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PHOTOINDUCED QUENCHING OF CHLOROPHYLL FLUORESCENCE IN INTACT CHLOROPLASTS AND ALGAE

RESOLUTION INTO TWO COMPONENTS

G.H. KRAUSE [a], C. VERNOTTE [b] and J.-M. BRIANTAIS [b]

[a] *Botanisches Institut der Universität Düsseldorf, Universitätsstrasse 1, D-4000 Düsseldorf 1 (F.R.G.) and*
[b] *Laboratoire de Photosynthèse, C.N.R.S., F-91190 Gif-sur-Yvette (France)*

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The light-induced decline of chlorophyll *a* fluorescence from a peak (P) to a low stationary level (S) in intact, physiologically active isolated chloroplasts and in intact *Chlorella* cells is shown to be predominantly composed of two components: (1) fluorescence quenching by partial reoxidation of the quencher Q, the primary acceptor of Photosystem II and (2) energy-dependent fluorescence quenching related to the photoinduced acidification of the intrathylakoid space. These two mechanisms of fluorescence quenching can be distinguished by the different kinetics of the relaxation of quenching observed upon addition of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU). The relaxation of quenching by addition of DCMU is biphasic. The fast phase with a half-time of about 1 s is attributed to the reversal of Q-dependent quenching. The slow phase with a half-time of about 15 s in chloroplasts and 5 s in *Chlorella* cells is ascribed to relaxation of energy-dependent quenching. As shown by fluorescence spectroscopy at 77 K, the energy-dependent fluorescence quenching essentially is not caused by increased transfer of excitation energy to Photosystem I. By analyzing the energy- and Q-dependent components of quenching, information on the energy state of the thylakoid membranes and on the redox state of Q under various physiological conditions is obtained.

Introduction

When isolated intact chloroplasts, algal cells or leaves of higher plants are illuminated, the rise of Chl *a* fluorescence to a peak (P) is followed by a decline of fluorescence emission to a steady state (S). In algae and leaves, the S level frequently is followed by a slower S → M → T transient [1]. The P → S decline has found rather different interpretations [2]. It has been attributed to the reoxidation of the primary electron acceptor of PS II, the 'quencher' Q, that is supposedly maximally reduced in the fluorescence peak. Other evidence has related the slow fluorescence quenching to energization of the thylakoid system by illumination, i.e., to the build-up of a transmembrane proton gradient and of secondary metal cation gradients [3–5]. Recently, a linear relationship between fluorescence quenching and intrathylakoid H^+ concentration was found [6,7]. Mg^{2+}, when added to broken chloroplasts that are depleted of divalent cations, drastically increases the fluorescence yield and strongly inhibits transfer of excitation energy to PS I [2]. Since illumination induces an efflux of Mg^{2+} from the thylakoids [8–10], one might attribute the fluorescence decline to a redistribution of excitation energy in favour of PS I (i.e.,

Abbreviations: Chl, chlorophyll; PS, photosystem; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Tricine, *N*-tris(hydroxymethyl)methylglycine.

to a 'State 1'-'State 2' transition). Such changes in excitation transfer were first described for algae by Murata [11] and Bonaventura and Myers [12]. However, our study on fluorescence of broken pea chloroplasts [6] did not reveal changes in the distribution of excitation energy related to the photoinduced fluorescence decline.

Williams et al. [13], who compared the fluorescence induction under preferential excitation of either PS I or PS II, did not find indications of State 1-State 2 transitions in intact chloroplasts, although they did observe such adaptation to the incident light quality in *Chlorella* cells. Based on a study of *Phaseolus* leaves, Bradbury and Baker [14] postulated that photoinduced fluorescence quenching was governed by the redox state of Q and in addition by thermal deactivation of excited PS II chlorophylls. In other recent reports [15–17] it has been suggested that a fluorescence decline is related to the phosphorylation of the light-harvesting protein-chlorophyll complex which is supposed to regulate the transfer of excitation energy to PS I.

The complex nature of the fluorescence quenching in vivo has been recognized before [1,18]. The present study is aimed at clarifying the mechanisms of the fluorescence decline in intact, physiologically active chloroplasts and *Chlorella* cells when they are illuminated with light that does not sensitize preferentially one of the two photosystems. We show that under physiological conditions the fluorescence decline from the peak to the steady state is composed predominantly of two components, quenching by reoxidation of Q and quenching by the decrease in the intrathylakoid pH. There appears to be no major contribution to the overall fluorescence decline by increased exciton transfer to PS I.

