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ATP-INDUCED INCREASE IN CHLOROPHYLL FLUORESCENCE

CHARACTERIZATION OF RAPID AND SLOW INDUCTION PHASES

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The biphasic rise of chlorophyll fluorescence induced in the dark (following activation of the latent ATP-ase) upon ATP-hydrolysis was investigated in detail, yielding the following main results: (1) The rapid phase is independent of artificial reductants or redox mediators. On the contrary, the slow phase requires such additions. (2) The slow phase is selectively eliminated by substances which collapse the transmembrane proton gradient, while the rapid phase may even be stimulated. (3) The ratio of rapid-to-slow phase is favored by a high degree of chloroplast integrity. The same factors which favor the rapid phase appear to be essential for a pronounced 'slow electrogenic reaction' in the flash-induced P 515 absorbance change. (4) For the rapid phase of the ATP-induced fluorescence increase, neither a ΔpH nor a $\Delta\psi$ are obligatory intermediates. (5) Hydroxylamine at about $5 \cdot 10^{-3}$ M causes a preferential stimulation of the rapid phase by about a factor 2. (6) There is selective inhibition of the slow phase by DBMIB, dinitrophenylether of iodonitrothymol, Bathocuproine and HQNO (2-heptyl-4-hydroxy quinoline-*N*-oxide) which are known to block at the level of the Cyt *b*/*f* FeS-complex. (7) The rapid phase is not affected by presence of 5 mM ferricyanide; however, there is substantial suppression if in addition a lipophilic redox mediator, like diamino-durene, is present. It is concluded that the two components of the reverse coupling reactions, reflected by the biphasic ATP-induced fluorescence rise, involve different coupling intermediates and different types of reverse electron flow. The rapid component appears to reflect close interaction between the coupling factor and a redox component in the vicinity of Photosystem II.

Introduction

When chloroplasts are illuminated in the presence of thiol reagents, the latent triadenosinephosphatase (ATPase), becomes activated [1–5]. In intact chloroplasts, the thioredoxin system appears to play an essential role, allowing light-activation of the hydrolase without addition of external sulfhydryl reagents [6–10].

Upon ATP – hydrolysis, a sequence of reactions is induced, which may be considered the reversal of those events leading to ATP-synthesis during photosynthetic electron flow (for a review, see Ref. 11). There is the build-up of a transmembrane proton gradient [12–14] and of a membrane potential [15–17], electrons are induced to flow in the reverse direction [8,11,13,14,18], and with en-

Abbreviations: PS II, Photosystem II; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea; PMS, phenazine methosulfate; HQNO, 2-heptyl-4-hydroxy quinoline-*N*-oxide; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; Cyt, cytochrome.

hancement of charge recombination at Photosystem II reaction centers there is stimulated light-emission (chlorophyll luminescence) [8,19,20].

The ATP-induced rise of chlorophyll fluorescence has been previously described as an indicator for ATP-driven reverse electron flow [13,14,18]: simultaneous recordings of the ATP-induced increase of chlorophyll fluorescence and of 9-aminoacridine fluorescence quenching (as indicator of ΔpH formation) [21] suggested a close relationship between the slow chlorophyll fluorescence rise component and formation of a proton gradient [13,14]. The rapid component, on the other hand, was found to precede ΔpH-formation and to persist under condition when ΔpH-formation was prevented [20].

In the present communication the properties of the ATP-induced fluorescence increase are analysed in more detail. Particular attention is paid to the different requirements and sensitivities of the two rise phases. It will be shown that the rapid rise component displays properties which are indicative of a close interaction between the coupling factor and an electron transfer step in the vicinity of PS II.

Materials and Methods

Intact chloroplasts were isolated from freshly harvested spinach leaves according to the method of Jensen and Bassham [22]. For optimal responses, it was found essential to preilluminate the leaves with strong light (about 300 W/m^2) shortly before chloroplast isolation. Intactness was between 75 and 90%, as estimated by the ferricyanide method [23]. If not stated otherwise, 10 μl aliquots of intact chloroplasts (at about 3 mg Chl/ml in buffer B) were suspended in 75 μl buffer C (pH 7.8) and illuminated for 15 s with intense white light (2000 W/m^2). The chloroplasts were then exposed for 30 s to hypotonic conditions by dilution (factor 4) in shock-buffer (5 mM $MgCl_2$/2.5 mM Hepes-KOH (pH 7.8)), or in water, and resuspended in buffer C (modified to give a final Mg^{2+}-concentration of 6 mM). The resulting chloroplast preparation (class D, according to the nomenclature in Ref. 24) had retained the capability of reducing added NADP at high rates (about 200 μmol per mg Chl per h) and displayed a pronounced slow phase in the flash-induced P 515 absorbance change (see also Fig. 3). Light-activation, hypotonic treatment and measurement of the ATP-induced changes were carried out at 10 °C with continuous stirring in a special cuvette which was described before [13,17]. Class D chloroplasts treated in the described way were used in all experiments, with the exception of parts of Figs. 3 and 7.

