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The carotenoid zeaxanthin and 'high-energy-state quenching' of chlorophyll fluorescence

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Abstract

The possibility that zeaxanthin mediates the dissipation of an excess of excitation energy in the antenna chlorophyll of the photochemical apparatus has been tested through the use of an inhibitor of violaxanthin de-epoxidation, dithiothreitol (DTT), as well as through the comparison of two closely related organisms (green and blue-green algal lichens), one of which (blue-green algal lichen) naturally lacks the xanthophyll cycle. In spinach leaves, DTT inhibited a major component of the rapidly relaxing 'high-energy-state quenching' of chlorophyll fluorescence, which was associated with a quenching of the level of initial fluorescence (F_0') and exhibited a close correlation with the zeaxanthin content of leaves when fluorescence quenching was expressed as the rate constant for radiationless energy dissipation in the antenna chlorophyll. Green algal lichens, which possess the xanthophyll cycle, exhibited the same type of fluorescence quenching as that observed in leaves. Two groups of blue-green algal lichens were used for a comparison with these green algal lichens. A group of zeaxanthin-free blue-green algal lichens did not exhibit the type of chlorophyll fluorescence quenching indicative of energy dissipation in the pigment bed. In contrast, a group of blue-green algal lichens which had formed zeaxanthin slowly through reactions other than the xanthophyll cycle, did show a very similar response to that of leaves and green algal lichens. Fluorescence quenching indicative of radiationless energy dissipation in the antenna chlorophyll was the predominant component of 'high-energy-state quenching' in spinach leaves under conditions allowing for high rates of steady-state photosynthesis. A second, but distinctly different type of 'high-energy-state quenching' of chlorophyll fluorescence, which was not inhibited by DTT (i.e., it was zeaxanthin independent) and which is possibly associated with the photosystem II reaction center, occurred in addition to that associated with zeaxanthin in leaves under a range of conditions which were less favorable for linear photosynthetic electron flow. In intact chloroplasts isolated from (zeaxanthin-free) spinach leaves a combination of these two types of rapidly reversible fluorescence quenching occurred under all conditions examined.

Abbreviations: DTT – dithiothreitol; F_0 (or F_0') – yield of instantaneous fluorescence at open PS II reaction centers in the dark (or during actinic illumination); F_M (or F_M') – yield of maximum fluorescence induced by a saturation pulse of light in the dark (or during actinic illumination); F_V (or F_V') – yield of variable fluorescence induced by a saturating pulse of light in the dark (or during actinic illumination); k_D – rate constant for radiationless energy dissipation in the antenna chlorophyll; SV – Stern–Volmer equation; PFD – photon flux density; PS I – photosystem I; PS II – photosystem II; Q_A – acceptor of photosystem II; q_N – coefficient of nonphotochemical chlorophyll fluorescence quenching; q_P – coefficient of photochemical chlorophyll fluorescence quenching.

Introduction

Under a variety of conditions we have previously observed a close correlation between the (non-photochemical) quenching of chlorophyll fluorescence indicative of the radiationless dissipation of excess excitation energy in the photochemical apparatus and the zeaxanthin content of intact leaves (Demmig et al. 1987, 1988, Demmig-Adams et al. 1989b,c, 1990d). Zeaxanthin can be formed rapidly, upon exposure to an excess of light, from violaxanthin as part of the xanthophyll cycle in most photosynthetic systems (Hager 1980, Siefermann-Harms 1977, Yamamoto 1979). The zeaxanthin-associated type of fluorescence quenching was usually accompanied by a quenching of instantaneous fluorescence at open PS II centers (Demmig-Adams et al. 1989b, see also Demmig and Björkman 1987). However, the induction and relaxation kinetics of this kind of fluorescence quenching differed considerably depending on the plant species and conditions to which the organism was subjected. We have demonstrated that a correlation exists between the zeaxanthin content and a sustained kind of fluorescence quenching which was induced by prolonged exposure of *Nerium oleander* to a combination of high light and water stress (Demmig et al. 1988, see also Björkman and Powles 1984), but have also recently shown a similar correlation between zeaxanthin and an extremely rapidly developing and relaxing kind of fluorescence quenching which could be induced in a few minutes upon exposure of leaves to an excess of light (Demmig-Adams et al. 1989b,c). A type of nonphotochemical fluorescence quenching with these properties, i.e., rapid induction and relaxation kinetics, had initially been characterized in chloroplast preparations (Briantais et al. 1979, Krause et al. 1982, 1983) and termed 'energy-dependent', 'pH-dependent', or 'high-energy-state quenching' (see also Oxborough and Horton 1987, Horton and Hague 1988). It was tacitly assumed that a type of fluorescence quenching with very similar properties in leaves (Schreiber and Bilger 1987, Weis and Berry 1987) was the same kind of quenching.

