Biochimica et Biophysica Acta, 423 (1976) 249–263

BBA 47030

ANALYSIS OF TEMPERATURE-JUMP CHLOROPHYLL FLUORESCENCE INDUCTION IN PLANTS

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(Received July 7th, 1975)

SUMMARY

A newly observed general chlorophyll fluorescence induction effect in plants is described. Fluorescence yield can rise through as many as four different phases (α, β, γ, δ) in the dark, when intact cells or leaves are rapidly heated (within approx. 2.5 s) from 20 to 40–50 °C. An analysis of this temperature-jump fluorescence induction in *Scenedesmus obliquus* leads to the following:

1. Phase α is due to removal of S-quenching and appears to be related to heat deactivation of the water-splitting enzyme system. With prolonged heating, irreversibility of α upon recooling reflects irreversible damage to the water-splitting enzyme system.

2. β is independent of the S-states and of the redox state of primary System II acceptor Q. It is suggested that β parallels functional separation of Q from the System II trapping centre. This effect is highly reversible.

3. γ and δ reflect reduction of primary System II acceptor Q by a heat-induced endogenous reductant, which is probably identical to hydrogenase.

Critical temperatures for pronounced α and β phases differ markedly in different plants. Possible correlations between temperature-jump fluorescence induction, thylakoid membrane lipid composition, lipid phase transition and lipid-protein interactions are discussed.

INTRODUCTION

Chlorophyll fluorescence is a sensitive indicator of energy conversion at Photosystem II in photosynthesis (for a review see ref. 1). In the first approximation there is a complementary relationship between fluorescence yield and rate of the primary photochemical reaction at System II centres. This rate undergoes characteristic transient changes when a plant is illuminated after a period of darkness. The accompanying transient changes in fluorescence yield are referred to as the "Kautsky effect" (Kautsky, 1931) [2–4], and have been associated primarily with the concentration of

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

the oxidized primary System II electron acceptor, so-called fluorescence quencher Q [5]. Under certain conditions there is fluorescence quenching which cannot be attributed to Q [6–10].

Changes in fluorescence yield are conventionally induced by a dark-light transition and are highly dependent on the state and functioning of all electron carriers between water and NADP. In presence of electron transport inhibitors such as 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), fluorescence rapidly increases to a high level in the light simultaneously with Q reduction. An increase in fluorescence yield also occurs during a dark period with treatments that result in inhibition of quenching mechanisms; it can then be monitored by a measuring beam, sufficiently weak not to affect fluorescence yield. We have reported previously a biphasic rise in fluorescence yield during dark anaerobic storage of *Scenedesmus*, where due to some endogenous electron donor the intermediates between System II and System I, including Q, become totally reduced [11, 12]. This effect is reversible; a biphasic decay to low fluorescence yield occurs with reoxidation of Q by System I.

Lavorel [13] first reported a heat-induced increase of the minimum fluorescence yield, characteristic of the dark-adapted state, in *Chlorella*. The high fluorescence level reached was described as transitory and not persisting at temperatures > 50 °C nor on return to room temperature. Later studies revealed some correlation between the heat-induced fluorescence rise and the removal of molecular oxygen from the vicinity of Photosystem II [11, 14]; in *Scenedesmus*, fluorescence returns to a low level upon recooling only if O_2 is present in the medium.

In this paper a more detailed study of the heat-induced fluorescence rise is reported, which indicates that this effect is general in a variety of plants. With the application of a temperature-jump, rather than conventional slow heating, several phases can be resolved in the fluorescence rise, related to loss of different forms of quenching.

Aspects of this temperature-jump chlorophyll fluorescence induction were previously reported by us in a short communication elsewhere [15]. We then suggested that the phenomenon is closely related to a transition of the physical phase of lipids in the thylakoid membrane.

Recently Murata et al. (ref. 16 and Murata, N., Troughton, J. H. and Fork, D. C., in preparation) have demonstrated a dependence of photosynthetic activities on the membrane lipid phase state in the blue-green alga *Anacystis nidulans*. Phase transition temperatures in this organism correlate with environmental growth temperatures and were found in the region from 12 to 24 °C. In contrast, higher plant thylakoid lipids do not appear to undergo phase transitions at physiological temperatures. We will show that, while the temperature-jump fluorescence rise curves occur in a much higher temperature region than the phase transitions reported by Murata et al., certain features of the temperature-jump curves could be related to factors which determine the phase transitions.

MATERIALS AND METHODS

Scenedesmus obliquus was grown as described previously [12]. *A. nidulans* was grown in the same medium and under the same conditions. The marine algae *Ulva lobata*, *Porphyra perforata* and *Fucus distichus* were collected from Brockton Point,

Vancouver, B.C., at a water temperature of approx. 7 °C. Leaf samples were from potted plants growing inside at room temperature (approx. 21 °C). *Parmelia perlata*, a lichen, was collected on Burnaby Mountain, B.C., at approx. 0 °C. All samples were kept for 1 h in the dark before the beginning of an experiment.

