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, CO₂ fixing chloroplasts isolated from spinach. Energy-dependent quenching (qₑ), 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 qₑ 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, qₑ. 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.

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 (L₁, see below) of different light flux densities. The reaction medium contained 0.33 mM sorbitol, 2 mM EDTA (disodium salt), 1 mM MgCl₂, 1 mM MnCl₂, 10 mM NaCl, 60 mM KCl, 0.5 mM KH₂PO₄, 40 mM (2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (pH 7.6 with NaOH), 2 mM KHCO₃ and 2000 U ml⁻¹ catalase (EC 1.11.1.6).

Quantum yields of CO₂-dependent O₂ 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 O₂ evolution, chlorophyll fluorescence was recorded with a pulse amplitude modulation fluorometer (PAM 101, H. Walz, Effeltrich, Germany) [14]. Non-modulated actinic light (L₁) was obtained with glass filters Calflex C, K65 (Balzers, Liechtenstein) and RG 645 (Schott, Mainz, FRG); λmax was 665 nm, half-band width 15 nm. The components of fluorescence quenching,
qE (ΔpH-dependent quenching) and qQ (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 qE and qQ. It is known from earlier studies [7, 8, 15] that under the conditions applied here, non-photochemical quenching (qN) is approximately equal to qE. Small contributions of other mechanisms to qN have been neglected. Light-saturation curves of qE and qQ in the presence of bicarbonate are depicted in Fig. 2a. With increasing light flux densities qE saturates at about 0.7 to 0.8, whereas qQ declines to a low value, indicating that the proportion of oxidized electron acceptor of PS II (Qa) decreases. Fig. 2b shows the light-dependence of rates and quantum yields of CO2-dependent O2 evolution, representing CO2 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 qE, 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 qE would indicate a low light...
rate-constant of thermal deactivation that allows a high quantum yield of photosynthesis. Extrapolation of the straight line in Fig. 3 to \( q_E = 0 \) 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 extend 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 \( \Delta pH \) 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.

**Conclusions**

The present communication shows a close linear correlation between \( \Delta pH \)-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.

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