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Enhancement of the ΔpH-dependent dissipation of excitation energy in spinach chloroplasts by light-activation: correlation with the synthesis of zeaxanthin

D. Rees, A. Young[+], G. Noctor, G. Britton[+] and P. Horton

Robert Hill Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN and [+]*Department of Biochemistry, University of Liverpool, Liverpool L69 3BX, England*

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The extent of energy-dependent quenching of chlorophyll fluorescence in broken spinach chloroplasts has been quantitatively related to the size of the thylakoid proton gradient as measured by the quenching of 9-aminoacridine fluorescence by titration at constant irradiance with the uncoupler nigericin or by change in irradiance. It was found that chloroplasts prepared from leaves that had been pre-illuminated with strong light for 30 min showed energy-dependent quenching at a lower proton gradient than chloroplasts prepared from dark-adapted leaves. Measurement of the carotenoid composition of the thylakoids showed that light treatment raised the ratio of zeaxanthin:violaxanthin. The possible dependence of energy-dependent quenching on xanthophyll composition and the physiological implications of this light-activation process to the regulation of photosynthetic electron transport are discussed.

1. INTRODUCTION

It is now recognised that the harmless dissipation of excitation energy in the thylakoid membranes of plant and algal cells is an important regulatory and protective process. Thus, assays of the non-photochemical quenching (qNP) of chlorophyll fluorescence, a measure of thermal de-excitation, have shown that dissipative processes regulate the quantum yield of photosystem II [1–3] and prevent photoinhibition of photosynthesis [4,5]. Three kinds of qNP have been described which function in the regulation/protection of the thylakoid: control of the absorption cross section of PS II via protein phosphorylation [6,7]; non-radiative dissipation dependent on the presence of the thylakoid ΔpH [4,8]; non-radiative dissipation correlated with the conversion of violaxanthin to zeaxanthin upon prolonged illumination [5,9]. The latter two have been designated qE and qI, respectively [2]. A number of investigations have clearly shown the quantitative relationship between qE and probes of the ΔpH such as the quenching of 9-aminoacridine fluorescence [3,10,11]. Although it has been suggested that qE results from the conversion of PS II reaction centres from an active to inactive state [1,12], the relationship between quenching of F_o and F_v levels of fluorescence suggest that qE occurs because of changes in the antenna chlorophyll [13,14]. A similar argument has been put forward to explain the mechanism of qI [5,9]. It therefore seems possible that dissipation observed as qE and qI arise from a common

Correspondence address: P. Horton, Robert Hill Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN, England

Abbreviations: PS II, photosystem II; qQ, photochemical quenching of chlorophyll fluorescence; qNP, non-photochemical quenching of chlorophyll fluorescence; qE, energy-dependent quenching of chlorophyll fluorescence; qI, slowly relaxing fluorescence quenching induced in high light; q9AA, quenching of the fluorescence from 9-aminoacridine; F_m, maximum level of chlorophyll fluorescence when PS II reaction centres are closed; F_o, minimum level of chlorophyll fluorescence when PS II reaction centres are open; F_v, F_m minus F_o; ΔpH, proton gradient across the thylakoid membrane