The measuring device was designed for rapid heating and cooling of plant samples, high sensitivity recording of fluorescence yield in a very weak measuring beam and with a provision for adding a high intensity actinic beam or saturating μs-flashes. Fig. 1 represents a cross section through the measuring chamber with the attached trifurcated fiber optics, and a flow circuit diagram for heating and cooling water. The measuring beam consists of a broad blue band selected from a projector lamp (Sylvania, Type DLG) by 8 mm Corning 9782 filter with an intensity of 0.2 $\mu W \cdot cm^{-2}$. Actinic light was isolated with a combination of a monochromator (Jarrel Ash, model 82-410) and interference filters (Balzers). Saturating flashes were delivered by a Stroboslave flash lamp (General Radio, type 1539-A) with peak intensity after 2 μs. The image of the discharge arc was focused on the slit-shaped end of one of the fiber optic branches, on which the actinic beam from the monochromator can also be focused. The flash light was filtered through a Balzers K6 broad-band interference filter, with the cut-off filter removed. The filter passes most of the light at $\lambda < 680$ nm and cuts out $\lambda > 700$ nm with high efficiency. Fluorescence was detected through a fiber strand carrying 80 % of the total fibers (measuring beam 2 % and actinic beam 18 %) by an EMI 9558 B photomultiplier. The photomultiplier was protected by a 2 mm Corning CS 2-64 filter, which was changed to a 2 mm Corning CS 7-69 when red actinic light or flashes were used. Fluorescence changes were recorded on a strip chart recorder (Metrohm, Type E478) or a storage oscilloscope (Tektronix, 5103 N). Measuring and actinic beams pass two electromagnetic shutters (Compur electronic-m) controlled by a pulse generator. This set up allows alternating, pulsed illumination by measuring or actinic beams. Light intensities were measured with a PIN-Photo-

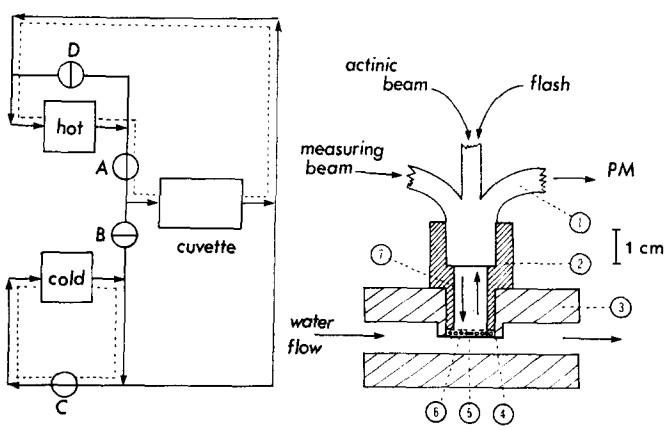

Fig. 1. Experimental set-up for temperature jump fluorescence measurements. Left: Flow diagram; A, B, C, D are valves. Dotted lines show water flow for valve positions where hot flow through cuvette and short circuit cold flow. Right: Cross-section through measuring chamber. 1, trifurcated fiber-optics; 2, black lucite adapter piece; 3, PVC-cuvette; 4, 0.2 mm thick stainless steel plate; 5, 0.1 ml algae suspension or leaf disk; 6, coverglass window; 7, silvered surface. PM, photomultiplier.

diode (Hewlett Packard 5082-4204) calibrated against a YSI-Kettering Radiometer (Model 65).

Fluorescence yield was measured in an extremely weak measuring beam (0.2 $\mu W \cdot cm^{-2}$) and the fluorescence changes are comparable to the changes in Lavorel's dark adapted state m-level [13]. There is a characteristic initial fluorescence yield for every healthy, dark-adapted photosynthetic plant, which generally constitutes the minimum fluorescence yield. The absolute value can vary from sample to sample, therefore all fluorescence curves in this paper are plotted in relative units. The curves are standardized at a relative yield of 1 unit for the initial fluorescence of a control at 20 °C.

Two thermoconstant circulators (Haake, type F 4291) were used, providing T_{max} and T_{min} for the temperature-jump experiments. Two valves (A, B) control heating or cooling, respectively, and two other valves (C, D) bypass the flow when not circulating through the cuvette. The tubing connecting valves A and B with the cuvette is as short as possible to enhance the rate of temperature change. The rate of the temperature change in a 0.1 ml water sample was determined by replacing the algae with a suspension of ground fluorescing glass, the fluorescence of which decays simultaneously with temperature. Test showed 95 % of a 30 °C temperature jump occurred in 2.5 s, 97 % in 3.5 s and 99 % in 6 s (see also Fig. 7).

Most experiments were carried out with continuously grown *Scenedesmus*. Old cells were removed by a 2-min centrifugation at $1000 \times g$ and discarded. After a second centrifugation for 5 min at $5000 \times g$ the pellet of mostly young cells was resuspended in fresh culture medium to approx. 10^5 cells/μl. For each experiment, 100 μl of the suspension was pipetted onto the stainless steel plate and spread out to a thin layer by the fiber optics adaptor piece. Then 5 min was allowed for the cells to settle on the plate and also for decay of the last traces of delayed light emission. In some experiments, instead of the coverglass window restricting gas exchange of the sample a cellophane membrane was used; artifacts due to water vapor were excluded by lining the silvered fiber optics adapter with wet filter paper. For leaf samples, uniform discs cut with a cork borer were covered with a disc of nylon net and gently pressed on to the steel plate by the fiberoptics adapter. Either 100 μl water was added or the sample kept dry, with the window removed from the adaptor piece (see Fig. 1). In the latter case heat transfer and thus rate of temperature-jump was somewhat slower.