Broken chloroplasts (class C) were isolated similarly to the intact chloroplasts, except that the leaves were macerated for 30 s (instead of 5 s) and centrifugation was for 2 min at $8000 \times g$ (instead of 1 min at $4000 \times g$). Before each experiment, aliquots of the class C chloroplasts were treated hypotonically, as described for intact chloroplasts. Class C chloroplasts were activated in the reaction medium (buffer C, 6 mM $MgCl_2$) in presence of 5 mM dithiothreitol by illumination (2000 W/m^2) for 2 min.

Chlorophyll fluorescence was monitored by an extremely weak 550 nm measuring beam (about 10^{-4} W/m^2) which by itself did not induce any appreciable fluorescence rise even in presence of DCMU and NH_2OH. Hence, the presented curves display changes of the dark-fluorescence level which is modified by factors different from light (ATP, DCMU and NH_2OH). The measuring system was based on a fiber optics design, as previously described [13,25,26]. ATP-induced changes of P 515 absorbance were measured in the same system, with a laboratory-built spectrophotometer [17,27] connected via appropriate fiber bundles to the cuvette, operated either in the single-beam or the dual-wavelength mode at an alternation frequency of 2500 Hz.

Results and Discussion

Different requirements for rapid and slow rise components

The ATP-hydrolase is effectively activated by preillumination of intact chloroplasts without addition of artificial sulfhydryl reagents [6–10,17,28]. Following light-activation and hypotonic treatment the hydrolase keeps active over several minutes [6–10] and ATP-induced reverse coupling reactions can be observed. Fig. 1 shows typical traces of the ATP-induced increase of chlorophyll

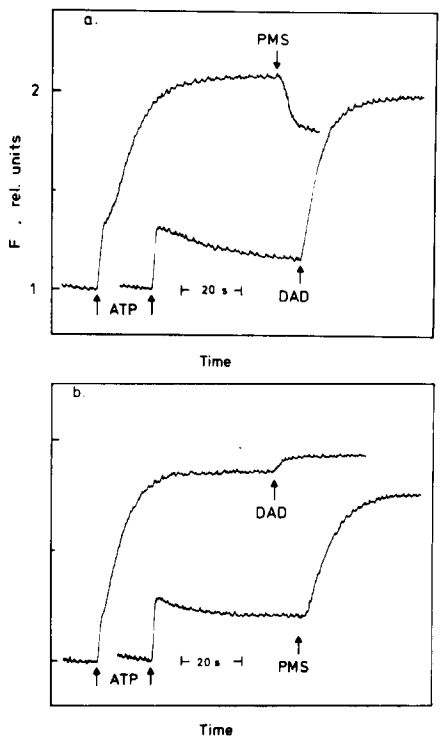

Fig. 1. ATP-induced increase of dark-level fluorescence in the presence and absence of external reductants and redox mediators. (a). First trace, presence of diaminodurene (10^{-4} M) and sodium ascorbate (10^{-3} M); essentially the same induction kinetics were observed with only diaminodurene added in the reduced form. Where indicated, PMS (final concentration, 10^{-7} M) was injected to the stirred sample. Second trace, absence of any artificial reductants and mediators; where indicated, 10^{-4} M diaminodurene was added. (b) First trace, presence of PMS (10^{-7} M) and dithiothreitol ($5 \cdot 10^{-3}$ M). Where indicated, diaminodurene (final concentration, 10^{-4} M) was added. Second trace, presence of $5 \cdot 10^{-3}$ M dithiothreitol; where indicated 10^{-7} M PMS was added. Fluorescence was monitored with an extremely weak measuring beam which by itself did not induce any fluorescence rise beyond the dark-level (see Methods). One relative unit of the ordinate scale corresponds to the fluorescence intensity shortly before ATP-addition. ATP was added (final concentration, $3 \cdot 10^{-4}$ M) 135 s following termination of activating illumination. Chlorophyll concentration, 35 μg/ml.

fluorescence, in dependence of the presence of added reductants and redox mediators. It is apparent that the slow phase of the fluorescence rise requires an artificial electron donation system (PMS/dithiothreitol or diaminodurene/ascorbate), while there is no such requirement for the rapid rise phase. The ratio between rapid and slow rise components is somewhat more favorable with diaminodurene as reductant. PMS also induced some fluorescence quenching. Hence, in most experiments, the diaminodurene donation system was preferred.

