+40
$F_{m(I)}/F_{m(II)}$[d]	1.50 ± 0.03	2.31 ± 0.09	+53

[a] Original values from leaf discs.
[b] Corrected values.
[c] Ratios calculated from uncorrected values.
[d] Ratios calculated from emission spectra of diluted leaf powder.

Table III. *Excitation with 480- and 430-nm Light*

$F_{m(I)}/F_{m(II)}$ values were obtained from preparations of diluted leaf powder. Conditions were as in Table II.

	Excitation	5°C Leaves	35°C Leaves	Difference
				%
$F_{m(I)}/F_{m(II)}$	480 nm (Chl *b*)	1.56	2.56	+64
	430 nm (Chl *a*)	1.89	2.63	+39
$F_{0(I)}$ (relative units)	480 nm (Chl *b*)	3.10	3.55	+15
	430 nm (Chl *a*)	3.56	3.77	+6

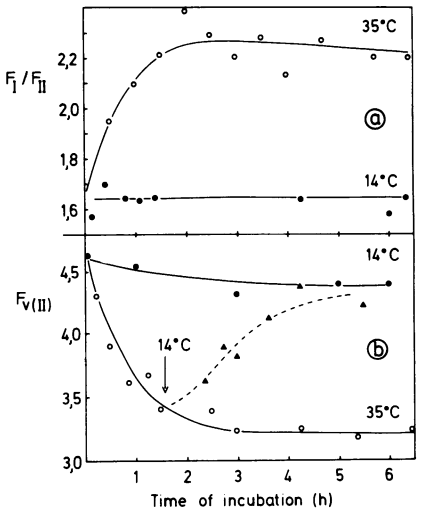

FIG. 4. a, F_I/F_{II} as a function of the preincubation time at 14 (●) and at 35°C (○). The values were obtained from emission spectra as shown in Figure 1. The leaves were preincubated in the dark. b, $F_{v(II)}$ (77K, relative units) as a function of the preincubation time at 14 (●), 35 (○), and 14°C after 1.5 h at 35°C (▲; recovery). The retransition of leaves from 35 to 14°C is indicated by the downward arrow. Measurements were as shown in Figure 3.

been increased rapidly (about 1.5°C/min) in order to demonstrate the maximum rate of the temperature transition. As shown, an incubation of 2 to 3 h (half-time, 0.5 to 1 h) was required for complete transition. The extent of changes in fluorescence properties was the same when the temperature was changed slowly, as occurs in the field (not shown).

All changes in fluorescence properties observed after incubation of leaves at 35°C were completely reversible. Figure 4b demonstrates the recovery of $F_{v(II)}$. After preincubating leaves for 1.5 h at 35°C, the temperature was lowered again to 14°C and subsequent recovery occurred with a half-time of about 1.5 h. However, the recovery time increased up to 24 h if the time of incubation at high temperature was extended to 8 h or more (not shown).

Thermal Stability of PSII Pigment System. Leaves preincubated for different times at 35°C were heated for 6 min at 45°C (heat shock) and then were measured at room temperature for F_0. The observed considerable heat-induced rise in F_0 which has been interpreted in terms of a breakdown in energy transfer from LHC/antennae pigments to PSII centers and related inhibition of photochemistry (19) has been taken as an indication of the thermal stability of the PSII pigment system. After incubation of leaves at 35°C, the heat-induced rise in F_0 was reduced from 70% to about 20% (Fig. 5). The half-time of the change was less than 1 h. The experiment demonstrates that the thermal stability of the PSII pigment structure increased almost in parallel with the change in fluorescence properties (*cf.* Fig. 4).