RESULTS

Phenomenology of the fluorescence rise following a rapid temperature increase

Fig. 2 shows the rise in fluorescence yield upon a rapid temperature increase in a variety of plants. A temperature-induced fluorescence rise was observed in all plants studied, a selection of which is presented in the figure. The shape of the rise curve in each plant is extremely sensitive to the value of T_{max}, shown here in each case for optimum resolution of the rise phases. The region for marked fluorescence rises in outdoors grown or marine species, as in the algae and the lichen, is 35–43 °C as compared to 48–53 °C in the indoor plants. All the rise curves consist of at least two main phases, resembling in shape light-induced changes in fluorescence yield (Kautsky effect) and the anaerobically induced fluorescence rise [11–12]. We will show below that the similarity between temperature-jump fluorescence rises, the anaerobically induced rise and the light-induced rise is not coincidental but that removal of the

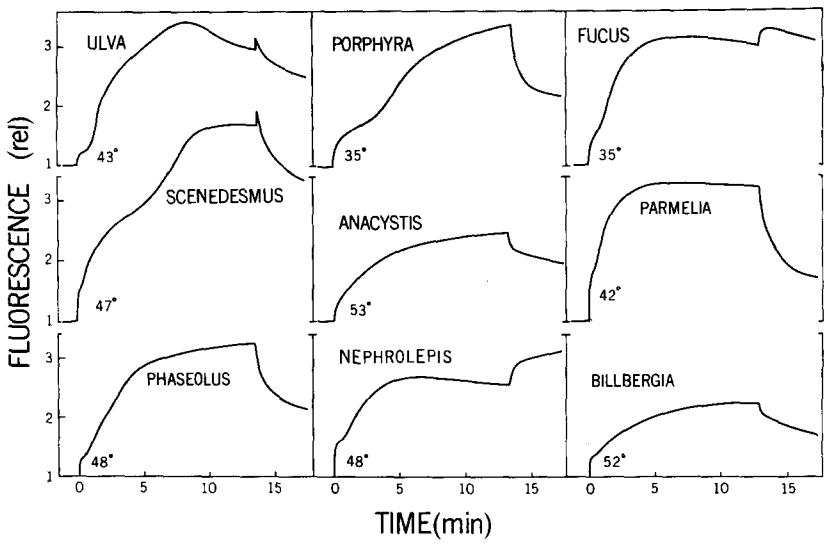

Fig. 2. Fluorescence induction curves in different plants with a temperature-jump from 20 °C to T_{max} indicated at beginning of each curve. After approx. 13 min at T_{max} each sample was rapidly recooled to 20 °C. *U. lobata*, *P. perforata*, *F. distichus* and *P. perlata* (a lichen species) were grown outdoors. *S. obliquus*, *A. nidulans*, *Phaseolus vulgaris* and *Billbergia* sp. (a bromeliad species) were grown indoors.

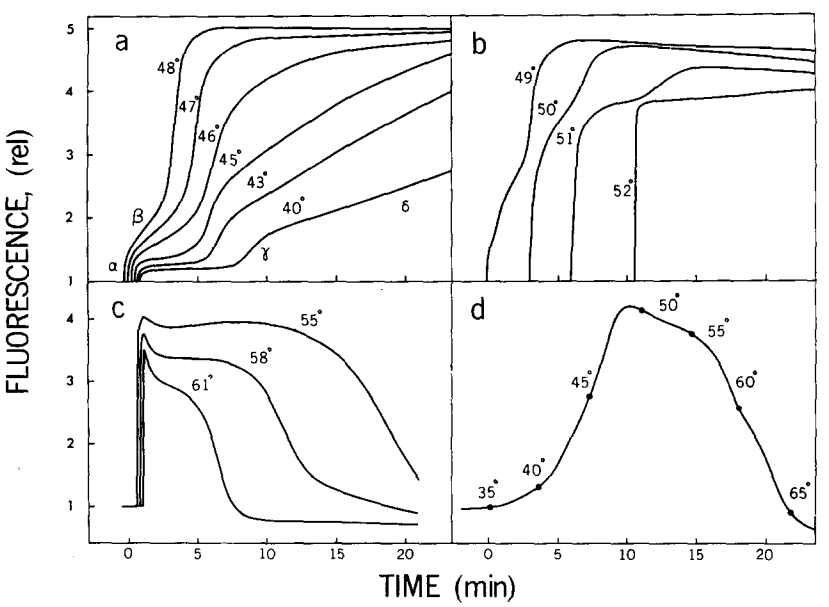

Fig. 3. Fluorescence rise upon heating in *Scenedesmus*. a–c, temperature-jump fluorescence induction for different values of T_{max}; starting temperature 20 °C; d, conventional, slow heating curve; the temperatures reached after the indicated times are marked on the fluorescence curve. Cell density for all curves, approx. 10^5 cells/μl. For the sake of clarity the curves are shifted horizontally and the time scales do not relate to the beginning of heating.

same quenching mechanisms is involved. While both the light- and the anaerobically induced effects are reversible, this is only partially true for the temperature effect. Several factors influence the degree of reversibility of the heat-induced increase in fluorescence, among them plant species, growth temperature, T_{max} of the temperature-jump, time of exposure to T_{max}, availability of O_2, and System I background light. These aspects are considered in more detail for *Scenedesmus* in the following sections.

Temperature-jump fluorescence rise in Scenedesmus

Fig. 3 shows series of temperature-jump fluorescence rise curves for *Scenedesmus* with T_{max} between 40 and 61 °C. For comparison, also shown is the fluorescence change occurring with slow heating rate of approx. 2 °C/min, as applied in earlier studies [13, 14]. Obviously most of the information inherent in the temperature-jump curves series is lost in the conventional slow heating curves. The temperature-jump curves reveal that at higher temperature a series of quenching-removing processes is initiated which take minutes to tens of minutes to be completed. In addition, at temperatures exceeding 52 °C, the fluorescence rise is overlapped by a decay, which is presumably not due to regeneration of photochemical quenching but to chlorophyll destruction. All these processes occur more or less simultaneously during slow heating.

