Biochimica et Biophysica Acta, 502 (1978) 138—151

BBA 47478

HEAT-INDUCED CHANGES OF CHLOROPHYLL FLUORESCENCE IN ISOLATED CHLOROPLASTS AND RELATED HEAT-DAMAGE AT THE PIGMENT LEVEL

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(Received August 22nd, 1977)

Summary

The heat-induced changes of chlorophyll fluorescence excitation and emission properties were studied in isolated chloroplasts of *Larrea divaricata* Cav. An analysis of the temperature dependency of fluorescence, under F_o and F_{max} conditions, of temperature-jump fluorescence induction kinetics, and of 77°K emission spectra of preheated chloroplasts revealed two major components in the heat-induced fluorescence changes: (1) a fluorescence rise, reflecting the block of Photosystem II reaction centers; and (2) a fluorescence decrease, caused by the functional separation of light-harvesting pigment protein complex from the rest of the pigment system. Preferential excitation of chlorophyll *a* around 420 nm, produces a predominant fluorescence rise. Preferential excitation of chlorophyll *b*, at 480 nm, gives a predominant fluorescence decrease. It is proposed that the overlapping of the fluorescence decrease on the somewhat faster fluorescence rise, results in the biphasic fluorescence rise kinetics observed in isolated chloroplasts. Both the rise component and the decay component are affected by the thermal stability of the chloroplasts, acquired during growth of the plants in different thermal environments. Mg^{2+} enhances the stability against heat-damage expressed in the decrease component, but has no effect on the rise component. Heat pretreatment leads to a decrease of the variable fluorescence in the light-induced 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) rise curve, but no change in half-rise time is observed. It is concluded that the block of Photosystem II reaction centers precedes the loss of the light-harvesting pigment protein complex. However, the approximately antiparallel heat-induced F_{max} decrease and F_o increase suggest

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Abbreviations: *P*-680, primary donor of Photosystem II; Q, primary acceptor of Photosystem II; DCMU, 3-(3,4-dichlorophenyll)-1,1-dimethylurea.

a common cause for the two events. A heat-induced perturbation of the thylakoid membrane is discussed.

Introduction

A heat-induced rise in chlorophyll fluorescence in *Chlorella* was first reported by Lavorel [1]. Studies performed with unicellular algae [2—4] and intact leaf tissue [2,5] have revealed several components in the heat-induced fluorescence changes. These changes of fluorescence do not require light and are unlikely to involve redox changes of the primary System II acceptor Q. In this respect, a different kind of information is gained than with measurements of the effects of temperature or heat pretreatment on light-induced fluorescence changes, reported in the literature (see e.g. refs. 6—8). While there primarily temperature effects or heat damage at the electron transport level is recorded, our heat-induced fluorescence changes reflect primarily heat-induced changes at the pigment level. The threshold temperature at which a marked fluorescence increase occurs was found to correlate with the onset of heat-induced inhibition of photosynthesis [3,5,9]. These studies showed that at least some aspects of primary heat damage are related to properties of the thylakoid membrane. Furthermore, observed shifts of the threshold temperature for the fluorescence transition with acclimation of plants to higher temperature [2,5,9] suggested that membrane properties can change when growth temperature is changed.

In the present study we investigated the mechanisms of heat-induced fluorescence changes by an analysis of the effect of heat on the chlorophyll fluorescence excitation and emission properties. It will be shown that the heat-induced fluorescence changes consist of two major components: (1) a fluorescence increase caused by inhibition of energy conversion at Photosystem II reaction centers, and (2) a fluorescence decrease, caused by a block of energy transfer between chlorophyll *b* (Chl *b*) and chlorophyll *a* (Chl *a*), and by functional separation of the light-harvesting pigment protein complex from the rest of the pigment system.

Materials and Methods

Chloroplasts were isolated from *Larrea divaricata* Cav., a desert shrub, which displays extraordinary heat- and water-stress resistance [10]. *L. divaricata* was chosen for this study, since its isolated chloroplasts show large heat-induced fluorescence changes almost identical to those of the intact leaf. The resinous components present in *L. divaricata* required a special isolation procedure, which is described elsewhere [9]. *L. divaricata* plants were grown in controlled temperature facilities at 20°C/15°C, 32°C/25°C or 45°C/33°C day/night temperatures. The results presented in the figures are for 32°C/25°C plants.

Heat-induced fluorescence changes were measured in a system which allowed rapid heating (within 2 s; *T*-jump curves) or slow heating (at about 1°C/min; *F-T* curves), as described previously [3—5]. If not stated otherwise, fluorescence was measured at wavelengths longer than 660 nm (Corning 2-64). The measuring apparatus was modified for monochromatic excitation with a mono-

chromator (Jarrel Ash, model 82-410) and a coupled fiberbundle. Liquid nitrogen temperature fluorescence emission spectra were recorded with a Perkin-Elmer fluorescence spectrophotometer model MPF-3L. Heat pretreatment was generally in the dark, or in extremely weak measuring light (1 erg · cm^{-2} · s^{-1}). If not stated otherwise, chloroplasts were containing about 10 μg chlorophyll/ml.