Separation of the rapid rise component is achieved by omission of artificial reductants and mediators. In the following chapters use of such separation will be made to study specific aspects of this phase. In intact chloroplasts, limited amounts of intrinsic reductants and redox mediators are available, allowing development of a secondary rise phase (albeit smaller and slower than in the artificial system [8]. Also in preparations of freshly shocked chloroplasts (class D), sometimes a small slow rise component can be observed in absence of PMS (or diaminodurene), provided dithiothreitol is present (data not shown).

It has been suggested by previous work [13,14] that the slow rise component requires the formation of an ATP-induced proton gradient. As shown in Fig. 2, the amplitude of the rapid phase is rather stimulated by up to 10 mM NH_4Cl, while the slow phase is suppressed.

The results of Figs. 1 and 2 emphazise two major differences in the requirements for the rapid and slow ATP-induced fluorescence rise components. Contrary to the slow phase, the rapid phase does not depend on addition of artificial reductants and redox mediators. And the rapid phase does not require formation of a ΔpH, which is essential for the slow phase.

Prerequisites for a pronounced rapid fluorescence response

All treatments which affect the activity of the ATP-hydrolase also suppress the rapid rise phase along with the slow phase. Hence, no response is found without proper light activation or in presence of ATP-ase inhibitors (Tentoxin, Dio-9, tri-*N*-butyl-tin) or in presence of uncouplers (Gramicidin, Desaspidin).

The rapid phase is particularly sensitive to factors which affect the state of the thylakoid membrane and, presumably, of the enzyme complexes associated with it. Generally, it is most pronounced in class D chloroplasts (intact chloroplasts freshly shocked). In Fig. 3, ATP-induced responses of classes D and C chloroplasts are

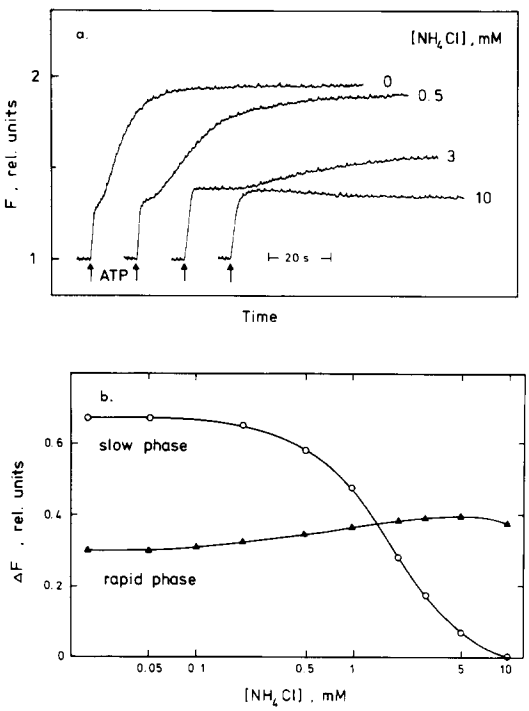

Fig. 2. Selective suppression of the slow component of the ATP-induced fluorescence increase by NH_4Cl. (a) Kinetic traces at indicated concentrations of NH_4Cl. All samples contained 10^{-4} M diaminodurene. Addition of NH_4Cl occurred only 15 s before ATP-injection. With longer incubation times of the uncoupler the rapid phase was somewhat suppressed at concentrations above 5 mM NH_4Cl. (b) Plots of the amplitudes of rapid and slow phases of the ATP-induced fluorescence rise kinetics, as derived from the traces in (a). The amplitudes are given in ΔF-units, one unit corresponding to the yield before ATP-addition.

compared. The figure also shows corresponding traces of the ATP- and light-induced carotenoid shift ($\Delta A_{525-535}$) and of the flash-induced P 515 change observed with the two different preparations. It is apparent, that the amplitudes of the ATP- and light-induced absorbance changes are smaller with class C chloroplasts as compared to class D chloroplasts. The amplitudes differ by about a factor 2.5, which is close to that by which the rapid fluorescence phases differ in the two preparations. There is considerably less difference in the slow phases of the ATP-induced fluorescence increases. Comparison of the flash-induced P 515 changes suggests that in the two types of chloroplasts Reaction I [28–30] is about equal, while Reaction II is distinctly more pronounced in Class D chloroplasts. Apparently, the occurrence of a large, ATP-induced P 515 change is correlated with the potential for the induction of Reaction II, which is considered to originate from an electrogenic Q-cycle involving the plastoquinone pool and the Cyt *b/f*-FeS complex [30–33].

