Energy-Dependent Chlorophyll Fluorescence Quenching in Chloroplasts Correlated with Quantum Yield of Photosynthesis

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Chlorophyll *a* fluorescence quenching was studied in intact, CO_2 fixing chloroplasts isolated from spinach. Energy-dependent quenching (q_E), which is correlated with the light-induced proton gradient across the thylakoid membrane presumably reflects an increase in the rate-constant of thermal dissipation of excitation energy in the photosynthetic pigment system. The extent of q_E was found to be linearly related to the decrease of quantum yield of photosynthesis. We suggest that this relationship indicates a dynamic property of the membrane to adjust thermal dissipation of absorbed light energy to the energy requirement of photosynthesis.

Introduction

When chloroplast pigments absorb light in excess of the energy turnover in photosynthetic reactions including carbon metabolism, photoinhibition of the electron transport system may occur. Photoinhibition is characterized by partial inactivation of photosystem (PS) II and to a lesser extent of PS I (see [1]). Various protective systems are known that minimize damage in excess light, *e.g.*, damage by active oxygen species, excited triplet states of chlorophyll (see [2, 3]), or "overreduction" of the electron transport chain [4, 5].

A possible way to cope with excess excitation energy in the photosynthetic apparatus before the onset of photochemical reactions would be a regulated non-destructive thermal deactivation of excited pigments. Such a regulated "valve" for excess energy appears to be indicated by "energy-dependent", ΔpH-related quenching of chlorophyll *a* fluorescence, q_E. The extent of this quenching was found to be linearly related to the intrathylakoid proton concentration and thus to the size of the light-induced proton gradient across the thylakoid membrane [6]. The quenching of fluorescence seems to be based on an increase in the rate-constant of thermal deactivation [7, 8]. This is probably caused by structural changes in the membrane due to intrathylakoid acidification and related cation exchange at the internal thylakoid surface.

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In a previous communication [9] it was shown that in the presence of q_E substantial protection against photoinhibition is provided. The present study gives evidence for a dynamic adjustment of thermal energy dissipation, as indicated by q_E, to the energy requirement of photosynthetic carbon assimilation (see also [10]).

Materials and Methods

Intact chloroplasts were isolated from freshly harvested leaves of spinach (*Spinacia oleracea* L.) [11, 12]. Samples containing 23 μg chlorophyll (Chl) ml^{-1} were illuminated in the cuvette of a Clark-type oxygen electrode (light path 6 mm) with red light (L 1, see below) of different light flux densities. The reaction medium contained 0.33 M sorbitol, 2 mM EDTA (disodium salt), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 10 mM NaCl, 60 mM KCl, 0.5 mM KH_2PO_4, 40 mM (2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (pH 7.6 with NaOH), 2 mM $KHCO_3$ and 2000 U ml^{-1} catalase (EC 1.11.1.6).

Quantum yields of CO_2-dependent O_2 evolution were determined according to Giersch and Heber [13]. Absorption by the chloroplasts measured with an integrating Ulbricht sphere was about 49% of incident red light. Simultaneously with O_2 evolution, chlorophyll fluorescence was recorded with a pulse amplitude modulation fluorometer (PAM 101, H. Walz, Effeltrich, Germany) [14]. Non-modulated actinic light (L 1) was obtained with glass filters Calflex C, K 65 (Balzers, Liechtenstein) and RG 645 (Schott, Mainz, FRG); λ_{max} was 665 nm, half-band width 15 nm. The components of fluorescence quenching,

q_E (ΔpH-dependent quenching) and q_Q (photochemical quenching) [7] were determined in the steady state of photosynthesis after 5 min actinic illumination at the indicated light flux densities (see Fig. 1).

Results and Discussion

Fig. 1 depicts an example of the fluorescence signals used for the determination of q_E and q_Q. It is known from earlier studies [7, 8, 15] that under the conditions applied here, non-photochemical quenching (q_N) is approximately equal to q_E. Small contributions of other mechanisms to q_N have been neglected. Light-saturation curves of q_E and q_Q in the presence of bicarbonate are depicted in Fig. 2a. With increasing light flux densities q_E saturates at about 0.7 to 0.8, whereas q_Q declines to a low value, indicating that the proportion of oxidized electron acceptor of PS II (Q_A) decreases. Fig. 2b shows the light-dependence of rates and quantum yields of CO_2-dependent O_2 evolution, representing CO_2 fixation in the steady state. The data demonstrate that at higher irradiances an increased part of the absorbed light energy is not utilized in photosynthetic carbon assimilation. If the quantum yield is plotted versus q_E, a linear relationship is seen (Fig. 3). We suggest that this reflects a regulation of thermal energy dissipation according to the energy requirement of photosynthesis. In low light, low q_E would indicate a low