Results and Discussion

Heat-induced fluorescence changes under F_o and F_{max} conditions

To study the effect of temperature at the pigment level, it is necessary to eliminate the influence of photosynthetic electron transport reactions on the fluorescence yield. Such influences are minimized: (1) with all Photosystem II reaction centers open (by use of an extremely weak excitation light [4], yielding minimum fluorescence, F_o), (2) with all Photosystem II reaction centers closed (by application of DCMU, NH_2OH and relatively strong excitation light [3,11], resulting in the maximum level of fluorescence, F_{max}). Fig. 1 compares heat-induced changes of chlorophyll fluorescence under F_o and F_{max} conditions, and for fluorescence excitation with 430 or 480 nm light during slow heating. Under F_o conditions, there is a marked, heat-induced fluorescence increase with 430 nm excitation, and a less pronounced increase, fol-

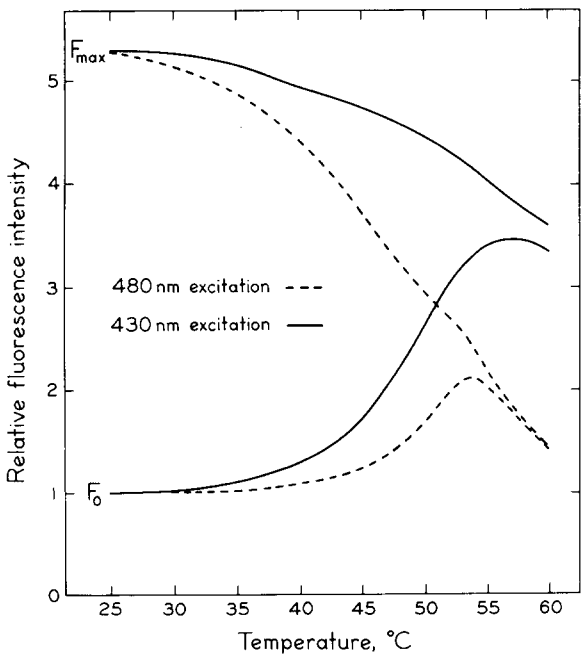

Fig. 1. Fluorescence-temperature dependency (*F-T* curves) measured under F_o and F_{max} conditions. Differences between 430 and 480 nm excitation. Chloroplasts were heated at a rate of about 1°C/min, and fluorescence ($\lambda > 660$ nm) was measured at the given temperatures. Monochromatic measuring light, 8 nm half-band width; intensity: for F_o, 1 erg · cm^{-2} · s^{-1}, for F_{max}, 300 erg · cm^{-2} · s^{-1}. F_o levels of all samples were normalized to give one relative unit. The F_{max} state was reached by addition of 10^{-5} M DCMU and 10^{-3} M NH_2OH, and 1 min illumination prior to heating.

lowed by a decay, with 480 nm excitation. In contrast, under F_{max} conditions, heating causes a decrease of fluorescence. This decrease of F_{max} is much more pronounced with 480 nm excitation than with 430 nm excitation. With both excitation wavelengths, convergence of the F_o and F_{max} curves at elevated temperatures is observed.

The approximately antiparallel course of the F_o-T and F_{max}-T curves suggests that the heat-induced rise in F_o and the decrease in F_{max} are caused by the same basic change within the chloroplast. The eventual convergence of the curves suggests that heating leads to a situation which is similar to that reached by illumination in the presence of DCMU, i.e. a "block" of Photosystem II centers.

The way by which this "block" is achieved upon heating is not evident. It is unlikely to involve reduction of the primary acceptor Q, as in the presence of DCMU in the light, because the measuring light is extremely weak. Also it was observed that far-red background illumination does not prevent the heat-induced rise of F_o [4]. Without knowledge of the specific mechanism involved, one may assume that heating causes a functional separation of Q from the primary donor *P*-680. With respect to fluorescence yield, a block of centers (i.e. of energy conversion) due to Q being reduced and a block caused by disconnection of Q from *P*-680 should be equivalent.

Besides this postulated effect of heat on the reaction centers, there is an additional effect that induces a decrease of fluorescence. Apparently, at elevated temperatures, 480 nm light absorbed preferentially by Chl *b* becomes much less effective in exciting Chl *a* fluorescence than 430 nm light, most of which is absorbed directly by Chl *a*.

Dependence of heat-induced fluorescence changes on wavelength of excitation

The excitation wavelength dependency of the heat-induced fluorescence changes is also evident in the *T*-jump fluorescence induction kinetics. This dependency is shown for F_o conditions in Fig. 2. When the fluorescence is excited by 430 nm light, heating induces a large biphasic fluorescence rise; 480 nm excitation only produces the first rise phase. The initial rise phases are practically identical for all excitation wavelengths. The extent of the second rise phase was measured as a function of excitation wavelength. The corresponding spectrum is depicted in Fig. 3 and shows a general decline with increasing wavelength and shoulders at 440 and 450 nm. The extent of the second rise phase is apparently determined by the degree of absorption by Chl *a* relative to that by Chl *b*. This interpretation explains the unusual shape of the spectrum, e.g. the increase from 420 to 400 nm appears to reflect an increase in the Chl *a*/Chl *b* absorption ratio.