The temperature-jump curves in *Scenedesmus* are characterized by the following features: (1) Depending on T_{max}, four separate phases are distinguished, designated α, β, γ and δ, starting at the beginning of the curve (see Fig. 3a). The highest fluorescence level reached (e.g. in the 48 °C curve) is approx. five times the initial value and close to F_{max} after anaerobic adaptation [11, 12] or after illumination in presence of DCMU. (2) β only developes as a separate rise phase at $T_{max} > 45$ °C; its amplitude increases rapidly with T_{max}. (3) Phases γ and δ are well developed even at low T_{max} values. γ and δ approach the maximum fluorescence yield F_{max}; the amplitude of γ increases to $T_{max} = 48$ °C, whereas the amplitude of δ decreases. There is a striking similarity between the γ, δ phases and the biphasic anaerobically induced fluorescence rise [11–13]. (4) At $T_{max} > 48$ °C the γ phase decreases due to two different developments, (a) most of the removable quenching is already lost during a speeded up β phase and (b) the rate of the γ phase is markedly slowed down, apparently reflecting inhibition of the process which brings about this part of the fluorescence rise. (5) At $T > 52$ °C the heat-induced rise is overlapped by a decay, the rate of which increases with T_{max}. The shoulder in the decay, shrinking with increasing T_{max}, is presumably due to a remainder of the γ phase.

These features of the temperature-jump curves are investigated further below and their relationship to photosynthetic partial reactions discussed.

Analysis of the polyphasic rise

In Fig. 4 the effect of 701 nm light on the temperature-jump curve is demonstrated. In the presence of practically continuous 701 nm illumination (only interrupted for the 10-ms measuring periods) the γ and δ phases are selectively suppressed. As soon as the 701 nm light is switched off there is a steep rise to the same maximum level as in the curve without 701 nm light. Once F_{max} is reached in the absence of 701 nm light, addition of the far-red beam lowers fluorescence yield again, but not to the initial level; fluorescence again quickly returns to F_{max} on removal of far-red.

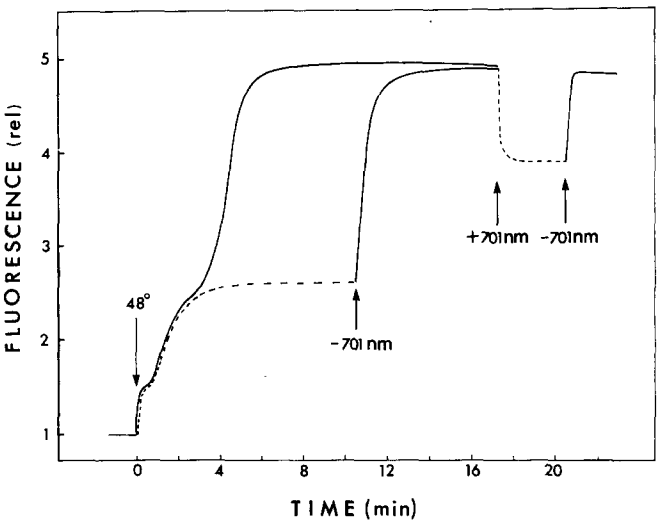

Fig. 4. Effect of far-red light on the temperature-jump (25–48 °C) fluorescence curve in *Scenedesmus*. Fluorescence measured during 10-ms pulses of a broad blue band measuring beam (5 $\mu W \cdot cm^{-2}$) at a frequency of 1 Hz. Dotted lines measured with almost continuous 701 nm background illumination (30 $\mu W \cdot cm^{-2}$) interrupted only during measuring flashes by use of alternating electronic shutters. Beginning of 701 nm illumination coincided with the temperature-jump. Solid lines measured without far-red background light. Cell density approx. 10^5 cells/μl.

These data suggest that the γ, δ part of the heat-induced rise is due to reduction of the electron transport intermediates between the two photosystems. On the other hand the α, β part of the rise appears to be independent of the redox state of Q or plastoquinone. As the temperature-jump fluorescence rise occurs in the dark, reduction of the intersystem intermediates must be due to some endogenous electron donor, which is highly stimulated at elevated temperatures. The fact that this endogenous reductant becomes inhibited at still higher temperatures (see Fig. 3) is characteristic for an enzyme. This enzyme could be identical with hydrogenase.

At physiological temperatures hydrogenase activity is induced only with prolonged periods of anaerobic conditions [17–20]. But, as observed by Gaffron [17], at 35 °C hydrogen metabolism of *Scenedesmus* starts rapidly with the removal of oxygen. It was pointed out above that the γ, δ part of the rise resembles that of the anaerobically induced fluorescence rise. Although here the experiments begin aerobically, it is conceivable that at elevated temperatures the samples become anaerobic in a very short time due to stimulated dark respiration. To check this hypothesis a series of temperature-jump curves was recorded with increasing dilution of a *Scenedesmus* suspension (Fig. 5). As the sample is isolated from atmospheric oxygen the time needed for removal of oxygen will be shortened with increased cell density. There is not only a delay of the γ phase but also a suppression of this "anaerobic phase" with lower cell densities. This is presumably due to denaturation of the enzymes involved in respiration and of hydrogenase during the extended times at high temperature. On the other hand α and β phases are rather stimulated at low cell density. Fig. 6a shows that addition of $Na_2S_2O_4$ (which rapidly removes oxygen) to a highly diluted suspension will restore a rapid rise curve. In Fig. 6b the effect of glucose on the temperature-jump