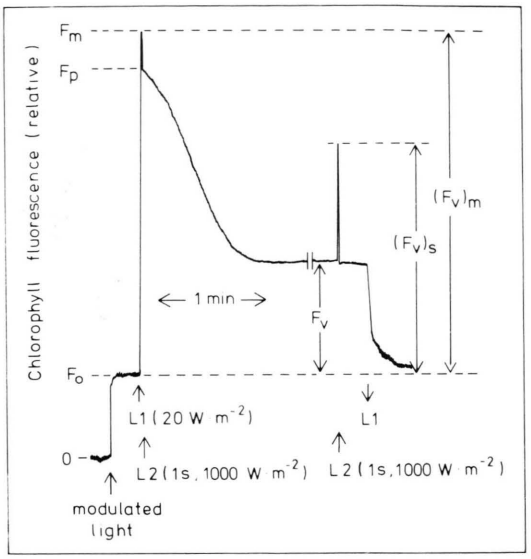

Fig. 1. Chlorophyll fluorescence recording ($\lambda > 700$ nm) of intact spinach chloroplasts excited by a weak modulated light. Non-modulated actinic light (L 1) causes a rise from initial fluorescence emission (F_o) to the peak (F_p), which is followed by slow quenching. Pulses (1 s) of saturating white light (L 2) cause full reduction of Q_A (electron acceptor of PS II) and reverse photochemical quenching (q_Q). When chloroplasts are dark-adapted, L 2 induces maximal fluorescence (F_m). The difference between F_m and the emission in L 2 after prolonged illumination with L 1 indicates non-photochemical quenching (q_N), most of which consists of energy-dependent quenching (q_E). The components of quenching are calculated as $q_E \approx q_N = [(F_v)_m - (F_v)_s]/(F_v)_m$; $q_Q = [(F_v)_s - F_v]/(F_v)_s$.

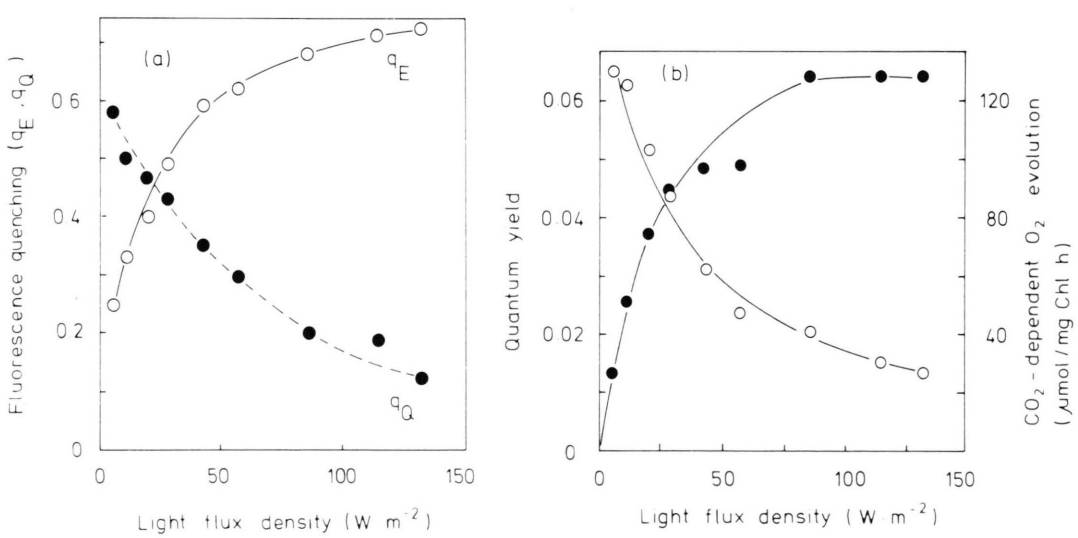

Fig. 2a, b. Light-dependence of energy-dependent (q_E) and photochemical (q_Q) fluorescence quenching (a) and of rate (●) and quantum yield (○) of CO_2-dependent O_2 evolution (b) in intact chloroplasts. Data represent the steady state after 5 min illumination with L 1 at the respective light flux density. For details of fluorescence recording see Fig. 1. The quantum yield is defined as moles O_2 evolved per mole photons absorbed.

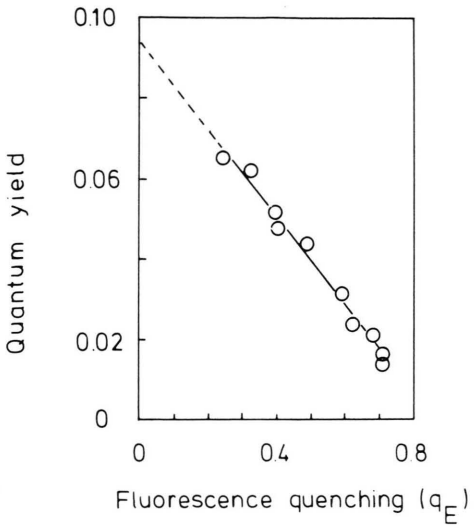

Fig. 3. Correlation between energy-dependent fluorescence quenching (q_E) and quantum yield of CO_2-dependent O_2 evolution (data from Fig. 2). The straight line was fitted by linear regression; correlation coefficient, r = −0.99.