It can be expected that the heat-induced fluorescence changes are to a certain extent also affected by heat-induced changes of absorption. Godnev and Efremova [12] reported shifts of the red absorption peak in vivo towards shorter wavelengths upon heating. We did observe heat-induced changes of absorption in the 400—700 nm region in the order of 10% (not in the figures). These absorption changes occur simultaneously with the fluorescence changes, and appear therefore to be caused by the same heat-induced changes within the pigment system. However, the large difference in the relative changes (10% in

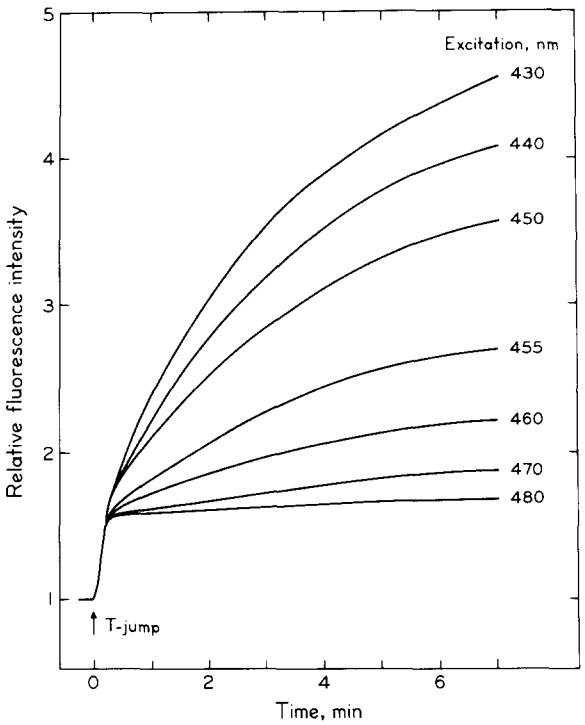

Fig. 2. Heat-induced rise in fluorescence induced by a temperature jump (*T*-jump). Effect of excitation wavelength. *T*-jump was from 25 to 52°C. Monochromatic measuring light, 3 nm half-band width. For all excitation wavelengths the F_0 level was adjusted at 25°C to one relative unit, by appropriate change of incident intensity in the region from 0.5 to 5 ergs · cm^{-2} · s^{-1}. This procedure gave about equal absorbed quanta at all wavelengths.

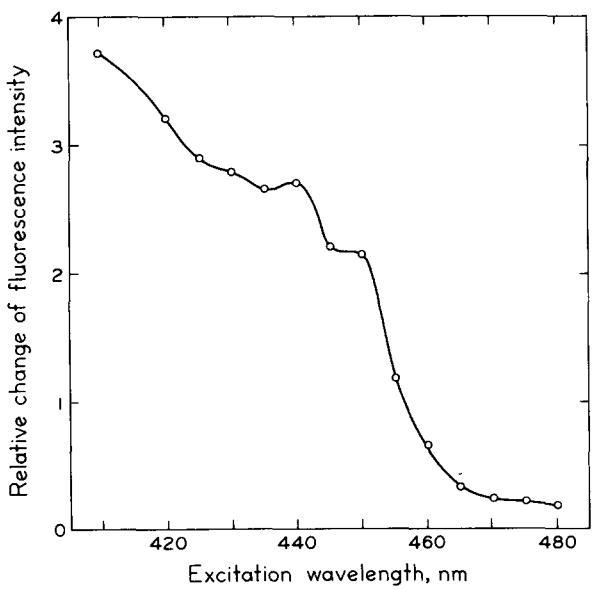

Fig. 3. Amplitude of the second heat-induced fluorescence rise phase as a function of excitation wavelength. Conditions as in Fig. 2.

TABLE I

RATIOS OF THE RELATIVE CHLOROPHYLL FLUORESCENCE INTENSITY UNDER F_0 CONDITIONS WITH 430 AND 480 nm EXCITATION, AS FUNCTION OF THE TEMPERATURE OF MEASUREMENT AND OF GROWTH TEMPERATURE

Fluorescence (F_0) measured at wavelengths longer than 660 nm. Measuring light intensities, about 1 erg · cm^{-2} · s^{-1}. Heating was performed as in Fig. 1; the temperatures of measurement correspond to the corresponding temperatures in a F_0-T curve. Ratios for non-heated chloroplasts at 25°C were 1.00 by normalization.

Growth temperature (°C)	Fluorescence excited by 430 nm / Fluorescence excited by 480 nm				
	Temperature of measurement				
	35°C	40°C	45°C	50°C	55°C
20	1.30	1.70	2.06	2.14	2.86
32	1.20	1.52	1.71	1.88	1.97
45	1.00	1.08	1.16	1.20	1.11

absorption; 300% in fluorescence) excludes the possibility that a substantial part of the fluorescence changes is due to changes in absorbed light intensity.