Materials and Methods

Intact chloroplasts were isolated from freshly harvested spinach leaves according to a modified version [19] of the method described by Jensen and Bassham [20]. Chlorophyll fluorescence at 20°C was excited with blue light (half-band width 405–490 nm) and recorded at 686 nm by means of a multiplier shielded by a suitable filter combination [3]. The level of initial fluorescence, F_0, was determined by recording fluorescence induction with an oscillograph. The intensity of exciting light (15 W/m^2) was strictly limiting for photosynthesis: Stationary rates of $KHCO_3$-dependent O_2 evolution were between 20 and 40 μmol/mg Chl per h (rates in saturating light, 100–200 μmol/mg Chl per h). A limiting intensity was chosen to prevent the electron-transport chain remaining in a strongly reduced state, blocking reoxidation of Q during substrate reduction. Chloroplast samples contained 50 μg Chl/3 ml of a sorbitol medium, pH 7.6 [21], in a stirred thermostatically controlled cuvette, equipped with a Clark-type O_2 electrode. When $KHCO_3$ was used as substrate, catalase (20 μg/ml) was added to obtain optimal rates of photosynthesis [21]. Corrected fluorescence emission spectra at 77 K were recorded with a Farrand MK 1 spectrofluorometer as described previously [22].

Cells of *Chlorella pyrenoidosa* were suspended in a medium containing 50 mM phosphate, pH 6.5, and 100 mM KCl. Chl *a* fluorescence was measured using an exciting beam at 480 ± 20 nm; the intensity was about 30 photons/reaction centre per s. At 20°C the fluorescence was detected through a Corning 2-64 filter or via a monochromator at 685 ± 3 nm. 2.5 ml of suspension (7 μg Chl/ml) were placed in a 1 ml cuvette and stirred during measurements for experiments where additions were made (i.e., DCMU, NH_4Cl). 0.2 ml of a suspension (50 μg Chl/ml) was layered on the bottom of a Dewar flask for experiments where liquid nitrogen was added at different points of fluorescence induction. The half-band width at 77 K was 3 nm for spectra and 10 nm for inductions at 685 and 720 nm; the emissions are not corrected for sensitivity of the apparatus.

For glutaraldehyde treatment, cell suspensions of *Chlorella* containing 10 μg Chl/ml were incubated with 0.2% glutaraldehyde; this treatment increased the permeability of the membranes to protons.

Results and Discussion

Two components of the reversal of fluorescence quenching

Addition of DCMU to illuminated intact chloroplasts increases the emission of Chl *a* fluorescence. This increase usually is biphasic, consisting of a fast phase with $t_{1/2} \approx 1$ s and a slow phase with $t_{1/2} \approx 15$ s. This is shown in Fig. 1 for different physiological states of the chloroplasts. The fast relaxation of