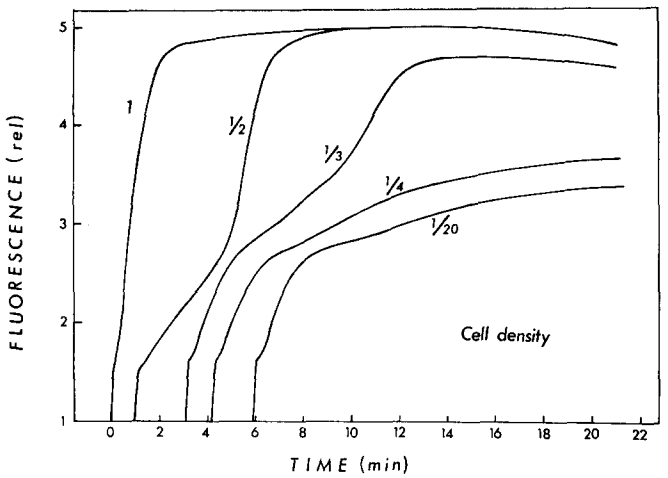

Fig. 5. Effect of cell density on temperature-jump (25–48 °C) fluorescence rise in *Scenedesmus*. Density 1 corresponds to approx. $2 \cdot 10^5$ cells/μl. Time scale with origin at temperature-jump applies only for density 1; other curves are horizontally shifted. All curves standardized at 1 relative fluorescence unit for the original yield before temperature-jump (see also Materials and Methods).

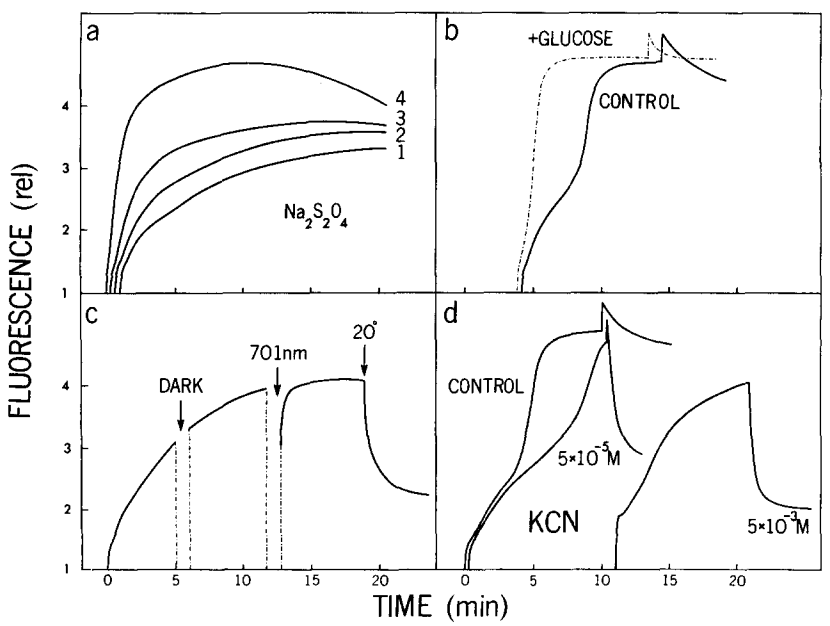

Fig. 6. Effect of various factors on the temperature-jump curves (25–48 °C) in *Scenedesmus*, which are related to the O_2 concentration in the sample. (a) Change of curve from a very dilute sample (approx. $5 \cdot 10^3$ cells/μl) with addition of $Na_2S_2O_4$ (as crystals) to final concentrations of (1) zero, (2) $2 \cdot 10^{-4}$ M, (3) $5 \cdot 10^{-4}$ M, (4) $2 \cdot 10^{-3}$ M. (b) Stimulation of fluorescence rise by 1 % glucose solution (broken line); approx. 10^5 cells/μl. (c) Free access of atmospheric O_2 through thin cellophane membrane; approx. 10^5 cells/μl; demonstration of fluorescence rise in the dark despite contact with air, and Q reoxidation in 701 nm light. (d) Effect of KCN on rise and decay curves upon recooling; approx. 10^5 cells/μl. Curves are shifted relative to time base.

fluorescence rise is demonstrated. Glucose can serve as substrate for respiration and hydrogenase activity [20]. There is a strong acceleration of the rise curve. In Fig. 6c a temperature-jump curve is recorded with a sample which is not separated from air by a window. The fluorescence rise is almost identical to that in a highly diluted sample in a closed volume (see Fig. 5). Despite the availability of oxygen from the air there is still reduction of Q by some endogenous donor, as is seen from the fact that 701 nm light restores quenching. That the fluorescence rise is not induced by the measuring beam can be concluded from the continued loss of quenching in a period of total darkness.

It is known that KCN inhibits hydrogenase activity and respiration in *Scenedesmus* [19, 20] and should therefore also inhibit the γ, δ phases. As shown in Fig. 6d there is a marked effect with $5 \cdot 10^{-5}$ M KCN on the γ, δ part of the temperature-jump curve. At higher concentrations the whole curve is affected, essentially resulting in a stronger β phase and total inhibition of γ, δ. The figure also shows the effect of KCN on reversibility with recooling. Reversibility upon recooling is greater in the presence of KCN, which could be explained by the assumption that KCN inhibits the uptake of O_2, which thus remains available for the reoxidation of intermediates.

From the experimental evidence in Figs. 5 and 6 it is apparent that an essential part of the temperature-jump fluorescence rise is caused by reduction of Q during a heat-stimulated, rapid anaerobic induction process. During this process, some endogenous electron donor becomes activated, presumably hydrogenase acting on organic hydrogen donors such as glucose. At the higher temperatures the O_2-consuming process (dark respiration) appears to attain a rate which is faster than the diffusion of O_2 into the chloroplast. This is surprising as the diffusion rate of O_2 also increases with temperature. There is a decrease of O_2 solubility in water with increased temperatures, but this effect is too small to account for the symptoms of anaerobiosis. It is conceivable, though, that oxygen lipid solubility is reduced at high temperatures. This point may be essential as chlorophyll and the Systems II electron acceptor pool (Q and plastoquinone) have lipid properties.