Fluorescence Induction at Room Temperature. Fluorescence at room temperature mainly represents fluorescence of PSII (for a review, see 12). In the presence of the inhibitor DCMU, the variable fluorescence (increase from F_0 to the maximum level) reflects the reduction of Q (7). Since in the absence of DCMU the redox state of Q rapidly equilibrates with that of PQ, the variable fluorescence under such a condition reflects the reduction of the PQ pool (15). In this study, the maximum level of fluorescence in the presence of DCMU (Q completely reduced) is defined as *F(max)*, the maximum level in the absence of DCMU is defined as P level. Figure 6, a and b, shows that, in leaves preincubated at 20 or 35°C, P (measured at 20°C) was almost as high as *F(max)* provided that the intensity of the actinic light was high, *i.e.* under such conditions Q and PQ, respectively, were almost completely reduced. In 35°C leaves, *F(max)* was lowered by about 30%, in agreement with the decline in $F_{m(II)}$ at 77K (Table II). F_0 remained unchanged or slightly decreased in 35°C leaves (5 to 10%; not shown). At low light

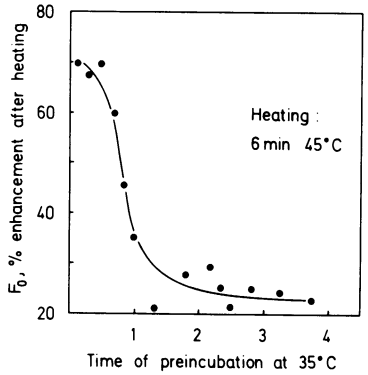

FIG. 5. The heat-induced rise in F_0 as a function of the time of incubation at 35°C. Leaves preincubated for the indicated times at 35°C were heated in a water bath (6 min at 45°C) and then taken for measurement of F_0 which had been determined from fluorescence induction at room temperature.

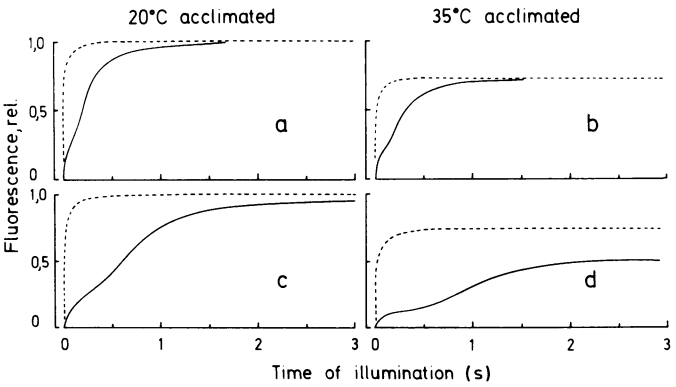

FIG. 6. Induction curves of variable fluorescence emitted at 20°C from the upper side of leaves as measured in the presence (- - -) and in the absence (——) of DCMU. The leaves were preincubated for 2 h in the dark at 20 and at 35°C, the infiltrated with 300 mM sorbitol, and measured for induction in the absence of DCMU. The same leaves then were bathed in sorbitol plus DCMU (3 min in the dark) and measured for *F(max)*. The values of *F(max)* of 20°C leaves were set at 1. F_0 was set at 0. The intensity of the actinic light was 40 $w \cdot m^{-2}$ (a and b) and 10 $w \cdot m^{-2}$ (c and d).

intensity, P in 20°C leaves was as high as *F(max)* but in 35°C leaves remained far below *F(max)* (Fig. 6, c and d) indicating that in 35°C leaves PQ was in a more oxidized state. Under low light conditions, a larger fraction of electrons can be withdrawn from PSI and then the redox state of PQ mainly depends on the rate of excitation of PSI relative to PSII. If a large fraction of the absorbed energy is delivered to PSII, PQ will be largely reduced as is shown with 20°C leaves (Fig. 6c). If PSI excitation is enhanced relative to PSII excitation, PQ remains in a more oxidized state, as appears to be the case in 35°C leaves (Fig. 6d). It should be noted that essentially the same result, namely a more oxidized PQ state in leaves preadapted at 35°C, has been obtained if leaves preadapted to 20 and 35°C were measured at 35°C (instead of 20°C) for fluorescence induction (not shown).