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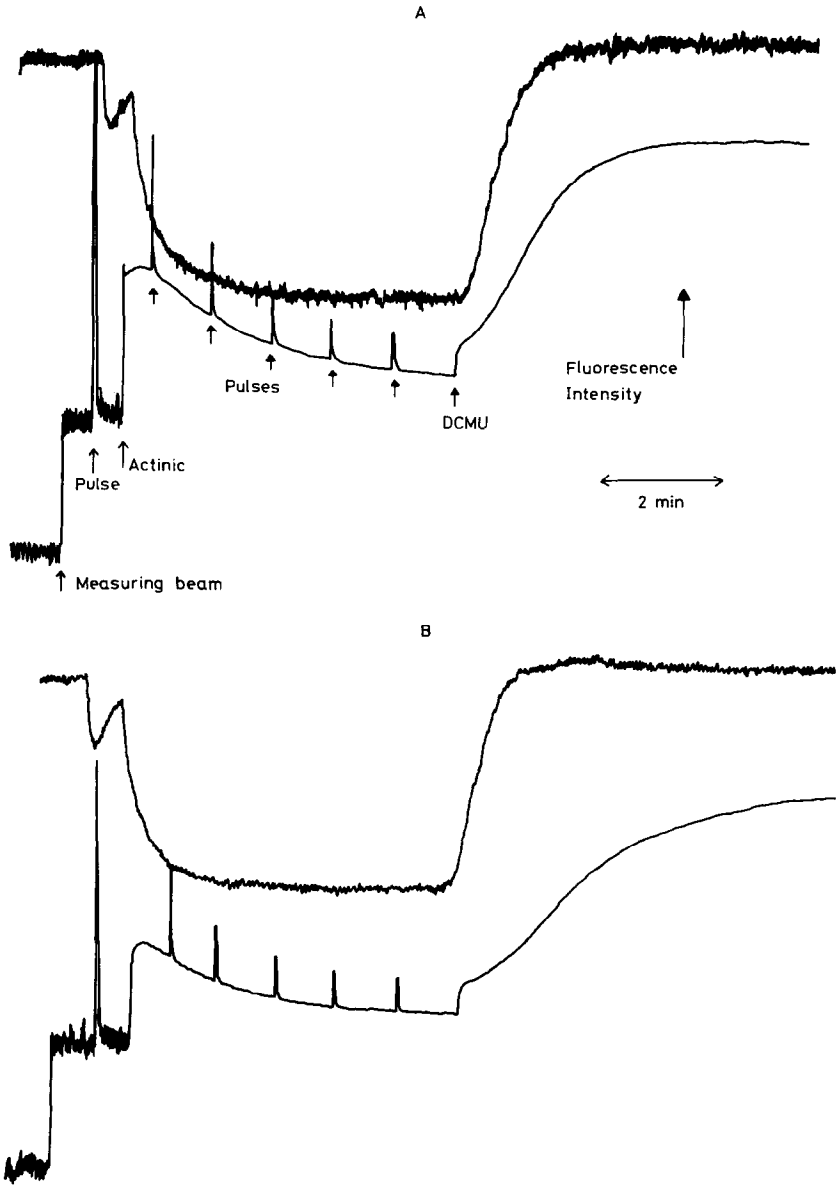
mechanism. In fact, it has been shown that pre-treatment of leaves to induce zeaxanthin formation accelerates the light-induced development of qNP [9], suggesting that both quenching processes are in some way related to the presence of this carotenoid in the thylakoid membrane. Based on this and other data [2], a dynamic model of the functioning of the light-harvesting complexes of PS II which were proposed to exist in various states of protonation, phosphorylation and zeaxanthin : violoxanthin content has been proposed [15]. This kind of model suggests that the capacity for qE would be dependent on the content of zeaxanthin. In this paper, data are presented which show that a pre-treatment of leaves which induces zeaxanthin formation increases the sensitivity of qE to the ΔpH. It is suggested that this 'light-activation' of qE allows photosynthesis and effec-



tive dissipation both to proceed together in high light without a large ΔpH that would inhibit linear electron transport.

2. MATERIALS AND METHODS

Spinach plants, grown under supplemented light in a greenhouse for 4–6 weeks were dark adapted for 24 h to deplete zeaxanthin levels. Leaves were cut at the base of the petiole and floated on water at 25°C for 320 min either in darkness or under 1000 μmol quanta$\cdot m^{-2}\cdot s^{-1}$ provided by a bank of 8 300-W tungsten halogen flood lamps filtered through 15 cm of circulating cold water. Immediately after treatment a leaf sample was frozen in liquid nitrogen. The remainder were immediately used for isolation of intact chloroplasts by a standard procedure [16]. Samples of chloroplasts at the beginning and end of the experiment were frozen in liquid nitrogen and, together with the leaf samples extracted and assayed for their carotenoid composition by HPLC [17]. The isolated chloroplasts were osmotically shocked in 20 mM $MgCl_2$, 25 mM Hepes buffer, pH 7.6, for 20 s and then diluted 1:1 with 0.66 M sorbitol, 20 mM KCl and 25 mM Hepes buffer before simultaneous assay of chlorophyll fluorescence and 9-aminoacridine fluorescence by procedures previously described [10,18]. Methyl viologen was used as electron acceptor. qE was determined by the DCMU method [19] and both qE and ΔpH were titrated either at constant light intensity (intensity 220 μmol quanta$\cdot m^{-2}\cdot s^{-1}$) by addition of small aliquots of nigericin or by change in light intensity.