The most simple explanation for these data would be that the generation of a membrane potential by ATP-hydrolysis is essential for the rapid phase of the ATP-induced fluorescence rise. However, this explanation is questioned by the finding that valinomycin does not suppress this rapid response, while it is known to dissipate effectively the membrane potential [34] and the Reaction-II – type response in particular [35]. In Fig. 4 the separated rapid phase (omission of artificial reductants and mediators) is shown under condition of dissipated membrane potential (+valinomycin), of dissipated proton gradient (+NH_4Cl) and of full uncoupling (+valinomycin, +NH_4Cl). Valinomycin as well as NH_4Cl somewhat slow down the ATP-induced response, without preventing it. There is strong synergism of the two substances, similar to the previously reported synergistic effect of valinomycin and nigericin [20]. These data do not support a straightforward association of the rapid fluorescence component with an ATP-induced membrane potential. It appears that neither $\Delta\psi$ nor ΔpH are obligatory intermediates for the reverse coupling reaction reflected by the rapid fluorescence component. The suppression of the reaction upon elimination of ΔpH and $\Delta\psi$ may be caused by the decay of the activated state [4,36,37], by dissipation of coupling intermediates, or by a combination of these two factors. In this context it may be important to note that a substantial part of the rapid ATP-induced fluorescence rise can be maintained in presence of valinomycin and NH_4Cl, provided the NH_4Cl is added only briefly (5–10 s) before ATP-injection (data not shown).

As shown in Figs. 5 and 6, hydroxylamine (NH_2OH) stimulates the rapid rise component by about a factor 2. Half-maximal effect is observed at $1.5 \cdot 10^{-3}$M NH_2OH. Also the slow phase is stimulated in amplitude, although to a lesser extent. Interestingly, NH_2OH itself induces a substantial dark-fluorescence increase. If NH_2OH is

