ΔpH-dependent chlorophyll fluorescence quenching indicating a mechanism of protection against photoinhibition of chloroplasts

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Intact isolated spinach chloroplasts were subjected to photoinhibitory conditions (high light and lack of CO_2). Photoinhibition of the electron transport system was considerably diminished when the chloroplasts were in a low-fluorescent state related to a high proton gradient across the thylakoid membranes, as compared to a high-fluorescent state in which ΔpH-dependent fluorescence quenching was abolished by addition of uncouplers. The hypothesis is discussed that in chloroplasts exposed to excess light, photoinhibition is partly prevented by increased thermal dissipation of excitation energy, as expressed by ΔpH-dependent ('energy-dependent') chlorophyll *a* fluorescence quenching.

Chlorophyll fluorescence *Chloroplast* *Photoinhibition* *Uncoupler* *Photosynthesis* *Electron transport*

1. INTRODUCTION

Under continuous illumination, plant leaves or isolated chloroplasts attain a state characterized by low chlorophyll *a* fluorescence emission (see fig.1). Quenching of fluorescence may be caused either by photosynthetic electron transport activity keeping the electron acceptor Q_A of photosystem (PS) II in a more or less oxidized state [1], or by events that are not directly related to photochemical reactions. There are several mechanisms of such non-photochemical quenching (see [2]); the most important one is related to the light-induced proton gradient across the thylakoid membrane and has been termed ΔpH- or energy-dependent quenching, q_E [3]. A linear relationship between q_E and the intrathylakoid H^+ concentration was found [4]. It is supposed that q_E reflects ultrastructural changes of the thylakoid membranes which are induced by the acidification of their internal spaces and lead to increased thermal deactivation of excited pigments [5].

When the photon flux is high and/or substrate CO_2 is lacking, photoinhibition of the electron transport system, particularly of PS II may occur [6,7]. Under such conditions, a high ΔpH is built up and q_E is the predominant component of fluorescence quenching [3,8]. It appears plausible that increased thermal deactivation, as reflected by q_E, represents a protective mechanism which partly converts excess light energy to heat in a non-destructive manner.

To prove this hypothesis, isolated intact chloroplasts were subjected to photoinhibitory treatment (high light, absence of substrate levels of CO_2) either in the low-fluorescent state characterized by high ΔpH or in a partially uncoupled high-fluorescent state with low ΔpH. If increased thermal deactivation in the presence of a high ΔpH indeed provides protection against damaging effects of light, less photoinhibition should be observed in the low-fluorescent (high-q_E) state.

2. MATERIALS AND METHODS

Isolation of intact chloroplasts from leaves of spinach (*Spinacia oleracea* L.), preparation of

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broken chloroplasts and composition of suspension media have been described in [7]. Chlorophyll *a* fluorescence emission at 20°C was recorded at 686 nm [3,9].

Chloroplast suspensions (50 μg chlorophyll per 2 ml air-saturated medium), placed into a temperature-controlled cuvette (20°C) of a Hansatech oxygen electrode, were treated as follows: a preillumination of 4 min with non-photoinhibitory red light (30 $W \cdot m^{-2}$) served to obtain the low-fluorescent state. This was followed by different periods of photoinhibitory irradiation with 660 $W \cdot m^{-2}$ white light (controls were kept in the dark). The light was passed through an infrared absorbing filter Calflex C (Balzers, Liechtenstein). For maintaining the chloroplasts in the high-fluorescent state, an uncoupler was added prior to this treatment at a concentration, which in separate tests caused almost complete inhibition of fluorescence quenching. Catalase (EC 1.11.1.6; 2000 $U \cdot ml^{-1}$) was added to all samples to avoid side effects of H_2O_2 formed during illumination.

The capacity of PS II-driven electron transport was measured after pretreatment as O_2 evolution in the presence of 1 mM 1,4-benzoquinone, a PS II electron acceptor that penetrates the chloroplast envelope [10]. An uncoupler was added, if not already supplied for pretreatment. Saturating red light [7], 300 $W \cdot m^{-2}$, was used. PS I-driven electron transport was measured after osmotic breakage of the chloroplasts as reported [7].

3. RESULTS

In fig.1 characteristic chlorophyll *a* fluorescence signals of intact chloroplasts are depicted. Most of the fluorescence quenching under the conditions applied here is ΔpH-dependent. This is indicated by the large slow phase of reversal of quenching that occurs when the ΔpH decays upon blocking of electron transport by addition of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) (see [3]). Accordingly, the fluorescence quenching can be reversed by addition of uncouplers. For photoinhibitory treatments of chloroplasts in the high-fluorescent state, various kinds of uncouplers were added (table 1) that lower the proton gradient by different mechanisms [11,12]. Their concentrations were chosen so as to obtain nearly full inhibition of quenching. In the absence of uncouplers,