It is the γ, δ part of the fluorescence rise which is mostly suppressed by both far-red light and molecular oxygen, whereas the α, β phases do persist under these conditions. The question arises as to whether α and β can be also correlated to known mechanisms of fluorescence changes. We did not find any temperature-jump fluorescence rise under the following conditions: (1) In *Scenedesmus* mutant 11, which lacks functional System II reaction centres [21] and at 20 °C is already in the maximum fluorescence yield state; (2) In dark anaerobically adapted *Scenedesmus*, which at 20 °C is also already in the state of maximum fluorescence yield [11–13]; (3) In DCMU-treated samples preilluminated until F_{max} was reached.

It is therefore clear that, however a temperature-jump induces the removal of fluorescence quenching during α and β, it can no longer do so if all known fluorescence-quenching mechanisms are already blocked. Consequently, the relationship between a heat-induced block of energy conversion at System II reaction centres and α and β should be considered. A form of quenching independent of the redox state of Q is the so-called S-quenching [9, 10], which therefore might be related to α, β. On the other hand Q-quenching can also be lost without reduction of Q, if the molecular arrangement of Q relative to the System II trapping centre is disturbed. Necessary conditions for efficient energy trapping (or quenching) at System II centres ZPQ are

that (a) Q is in a state to accept an electron, i.e. it has to be oxidized and in functional contact with P, (b) Z is in a state to donate an electron to Q, and (c) P has to be in a state to sensitize the transfer.

The integrity of the ZPQ complex is undoubtedly linked to the integrity of the thylakoid membrane on which it is located. Thus it is not unlikely that a structural change of this membrane with heat will be reflected by a loss of trapping efficiency in ZPQ. From the work of Döring et al. [22] it is known that P is not deactivated in the 40–50 °C region, whereas there is an irreversible deactivation of the water-splitting enzyme system, which includes Z. Part of the S-quenching (which must involve a functional Z) can be removed selectively by preillumination in a weak continuous beam [9, 10, 23] or by a μs-saturating flash [9]. As shown in Fig. 7 the increase in fluorescence due to removal of S-quenching does cut down the amplitude of the α phase, without much effect on the β phase. With the higher time resolution used in this experiment two subphases α_1 and α_2 can be distinguished and it is α_1 which is almost completely removed. The flash preillumination further appears to cause a delay in the α_2 rise which thus becomes even better separated from the small remaining α_1. For these relatively fast rise phases it must be taken into account that the temperature-jump is not instantaneous (as indicated in the insert of Fig. 7a). α_1 may only be distinguishable from α_2 because it is initiated at a lower temperature than α_2. Preliminary experiments (not included in the figure) with a series of saturating flashes showed variations in the size of the remaining α_1 and α_2 phases. We conclude that the α phase does reflect removal of S-quenching by heat. The α phase hence can be considered an indicator for heat deactivation of the water-splitting enzyme system. Such an indicator appears extremely useful for the study of heat tolerance in plants, as water splitting is known to be the most sensitive part of the photosynthetic apparatus.

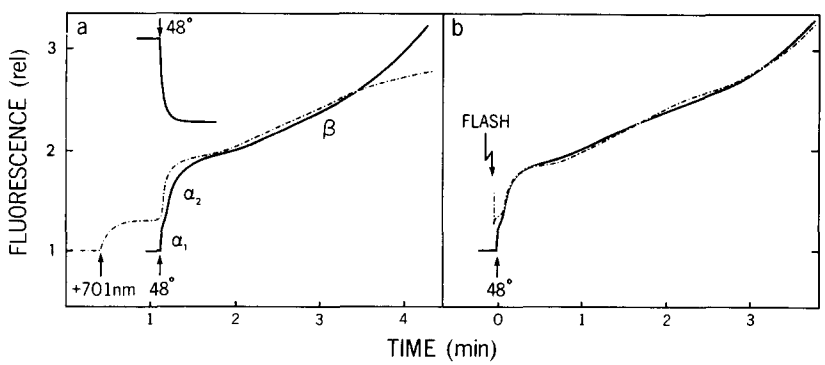

Fig. 7. Effect of preillumination, which selectively removes S-quenching, on the temperature-jump (25–48 °C) fluorescence rise. (a) Dotted line, 701 nm background light (30 $\mu W \cdot cm^{-2}$) interrupted for 10-ms periods simultaneous with applying 10-ms pulses of the blue measuring beam (10 $\mu W \cdot cm^{-2}$); light control by alternating electronic shutters; beginning of illumination approx. 40 s before temperature-jump. Solid line, without 701 nm background light. Insert: Decay of fluorescence yield from ground fluorescing glass upon temperature-jump; same volume of 0.1 ml water present as in *Scenedesmus* suspensions. (b) Broken line, saturating μs-flash triggered approx. 1 s before the temperature-jump. Solid curve without flash preillumination. Same measuring pulses as in (a). Cell density for (a) and (b) approx. $5 \cdot 10^4$ cells/μl. Traces recorded on storage oscilloscope from where they were redrawn.

The part of the temperature-jump fluorescence induction which remains to be explained is the β phase. β has been shown above to be neither related to Q reduction nor to removal of S-quenching. A possible explanation for β is that it reflects separation of Q from the System II trapping centre. Efficient Q-quenching must depend on a close interaction of Q with *P*-680, which appears to become disturbed in the heat. As will be shown below, this perturbation, reflected by β, is practically completely reversible upon recooling.