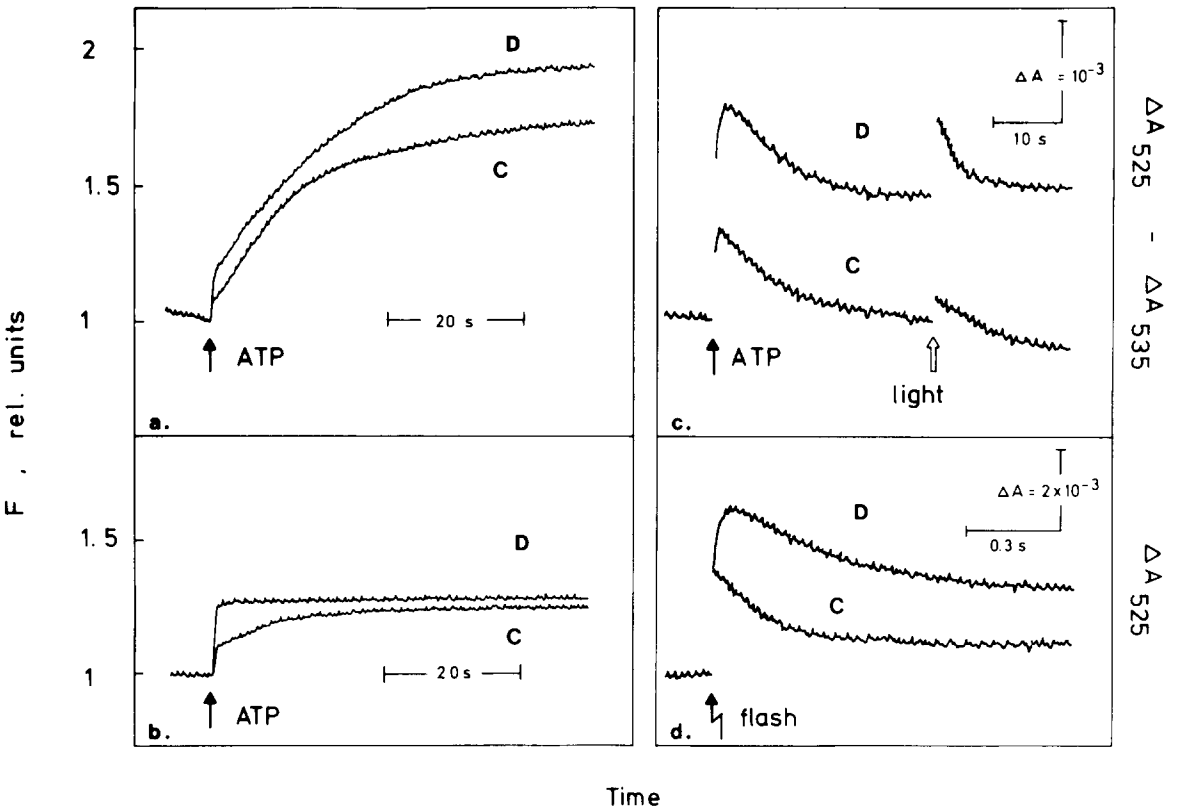

Fig. 3. Comparison of classes C and D chloroplasts with respect to their capacity for ATP-induced changes of chlorophyll fluorescence and P 515 absorbance, as well as for the flash-induced P 515 change. (a) Fluorescence induction in presence of 5 mM dithiothreitol and $5 \cdot 10^{-8}$ M PMS. (b) As in (a), but without PMS. (c) P 515 absorbance change, measured dual-wavelength at 525 vs. 535 nm, to eliminate non-specific absorbance changes and stirring noise. Conditions as in (b). Where indicated, continuous red light ($\lambda > 650$ nm; Schott RG 665) was given at an intensity of 10 W/m^2. (d) Flash-induced change in P 515 absorbance, measured single beam at 525 nm. Where indicated, a saturating flash from a General Electrics FT 230 xenon flash tube (about 10 μs duration) was given. Chlorophyll concentration was 35 μg/ml in the fluorescence and 70 μg/ml in the P 515 experiments, adjusted to be identical for the classes C and D chloroplast preparations.

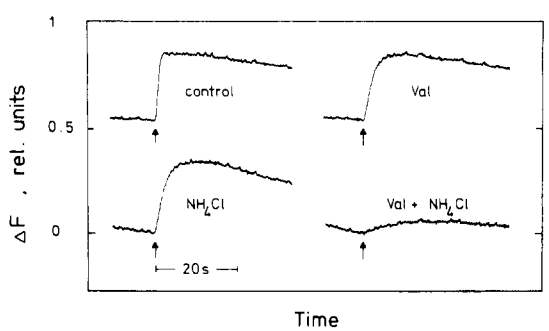

Fig. 4. Separated rapid phase under conditions of suppressed transmembrane gradients. Absence of artificial reductants or mediators. Valinomycin was added to a final concentration of 10^{-7} M at 1 min before ATP-injection. 5 mM NH_4Cl was added 45 s before ATP.

added after ATP, the NH_2OH-induced change is enhanced, such that the combined effect of ATP and NH_2OH is about the same, irrespective of the order of addition. Hydroxylamine is known to act at the PS II donor site [38–40]. It blocks watersplitting by reducing the oxidized secondary donor of PS II [40]. For the same reason it is a potent inhibitor of charge recombination at PS II reaction centers and of the resulting emission of delayed chlorophyll fluorescence [39]. NH_2OH also inhibits the ATP-induced luminescence burst which normally is correlated with occurrence of the rapid fluorescence response [19,20] (data not shown). Measurements of ATP-induced P 515 absorbance changes and of ATP-induced 9-aminoacridine

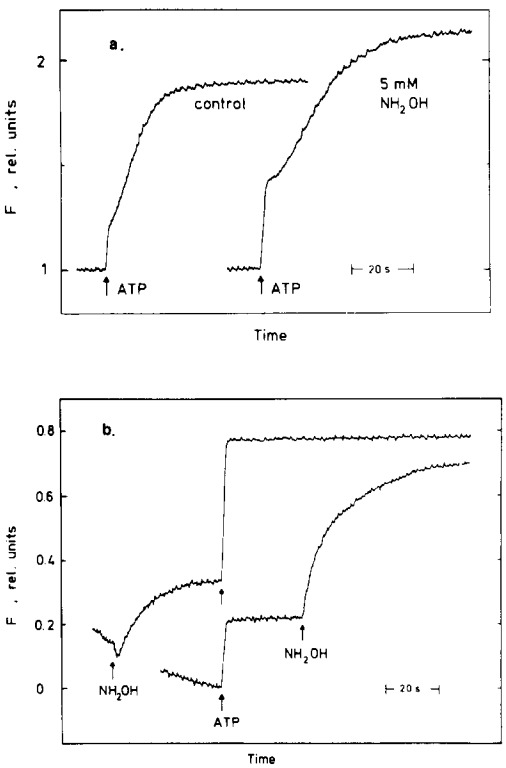

Fig. 5. Effect of NH_2OH on the kinetics of the ATP-induced fluorescence rise. (a) Presence of 10^{-4} M diaminodurene. In the experiment of the second trace 5 mM NH_2OH was added 1 min before ATP-injection. (b) Absence of artificial reductants or mediators. Where indicated, aliquots of a neutralized 1 M $NH_2OH \cdot HCl$ solution is added to give final concentrations of $5 \cdot 10^{-3}$ M.