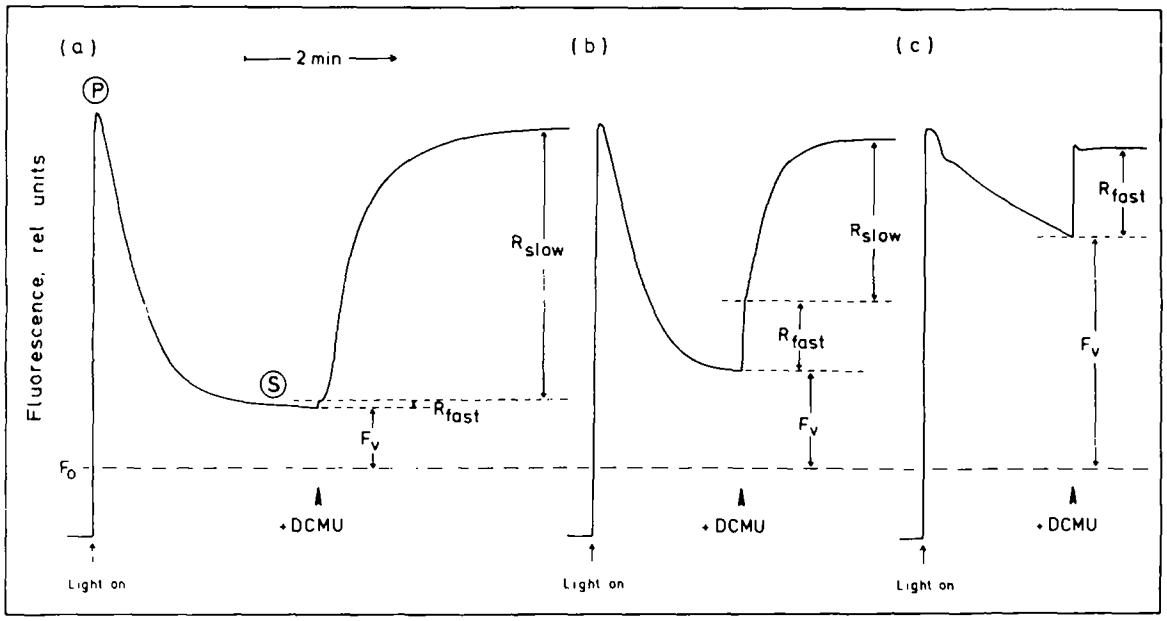

Fig. 1. Induction of chlorophyll fluorescence in isolated intact chloroplasts at 20°C and relaxation of quenching by addition of DCMU ($4 \cdot 10^{-5}$ M). Illumination was started after 3 min of dark adaptation. DCMU was added in the light as indicated, causing a fast and total block of photosynthetic O_2 evolution. F_0 denotes constant fluorescence; F_v, variable fluorescence, R_{fast} and R_{slow}, fast and slow phases of fluorescence increase upon DCMU addition; P and S, peak and steady-state level of fluorescence emission, respectively. (a) Absence of bicarbonate; net O_2 evolution was not detectable. (b) Presence of 2 mM $KHCO_3$; stationary rate of O_2 evolution, 28 μmol/mg Chl per h. (c) Presence of 2 mM $KHCO_3$ and 2 mM NH_4Cl; stationary O_2 evolution, 26 μmol/mg Chl per h.

quenching, R_{fast}, can be attributed to reduction of that part of Q which was in the oxidized state before the electron transport was blocked by the inhibitor. As demonstrated in Fig. 1, the slower phase, R_{slow}, can be ascribed to relaxation of 'energy-dependent' quenching which is related to the intrathylakoid H^+ concentration [6,7]. When chloroplasts are illuminated in the absence of efficient electron acceptors, Q stays in a largely reduced state indicated by the very small fast phase of relaxation in Fig. 1a. Since in CO_2-depleted chloroplasts phosphorylation energy cannot be utilized in the Calvin cycle, an increased proton gradient develops which is responsible for the large energy-dependent fluorescence quenching represented by R_{slow}. The presence of bicarbonate (Fig. 1b) leads to a steady rate of photosynthesis after a few minutes of illumination. The electron acceptor Q then becomes more oxidized and the proton gradient at the thylakoid membrane is partly degraded by phosphorylation. Then oxidized Q and the proton gradient contribute about equally to the overall fluorescence quenching. The proton gradient can be further diminished by addition of NH_4Cl (2 mM) in the presence of bicarbonate without inhibiting CO_2 fixation and the affiliated electron transport [23]. Fig. 1c shows that then only a fast phase of relaxation is visible upon addition of DCMU. This means that energy-(ΔpH)-dependent quenching is abolished by NH_4Cl, whereas Q-dependent quenching remains.

That the above considerations are relevant to the situation in vivo is demonstrated by similar experiments with intact *Chlorella* cells [24]. The fluorescence induction signal in the presence of CO_2 (Fig. 2a), exhibiting a much faster P → S decline than chloroplasts, resembles that observed by Mohanty and Govindjee [25]; the slow fluorescence increase seen subsequent to the P → S decline denotes the onset of the M phase. Addition of DCMU also causes a biphasic relaxation of quenching. The slow phase, R_{slow}, ($t_{1/2} \approx 5$ s), is faster than that observed with intact