Reversibility of the heat-induced fluorescence increase

The change of fluorescence yield upon recooling depends on a variety of factors, the most important of which are T_{max}, the time of exposure to T_{max} and the availability of O_2. Particularly the last factor has to be appreciated in any discussion of the reversibility of the heat-induced fluorescence phenomenon, as can be seen from Fig. 8. This figure shows the fluorescence change upon recooling of samples kept for 25 min at the indicated temperature. The corresponding temperature-jump heating curves are as in Fig. 3. Reversibility, if judged by the degree of fluorescence decay, appears greater the higher the previous T_{max}, a response which is opposite to what one might expect. The solution for this apparent discrepancy lies in the fact (described in Figs. 4–6) that there is reduction of Q during the γ, δ phases by some heat-stimulated endogenous donor, which is probably identical to hydrogenase. In Fig. 6d it was already shown that in presence of KCN, which inhibits hydrogenase and O_2 uptake as well, reversibility is greater. The fact that, in Fig. 8 reversibility becomes higher with $T_{max} > 48$ °C, appears to be related to a heat denaturation of the enzymes involved in dark respiration and of hydrogenase. A surprising feature is the rapid positive spike accompanying a negative temperature-jump, in Fig. 8 for $T_{max} < 52$ °C. This spike is likely to be a physical transient reflecting the negative temperature

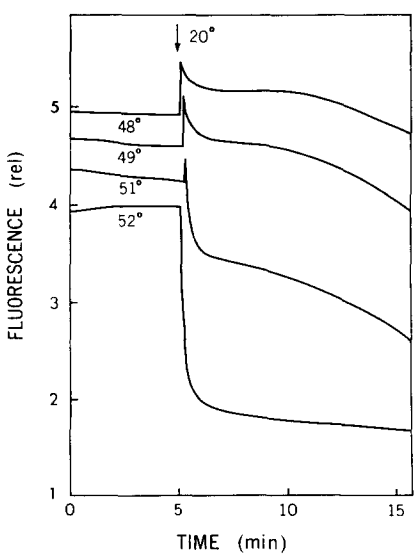

Fig. 8. Fluorescence yield changes upon rapid recooling (negative temperature-jump) after 25 min at indicated T_{max}. Same conditions as in Fig. 3.

coefficient of fluorescence yield, a general phenomenon of all fluorescing substance (see e.g. insert in Fig. 7). This spike does not appear in Fig. 8 for $T_{max} > 51$ °C because of an equally rapid overlapping decay of fluorescence yield. Obviously a negative spike should occur for the same reason upon a positive temperature-jump But it has to be considered that the amplitude of the spike is proportional to the fluorescence yield in the moment of the temperature-jump. As fluorescence yield of a normal sample at room temperature is approx. one-fifth of the maximum yield, the negative spike is relatively small and in most cases obscured by a bigger fluorescence rise during the α phase. In samples, which are in a state of high fluorescence yield when the temperature-jump is applied (as in presence of DCMU after preillumination or in *Scenedesmus* mutant M 11, which lacks a functioning System II) a rapid negative transient does occur (Schreiber, U., unpublished).

Anaerobic effects are excluded when only a short heat pulse is given. As shown in Fig. 9a, in this case the heat-induced fluorescence increase is almost completely reversible upon recooling in the whole range of $T_{max} \leqslant 58$ °C. In a highly diluted sample, anaerobic effects are minimized and the effect of longer heating on the reversibility can be studied. Fig. 9b suggests that it is the part corresponding to the α phase which becomes more and more irreversible the longer the sample is kept at 48 °C Additional 701 nm illumination did not further decrease fluorescence yield after recooling, which confirms that the irreversible part of the rise is not due to lack of oxidant for Q reoxidation. That it is only α which is irreversible is substantiated by Fig. 10, which compares a temperature-jump (20–48 °C) curve of a normal and of a heat-pretreated sample (after 10 min at 52 °C). In the heat-pretreated sample fluores

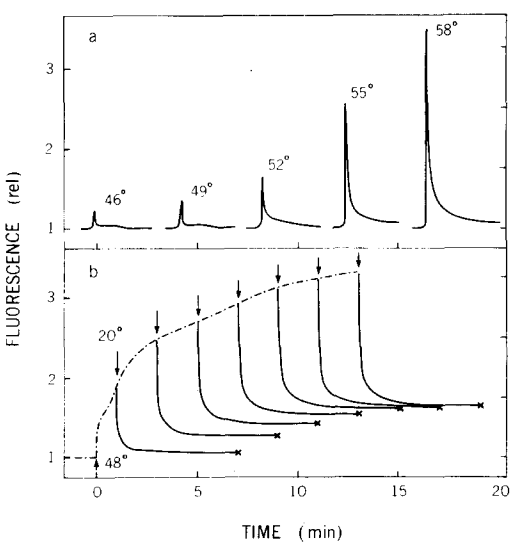

Fig. 9. Reversibility of heat-induced fluorescence increase upon recooling. (a). Application of short (5 s) heat pulses with T_{max} at the indicated value. (b) Solid lines represent fluorescence decay curves upon negative temperature-jump after different times of exposure to $T_{max} = 48$ °C. Each decay measured in fresh sample; recorded over 6 min, such that line composed of end values (crosses) relates to increase of irreversibility. Dotted line, course of temperature-jump (20–48 °C) fluorescence induction. Cell density for (a) approx. 10^5 cells/μl and (b) approx. $5 \cdot 10^3$ cells/μl.

