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Hypothesis

Control of the light-harvesting function of chloroplast membranes by aggregation of the LHCII chlorophyll–protein complex

P. Horton[1], A.V. Ruban[1], D. Rees[1], A.A. Pascal[1], G. Noctor[1] and A.J. Young[2]

[1]*Robert Hill Institute, PO Box 594, Department of Molecular Biology and Biotechnology, Firth Court, University of Sheffield, Sheffield S10 2UH, UK* and [2]*School of Natural Sciences (Plant Sciences Division), Polytechnic of Liverpool, Byrom Street, Liverpool L3 3AF, UK*

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A new hypothesis is presented to explain the major molecular process that regulates the efficiency of light harvesting by chloroplast membranes. It is proposed that in excess light the decrease in the thylakoid lumen pH causes an increase in aggregation of the light harvesting complexes of photosystem II resulting in formation of an efficient pathway for non-radiative dissipation of excitation energy. The aggregation is potentiated by the conversion of violaxanthin to zeaxanthin. This hypothesis is based upon (i) similarity between the spectroscopic changes associated with energy dissipation and those observed upon aggregation of isolated light harvesting complex; and (ii) the link between changes in light scattering and increased energy dissipation.

Photosystem II; Thylakoid membrane; Chlorophyll fluorescence; Light harvesting

1. INTRODUCTION

Photosynthetic O_2 evolution involves a series of electron transfer and enzymic reactions driven by photochemical charge separation in photosynthetic reaction centres. The quantum efficiency of photosynthesis declines as the light intensity increases because of increasing limitation by these electron transfer and carbon assimilation capacities; this results in 'closing' of the reaction centres (i.e. Q_A reduction in photosystem II). However, it has been demonstrated that the 'open' PSII centres themselves operate less efficiently in high light [1–3], see [4] for a review. A major factor responsible for this decrease in intrinsic PSII efficiency is an increase in non-radiative dissipation in the thylakoid membrane, detected as a non-photochemical quenching of chlorophyll fluorescence [5]. This dissipation results from acidification of the lumen associated with an increased transmembrane ΔpH and is termed q_E [6]. It is formed as light-dependent ΔpH production exceeds metabolic capacity for ΔpH consumption via utilization of ATP. Thus, as light intensity becomes increasingly saturating, there is a switch from efficient utilization of absorbed light to effective dissipation, which functions to counteract excess excitation. Hence q_E plays an important physiological role in matching the rate of PSII excitation to the rate of photosynthesis [1,4], so providing protection against photo-inhibition [7]. However, mechanistic details of events linking lumen acidification to increased energy dissipation rate are lacking. Two models for q_E have been proposed. The first suggests that quenching results from inactivation of PSII reaction centres [1]. The second suggests that dissipation occurs within the PSII antenna by the formation of a special quenching carotenoid, zeaxanthin [8]. In this paper we propose an alternative model suggesting that quenching results from an organisational change in the thylakoid, involving aggregation of the major light harvesting complex, LHCII. This aggregation would be induced by protonation of the lumen surface and promoted by the presence of zeaxanthin. This hypothesis is based upon spectral changes associated with q_E, on properties of isolated LHCII, and on the linkage between q_E and light scattering changes.

Abbreviations: F_m, maximum level of chlorophyll fluorescence when all PSII reaction centres are closed; PSII, photosystem II; LHCII, the major light harvesting complex of photosystem II; q_E, non-photochemical quenching of chlorophyll fluorescence dependent upon the thylakoid proton gradient.

Correspondence address: P. Horton, Robert Hill Institute, PO Box 594, Department of Molecular Biology and Biotechnology, Firth Court, University of Sheffield, Sheffield S10 2UH, UK. Fax: (44) (742) 728697.

2. THE QUANTITATIVE RELATIONSHIP BETWEEN q_E AND ΔpH

The quantitative relationship between q_E and ΔpH measured in isolated thylakoids using indicators such as