The differences in heat-induced fluorescence changes observed with 430 or 480 nm excitation are dependent upon the growth temperature of the plants from which chloroplasts were isolated. Table I gives the ratios of relative fluorescence intensity excited by 430 and 480 nm light, in relation to the heating temperature, for chloroplasts isolated from plants grown at different temperatures. In general, the excitation wavelength dependency, and presumably also the correlated heat damage, are less pronounced at higher growth temperatures.

The kinetics of the T-jump induction under F_o conditions

An analysis of the polyphasic T-jump induction curves in whole cells of *Scenedesmus obliquus* has been previously presented [4]. The T-jump kinetics observed in isolated chloroplasts for F_o fluorescence are less complex, primarily because anaerobic induction [13,14] is prevented in the absence of heat-stimulated dark respiration. In isolated chloroplasts the kinetics are essentially biphasic.

In Fig. 2 it was shown that with 480 nm excitation there is practically no second rise phase. However, when the T-jump is to temperatures exceeding 52°C, even with 480 nm excitation more than a single rise phase is observed. As depicted in Fig. 4, with T-jumps to 55–61°C, the rapid first phase is followed by a dip and a much slower second rise phase. Presumably, the decrease of fluorescence expressed in the dip of the T-jump curve, and the decrease of fluorescence observed in an F_{max}-T curve, reflect the same heat-induced process. The existence of such a fluorescence decrease component, which is most prominent with 480 nm excitation, suggests that the two phases in the T-jump curves are caused by the overlapping of a fluorescence rise and a fluorescence decrease. The rise is predominant with Chl *a* excitation, whereas with excitation of Chl *b* the decay is predominant. If this interpretation is correct, the event

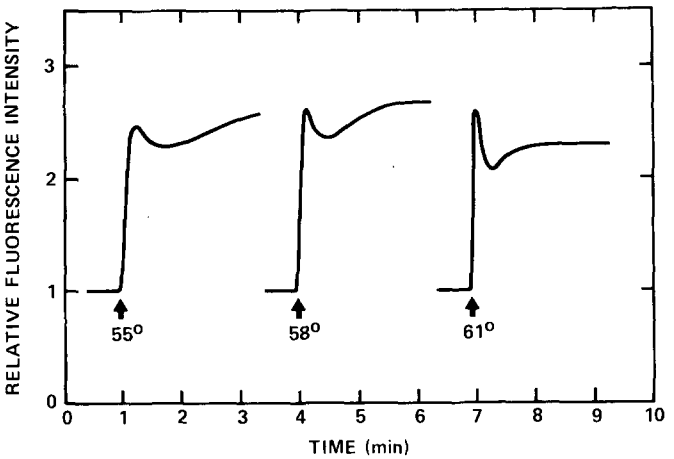

Fig. 4. Kinetics on heat-induced fluorescence changes upon T-jump to 55, 58 and 61°C. Fluorescence is excited by 480 nm measuring beam. Other conditions as in Fig. 2.

which causes the fluorescence rise occurs somewhat earlier than the one which causes the fluorescence decay.

Fig. 5 shows the effect of Mg^{2+} depletion on the T-jump induction kinetics. In the absence of Mg^{2+}, the second rise phase is depressed, while the rapid rise phase is not affected. We conclude that the presence of Mg^{2+} provides some stabilization against that component of heat damage which is expressed in the heat-induced fluorescence decrease. On the other hand it appears that Mg^{2+} has no particular effect on the component of heat damage which is reflected in the fluorescence rise.

Growth temperature of the plants from which the chloroplasts were isolated have a marked influence on the kinetics of the F_o-T-jump curves. In chloro-

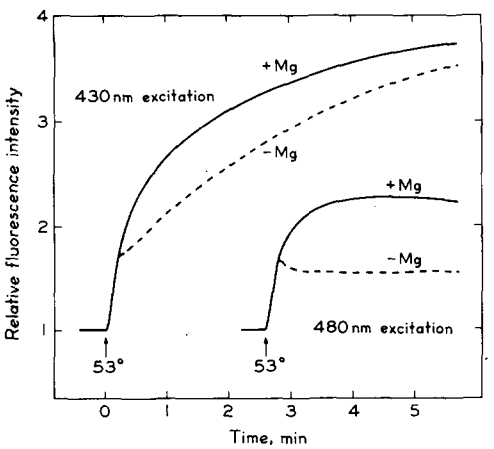

Fig 5. Effect of Mg^{2+} depletion on the kinetics of the heat-induced fluorescence rise upon a T-jump to 53°C. Mg^{2+} depletion was by washing the chloroplasts in 10 mM EDTA, and resuspending them in Mg^{2+}-free buffer. Re-addition of Mg^{2+} (5 mM) gave the $+Mg^{2+}$ sample. Other conditions as in Fig. 2.