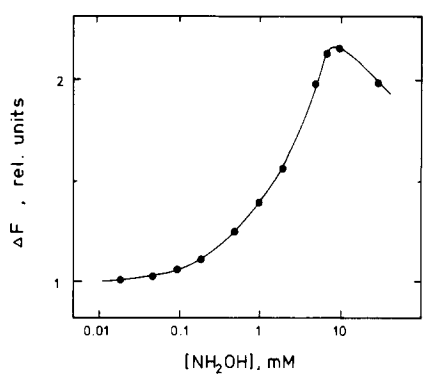

Fig. 6. Dependency of the separated rapid phase on the concentration of NH_2OH. Conditions as in Fig. 5b. One relative unit of ΔF corresponds to the ATP-induced increase of fluorescence in a control sample.

quenching did not reveal any significant effect of NH_2OH on formation of the transmembrane potential and of the proton gradient (data not shown).

So far, the mechanisms of the ATP-induced rapid fluorescence increase and of its stimulation by NH_2OH are not clear. If the rapid component reflects a type of reverse electron flow involving reduction of PS-II-acceptor molecules, one could suspect that NH_2OH enhances this response by preventing reoxidation of the reduced acceptor via the PS-II backreaction.

As pointed out above (see Fig. 3), the rapid phase is particularly sensitive to modifications of the state of the thylakoid membrane. In some cases, such modifications do not affect the amplitude of the rapid phase in controls, but are clearly expressed in experiments where ionophores or uncoupling reagents are present. This aspect is demonstrated by the results of Fig. 7. It is shown that the protonophore nigericin can have rather opposing effects on the rapid-rise phase, depending on whether chloroplasts were activated intact (stimulation) or envelope-free in presence of dithiothreitol (suppression). It should be pointed out that these two chloroplast preparations were obtained from the same stock, exposed to the same shock medium and resuspended in the same reaction mixture. The only difference was that in one case activation was while chloroplasts were still intact, whereas in the other case activation was following hypotonic treatment. Similar results were obtained with a number of other ionophores and uncoupling reagent. For some reason, the activated state created by illumination of the intact chloroplasts appears to be more stable, or more resistant against dissipative agents, than that formed by illumination of the envelope-free chloroplasts.

Selective suppression of the slow rise by electron transport inhibitors

A number of electron transport inhibitors selectively suppress the slow ATP-induced fluorescence rise, without affecting the rapid phase, and without preventing the build-up of a proton gradient or of a membrane potential. Such substances are dinitrophenylether of iodonitrothymol ($1.5 \cdot 10^{-6}$ M); DBMIB ($2 \cdot 10^{-7}$ M), HQNO ($4 \cdot 10^{-5}$ M) and Bathocuproine ($8 \cdot 10^{-6}$ M). The numbers in