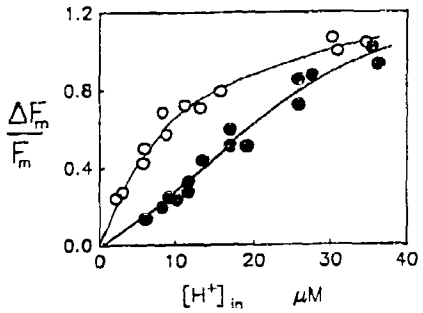

Fig. 1. Dependency of the rate of excitation dissipation ($\Delta F_m/F_m$) on the estimated $[H^+]$ of the thylakoid lumen ($[H^+]_m$) in thylakoids prepared from light-treated (○) and dark-adapted (●) spinach leaves as in [10]. The parameter $\Delta F_m/F_m$ has been shown to be directly proportional to the increase in rate of energy dissipation [8]. It was determined from the relaxation in F_m upon collapse of the ΔpH by adding DCMU to thylakoids illuminated for 5 min as in [10]. F_m(relaxed) − F_m(quenched)/F_m(quenched). An estimate of $[H^+]_m$ was obtained from the quenching of 9-aminoacridine fluorescence [10]. For light-treated thylakoids the zeaxanthin/violaxanthin was 1.2, and 0 for dark-adapted.

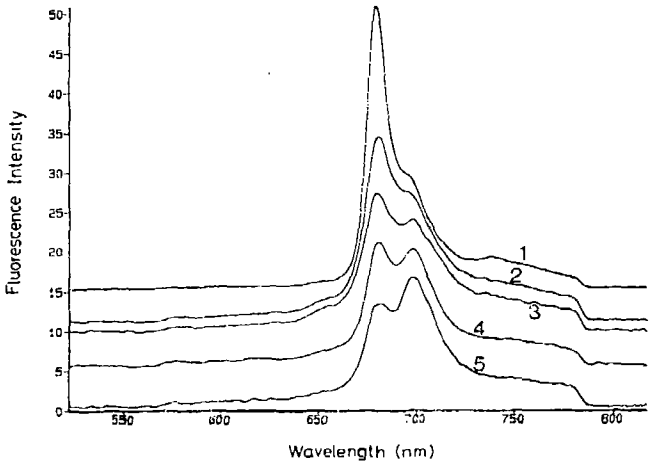

Fig. 2. Fluorescence emission spectra at 77K for LHCII isolated from spinach leaves as described in [16] after solubilisation in 0.1% octyl glucoside + 0.1% digitonin at a chlorophyll concentration of 35 μg/ml (1) and following subsequent dialysis for 16 h at 25°C against 5 mM Tricine buffer, pH 7.8, containing 10 μM (2), 0.5 μM (3), 0.1 μM (4) and 0 (5) antimycin A. Fluorescence spectra were recorded as in [12]. Spectrum 1 is divided by a factor of 3. The concentration of antimycin A required for half-maximum saturation of the change in F680/F700 was approx. 200 nM, similar to that required to inhibit q_E [15].

9-aminoacridine is not constant; for example, the inhibition of q_E by antimycin A is seen as an increase in the ΔpH requirement for q_E [3,9]. The titration curve of q_E vs. ΔpH can be shifted to the left or right, suggesting that q_E may be subject to *reversible activation/inactivation*. Indeed, if leaves are pretreated with saturating light prior to chloroplast isolation, q_E can be observed at lower ΔpHs than the control [10,11]. In Fig. 1, energy dissipation rate is plotted as a function of calculated lumen $[H^+]$ for chloroplasts isolated from dark-adapted and light-treated spinach leaves.

Demming-Adams and co-workers have shown that conversion of violaxanthin to zeaxanthin is correlated with the formation of q_E in vivo and have proposed that zeaxanthin has a direct and obligatory role in non-radiative dissipation [8]. Chloroplasts prepared from leaves pretreated as described above contain different ratios of violaxanthin/zeaxanthin [10]. In all cases, activation of q_E (i.e. the shift towards a lower ΔpH requirement shown in Fig. 1) was correlated with zeaxanthin formation. However, in thylakoids with and without zeaxanthin, the maximum extent of q_E at saturating ΔpH was nearly identical and quenching appeared to occur by the same mechanism (e.g. complete sensitivity to inhibition by antimycin A, and an identical relationship between quenching of F_o and F_m). It was concluded that conversion of violaxanthin to zeaxanthin *amplifies* q_E [10,11]. It is this amplification process that is hard to reconcile with existing models for q_E but which is so readily explained by the model we present below.