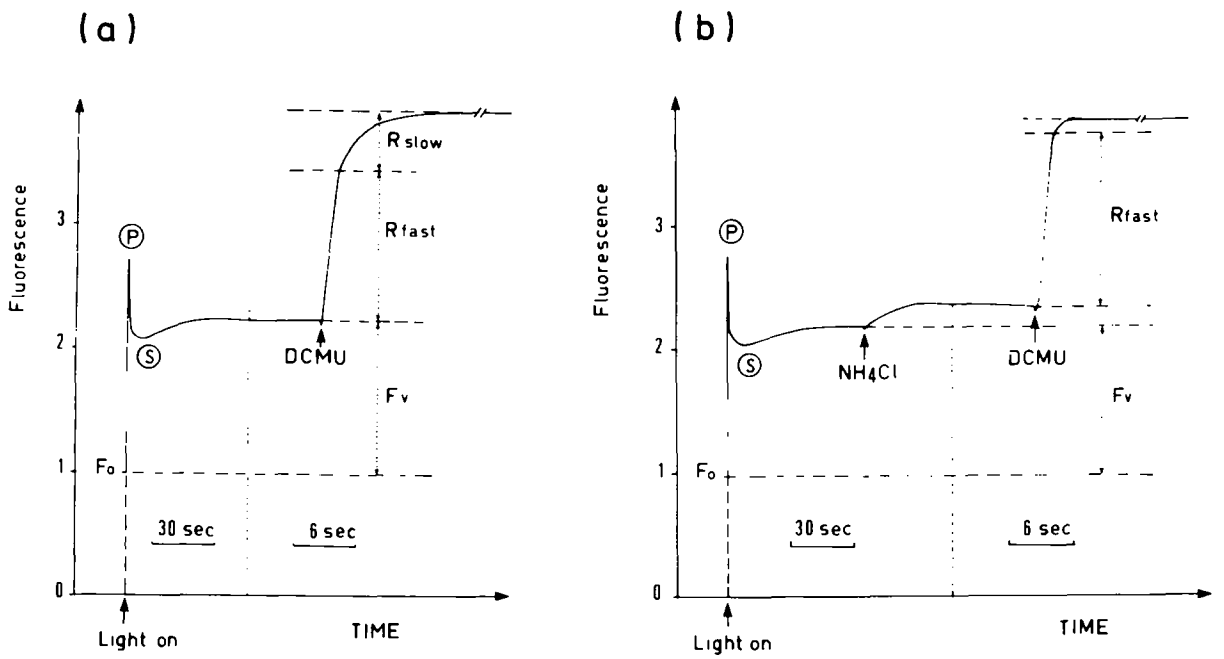

Fig. 2. Effects of DCMU (10^{-5} M) addition (a) and of successive addition of NH_4Cl (20 mM) and DCMU (10^{-5} M) (b) on room-temperature fluorescence of *Chlorella* cells illuminated with blue light. Symbols as for Fig. 1.

chloroplasts. The final fluorescence level attained after DCMU addition is considerably higher than the peak of fluorescence induction. As discussed below, this is probably due to fast onset of noncyclic electron transport that prevents strong transitory reduction of Q. In Fig. 2b the effect of partial uncoupling on the fluorescence emission is shown. Addition of NH_4Cl causes partial relaxation of quenching. When DCMU is added subsequently, R_{slow} is strongly diminished as compared to Fig. 2a. From this, it appears that like in isolated chloroplasts, the fast phase represents relaxation of Q-dependent fluorescence quenching and the slow phase that of energy-dependent fluorescence quenching.

By adding DCMU to samples of chloroplasts or *Chlorella* cells at various points of the fluorescence induction curve, the kinetics of the two components of quenching can be resolved (Figs. 3–5). As depicted in Fig. 3 for intact chloroplasts, the ΔpH-dependent quenching, Q_E, is strongly affected by the presence of substrates of photosynthesis. When bicarbonate is present, energy-dependent quenching is the predominant component in the first minute of illumination, i.e., during the lag phase of CO_2-dependent O_2 evolution (see inset of Fig. 3). Toward the end of the lag phase, the two components become about equal. In the presence of 3-phosphoglycerate the lag phase is much shorter [26]. The onset of electron transport is reflected by strong Q-dependent quenching within about the first 20 s of illumination, followed only later by the energy-dependent fluorescence decline. The final amplitude of the energy-dependent quenching is higher in the presence of 3-phosphoglycerate than of bicarbonate. This is consistent with the lower requirement of phosphorylation energy by 3-phosphoglycerate reduction as compared to CO_2 assimilation. From the extent of R_{fast}, and the corresponding level of variable fluorescence, F_v, the approximate redox state of Q can be calculated (Fig. 4). It can be seen that in intact chloroplasts about 90% of Q is in the reduced state in the peak of the induction curve. During continued illumination, considerable reoxidation of Q takes place in the presence of substrates, particularly of 3-phosphoglycerate. In Fig. 5, the contributions of the two components of the fluorescence decline during the induction period of *Chlorella*, illu-

