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ATP-induced photochemical quenching of variable chlorophyll fluorescence

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ATP induces rapid quenching of fluorescence yield in light-activated class D chloroplasts, distinguishable from the slower quenching caused by protein phosphorylation. The mechanism of the rapid quenching is shown to be photochemical, by application of the saturation pulse method with a modulated measuring system [(1986) Photosynth. Res. 10, 51–62]. The effect is most pronounced at relatively low light intensities (optimum 2 W/m^2) without addition of electron acceptors. The properties of the ATP-induced quenching with respect to ionophores, uncouplers and ATPase inhibitors suggest involvement of the reversible, light-activated thylakoid ATPase. As a working hypothesis, it is proposed that part of PS II reaction centers, which are stroma-exposed and in close proximity to CF_0-CF_1, are modified by ATPase activity. Transformation of cytochrome *b*-559 high-potential form to low-potential form by membrane acidification is discussed as a possible mechanism.

Chlorophyll fluorescence; Photosystem II; Coupling factor; ATP hydrolysis; Cytochrome *b*-559

1. INTRODUCTION

Chlorophyll fluorescence in vivo is controlled by photochemical and non-photochemical quenching mechanisms (reviews [1–3]). Photochemical quenching is caused by energy transformation at PS II reaction centers, depending primarily on the concentration of primary electron acceptors [4]. Non-photochemical quenching is heterogeneous, with a major component which is correlated with the acidification of the internal thylakoid space [5], which is generally referred to as 'energy-dependent quenching'. Methods have been developed to differentiate between photochemical and non-photochemical quenching [5–9]. With the help of brief, intense pulses of light, all PS II acceptors can be temporarily closed, eliminating photochemical quenching and thus allowing determination of the non-photochemical component. Recently a modulation fluorometer became available with which photochemical and non-photochemical quenching components can be recorded repetitively by application of saturation pulses [9–11].

ATP is known to change chlorophyll fluorescence in various ways. Dark fluorescence yield, F_0, displays a biphasic rise induced by a reverse coupling reaction driven by ATP hydrolysis when the latent ATPase is light-activated [12,13]. This has been thoroughly analysed in previous work [14,15]. Phosphorylation of thylakoid membrane proteins, in particular of the

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Abbreviations: PS I and II, photosystem I and II; CF_0 and CF_1, subunits of the thylakoid coupling factor; TNBT, tri-*n*-butyl-tin; DCCD, dicyclohexylcarbodiimide; DCMU, dichlorophenyldimethylurea

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light harvesting chlorophyll (chl) *a/b* complex, cause a decrease in fluorescence yield, which has been correlated with a shift of energy distribution in favor of the non-fluorescing PS I [16–18].

Here we wish to report on ATP-induced quenching of variable fluorescence (i.e. at light intensities which induce an increase of fluorescence yield beyond the dark level, F_0) which depends on ATPase activity. This type of quenching can be well distinguished from protein phosphorylation-induced quenching by its photochemical nature and fast rate.

2. MATERIALS AND METHODS

Intact chloroplasts were isolated from greenhouse grown spinach as described [14]. For activation of the latent ATPase intact chloroplasts were illuminated with strong white light (2000 W/m^2) for 15 s at 15°C. Then chloroplast envelopes were ruptured by a 30 s hypotonical treatment with 5 mM $MgCl_2$, 5 mM Tricine (pH 7.6) and the resulting class D chloroplasts were resuspended isotonically as described in [14]. ATP was added 3 min following light activation.

Chlorophyll fluorescence was measured with a modulation fluorometer (basic system PAM 101, actinic illumination unit 102, saturation pulse unit 103, Walz, Effeltrich, FRG). The properties of this measuring system have been described [9–11]. Chloroplasts were contained in a thermostatted cuvette at 15°C, equipped with a stirring mirror and a port for chemical additions (KS 101 suspension cuvette, Walz). The chlorophyll concentration was about 50 μg/ml.