plasts isolated from 45°C grown plants, a predominant first phase and only a small second rise phase or none is observed with 430 nm excitation. The situation is reversed with chloroplasts from 20°C grown plants, i.e. the first phase is small and the second phase is large. In 32°C chloroplasts an intermediate situation was found (see Fig. 2). This behavior is in agreement with the above interpretation of the two rise phases (an overlapping of a fluorescence increasing and a fluorescence decreasing process). Adaptation to higher growth temperatures appears to involve an increased stability with respect to the fluorescence decreasing process. In the chloroplasts of the 45°C grown plants, the decay component appears to be almost absent (see also Table I). However, the heat damage expressed in the fluorescence rise is also affected by adaptation to different growth temperature, as indicated by the observed shifts of the threshold temperatures for the fluorescence transition in F_o-T curves to higher values with increased growth temperatures [3,5,9].

We observed that in most plants the heat-induced fluorescence rise is much smaller in isolated chloroplasts than in the intact leaf. On the other hand the heat-induced fluorescence decay is more pronounced in isolated chloroplasts than in the leaves. We believe that this finding can be explained by a decrease in heat stability of the chloroplasts upon isolation. As shown above, heat stability protects from the fluorescence decay component. In spinach chloroplasts, even with 420 nm excitation, the heat-induced fluorescence rise is only one-fourth of that found in the intact leaf. We choose *Larrea* chloroplasts for our study, because the isolated chloroplasts show approximately the same heat-induced fluorescence changes as in the intact leaf. It appears that *Larrea* chloroplasts exhibit extraordinary intactness and heat stability, presumably due to the original desert habitat of the plant.

Heat-induced changes of the low temperature emission spectra

To investigate further the heat-induced changes at the pigment level, chloroplasts were first heated, then frozen in liquid nitrogen and the chlorophyll emission spectra measured. In contrast to the behavior of intact cells [2—5], only a small part of the heat-induced fluorescence changes in isolated chloroplasts is reversible upon recooling. After 5 min heat pretreatment at 52°C, the changes are completely irreversible (not shown in the figures) and thus can be analyzed at 77 K. Fig. 6 shows 77 K emission spectra with 420 nm (Fig. 6A) and 480 nm excitation (Fig. 6B) for heated and non-heated chloroplasts, and the resulting difference spectra (Fig. 6C). Heat treatment caused the following spectral changes: (1) Long wavelength emission near 735 nm is significantly suppressed. Brown [15] observed a similar heat-induced suppression of long wavelength emission in *Ochromonas danica*. (2) The composition of short wavelength emission is changed; 685 nm emission is suppressed, particularly with 480 nm excitation; 693 nm emission is stimulated, particularly with 420 nm excitation; new emission bands emerge at shorter wavelengths, leading to a shoulder at 673 nm with 420 nm excitation, and a peak at 660 nm with 480 nm excitation. (3) Emission is stimulated in a broad band centering around 705 nm, both with 420 and 480 nm excitation. (4) The overall fluorescence yield is reduced by the heat treatment, particularly with 480 nm excitation.

To characterize the dependency of the heat-induced spectral changes with

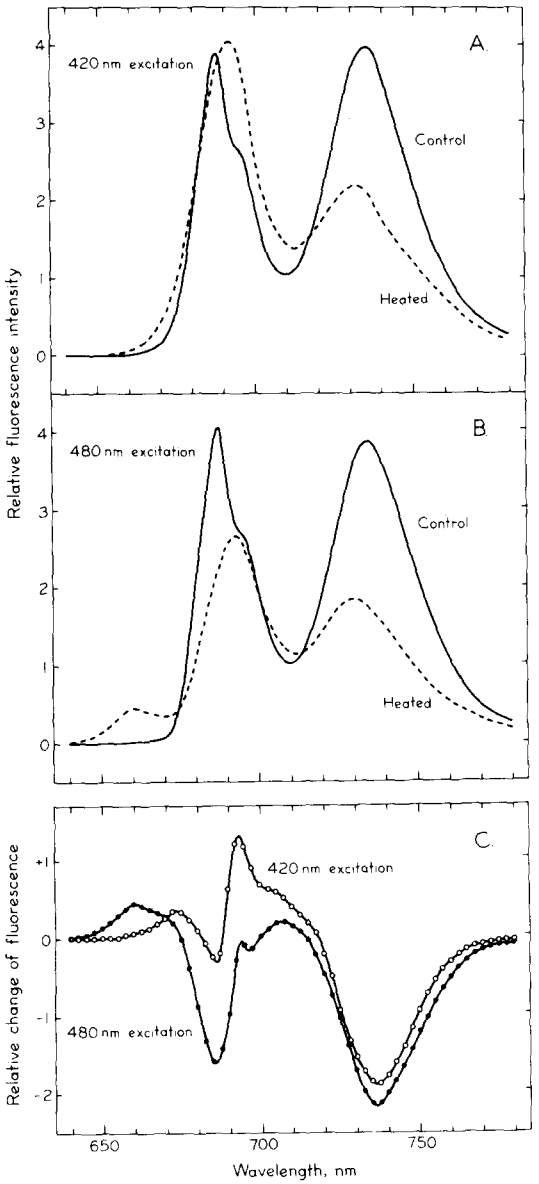

Fig. 6. Effect of heat pretreatment on the emission spectra at 77 K. The chloroplasts were heated for 5 min at 52°C. Spectra of control and heated samples (A, B) were recorded with identical measuring sensitivity. Half-band width of excitation, 5 nm; half-band width of emission, 3 nm. The difference spectra (C) were calculated from the emission spectra (A, B). Chlorophyll concentration, 2 μg Chlorophyll/ml.