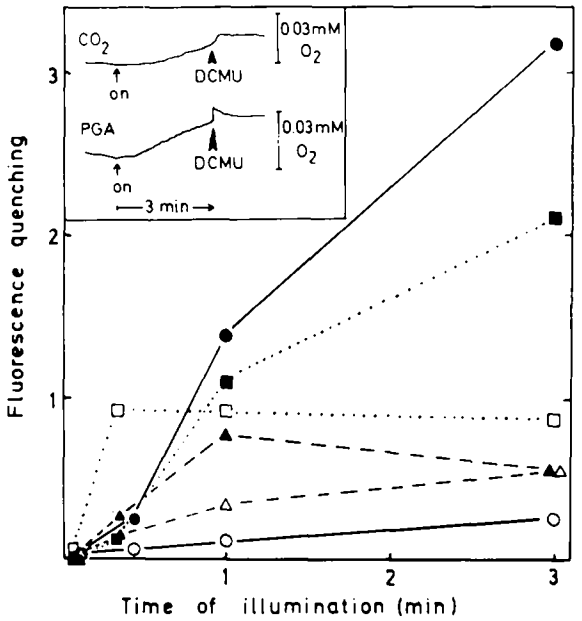

Fig. 3. Q-dependent fluorescence quenching, $Q_Q = R_{fast}/F_v$, and energy-dependent quenching, $Q_E = R_{slow}/(F_v + R_{fast})$, of intact isolated chloroplasts illuminated at 20°C in the absence of substrate (○, ●) and in the presence of 2 mM $KHCO_3$ (△, ▲) or 2 mM 3-phosphoglycerate (□, ■). Open symbols denote Q_Q; closed symbols, Q_E. Values were calculated with the above equations from slow and fast phases of relaxation of quenching by adding $2 \cdot 10^{-5}$ M DCMU after different times of illumination. The inset shows oxygen traces recorded in the presence of $KHCO_3$ or 3-phosphoglycerate (PGA), with DCMU added after 3 min in the light. Stationary rates of O_2 evolution (in μmol/mg Chl per h) were 33 for $KHCO_3$ and 37 for 3-phosphoglycerate as substrate.

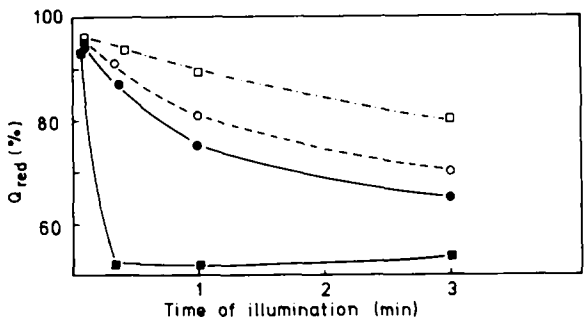

Fig. 4. Changes in the redox state of Q in intact chloroplasts during the slow fluorescence decline recorded at 20°C in the absence of substrate (□ – · – · □) or with 2 mM $KHCO_3$ (●——●), 2 mM $KHCO_3$ + 2 mM NH_4Cl (○- - - - -○), or 2 mM 3-phosphoglycerate (■——■). The stationary rate of O_2 evolution with $KHCO_3$ + NH_4Cl was 42 μmol/mg Chl per h. For other rates see legend to Fig. 3. The approximate portion of reduced Q was obtained from the fast phase of fluorescence relaxation and the level of variable fluorescence emission at the time of DCMU ($2 \cdot 10^{-5}$ M) addition, according to $Q_{red}(\%) = [F_v/(R_{fast} + F_v)] \times 100$.

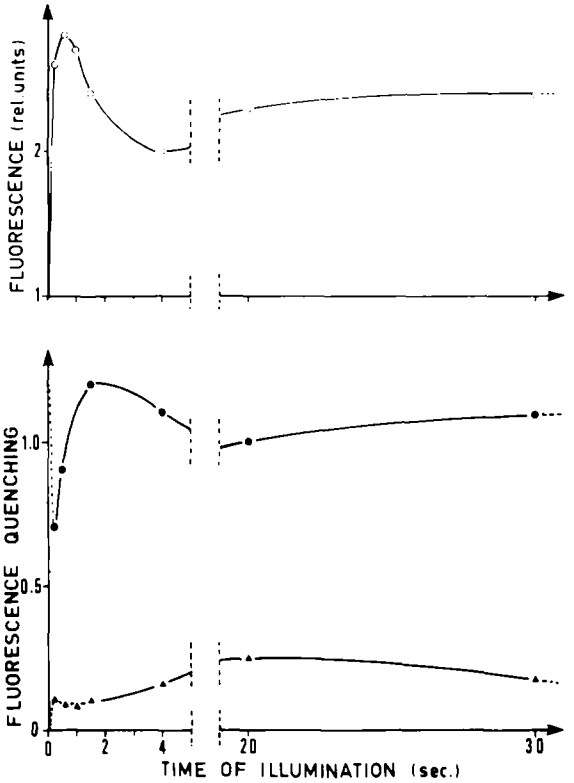

Fig. 5. Kinetics of the photoinduced variations of fluorescence of dark-adapted *Chlorella* cells (○) and corresponding Q-dependent (●) and energy-dependent (▲) fluorescence quenching at 20°C. Values of quenching were calculated as for Fig. 3.

minated in the presence of CO_2, are given. Like in intact chloroplasts in the presence of 3-phosphoglycerate, Q-dependent quenching predominates during the first seconds of illumination. Some energy-dependent quenching is observed within less than 1 s and then increases slowly. The slow phase of Q_E is accompanied by a decrease in Q_Q which at present is not fully understood but might indicate a control of electron transport by decreasing intrathylakoid pH.