3. RESULTS

Illumination of chloroplasts isolated from the control (dark) leaves gave rise to the expected

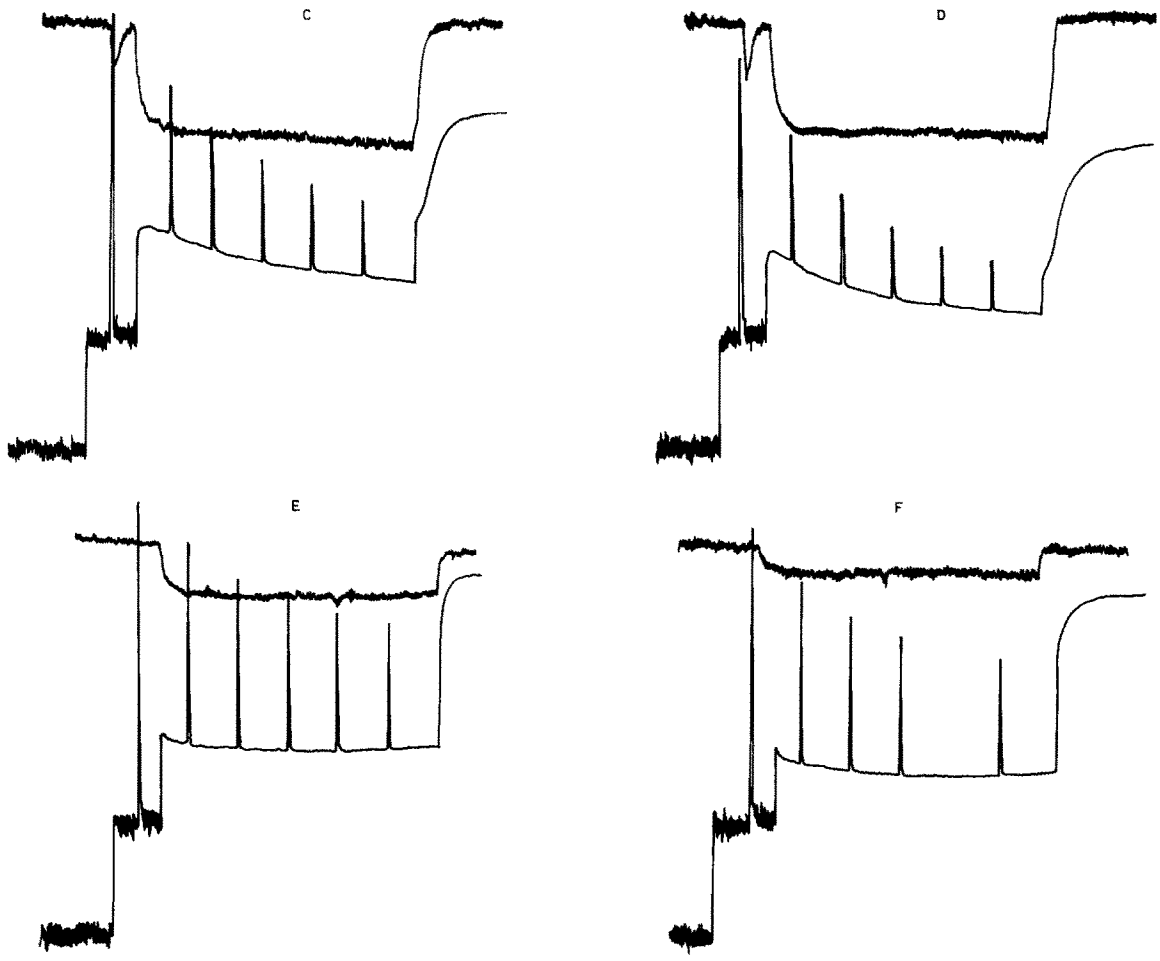


Fig.1. Chlorophyll fluorescence (bottom) and 9-aminoacridine fluorescence (top) upon illumination with 220 μmol quanta$\cdot m^{-2}\cdot s^{-1}$ in coupled broken chloroplasts (A,B) and in the presence of 2 nM (C,D) and 100 nM nigericin (E,F). Chloroplasts were prepared from light-treated (B,D,F) or dark-adapted (A,C,E) leaves.