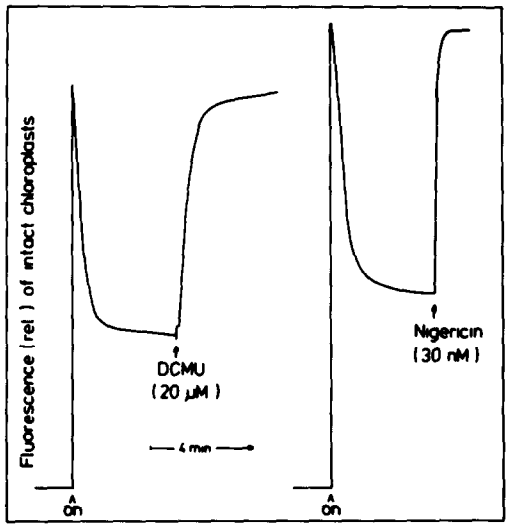

Fig.1. Chlorophyll fluorescence signals (686 nm, 20°C) of isolated chloroplasts; effects of addition of DCMU and nigericin on fluorescence quenching. Chloroplasts equivalent to 50 μg chlorophyll in 2.6 ml suspension (without added bicarbonate) were illuminated with 13 $W \cdot m^{-2}$ blue light. Right-hand trace, 30 mM KCl was added to the medium.

Table 1

Chlorophyll fluorescence quenching (in % of the fluorescence peak, F_p) in isolated chloroplasts

Chloroplasts	Quenching (%)		Uncoupler
	Coupled	Uncoupled	
Intact	57.5	4.3	5 mM NH_4Cl
	53.0	1.9	8 μM A23187 (+ 5 mM $MgCl_2$)
	58.4	2.3	30 nM nigericin (+ 30 mM KCl)
	49.4	2.4	2 μM gramicidin D (+ 30 mM KCl)
Broken	21.4	2.4	8 μM A23187 (5 mM $MgCl_2$)

Fluorescence was recorded during illumination periods of 4 min under conditions as for fig.1, which caused maximum ΔpH-dependent quenching ('coupled' chloroplasts). Quenching was inhibited in the presence of an uncoupler ('uncoupled' chloroplasts). Suitable electrolytes to facilitate the action of the ionophores were added to both types of chloroplasts as indicated

usually 50–60% of the fluorescence emission of the peak (F_p) was quenched in intact chloroplasts.

The degree of photoinhibition of PS II observed in the absence and presence of an uncoupler, i.e. in the low- and high-fluorescent states, is given in fig.2. In the absence of uncouplers, the capacity of PS II-driven electron transport was inhibited upon pre-irradiation by about 20%. The extent of photoinhibition was about doubled when an uncoupler was present during pretreatment. All 4 uncouplers tested gave similar results. The increased photoinhibition of PS II in the high-fluorescent state was well reproducible in repeated tests (see legend to fig.2). Also PS I-driven electron transport was more affected in the high- as compared to the low-fluorescent state of the chloroplasts (not shown).

Electron transport measurements were corroborated by chlorophyll *a* fluorescence induction signals recorded in the presence of DCMU. Depending on the time of pre-irradiation, variable fluorescence, F_v, was strongly decreased, while initial fluorescence, F_0, was little affected (fig.3a). The decline in F_v can be interpreted to indicate damage to the PS II reaction centers (see [7,13,14]). This effect was considerably stronger when irradiation occurred in the high-fluorescent state. Fig.3b shows that the loss of F_v is accompanied by a decrease in the capacity of PS II-driven electron transport.

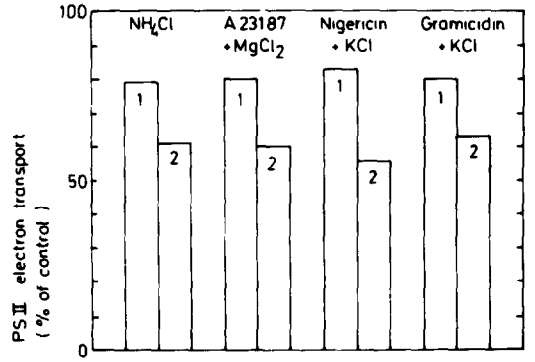

Fig.2. Photoinhibition of PS II electron transport of intact chloroplasts. Bars denote the capacity of electron transport (in % of control rates) measured subsequent to 10 min photoinhibitory irradiation without added bicarbonate in the absence (1) and presence (2) of uncoupler as indicated (concentrations as for table 1). In 17 experiments the extent of photoinhibition after 10 min irradiation in the absence of uncoupler was 20 ± 8% (SD), which increased to 43 ± 10% in the presence of A23187. Control rates of O_2 evolution were between 280 and 560 μmol·(mg chlorophyll)$^{-1}\cdot h^{-1}$.