3. RESULTS

In fig.1 the basic effect of ATP-induced fluorescence quenching is shown in an original recording with the modulation measuring system and repetitive application of saturation pulses. The modulated measuring light (integrated intensity, 10 mW/m^2) is turned on about 90 s following light activation. The intensity of the measuring light is sufficiently low to monitor the dark-fluorescence level, F_0. Upon application of a saturation pulse the maximal fluorescence yield with all PS II centers being closed is recorded. When a relatively weak actinic light (2 W/m^2) is turned on,

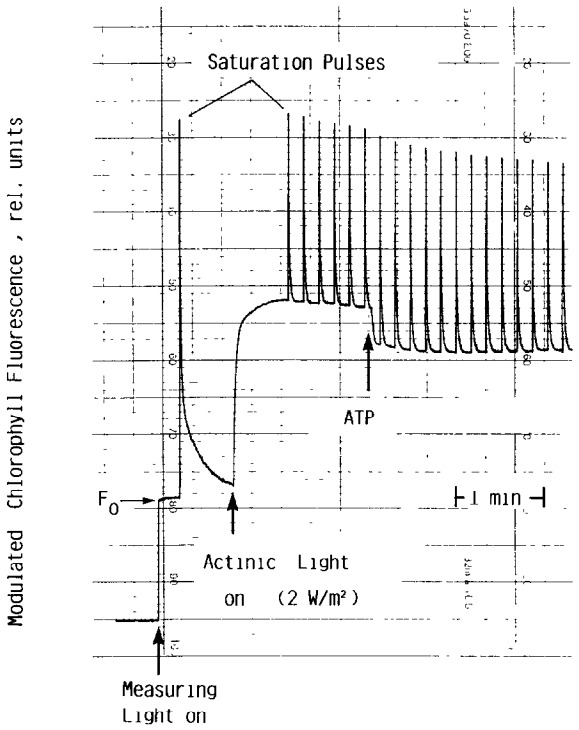

Fig.1. ATP-induced photochemical quenching of chlorophyll fluorescence. Fluorescence is measured with weak modulated light (integrated intensity, 10^{-2} W/m^2). Where indicated, 500 ms pulses of strong white light (2000 W/m^2) were applied to determine the maximal fluorescence yield upon complete saturation of PS II centers. Actinic light, peak wavelength 650 nm, 2 W/m^2. An increase in non-photochemical quenching is indicated by a decrease of the fluorescence yield during a saturation pulse. ATP was added to give final concentrations of 10^{-4} M. For further details, see section 2.

fluorescence yield increases, as photochemical quenching is decreased. Repetitive application of saturation pulses reveals a slow decrease of maximal fluorescence, as non-photochemical quenching is increasing with the light-driven acidification of the thylakoid internal phase. Upon addition of ATP there is a rapid decrease in fluorescence yield followed by a slower decline. There is no rapid decrease of the saturation pulse fluorescence when ATP is added, suggesting that the rapid phase reflects increased photochemical quenching. The slow decrease in saturation pulse fluorescence already induced by illumination is somewhat accelerated after ATP addition,

presumably due to additional internal acidification by ATP hydrolysis [13].

The extent of ATP-induced quenching strongly depends on the quality of the chloroplast preparation. Freshly isolated chloroplasts showing high rates of HCO_3^--dependent electron transport when intact and of NADP-dependent flow when osmotically shocked are required for a pronounced effect. Ageing of such chloroplasts, while not affecting methylviologen-dependent electron flow, causes strong suppression of ATP-induced quenching. Even with freshly prepared chloroplasts, significant variability was observed as to the extent of the ATP effect, for reasons so far unknown. The largest effects (up to 70% quenching of variable fluorescence) were observed with summer spinach. With the types of chloroplasts used in the experiment of fig.1, the amplitude of the ATP-induced quenching was half-saturated at 8×10^{-5} M ATP. This value is close to the K_m determined for other ATP-induced reactions [12,19,20].

Table 1 contains a summary of observations relating to inhibition and stimulation of the ATP-induced increase of photochemical quenching.