In contrast to intact chloroplasts, in *Chlorella* cells a nearly complete reduction of Q in correlation with the fluorescence peak is not observed; about 60% of

Q is reduced at the P level, as compared to about 50% in the steady state (see Figs. 2 and 5). This can only be explained by fast onset of noncyclic electron transport in the algae, as compared to isolated chloroplasts. It should be noted that incomplete reduction of Q during fluorescence induction was similarly observed with isolated protoplasts from *Valerianella locusta* leaves illuminated in the presence of bicarbonate (Singhal, G.S. and Krause, G.H., unpublished data). In intact spinch leaves [18] the fluorescence peak was strongly decreased by the presence of CO_2. Fast onset of O_2 evolution in algae has been described and attributed to a fast O_2 reduction (Mehler reaction) taking place during the induction period of CO_2 fixation [27,28]. In isolated chloroplasts, fast electron transport to O_2, mediated by methyl viologen, strongly lowers the fluorescence peak (not shown). In the presence of methyl viologen, about 50% of Q was in the oxidized state at the P level of fluorescence. Prolonged illumination only slightly increased the percentage of oxidized Q, while extremely strong energy-dependent quenching developed due to the absence of significant ATP consumption in the chloroplasts. It was observed previously [25,29] that incubation of *Chlorella* cells with methyl viologen drastically decreased the transitory P level.

The two components of quenching in isolated chloroplasts have been also distinguished by their kinetics of dark relaxation that exhibits two phases [30].

Nature of the energy-dependent fluorescence decline

In the experiment of Fig. 6, we show that in glutaraldehyde-treated *Chlorella* cells which are permeable to protons, the maximal fluorescence level observed in the presence of DCMU and NH_4Cl can be quenched by acidification of the medium. This suggests a relationship between chlorophyll fluorescence emission and intrathylakoid proton concentration similar to that which has been found with isolated thylakoids [6,7]. To answer the question as to the relationship between energy-dependent fluorescence quenching and changes in the distribution of excitation energy, the fluorescence emission of intact chloroplasts and *Chlorella* cells at 77 K was studied. Intact chloroplasts were frozen in the light at different points of the fluorescence induction curve and at the P level after different times of dark relaxation

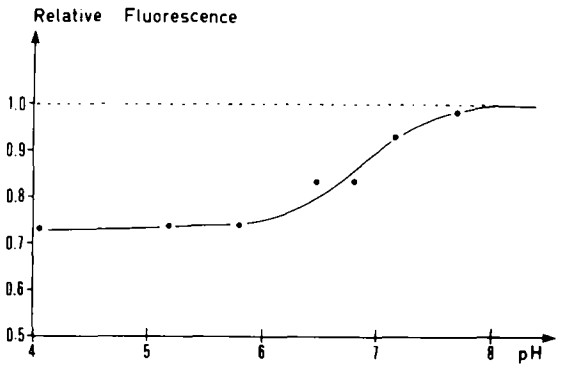

Fig. 6. Decrease in fluorescence emission at 20°C of glutaraldehyde-treated *Chlorella* induced by chemical acidification. For experimental conditions see Materials and Methods. The dashed asymptote is the fluorescence amplitude of *Chlorella* cells resuspended in Tricine buffer (0.5 mM), pH 7.8, in the presence of DCMU (10^{-5} M) plus NH_4Cl (10^{-2} M), before glutaraldehyde addition.