increasing pretreatment temperature, chloroplasts were slowly heated from 25 to 65°C (at about 1°C/min), aliquots removed at given temperatures, rapidly frozen in liquid nitrogen and the emission spectra measured. With this procedure, heat treatment of the chloroplasts was essentially identical to that used for the measurements of the *F-T* curves (see e.g. Fig. 1). In Fig. 7 the observed

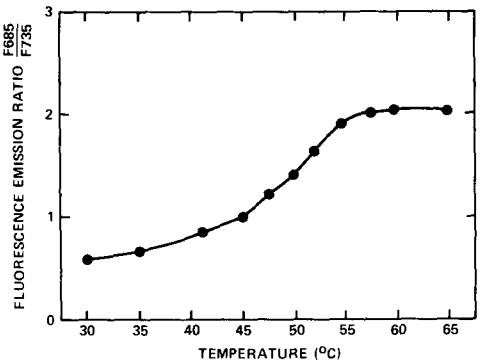

Fig. 7. Temperature dependency of the heat-induced changes in emission characteristics. Chloroplasts were slowly heated (about 1°C/min) from 25 to 60°C. Aliquots were removed at given intervals and rapidly frozen in liquid nitrogen. The emission spectra were recorded and the emission ratios determined. Excitation wavelength, 430 nm. Other conditions as in Fig. 6.

emission ratio F_{685}/F_{735} is plotted vs. pretreatment temperature. By comparison with Fig. 1 it is evident that the heat-induced spectral changes observed at 77 K are caused by the same heat-induced changes within the chloroplast as the fluorescence changes observed at the elevated temperatures measured in an *F-T* curve.

Interpretation of the heat-induced changes in the emission spectra

To interpret heat-induced changes in the 77 K emission spectra, it is important to note that the conditions of the spectra recording are such to induce a complete block of Photosystem II reaction centers. This block is caused by the irreversible reduction of the Photosystem II acceptor Q in the relatively strong excitation light [16,17]. Since the measuring conditions as such induce a rise of fluorescence to F_{max}, 77 K emission spectra are particularly suited to characterize the heat-induced changes expressed in F_{max}-T curves (see Fig. 1). It is apparent from the spectra in Fig. 6 that heat pretreatment, which about tripled the F_o yield at the elevated temperature (Fig. 2), rather decreased the overall emission at 77 K. In agreement with conclusions drawn above (see discussion of Fig. 1), this finding can be explained by the assumption that the heat-induced increase in F_o reflects inhibition of energy conversion at System II centers, which may be caused by a functional separation of the primary electron acceptor Q from the primary electron donor *P*-680. As the 77 K spectra are recorded under conditions of System II centers being per se completely blocked, a block of centers by heat pretreatment should be difficult to distinguish, unless this is accompanied by some specific spectral changes. In principle, heating could also cause a fluorescence increase by physical changes in the de-excitation rate constants. Such changes would, however, be expected to affect F_o and F_{max} in the same direction.

The data in Figs. 1 and 6 demonstrate that when all Photosystem II reaction centers are blocked, heating induces a substantial decrease in fluorescence, both at the elevated temperature and when assayed at 77 K after heat pretreatment. The 77 K spectra reveal that the loss of fluorescence emission is centered

around 685 and 735 nm. These are the predominant changes, when excitation is at 480 nm; with 420 nm excitation, there is also a sizeable gain in emission around 693 and 705 nm. According to Butler and Kitajima [18], F 685 originates from the light-harvesting pigment complex, F 693 from pigment System II and F 735 from pigment System I. The origin of F 705 is not clear, but it could derive from non-functional System II centers. The heat-induced short wavelength emission peak at 660 nm, which is only observed with 480 nm excitation, represents Chl *b* fluorescence [19]. The concomitantly appearing new emission band around 673 nm appears to originate from some short wavelength absorbing Chl *a* species, likely to be associated with Chl *b*.