Table 1

Extent of rapid ATP-induced quenching in dependence of a variety of conditions

Condition	Relative size of rapid phase (% of control)
Control	100
2×10^{-5} M tentoxin	42
2×10^{-4} M DCCD	16
3×10^{-5} M TNBT	37
Activation omitted	28
10^{-7} M FCCP	125
2×10^{-6} M FCCP	27
2×10^{-6} M gramicidin	38
3×10^{-8} M nigericin	223
3×10^{-7} M valinomycin	210
3×10^{-8} M nigericin plus 3×10^{-7} M valinomycin	24
3×10^{-5} M DCMU	6
3×10^{-5} M DNP-INT	5
5×10^{-3} M NH_2OH	3

Control conditions: 15 s light activation, 2 W/m^2 actinic light intensity

ATPase inhibitors acting on CF_1 (tentoxin) and CF_0 (DCCD and TNBT) cause suppression of the reaction. Some ATP-induced quenching is also found with chloroplasts which were not given special light activation while still intact. However, the effect is clearly stimulated by light activation. Low concentrations of uncouplers stimulate, while high concentrations inhibit, although less than expected. Nigericin and valinomycin, when only one of the two ionophores is applied, cause substantial stimulation, while there is inhibition when the two are applied together. The electron transport inhibitors DCMU and DNP-INT cause suppression of ATP-induced photochemical quenching. So does hydroxylamine which is known to affect the PS II donor side.

In figs 2 and 3 the effects of the ionophores, of tentoxin and of hydroxylamine are dealt with in more detail showing the original recordings. Addition of nigericin (fig.2A) causes a pronounced decrease in photochemical quenching. ATP, added after nigericin, increases photochemical quenching again, the effect being distinctly enhanced with respect to a sample without nigericin (see fig.1 and table 1). Similarly, valinomycin decreases photochemical quenching and consequently added ATP causes enhanced quenching again (fig.2B). When valinomycin and nigericin are both added (fig.2C) the ATP effect is markedly reduced. Addition of tentoxin causes some photochemical quenching (fig.2D), while the effect of ATP is slowed down and diminished.

Hydroxylamine, when added to a final concentration of 5 mM, induces a biphasic decrease of variable fluorescence, similar to the decrease induced by ATP (fig.3A). The rapid phase involves photochemical quenching while the slow phase constitutes an increase in non-photochemical quenching. ATP addition after hydroxylamine does not lead to further quenching. When ATP is added first, inducing a rapid increase of photochemical quenching, hydroxylamine addition causes no further photochemical quenching, while the slow stimulation of non-photochemical quenching is still observed (fig.3B).

A particular role for the occurrence of ATP-induced photochemical quenching is played by the intensity of light and the redox poising of the system. The present results were obtained at a rather low actinic intensity of 2 W/m^2, which was