behaviour observed previously in spinach [18]; ΔpH developed within 30 s whereas fluorescence quenching took several minutes to reach a steady state (fig.1A). Addition of DCMU shows the extent of *q*Q (the fast rise, equivalent in amplitude to the rise induced by the light saturation pulses) and *q*E (the slow rise) as described by Krause et al. [19]. Chloroplasts isolated from light-treated leaves exhibited exactly similar behaviour (fig.1B); both the extent and rate of formation of *q*E are indistinguishable in fig.1A and B. However, if the steady-state ΔpH was lowered with small aliquots of the uncoupler nigericin, a marked difference between 'light' and 'dark' chloroplasts was observed. Fig.1C–F shows that the chloroplasts from light-treated leaves showed a significantly larger *q*E for the same ΔpH compared to the dark control. For example, at 2 nM nigericin, in light chloroplasts *q*9AA was 0.27 and *q*E 0.69, whereas in dark chloroplasts, the same *q*9AA only gave a *q*E of 0.48 (fig.1C,D).

Titration curves for the two chloroplast samples are shown in fig.2. Confirming the observation in fig.1A,B it is clear that the maximum *q*E (approx. 0.75) is the same in both light and dark chloroplasts. However, as predicted from the data shown in fig.1C–F, the slope of the curve relating *q*E to ΔpH is increased in the light chloroplasts. Thus, the *q*9aa giving half-maximal *q*E is approx. 0.2 in dark chloroplasts, falling to 0.1 in light chloroplasts. At a *q*9aa of 0.1, the *q*E increased

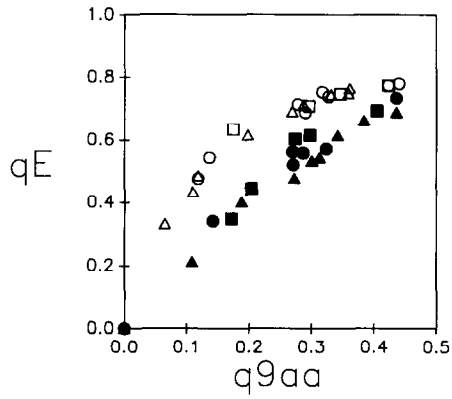


Fig.2. Titration of *q*E and ΔpH (*q*9aa) by increasing nigericin concentration (circles and triangles) or light intensity (squares) for chloroplasts prepared from light-treated (open symbols) or dark-adapted leaves (closed symbols). Different symbols represent different chloroplasts preparations.

from 0.2 to 0.4 upon light pre-treatment. It should also be noted from fig.1 that the rate of relaxation of *q*E following DCMU addition is much slower in the chloroplasts prepared from light-treated leaves.

Table 1 shows that the illumination of spinach leaves increases the zeaxanthin content from 1% total carotenoid to 12%; a ratio of violaxanthin:zeaxanthin of 19:1 was observed in dark-adapted leaves compared to 0.7 after pre-illumination. It is noted that the total content of violaxanthin + zeaxanthin is unchanged by this

Table 1

Carotenoid composition of spinach leaves and isolated chloroplasts following light and dark treatment Ch(1) and Ch(2) refer to chloroplasts samples taken at the start and end of an experiment as in fig.1. neo, neoxanthin; viol, violaxanthin; anth, antheraxanthin; lut, lutein; zea, zeaxanthin; β-car, β-carotene; v/z, violaxanthin:zeaxanthin ratio. F_v/F_m values were recorded at the beginning of each experiment from the F_o and F_m as shown in fig.1 and are the mean of 4 replicate (S.E. was ± 0.002). Carotenoid values are means of 3 determinations ± S.E.

Sample		Carotenoid composition (%)							F_v/F_m
		neo	viol	anth	lut	zea	β-car	v/z	
Leaf	D	12.7 ± 0.8	20.1 ± 2.8	1.8 ± 0.2	37.6 ± 2.5	1.0 ± 0.03	26.4 ± 0.7	19.1	n.d
	L	12.1 ± 0.4	8.3 ± 0.2	3.2 ± 0.2	36.4 ± 0.5	12.2 ± 0.9	27.8 ± 0.1	0.68	n.d
Ch(1)	D	11.6 ± 0.1	18.4 ± 1.3	1.5 ± 0.1	40.9 ± 0.7	1.5 ± 0.05	27.0 ± 1.1	12.3	0.75
	L	12.0 ± 0.8	8.6 ± 0.7	2.3 ± 0.2	40.7 ± 0.5	8.9 ± 0.6	26.9 ± 1.8	0.95	0.71
Ch(2)	D	12.9 ± 0.4	19.7 ± 0.3	2.0 ± 0.1	40.2 ± 0.1	2.3 ± 0.1	22.6 ± 0.3	8.86	n.a.
	L	12.8 ± 0.3	9.5 ± 0.1	3.8 ± 0.3	39.8 ± 0.3	8.6 ± 0.1	25.2 ± 0.9	1.09	n.a.