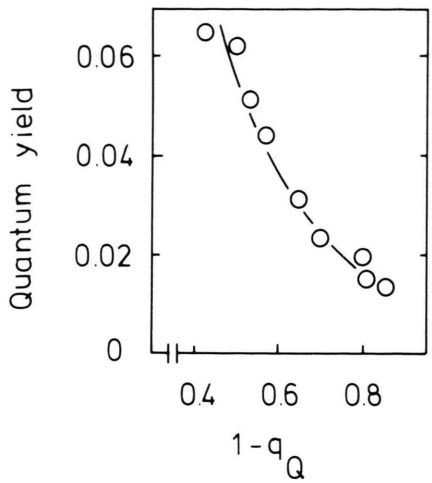

Fig. 4. Relationship between photochemical quenching (q_Q) and quantum yield of CO_2-dependent O_2 evolution (data from Fig. 2). The quantum yield is plotted versus $1-q_Q$; the latter term indicates the approximate proportion of reduced Q_A in the steady state.

rate-constant of thermal deactivation that allows a high quantum yield of photosynthesis. Extrapolation of the straight line in Fig. 3 to $q_E = O$ denotes an "optimal" quantum requirement of about 10 photons per O_2 molecule evolved. When with increasing irradiance CO_2 fixation approaches light-saturation and the quantum yield declines, the rate-constant of thermal deactivation, as indicated by q_E, appears to increase. This would avoid or minimize overenergization of the photosynthetic apparatus and thereby protect it from photoinhibitory damage. It should be noted that the linear relationship depicted in Fig. 3 does not extent beyond light saturation of photosynthesis, because then also q_E becomes light-saturated. In this respect different responses were observed from the more complex system of intact leaves [16].

One may argue that the quantum yield of CO_2 fixation is solely regulated by the redox state of Q_A, as a correlation between quantum yield and q_Q is indeed observed (Fig. 4). A high ΔpH might control reoxidation of Q_A by restricting electron flow through the plastoquinone pool. However, this is not the case, as shown in Table I. Addition of moderate concentrations of the uncoupler NH_4Cl (5 mM) to the chloroplasts does not inhibit CO_2 fixation (and thus does not lower the quantum yield) but drastically diminishes q_E due to partial uncoupling [17]. It can be seen that under this condition Q_A does not become more oxidized (as q_Q does not increase) but rather more reduced. Thus, an increased excitation of PS II due to diminished thermal energy dissipation appears to be the predominant effect of partial uncoupling.

Table I. Effects of partial uncoupling with NH_4Cl (5 mM) on fluorescence quenching in intact chloroplasts (presence of 2 mM $KHCO_3$). Quenching was determined as for Fig. 1 after 5 min illumination with L 1 (light flux densities as given).

Conditions		CO_2-dep. O_2 evoln.	Quenching	
Light (L 1)	NH_4Cl	[μmol/mg Chl·h]	q_E	q_Q
20 W m^{-2}	absent	13	0.58	0.34
	present	18	0.03	0.21
70 W m^{-2}	absent	74	0.72	0.27
	present	74	0.13	0.22

Conclusions

The present communication shows a close linear correlation between ΔpH-related quenching (q_E) and quantum yield of photosynthetic CO_2-dependent O_2 evolution. We view this as evidence for a regula-

tion of thermal energy dissipation according to the energy requirements of photosynthesis. Protection against the damaging effects of excess excitation would be the consequence of such regulation. The effectiveness of this protective mechanism can be judged from the data in Fig. 3. An increase of q_E from about 0.3 to 0.7 is related to an approximate increase in quantum requirement from 15 to 70 (quantum requirement is defined here as moles quanta absorbed per mole O_2 evolved). We do not suppose that the increase in the thermal dissipation limits photosynthesis. There is strong evidence that CO_2 assimilation is limited by the activity of the carbon reduction cycle even at low, non-saturating irradiances [18]. Rather, we suggest that thermal energy dissipation is dynamically adjusted in accordance to the proportion of excess excitation energy. This adjustment would be limited, however, by the light-saturation of the processes responsible for q_E. At irradiances below saturation, overexcitation of the membrane and related damage should be largely avoided. But even at light-saturation, partial protection should be provided due to the high rate-constant of thermal dissipation, as indeed indicated by previous experiments [9, 10]. The mechanism discussed here allows relatively fast responses (in the range of one minute) of the photosynthetic system to changing light regimes. As recently reported [19], there seems to be a second, long-term mechanism of protection that is also based on increased thermal dissipation and would be particularly effective in light above saturation of photosynthesis.

Acknowledgements

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