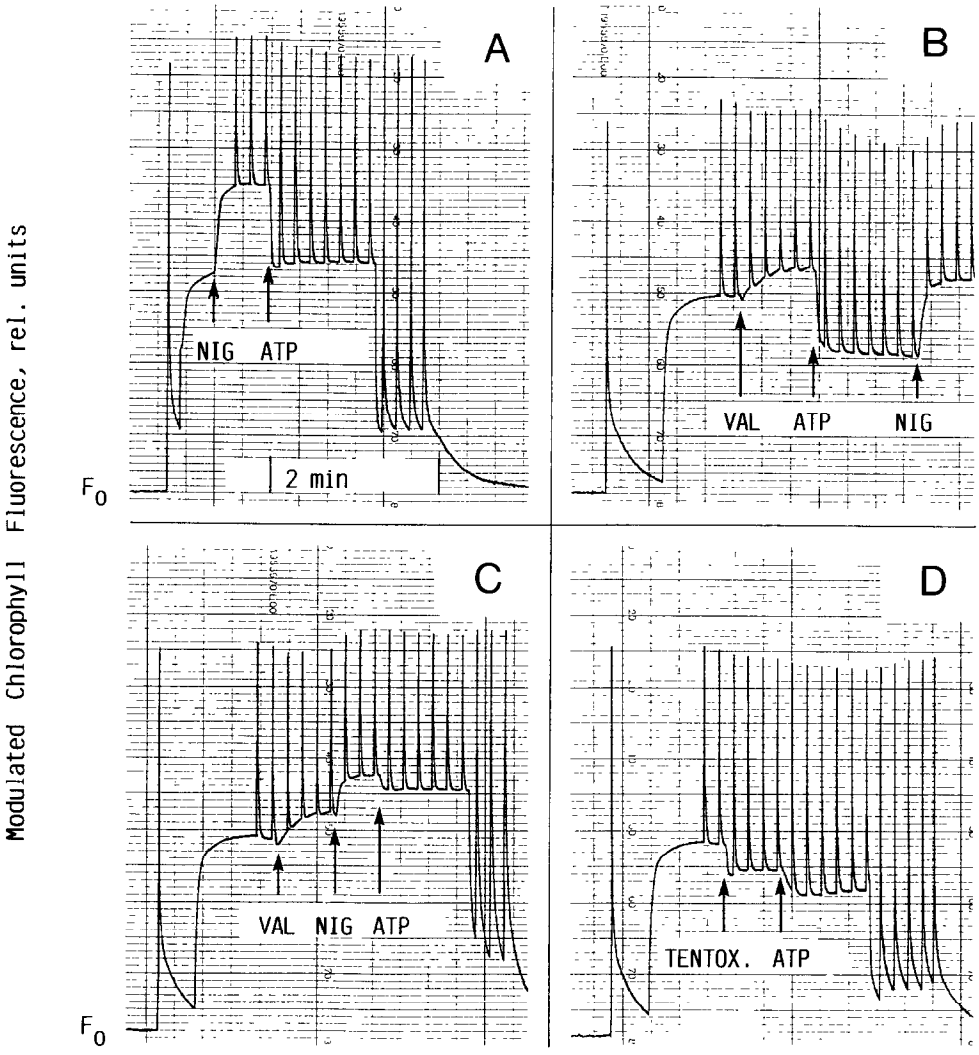

Fig.2. ATP-induced quenching in the presence of ionophores and of tentoxin. Concentrations: ATP, 10^{-4} M; nigericin, 3×10^{-8} M; valinomycin, 3×10^{-7} M; tentoxin, 2×10^{-5} M. In panels A, C and D about 1 min after ATP addition the actinic light was switched off. For other details see fig.1.

optimal under the given conditions. 50% decrease of the effect was apparent at 0.8 W/m^2 and at 7 W/m^2. However, these values should be considered in connection with the fact that no electron acceptor was added to the chloroplasts, i.e. molecular O_2 served as acceptor in the Mehler reaction. Actually, when O_2 was removed by an enzymatic trap (glucose oxidase + glucose) the fluorescence yield at 2 W/m^2 increased somewhat beyond the DCMU level and ATP-induced quenching was completely abolished. The presence of efficient electron acceptors, such as methylviologen or NADP, caused strong photochemical quenching, eliminating the ATP effect. Electron transport rates in the presence of these acceptors, as measured with an oxygen electrode, were slightly decreased (about 10%) when ATP was added.