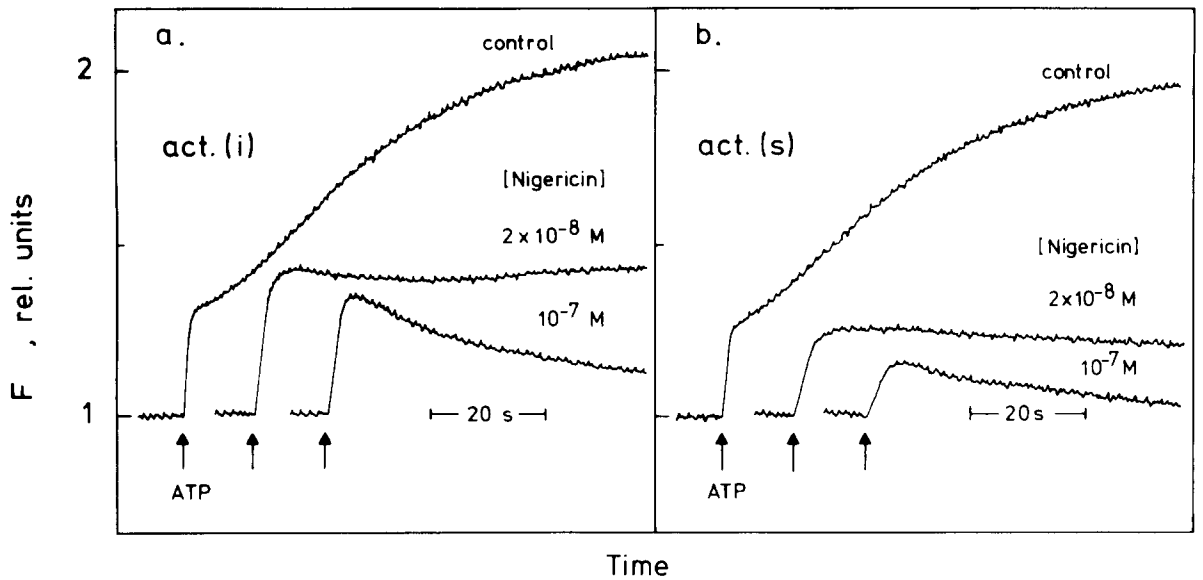

Fig. 7. Effect of the protonophore nigericin on the ATP-induced fluorescence rise in dependence of the way of ATPase light-activation. (a) Activation of chloroplasts while still intact. Following hypotonic treatment, chloroplasts were resuspended in a medium containing $5 \cdot 10^{-3}$ M dithiothreitol and $5 \cdot 10^{-8}$ M PMS. Nigericin was added at the indicated final concentrations 45 s before ATP-injection. (b) Activation of chloroplasts following hypotonic treatment and resuspension in a medium containing $5 \cdot 10^{-3}$ M dithiothreitol and $5 \cdot 10^{-8}$ M PMS (see also Methods). Other conditions identical to (a).

the parentheses correspond to the concentrations for 50% suppression of the slow phase. Fig. 8 shows examples of selective suppression of the slow phase by DBMIB and Bathocuproine. With dinitrophenylether of iodonitrothymol and HQNO essentially the same type of changes were observed (not shown). These inhibitors have a mechanism of action which is different from that of DCMU (see Refs. 40–42). At concentrations which are sufficient to completely suppress the slow ATP-induced fluorescence rise these substances do not induce any dark-fluorescence rise (not shown) and, hence, do not appear to bind to the B-protein at the PS-II-acceptor complex. A common property of these inhibitors is their interference with electron transport steps at the cyt *b/f*-FeS complex [31–33,43]. One may conclude that electron flow through this complex is essential for the type of reverse electron flow expressed in the slow phase of the ATP-induced fluorescence rise. On the other hand, the rapid phase does not appear to involve such electron flow.

Effect of strong oxidants

The data presented above confirm previous conclusions that the slow ATP-induced fluorescence rise reflects ATP-induced reverse electron flow. However, so far it is not clear whether this is also true for the rapid phase. If the rapid phase is to reflect reverse electron flow, this should be suppressed by preoxidizing that component from

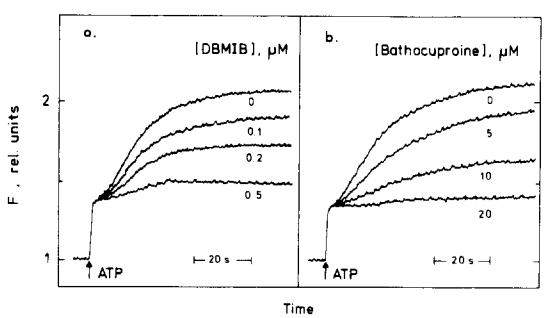

Fig. 8. Selective suppression of the slow phase by inhibitors acting at the Cyt *b/f* Fe-S complex. (a) Concentration dependency of DBMIB. (b) Concentration dependency of Bathocuproine. The inhibitors were added 1 min before ATP-injection in form of small aliquots of methanolic solutions. Methanol additions did not exceed 0.3 volume percents, to avoid uncoupling effects. All samples contained $5 \cdot 10^{-5}$ M diaminodurene, 10^{-3} M sodium ascorbate and $3 \cdot 10^{-3}$ M NH_2OH.

which electrons are driven back to PS-II acceptors.

In Fig. 9 the effect of ferricyanide on the rapid phase is shown. It is observed that even 5 mM of this strong oxidant, if added without mediator, will not appreciably affect the response. This appears remarkable also from the activation point of view, as the ATPase is supposed to be rapidly deactivated under oxidizing conditions [7,9]. If, however, a lipophilic redox mediator like diaminodurence (or phenylenediamine) is present, there is the expected suppression of the ATP-induced response. Strong suppression of the rapid phase is also observed with the lipophilic oxidant dimethylbenzoquinone (DMQ) (50% suppression at $6 \cdot 10^{-4}$ M) and with H_2O_2 (50% effect at $2 \cdot 10^{-5}$ M) (not shown in the figures).

While these effects of strong oxidants could indicate oxidation of the donor component in ATP-induced reverse electron flow, also oxidative deactivation of the ATP-hydrolase may be involved. To differentiate between these two possibilities the effect of ferricyanide diaminodurene on ATP-induced membrane potential formation was measured. Fig. 10 shows that oxidizing conditions somewhat slow down the build-up of $\Delta\psi$ and suppress the maximal amplitude, however, without much affecting the stationary level. Interestingly, oxidizing conditions appear to favor the light-induced P 515 change.