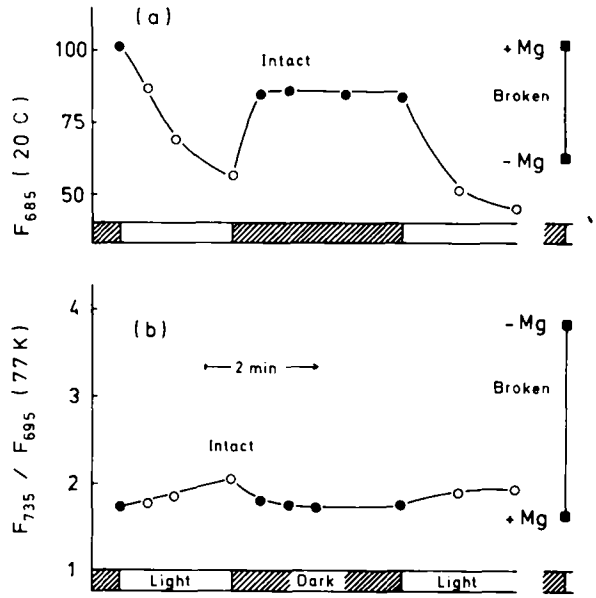

Fig. 7. Relationship between chloroplast fluorescence at room temperature and at 77 K. (a) Variations in fluorescence at 20°C of intact chloroplasts induced by light (○) and dark (●) adaptation, and P levels of broken, dark-adapted chloroplasts (■) in the presence and absence of 5 mM $MgCl_2$. Closed symbols denote P levels of fluorescence. (b) Ratio of 77 K fluorescence emission in the band peaks at 735 and 695 nm ($F_{735/695}$) derived from emission spectra of samples frozen in the different states of fluorescence emission at 20°C, as marked in (a). Note that all samples were frozen in the light either at the P level (closed symbols) after different times of dark adaptation, or after prolonged illumination (open symbols).

(Fig. 7a). Because substrates were absent as in Fig. 1a, Q-dependent fluorescence changes were negligible. For comparison, dark-adapted broken chloroplasts were frozen in the presence and absence of $MgCl_2$. Fig. 7b shows the ratio of the bands at 735 nm (emission by PS I) and 695 nm (emission by PS II) obtained from the fluorescence spectra at 77 K. It can be seen that in intact chloroplasts only small, although reproducible changes in these ratios occur when large changes of room-temperature fluorescence take place. In contrast, a large increase in the F_{735}/F_{695} ratio accompanies the lowering of room-temperature fluorescence of broken chloroplasts caused by removal of Mg^{2+}. Thus, Fig. 7 suggests that at least the major part of the energy-dependent fluorescence decline in intact spinach chloroplasts is not due to a change in the distribution of excitation energy.

In *Chlorella* cells, as in leaves of *Phaseolus* [31], the fluorescence quenching is not reversed by cooling to 77 K (Fig. 8). The ratio between emission at 720 nm (PS I) and 685 nm (PS II and light-harvesting complex) is increased by preillumination. But this increase is not necessarily due to an increased exciton transfer to PS I. There is a large change in the ratio of PS I to PS II emission during the fluorescence induction at 77 K, as was first observed in chloroplasts [32]. In Fig. 8, this ratio for F_0 and F_M is 2.4 and 1.04, respectively, for dark-adapted algae. A quenching of only PS II fluorescence will give points of F_{720} vs. F_{685} located on the slope obtained for the induction, as is indeed observed in the experiment of Fig. 8. In contrast, an increased energy transfer to PS I should induce a decrease in PS II fluorescence and an increase in PS I fluorescence; the points of the F_{720} vs. F_{685} relationship should be situated above the slope of the relationship obtained for the 77 K induction of dark-adapted algae. We conclude from Fig. 8 that in *Chlorella* cells, frozen in various states between P and S levels of fluorescence, there is no detectable change in the excitation energy distribution compared to dark-adapted cells.

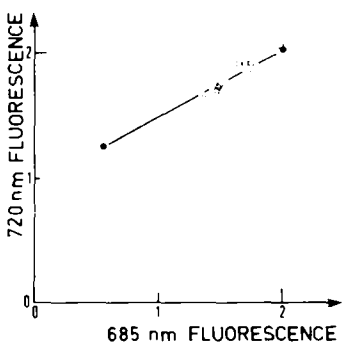

Fig. 8. Relationship between 685 and 720 nm fluorescence at 77 K of F_0 and F_M levels during induction of dark-adapted *Chlorella* (●——●) and of total fluorescence of illuminated cells (○) frozen at various points of the P → S decline.

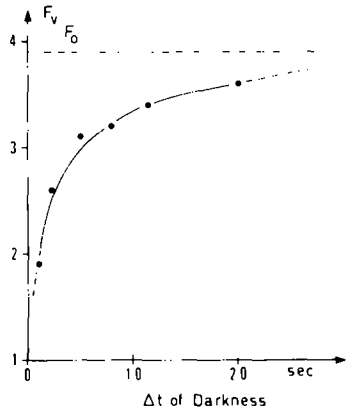

Fig. 9. Dark relaxation of quenching of 77 K variable PS II fluorescence after preillumination at room temperature. Δt denotes the dark time allowed after 1 min light, before liquid nitrogen was added. On the ordinate, the ratio of variable to constant fluorescence at 77 K and 685 nm, F_v/F_0, is given.

Fig. 9 depicts the kinetics of dark relaxation at room temperature of the quenching observed at 77 K and 685 nm. After 1 min of light adaptation at 20°C, 1 s of darkness is sufficient to restore the F_0 level of dark-adapted *Chlorella*, but not to restore the F_M level. The quenching affects variable fluorescence and its dark relaxation shows approximately the same kinetics as the slow phase of reversion by DCMU.