3. SPECTRAL CHANGES ASSOCIATED WITH q_E

The fluorescence emission spectrum of thylakoids at 77K has a number of clearly discernible bands originating from particular protein complexes. We have examined in detail the changes within the PSII band that occur upon q_E formation and found that a normalised q_E spectrum had a peak at 680 nm and a shoulder at 700 nm [12]. Although neither of these correspond to the major PSII maxima (at 685 nm and 695 nm) they have been found in the spectrum of isolated LHCII [13]. Furthermore, the spectrum for q_E in thylakoids containing zeaxanthin had a peak at 700 nm and a shoulder at 680 nm. As discussed below the 700 nm emission is associated with LHCII aggregation. We therefore suggest that q_E results from energy dissipation in an aggregated form of LHCII and that aggregation is favoured both by protonation of its lumenal surface and by conversion of violaxanthin to zeaxanthin. In support, the formation of zeaxanthin is associated with the appearance of a long wavelength chlorophyll species absorbing 685 nm [12] and preliminary data indicates that this species is also formed during q_E (data not shown).

4. AGGREGATION STATES OF LHCII

Dialysis of detergent-solubilised LHCII has been shown to result in the formation of 2-dimensional aggregates of LHCII [13] that can be crystalline [14]. This aggregation brings about a 60–90% decrease in fluorescence yield and a pronounced increase in the 700/680 nm emission ratio observed at 77K (Fig. 2). Although brought about by an entirely different mechanism, this in vitro aggregation of LHCII shows relationships to q_E. Firstly, aggregation is associated with a red shift in the chlorophyll absorption maximum (data not shown and [13]). Secondly, it was found that decreasing the pH from 7.6 to 4.5 quenched the fluorescence yield of unag-

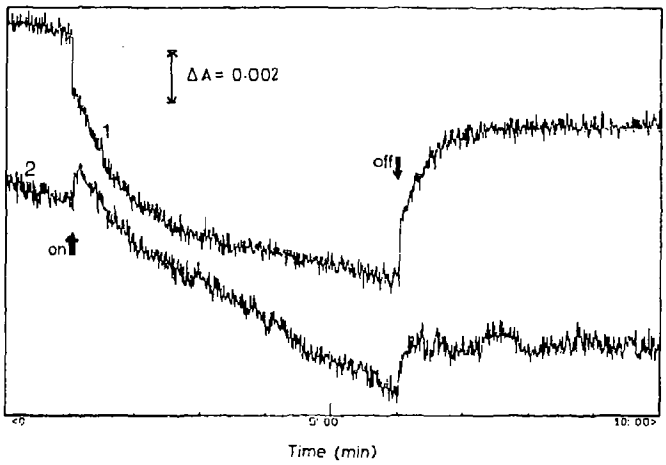

Fig. 3. Changes in light-scattering induced by illumination of spinach thylakoids in the absence (1) and presence (2) of 1 μM antimycin A. Scattering was recorded at 180° to the measuring beams using a SLM DW2000 spectrophotometer at 505 nm relative to a reference at 540 nm to select the q_E related component (see text). Downward deflections on the chart therefore indicate increases in scattering. The photomultiplier was protected by Corning 4-96 and Cyan-mirror T400–570 filters. The actinic light intensity was 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of red light defined by a Corning 2-58 long-pass filter and the chlorophyll concentration was 35 $\mu g/ml$. (↑) light on, (↓) light off.

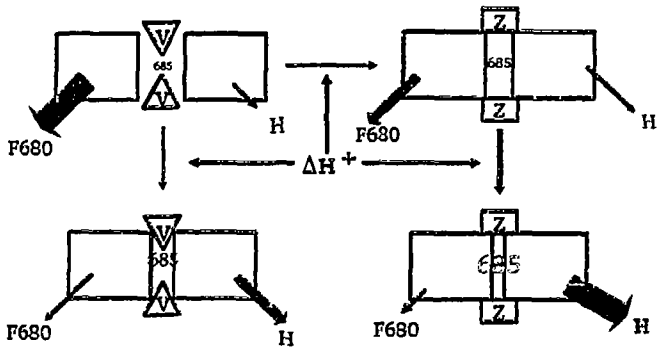

Fig. 4. Scheme to describe the role of LHCII aggregation in the dissipation of excitation energy. In the unenergised state, the xanthophyll violaxanthin (V) prevents LHCII aggregation. This hindrance necessitates a high [H+] to induce aggregation. Conversion to zeaxanthin (Z), also stimulated by the increase in acidification of lumen (ΔH^+), causes some increase in LHCII interaction, and this state now requires a smaller increase in [H+] to cause aggregation. The width of the arrows indicates the relative rates of fluorescence emission (F680) and heat evolution (H). The extent of aggregation is indicated by the proximity between the LHCII 'boxes' and the size of '685', representing chlorophyll absorption at 685 nm. For further explanation refer to the text.