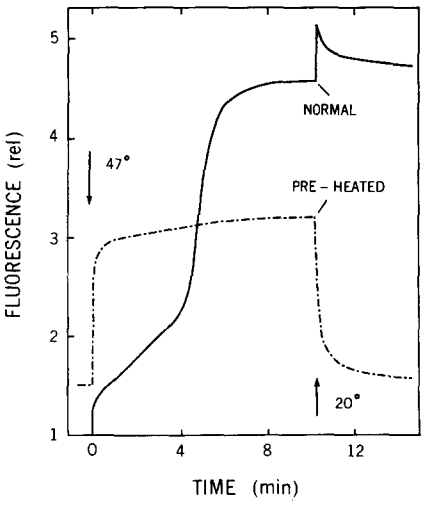

Fig. 10. Comparison of temperature-jump (20–47 °C) fluorescence curves in a preheated (10 min at 52 °C) and a normal sample. Cell density approx. 10^5 cells/μl.

cence yield already at 20 °C is at the level reached in the control during the α phase, and there is no α phase in the subsequent temperature-jump curve. This supports the conclusion drawn in the previous section that α reflects loss of S-quenching during deactivation of the water-splitting enzyme system. Irreversibility of this deactivation increases with exposure to T_{max} and is (as concluded from Fig. 9b) completed after approx. 10 min at 48 °C, a result which agrees with other determinations using different methods [22, 24]. The temperature-jump fluorescence rise which remains after heat pretreatment should then reflect loss of Q-quenching during an isolated β phase. This is highly reversible as shown in Fig. 10, because the pretreatment prevents anaerobic reduction of Q. If our hypothesis is right that Q becomes functionally separated from photocentre II at high temperature because of structural changes in the thylakoid membrane, the efficiency of recombination upon recooling is very high and the decay is an indicator for the kinetics of this process.

DISCUSSION

The analysis of the temperature-jump chlorophyll fluorescence rise curves has shown that a large part of the fluorescence increase is due to the rapid induction of anaerobic conditions by heat. When anaerobic effects are minimized by increased supply of O_2 or reoxidation of Q by System I light, temperature-jump fluorescence induction consists of two well separable phases α and β, which can be related to the loss of S-quenching and Q-quenching, respectively.

Temperature-jump fluorescence induction curves have the potential of yielding valuable information in several respects:

(a) The presence of γ and δ phases in any particular plant indicates that it contains a strong endogenous reductant, presumably identical to hydrogenase, which feeds electrons into the electron transport chain under anaerobic conditions.

(b) The α phase reflects the deactivation of the water-splitting enzyme system the nature of which remains the least understood part of photosynthesis.

(c) The critical temperatures at which α and β develop in a particular plan indicate the region in which structural changes occur in the thylakoid membrane

(d) With the isolated β phase after preheating a reversible transient is given which can function as an indicator for temperature-induced changes in thylakoi membrane properties.

At the present state of investigations no evidence is available that the temper ature-jump fluorescence curves are directly related to a lipid phase transition in th thylakoid membrane. The data reported by Murata et al. (ref. 16 and unpublished suggest such a phase transition in *Anacystis* at 13 °C (if grown at 28 °C) while ou temperature-jump fluorescence induction in the same organism (grown at 25 °C occurs at 53 °C (see Fig. 2). Their explanation for an increased chlorophyll fluores cence yield with a phase transition is based exclusively on the lipid properties of th thylakoid membrane. Their fluorescence increase is approx. 10 % and is observed after all quenching at System II centres is already blocked by addition of DCMU. Thus this effect appears to result primarily from a change in the state of the bulk chlorophyll. On the other hand after a temperature-jump to 40–55 °C the fluorescence increase can amount to as much as 400 %, as the system goes from maximum to minimum quenching. Although some contribution of changes in bulk chlorophyll cannot be excluded, the main effect appears to result from a change in the state of the reaction centre II. Much of the chlorophyll in the thylakoid membrane, and particularly the molecules associated with the reaction centres, is attached to protein [25]. Therefore one may suggest that the state of the centres and the temperature-jump fluorescence induction is governed by the properties of the protein boundary lipids and the protein-protein interaction in a given lipid environment. Protein boundary lipids have been shown to become mobilized at higher temperatures than bilayer lipids [26, 27]. The data presented above and in a previous paper [15] show substantial differences for outdoor and indoor plants in the temperatures for pronounced temperature-jump fluorescence induction. It has been reported that plants can regulate membrane lipid composition, such as hydrocarbon chain length and unsaturation, in response to growth temperature (refs. 16, 28, 29 and Murata, N., Troughton, J. H. and Fork, D. C., in preparation). Lipid composition will affect in a similar way the critical temperatures for the liquid-crystalline phase transition and the mobilization of the protein boundary lipids, i.e. critical temperatures will be lowered by unsaturation and shorter hydrocarbon chains. This explanation seems more likely than to invoke differences in enzymes due to a different thermal environment. While the technique used by Murata et al. (ref 16 and Murata, N., Troughton, J. H. and Fork, D. C., in preparation) seems to yield information concerning thylakoid lipids only for plants with an extremely low content of unsaturated lipids (like *Anacystis*), the temperature-jump method (with T_{max} at 40–55 °C) applies for all plants. Little is known about the correlation between growth temperature and membrane lipid composition in higher plants. Temperature-jump fluorescence studies with plants of a single species grown under different temperatures, currently under way in our laboratory, should provide more information.

ACKNOWLEDGEMENTS

We gratefully acknowledge support by the National Research Council of Canada operating grants A 2908 and A 9418. The staff of Simon Fraser Machine Workshops is thanked for their help in building the cuvette.

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