brief light treatment and that no other changes in carotenoid composition were observed, apart from a small increase in antheraxanthin, an intermediate in the xanthophyll cycle. Analyses of the chloroplasts isolated from the dark and light leaves show that the in vivo alterations in xanthophyll content are preserved. Ratios of violaxanthin:zeaxanthin of 0.95 and 12.25 were observed for the light and dark chloroplasts, respectively. It is important to note that there was little change in this ratio upon storage of chloroplasts on ice for the duration of the experiment (3–4 h, data not shown) or during the experimental assay of qE and q9AA. It is significant that the light-treated samples have a longer F_v/F_m than the dark controls, indicating development of qI expected to be associated with the synthesis of zeaxanthin [5].

4. DISCUSSION

The data presented here show that chloroplasts prepared from light-treated leaves can form qE at lower ΔpH than those isolated from dark-adapted leaves. Light treatment will elicit a series of photosynthetic changes. The assay of qE and ΔpH in isolated thylakoids would not be affected by changes in carbon metabolism or in the light activation of the ATPase or NADP reductase. The only identified effects of high light are protein phosphorylation, photoinhibition or zeaxanthin formation. Previous data indicate minimal in vivo phosphorylation in strong light [20] and we have no evidence for inhibition of electron transport activity in the light-treated chloroplasts. Conversely, there is clearly a large increase in the level of zeaxanthin in the chloroplasts isolated from the light-treated leaves which is associated with a decrease in F_v/F_m as previously described [5]. However it cannot be discounted that the light treatment had other unknown effects on the thylakoid which altered the relationship between qE and ΔpH, and which may occur in parallel with the promotion of zeaxanthin formation or indeed by a pre-requisite for it.

Nevertheless, the results strongly indicate that when zeaxanthin is present in the thylakoid, the ΔpH required for qE is lowered. This has important implications. Firstly, it provides strong evidence that the process involved in qE is in some way related to xanthophylls. Clearly, qE can develop without zeaxanthin. However, the conversion of violaxanthin to zeaxanthin raises the pK (of the presumed protonation site in the thylakoid lumen) for qE formation. Whether the xanthophyll is involved in quenching directly in a process with differential pH dependency or whether the xanthophyll composition influences the conformation of a chlorophyll protein complex whose pK is thereby changed cannot be ascertained. However, the data do indicate that the sustained quenching qI is perhaps exactly the same as qE. The prediction of the data in fig.2 is that with high levels of zeaxanthin (as in [9]) a small ΔpH of the size that could persist in darkness for a long time following illumination could give significant qE.

The light activation of qE observed here is not unexpected. Previous studies have shown that the slope of the titration of qE against ΔpH can be reduced in the presence of antimycin A [3], or raised by the presence of the electron acceptor, methyl viologen [12] or diaminodurene (Noctor, G.D., personal communication).

Apart from these mechanistic implications, the present data indicate that in vivo high qE values could be established without the necessity for a large ΔpH, if light activation (through zeaxanthin formation) has occurred. This light activation of qE would allow high qE to exist without the simultaneous restriction of linear electron transport rate by ΔpH control of plastoquinol oxidation. Thus, high rates of photosynthesis and energy dissipation could both proceed simultaneously, since an inhibitory ΔpH could be avoided. Equally, the turning-off of qE in low light means that a ΔpH sufficient to drive ATP synthesis could be maintained without causing wasteful energy dissipation. The light activation of qE is in many ways analogous to the reductive activation of the thylakoid ATPase [21]: there may be functional similarity also since in both cases the effect of activation is to lower the ΔpH requirement for functional activity. (It should be emphasised that harmless dissipation in the light-harvesting system is a major function of the protein complexes in high light.)

Finally, the linking of qE, which is a major factor in the control of PS II, with the xanthophyll cycle, which is dependent on ascorbate supply, PS I activity, and the processes involved in O_2 metabolism is an attractive and perhaps predic-

table possibility that could provide integrated protection of the thylakoid from high light.

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