gregated LHCII by approx. 30% and simultaneously induced some aggregation as evidenced from the increased 700/680 nm ratio. Thirdly, we find that 'light' LHCII with a zeaxanthin/violaxanthin ratio of 1.2 (isolated from light-treated spinach leaves) differs quantitatively from the control, zeaxanthin-deficient, 'dark' complex; the fluorescence yield of the aggregated state is approx. 25–30% lower for the 'light' complex and the 700/680 nm ratio is 15% higher. The fluorescence yield increase upon disaggregation was found to be 72% larger for the 'light' LHCII. Lastly, and most significantly, antimycin A prevents LHCII aggregation (Fig. 2). This reagent is a well-known inhibitor of q_E whose action has been difficult to explain previously since it had been assumed to bind only to a component involved in cyclic electron transport around PSI [15]. However, the data in Fig. 2 indicates that antimycin A inhibits q_E by preventing formation of a ΔpH-induced aggregated state of LHCII. It should be pointed out that the exact nature of the aggregated state that we propose to be associated with q_E is not known and is probably not identical to that formed in vitro upon dialysis.

5. LIGHT-SCATTERING CHANGES ASSOCIATED WITH q_E

LHCII aggregation is associated with an increase in light scattering [13]. The occurrence of light-scattering changes upon formation of the ΔpH are well known [16]. The kinetics of these changes are similar to those of q_E upon illumination of leaves [17], although it is impossible to know if this merely reflects separate effects of the ΔpH. In isolated chloroplasts there is a time lag between changes in ΔpH and q_E and it is possible to specifically eliminate q_E with antimycin A [15,18]. Thus it is possible to test whether increased light-scattering is associated with q_E or with ΔpH per se. The data in Fig. 3 shows that a component of light-scattering change is blocked in the presence of antimycin A; this component of scattering forms within 1 min of illumination and relaxes in darkness within 2 min, kinetics identical to those of q_E, but slower than ΔpH [10,11]. In contrast, antimycin-insensitive scattering change is much slower to form and is mostly irreversible. We have found that this q_E-related scattering has rather specific spectral characteristics, showing a maximum at about 535–540 nm (data not shown). Furthermore, at limiting ΔpH values, the amplitude of the light scattering is greater in thylakoids containing zeaxanthin, correlating with their larger q_E (data not shown). We conclude from these observations that formation q_E is accompanied by significant conformational changes in the thylakoid membrane. This origin for a component of the light scattering change is consistent with light-induced changes in membrane thickness [16] and theoretical studies which suggest that scattering in this wavelength range is indicative of the formation of large domains in the membrane complexes [19]. It is important to note that light-induced changes in circular dichroism have also been observed. Again, these have been attributed to macro-domains of LHCII [20]. In a recent study, the amplitude of the circular dichroism spectrum was found to increase during a diurnal cycle and to correlate with the extent of non-photochemical fluorescence quenching [21].

6. LHCII AGGREGATION MODEL FOR q_E

It is proposed that q_E results from a H^+-linked conformational transition in the LHCII complex which results in its aggregation (Fig. 4). In the aggregated state the electronic state of chlorophyll is modified dramatically such that heat dissipation is favoured over fluorescence or energy transfer. This dissipative state has an absorption maximum at 685 nm and at 77K emits at 700 nm. In this scheme, zeaxanthin formation, itself also induced by lumen acidification, promotes the adoption of the aggregated state and may not be directly involved in the quenching of excitation energy. Agents or conditions known to stimulate or inhibit q_E do so by enhancing or disrupting aggregation respectively. This model is consistent with all previous observations on q_E and explains simply the properties of light activation and antimycin A inhibition. The physiological significance of this mechanism for regulation is that only small changes in ΔpH may be required to greatly alter the rate of energy dissipation; this is necessary since ΔpH has to be optimised so as both to allow electron transport and drive ATP synthesis [22].

There are several important implications of this model, both in terms of future experiments and in terms of biological function. The model specifically suggests a role for LHCII in the control of energy dissipation; with the structural information now available, manipulation of q_E by protein engineering becomes a possibility. The scheme also suggests that q_E would be sensitive to the boundary lipid environment; it is perhaps in this sense that the role of xanthophylls should be best regarded, a role entirely consistent with a xanthophyll requirement for reconstitution of LHCII [23]. The significance of other phenomena, such as the involvement of specific lipids in interacting with LHCII upon acclimation to low temperature [24] will perhaps become clear in terms of a role in the regulation of energy dissipation.

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