All of Chl *b* is known to be in an approx. 1 : 1 complex with Chl *a* in the light-harvesting pigment protein complex [20,21]. In a non-heated chloroplast the efficiency of energy transfer from Chl *b* to Chl *a* is close to 100%, and no Chl *b* fluorescence is observed. The emergence of 660 nm fluorescence indicates that one aspect of heat damage is a loss of energy transfer efficiency between Chl *b* and Chl *a*. It is important to point out that substantial disturbance of energy transfer efficiency occurs in a temperature region where light-saturated whole chain electron transport in the isolated chloroplasts is still unaffected [9]. A block between Chl *b* and Chl *a* explains: (a) emergence of F 660; (b) loss of F 735; as less energy is reaching the Photosystem I pigment complex; (c) loss of F 685; as less energy is reaching Chl *a*. However, with a block of energy transfer between Chl *b* and Chl *a* alone it is difficult to explain: (1) The stimulation of F 693 and F 705, observed primarily with Chl *a* excitation; loss of Chl *b* to Chl *a* transfer should rather lead to a decrease of F 693. (2) The fact, that suppression of F 735 occurs to almost the same extent, regardless of whether excitation is at 420 or 480 nm; if there were only loss of Chl *b* to Chl *a* transfer, one would expect a substantially larger suppression of F 735 with 480 nm excitation than with 420 nm excitation. We believe that these two aspects can be accounted for in the model proposed by Butler and Kitajima [18]. According to this model a predominant part of F 735 is excited by energy transfered from Photosystem II, when the System II centers are closed. Possibly, pigment System II units, the reaction centers of which are blocked by heat, have lost the capacity of transferring energy to pigment System I. Instead, the energy may be emitted from pigment System II around 693 nm, or possibly by the centers themselves, which could be the origin of F 705.

It appears likely that not only Chl *b* to Chl *a* energy transfer, but also energy transfer between the light-harvesting pigment complex and the reaction center complexes is impaired by heat treatment. This conclusion is particularly supported by the data in Fig. 5, where it was shown that Mg^{2+} depletion enhances the differences in heat-induced fluorescence between 430 and 480 nm excitation. In a model of the photosynthetic unit advanced by Armond et al. [22], the light-harvesting complex is closely associated with Photosystem II, and Mg^{2+} is believed to regulate the extent of interaction between the two photosystems [23]. Mg^{2+} appears to stabilize the association of the light-harvesting complex with the pigment System II complex, and thus counteracts the postulated effect of heat. Whether there is a true release of the light-harvesting complex upon heating remains uncertain. However, it can be assumed that the

observed block of energy transfer between Chl *b* and Chl *a* leads to a situation which is equivalent to a practical loss of the light-harvesting complex, i.e. the functional separation of the light-harvesting complex from the reaction center complexes.

If the above conclusions are correct, two major heat-induced events can be identified by the analysis of the heat-induced fluorescence changes: (1) the inhibition of energy conversion at System II reaction centers, and (2) the functional separation of the light-harvesting complex from the rest of the pigment system. It is the first event which causes the heat-induced fluorescence rise. The second event causes the heat-induced fluorescence decrease.

Effect of heat pretreatment on kinetics of light-induced fluorescence rise in the presence of DCMU

When dark-adapted chloroplasts are illuminated in the presence of DCMU, fluorescence rises from an initial level F_o to a maximum level F_{max}. At low temperatures (0—10°C) and in the presence of NH_2OH, this fluorescence rise reflects the light-driven, irreversible block of System II reaction centers by reduction of the primary acceptor Q [16,24]. As shown in Fig. 8, heat pretreatment causes substantial changes of extent and kinetics of the light induced fluorescence rise in the presence of DCMU. The heat pretreatment was the same as in the recording of an *F-T* curve (see Fig. 1) with rapid recooling at various points in the curve. The effect of heat pretreatment on the DCMU rise curves can be summarized as follows: (1) F_o levels undergo essentially the same change as in the F_o-*T* curves, for both 430 and 480 nm excitation. (2) F_{max} levels decline, but much more with excitation at 480 nm than at 430 nm. (3) The amplitude of the light-induced variable fluorescence decreases with increasing pretreatment temperatures. The extent to which the variable fluorescence is reduced is about the same for 430 and 480 nm excitation. (4) Sigmoidicity of the rise is lost between 45 and 50°C pretreatment temperatures. (5) The half-rise time of the curves is virtually unaffected by the heat pretreatment.

These results complement the information gained from the *F-T* curves (Fig. 1), *T*-jump curves (Figs. 2, 4 and 5) and emission spectra (Fig. 6). The increase of F_o and the loss of variable fluorescence, which were also observed by Murata [7], indicate heat-induced block of System II reaction centers. The decrease in F_{max}, particularly pronounced when Chl *b* is excited, reflects the functional disconnection of the light-harvesting complex from the reaction center complexes. The loss of sigmoidicity suggests that the cooperation between System II reaction centers by interunit energy transfer [22] is disturbed. But it is also possible that the loss of sigmoidicity is a consequence of the increase in F_o, by which the starting point of the induction curve is raised above the inflexion point in a control curve.