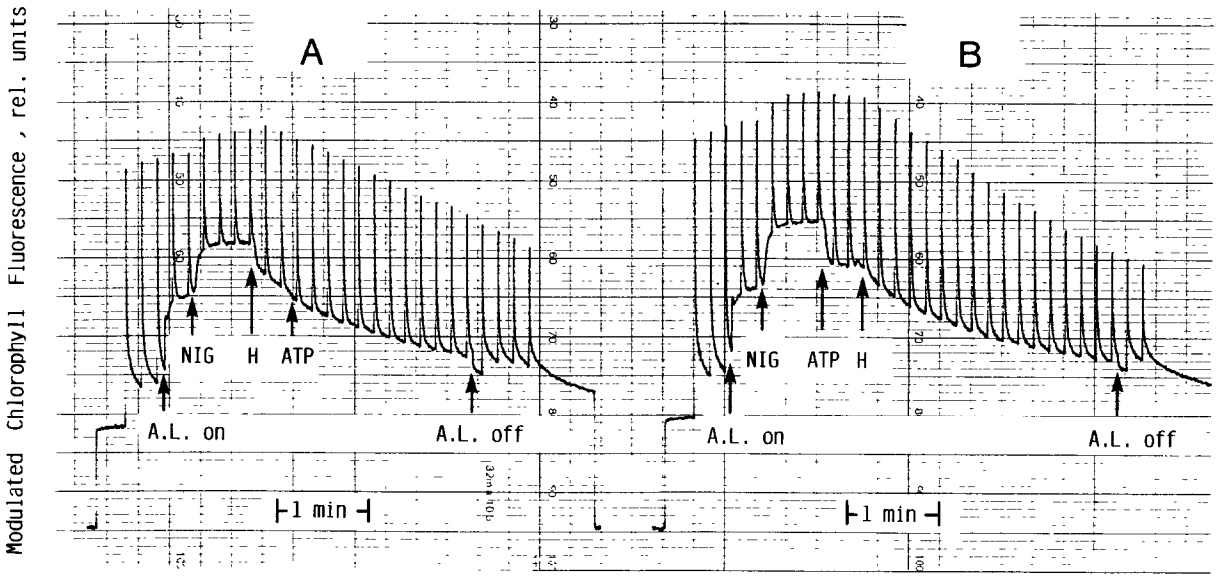

Fig.3. Complementarity of ATP- and hydroxylamine-induced photochemical quenching. Concentrations: ATP, 10^{-4} M; nigericin, 3×10^{-8} M; hydroxylamine, 5×10^{-3} M (addition indicated by H). In the given chloroplast preparation, the initially observed increase of saturation pulse fluorescence is related to a particularly slow dark-relaxation of energy-dependent quenching following light activation. For details see section 2 and fig.1.

4. DISCUSSION

The ATP-induced fluorescence changes described above display properties which allow clear distinction from previously reported ATP-induced fluorescence quenching following protein phosphorylation [16–18]. Contrary to the latter, the ATP-induced quenching reported here is photochemical, is quickly established (half-response time in the range of seconds as compared to minutes in the case of protein phosphorylation) and depends on ATPase activity. So far, the fluorescence measurements intended to monitor protein phosphorylation did not reveal the rapid photochemical quenching [16–18] because of the high light intensities used in these studies (see discussion on the role of light intensity below).

The present results should be discussed in connection with results from previous work on the ATP-induced increase of dark-level fluorescence, F_0 [12–15]. It was shown in this previous work that ATP hydrolysis induces a small, rapid F_0 rise, the properties of which differ considerably from those of a larger, slow F_0 rise. It has been proposed that this change is due to a population of CF_0-CF_1 closely interacting with that part of PS II reaction centers which is located in the stroma-exposed region of the thylakoid membrane [14,15]. The same type of interaction may well be involved in the phenomenon of ATP-induced photochemical quenching reported here. Both phenomena depend on the activity of the ATP hydrolase, are not inhibited but rather stimulated by nigericin, persist in the presence of valinomycin, but are suppressed by the combination of the two ionophores which results in strong uncoupling, causing deactivation of ATPase. Obviously, however, the two phenomena differ in the direction of the fluorescence change, i.e. the hypothetical PS II acceptor, affected by interaction with CF_0-CF_1, appears to be delicately redox poised.

The data of fig.3, showing 'complementarity' of the effects of hydroxylamine and ATP, may help one to understand the possible mechanisms of interaction between ATPase and PS II. It was previously found that hydroxylamine stimulated the ATP-induced F_0 rise considerably [14]. Hence, some property of PS II centers involving the action site of hydroxylamine appears to play a role in the observed phenomena. Hydroxylamine is known to

cause transformation of cytochrome (cyt) *b*-559 high-potential form to low-potential form [21], and upon hydroxylamine addition auto-oxidation of the cytochrome was observed. Also low pH conditions favor formation of the low-potential form of cyt *b*-559 [22]. Interestingly, Horton and Cramer [23] found a shift towards the low-potential form also by 'light activation'. These authors suggested that electron transport coupled proton liberation causes membrane acidification in the vicinity of cyt *b*-559. As the effect on cyt *b*-559 persisted in the presence of NH_4Cl, intramembrane acidity was considered decisive.

Hence, the observed ATP-induced photochemical quenching could be caused by membrane acidification resulting from ATP hydrolysis, and consequent formation of cyt *b*-559 (LP) which may induce a quenching cycle around PS II [24]. As in the presence of nigericin the bulk phase cannot play a role in the transport of protons from the ATPase to PS II, the proposed acidification should be considered as intramembranal and as being most pronounced in the vicinity of the coupling factors, i.e. in the stroma-exposed region of the membrane.

Hodges et al. [25] reported photooxidation of cyt *b*-559 (LP) by incubating chloroplasts under reducing conditions with 5×10^{-4} M ATP. These authors interpret their data in terms of thylakoid protein phosphorylation and a resulting redistribution of excitation energy in favor of PS I. In this context it may be noted that, so far, we did not succeed in separating absorbance changes due to cyt *b*-559 oxidation from those caused by cyt *f* oxidation [12] and by scattering changes under the conditions of the fluorescence experiments. This may be a particularly difficult task, if only part of PS II centers are involved.

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