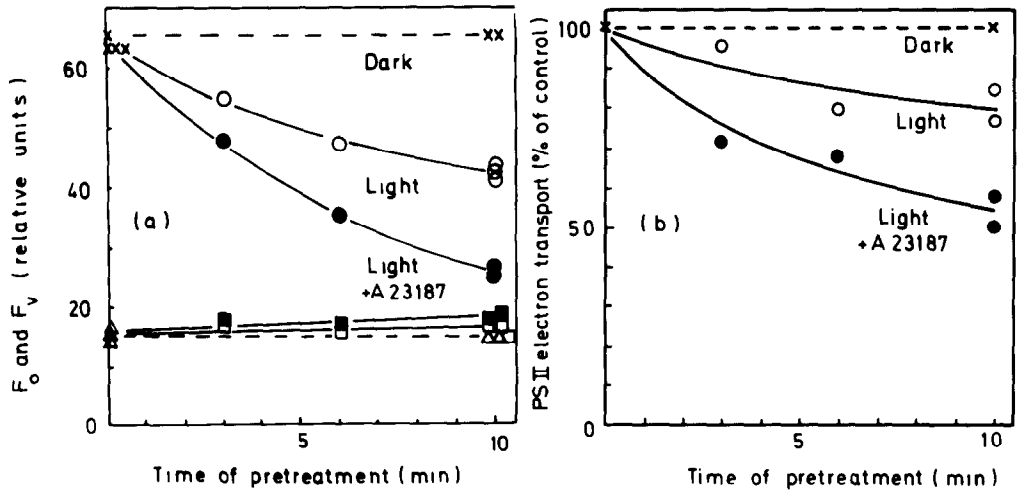

Fig.3. Photoinhibition of PS II of intact chloroplasts in the low-fluorescent state (absence of uncoupler: ○, □) and high-fluorescent state (presence of 8 μM A23187: ●, ■). The medium was supplied with 5 mM $MgCl_2$; no bicarbonate was added. Controls kept in the dark (– – –) were not affected by the presence of uncoupler. (a) Effects of photoinhibitory treatment (660 W·m^{-2}) on initial fluorescence, F_0 (□, ■), and on maximum variable fluorescence, F_v (○, ●) recorded in the presence of 20 μM DCMU (actinic blue light, 0.9 W·m^{-2}; samples of 50 μg chlorophyll in 2.6 ml medium). (b) Effects of pretreatment on PS II electron transport (○,●) measured as for fig.2. Control rate of O_2 evolution (= 100%), 189 μmol·(mg chlorophyll)$^{-1}\cdot h^{-1}$.

In broken chloroplasts, the protecting effect related to the high ΔpH was not found previously [7], but under the conditions applied here was observed; however, the effect was usually less pronounced than in intact chloroplasts (not shown). The reason for this difference may be that by breakage of chloroplasts the capability of the thylakoids to undergo the conformational changes expressed by ΔpH-dependent quenching may be easily lost. As shown in table 1, even in thylakoids freshly prepared from intact chloroplasts by osmotic shock in the presence of $MgCl_2$ (which maintains the grana structure), fluorescence quenching was strongly diminished.

4. DISCUSSION

In chloroplasts, various reaction systems are known that protect the photosynthetic apparatus against destructive light effects [6]. However, when the protective systems are overburdened by excess light, damage expressed by photoinhibition will occur. Conditions of excess light are created not only by high photon fluence density exceeding the light normally experienced by the plant, but also by restriction of CO_2 assimilation. It has been shown [7] that orderly utilization of photosynthetic energy in carbon metabolism provides protection; lack of CO_2 or environmental factors that affect the carbon reduction cycle therefore promote photoinhibition [6]. The possibility that increased thermal dissipation of excitation energy represents a mechanism (amongst others) to avoid photoinhibition has attained little attention so far. Such increased thermal deactivation of excited pigments seems to be indicated by the ΔpH-dependent fluorescence quenching [5]. This effect is very large in excess light (see section 1) and heretofore no physiological function has been related to it. The present investigation provides evidence that inhibition of photosynthetic electron transport by excess light is considerably less severe when strong q_E occurs, as compared to partially uncoupled conditions that prevent fluorescence quenching (figs 2,3).

It might be argued that the protection of PS II observed in the absence of uncouplers is related to phosphorylation of the light-harvesting complex (LHC) of PS II. This regulatory process may contribute to non-photochemical fluorescence quenching due to increased excitation energy transfer to PS I [15,16]. Partial protection based on this effect has been described [17]. However, the contribution of this ATP-dependent fluorescence quenching seems to be small (see [3,18]). This is apparent from the nearly complete reversion of quenching occurring within less than 1 min upon addition of an uncoupler (fig.1). It was shown, furthermore, that a high ΔpH partly inhibits phosphorylation of the LHC [19]. As endogenous ATP is present in the intact chloroplasts, we cannot exclude that phosphorylation of the LHC, which was not determined in our study, slightly contributes to protection; but the protecting effect reported here seems to be related predominantly to the high proton gradient causing increased thermal deactivation.

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