The fact that the half-rise time of DCMU rise curves is not affected by the heat treatment may appear to be in contradiction to the hypothesis of a heat-induced loss of the light-harvesting complex. One could argue that the constancy of $t_{1/2}$ reflects unchanged photosynthetic unit size. However, the two aspects can be reconciled with each other, if it is assumed that within a single unit (i.e. pigment system and reaction center) the heat-induced block of Photo-

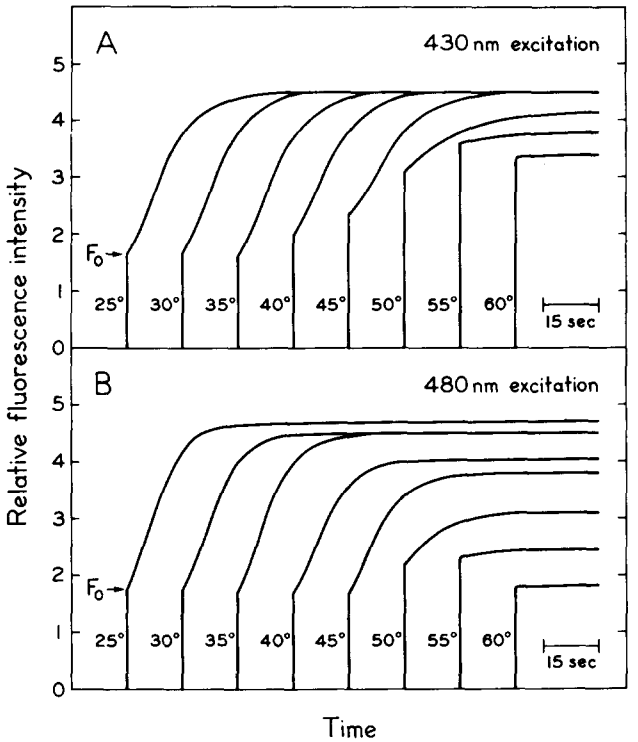

Fig. 8. Kinetics of the light-induced fluorescence rise in the presence of DCMU as a function of pretreatment temperature. DCMU concentration, $5 \cdot 10^{-6}$ M; also NH_2OH was present at 10^{-3} M; the cuvette temperature was maintained at 5°C. Chloroplasts were slowly heated (about 1°C/min) from 25 to 60°C. Aliquots were removed at given intervals and rapidly cooled to 5°C. The inhibitors were added in complete darkness and the light-induced fluorescence rise measured after 5 min incubation of the inhibitors. F_0 levels for the controls, without inhibitors present, were adjusted to be one relative unit. Measuring light intensity, for 430 and 480 nm excitation, about 300 ergs · cm^{-2} · s^{-1}.

system II centers precedes the loss of the light-harvesting complex. The results presented above in Figs. 4 and 5 suggest that the block of Photosystem II centers is indeed initiated somewhat earlier than the disconnection of the light-harvesting complex. This conclusion was further confirmed by the finding that 77 K emission spectra were nearly identical with 420 and 480 nm excitation, as long as heating did not exceed the first phase in a *T*-jump curve (not shown in the figures).

Conclusions

The above analysis of the heat-induced changes in chlorophyll fluorescence excitation and emission properties has revealed two major components of primary heat-damage within the chloroplast. The first is a heat-induced block of Photosystem II reaction centers, which is reflected in: (1) the heat-induced fluorescence rise under F_0 conditions (Figs. 1, 2 and 8); (2) the stimulation of F 693 at 77 K (Fig. 6); (3) the loss of variable fluorescence, observed in the light-induced DCMU rise curves (Fig. 8). The second is a heat-induced block of

Chl *b* to Chl *a* energy transfer, resulting in a functional disconnection of the light-harvesting complex from the reaction center complexes, which is reflected in: (1) the heat-induced decrease of fluorescence under F_{max} conditions (Fig. 1); (2) the suppression of the heat-induced rise in F_o (Figs. 2—5, and Table I); (3) the stimulation of Chl *b* fluorescence at 660 nm, measured at 77 K (Fig. 6); (4) the loss in emission around 685 nm and 735 nm at 77 K. The fact that F_o-T and F_{max}-T curves are approximately antiparallel, suggests that these two major components of primary heat damage are induced within the same temperature region. Therefore, it appears likely that both have a common cause, presumably some heat-induced perturbation of the thylakoid membrane, with which both the reaction centers and the light-harvesting complex are closely associated. This conclusion is not contradicted by our interpretation of the *T*-jump fluorescence rise kinetics (Figs. 2, 4 and 5), and our interpretation of the constancy of $t_{1/2}$ in the DCMU rise curves (Fig. 8), where it was argued that the heat-induced block of Photosystem II centers does slightly precede the loss of the light-harvesting complex. It is conceivable that a common cause, e.g. a thylakoid membrane conformational change, can affect the two events with somewhat different rates. While our experimental approach detects specifically heat damage at the pigment level, the same heat-induced changes of thykaloid membrane properties which cause these pigment changes, may also lead to denaturation of associated enzymes. In this sense, the measurement of F_o-T and F_{max}-T curves constitute a rapid and easy assay for evaluating heat damage and heat stability in plants, and also can be expected to give additional insight into the mechanism of thermal adaptation.

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