Conclusions

The present investigation shows that the fluorescence decline from the peak (P) to the steady state (S) in isolated chloroplasts or *Chlorella* cells essentially consists of two components. One is the quenching by reoxidation of the electron acceptor Q of PS II. The other component is the energy-dependent quenching, which, according to our results of this and a previous study [6], is not related to a transition

from State 1 to State 2. A possible alternative mechanism is increased thermal deactivation of excited states that may be caused by ultrastructural changes of the membrane occurring upon displacement of metal cations (particularly of Mg^{2+}) by protons at the inner thylakoid surface.

Ley and Butler [33] have argued that State 2 may only be stable when freezing is carried out in the light whereas freezing in the dark could cause reversion to State 1, and that this was the cause of the inability of Briantais et al. [6] to detect State 2 adaptation in the broken chloroplasts at the S fluorescence level. However, in the experiments of Ref. 6, as in this paper, samples were frozen under illumination (besides those used to record fluorescence induction at 77 K in Figs. 8 and 9). The slight increase in the F_{735}/F_{695} ratio observed at 77 K when chloroplasts were illuminated before freezing (Fig. 7) might indicate a minor contribution of State 2 adaptation to the fluorescence decline. But, according to preliminary results from current experiments, this change probably is caused, as in *Chlorella* cells (Fig. 8), by quenching of variable PS II fluorescence at 77 K without concomitant increase in fluorescence of PS I. Such quenching could be caused by decreased electron donation to PS II, occurring when at the time of freezing the intrathylakoid pH is low and part of Q is in the oxidized state. The accessory donor to PS II operating at 77 K, high-potential cytochrome *b*-559 [34,35], has indeed been shown to be transformed to its low-potential form and oxidized when thylakoids are acidified [36].

According to Horton and Black [15], a slow light-dependent fluorescence quenching at room temperature takes place upon addition of ATP to uncoupled chloroplasts. The kinetics of this quenching and its reversion are considerably slower than those of the energy-dependent fluorescence decline reported here. In contrast to the latter, ATP-induced quenching seems to result from increased exciton transfer to PS I. It occurs in close parallel with phosphorylation of the light-harvesting complex and supposedly is regulated by the redox state of the plastoquinone pool [37]. At first sight, it seems puzzling why in our experiments with intact chloroplasts, as in those of Williams et al. [13], a contribution of the ATP-induced quenching is not apparent, although under illumination in the absence of CO_2 the ATP level is high and Q and plastoquinone are strongly reduced. Possibly, in coupled chloroplasts, an ATP-dependent component is concealed by the faster and stronger energy-dependent quenching occurring upon acidification of the intrathylakoid space. Similarly, in *Chlorella* cells, the State 2 adaptation due to its slow kinetics does not contribute significantly to the P → S decline depicted in Fig. 2; a longer time and/or a preferentially PS II-sensitizing light [12,13,38] seem necessary to induce a detectable change in the distribution of absorbed light energy.

Thus, we can conclude that the major part of the 20°C quenching observed after 2–3 min illumination in chloroplasts and after 1 min in *Chlorella* cells corresponds to Q reoxidation and to acidification of the intrathylakoid space, and only an insignificant part may correspond to State 2 adaptation. From the experiments with chloroplasts it follows that energy-dependent quenching is predominant when electron transport is restricted due to lack of efficient electron acceptors beyond PS I. On the other hand, in the uncoupled state, only Q-dependent quenching can be observed. Under physiological conditions, both mechanisms contribute to the overall fluorescence decline to varying extents. In *Chlorella* and probably in higher plant leaves in the presence of CO_2, fast quenching by reoxidation of Q upon illumination is followed by slower energy-dependent quenching. Reoxidation of Q can actually be fast enough to prevent the nearly complete reduction of Q in the fluorescence peak (yielding a low peak as seen in Fig. 2). In intact isolated chloroplasts, reoxidation of Q is – for reasons that are not apparent at present – too slow to lower the fluorescence peak, except when an artificial electron acceptor such as methyl viologen is added.

The two phases of fluorescence relaxation observed upon addition of DCMU are convenient indicators of the approximate redox state of Q and the energy state of the thylakoid system at a given physiological condition. If energy-dependent quenching essentially is caused by the intrathylakoid H^+ concentration, this component can be calibrated to be used as an intrinsic probe of ΔpH [6,7]. From the pH calibration with glutaraldehyde-treated *Chlorella* (Fig. 6) and from the slow phase of fluorescence relaxation by addition of DCMU to intact cells illuminated for 1 min (Fig. 2), a ΔpH of 1.1 units can be calculated.

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