These data suggest that at least part of the rapid ATP-induced fluorescence response may be related to a type of reverse electron flow which involves some lipophilic high potential PS-II acceptor. In the following communication (ref. 44) it will be shown that this PS-II acceptor displays 'non-B properties', according to the definition of Lavergne [45,46].

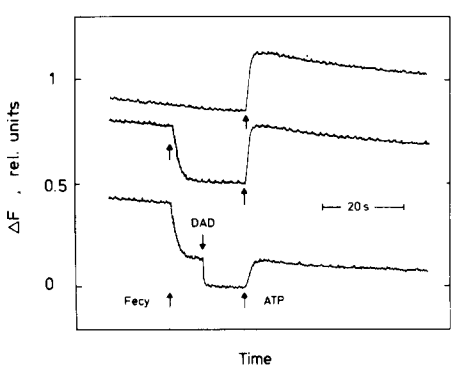

Fig. 9. Effect of oxidizing conditions on the rapid phase of the ATP-induced fluorescence increase. (a) Control; reaction medium without further additions. (b) Addition of $5 \cdot 10^{-3}$ M ferricyanide about 20 s before ATP-injection. (c) As (b), but addition of $3 \cdot 10^{-4}$ M diaminodurene on top of 5 mM ferricyanide. Similar results were obtained with $3 \cdot 10^{-4}$ M phenylenediamine substituting for diaminodurene (data not shown).

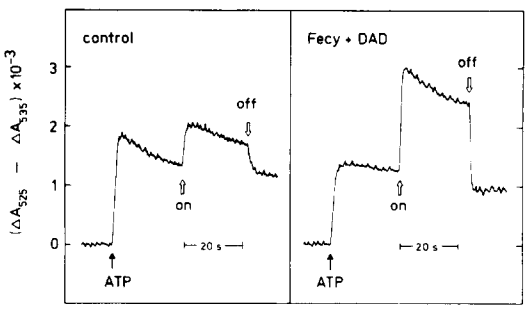

Fig. 10. Effect of oxidizing conditions on the ATP-induced rise of P 515 absorbance. Conditions as described for the fluorescence experiment of Fig. 9. Where indicated, continuous red light (10 W/m^2) was switched on and off.

Conclusions

The presented data display the following fundamental differences between the rapid and slow ATP-induced fluorescence rise components.

(1) The slow phase requires addition of reductants and redox mediators (Fig. 1), while the rapid phase persists without any additions and is only suppressed by strong lipophilic oxidants (Fig. 9). Obviously, if both phases are to reflect ATP-induced reverse electron flow, the electron sources differ vastly in redox potential and accessibility to oxidants.

(2) The slow phase depends on ΔpH-formation, whereas the rapid phase may be even stimulated by ΔpH-dissipation (Figs. 2, 4 and 7), provided the state of the thylakoid membrane and of its associated enzyme complexes is kept close to that of intact chloroplasts. Hence, the slow phase, with a ΔpH as an obligatory intermediate, follows the predictions of the chemiosmotic hypothesis [34,47–49]. This is not necessarily true for the rapid phase, as neither ΔpH nor $\Delta\psi$ are obligatory (Fig. 4).

(3) A number of inhibitors which are known to

block electron transport at the level of the Cyt *b/f*-FeS complex, suppress the slow phase without affecting the rapid one (Fig. 8). This difference in inhibitor sensitivities may be interpreted in terms of differing pathways of reverse electron flow. The reverse flow expressed in the slow phase, depends on electrons fed into the Cyt *b/f*-FeS complex by a suitable external reductant. It appears likely that the inhibitors block the ΔpH-driven transfer of electrons from the complex to the plastoquinone pool by occupying the plastoquinone binding sites. The electron-transfer step involved in the rapid phase should be more close to the PS II reaction centers. As will be shown in the following paper, even DCMU at concentrations which are saturating with respect to the displacement of the plastosemiquinone anion, does not prevent the rapid ATP-induced fluorescence rise. Inhibition occurs, however, at concentrations exceeding 10^{-4} M DCMU [44].

In conclusion, analysis of the ATP-induced fluorescence rise reveals two phases of reverse coupling reactions with vastly differing properties. The two phases appear to involve different coupling intermediates and different steps of reverse electron flow. The rapid phase may be particularly important with respect to the current controversy on 'chemiosmotic' and 'localised' coupling mechanisms [28,47–60]. Obviously, this rapid phase reflects some very close interaction between the coupling factor and a redox component in the vicinity of PS II. In the following contribution (Ref. 44) this aspect will be further analysed by a comparative study of the ATP-induced and the DCMU-induced fluorescence changes.

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