DISCUSSION

In this study, a technique has been described (diluted leaf powder) which permits measurements of 77K fluorescence emission spectra from leaf tissue less affected by reabsorption artifacts. This technique appears to provide a useful method for analyzing the distribution of excited states between the three pigment structures in whole leaves. Using this method, it has been concluded that, in leaves which were allowed to adapt to high temperatures, the distribution of excitation energy is changed in favor of PSI at the expense of PSII excitation. Although the fraction of energy spilled over from PSII to PSI increases, the amount of energy transferred from PSII to PSI appears to be somewhat reduced, probably due to an increased rate of thermal deactivation of excited states. However, the overall amount of excitation energy initially distributed to PSI appears to be increased (increase in $F_{0(I)}$; data in Table II). The possibility that the decrease in $F_{v(II)}$ is related to a reversible heat-induced inactivation of PSII-centers (*e.g.* Ref. 19) and/or inactivation of the water-splitting system is rejected on the ground that after 35°C treatment the electron transport from H_2O to PQ appears to remain intact (Fig. 6, curves a and b).

A change in the distribution of excited states in favor of PSI as suggested from pure 77K fluorescence data should increase electron flow through PSI relative to PSII, as indeed, is evidenced by a decreased reduction level of the PQ pool (experiment in Fig. 6, curves c and d). In essence, the data suggest that short term acclimation to high temperatures gives rise to an increase in the effectiveness of light absorbed by PSII/LHC to drive electron transport (*cf.* also discussion in Ref. 8). The observed temperature effect resembles that one of a light-induced state 1-state 2 transition as was shown with spinach leaves (16). However, leaves treated with high temperatures were already in a state 2 (blue-green light-adapted state; data in Table I) and the adaptation to high temperatures appears to enhance the extent of maximum spill-over from LHC and PSII to PSI in state 2. In nature, light-dependent state 1-state 2 regulation and temperature acclimation would cooperate. It is obvious from the present data that energy distribution between the photosystems in leaves can be modulated by both light conditions and temperature. This would help to maintain an optimal balance between stromal energy charge, redox power, and photosynthetic electron transport during short term fluctuations of environmental variables such as light and temperature.

Temperature-induced changes in the efficiency of excitation energy transfer between different pigment systems has also been observed with the blue-green alga *Anacystis nidulans* (17, 18, 23). They have been attributed to lipid phase transitions, which in turn, are considerably modulated by ionic effects of the environment of photosynthetic membranes (17, 18).

The close correlation between fluorescence characteristics and changes in thermal stability of the pigment system (Figs. 4 and 5) suggests that the temperature effects described here for spinach involve a reversible transition in membrane conformation. However, phase transitions of matrix lipids as observed in *Anacystis* are not expected to occur in thylakoids of higher plants at physiological temperatures above the freezing point (11).

In higher plants, essentially two different molecular mechanisms are known to induce changes in excitation energy transfer between the two photosystems. They include membrane-stacking and lateral movement of the three pigment structures (1) initiated either by ATP-dependent enzymic phosphorylation-dephosphorylation of LHC (*e.g.* Ref. 4) or by changes in the electrolyte conditions of the environment of thylakoids (for a review, see Ref. 3). For the temperature-induced state transition described in this paper, an ATP-dependent mechanism cannot be excluded but appears to be unlikely because the transition occurred in the dark and in the light as well. As an alternative explanation, it may be assumed that a reversible loss of membrane-membrane interaction and movement of PSII/LHC complexes from the appressed to exposed regions of membranes is caused by changes in the electrolytic conditions of the aqueous phase in the cells and changes in the electrostatic forces at the surface of thylakoid membranes, occurring during short term acclimation of leaves to high temperatures. As a consequence, a more intimate mixing of the three pigment protein complexes would occur and the probability of excitation energy transfer from LHC and PSII to PSI would be increased. Further work is underway to get more insight into the relation between fast photosynthetic adaptation to light and adaptation to temperature.

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