We should like to briefly review the various forms in which the relationship between zeaxanthin and chlorophyll fluorescence quenching has been expressed to date. Fluorescence quenching yielded a good linear relationship with zeaxanthin for either F_V, q_N, or k_D, the rate constant for radiationless energy dissipation in the pigment bed as calculated from the bipartite model of Butler and coworkers (Butler 1978, Butler and Kitajima 1975, Kitajima and Butler 1975), as long as the quenching was not too strong (up to a q_N of 0.7, Demmig-Adams et al. 1989c). A linear correlation between fluorescence quenching and the zeaxanthin content over the entire range of quenching has, however, typically been obtained when fluorescence quenching was expressed as k_D (Demmig et al. 1988, Demmig-Adams et al. 1989b,c, 1990c). This was the expected response, since the rate constant k_D which is proportional to $1/F'_M$ is also proportional to the concentration of e.g., an added quencher of fluorescence (Kitajima and Butler 1975), whereas F_V or q_N approach a finite value (of 1 in the case of q_N) and are not proportional to the quencher concentration at high concentrations. In our initial studies which considered only one (small) portion of fluorescence quenching (the slowly relaxing one, Demmig et al. 1987), F_V was plotted against the zeaxanthin content and gave a linear relationship, but k_D has been used in subsequent studies in which all of the strong fluorescence quenching during actinic illumination was considered (e.g., Demmig-Adams et al. 1989b,c).

The Stern–Volmer equation has recently been used by W. Bilger to quantitate the dissipation activity in the photochemical apparatus from chlorophyll fluorescence quenching (see Bilger and Björkman 1990). The calculated dissipation activity after SV is proportional to k_D as calculated by us from the Butler model (see Demmig-Adams 1991 and Bilger and Björkman 1990). The Stern–Volmer equation uses $SV = F_M/F'_M - 1$ and we are using $k_D = F_M/(F'_M \cdot 0.074) - 1$. For further explanations see Demmig-Adams et al. (1990c) and Demmig-Adams (1991). Therefore the conclusion that a linear correlation exists between the zeaxanthin content and chlorophyll fluorescence quenching indicative of radiationless energy dissipation is independent of any assumptions in the Butler model.

Whereas a close correlation existed between zeaxanthin and the rapidly reversible kind of

chlorophyll fluorescence quenching present during illumination of leaves with actinic light under various conditions (Demmig-Adams et al. 1989b,c), the kinetics of the relaxation of fluorescence quenching and those of the removal of zeaxanthin upon darkening do not appear to match. Upon return to darkness the kinetics of the re-conversion of zeaxanthin to violaxanthin are slower than those of the relaxation of this kind of quenching. Futhermore, leaves may contain a background level of zeaxanthin without exhibiting any quenching of fluorescence in the dark or in low, nonexcessive light (Demmig-Adams et al. 1989c). However, under the experimental conditions used, leaves which contained no zeaxanthin prior to the exposure to excessive light did show induction kinetics of nonphotochemical fluorescence quenching which matched those of the formation of zeaxanthin in the xanthophyll cycle, and only leaves which contained background levels of zeaxanthin exhibited an extremely rapid onset of nonphotochemical fluorescence quenching upon transfer to excessive light (Demmig-Adams et al. 1989c). We concluded that either there was no causal relationship between zeaxanthin and fluorescence quenching, or that an additional mechanism was involved which led to an activation of zeaxanthin as a fluorescence quencher in excessive light and a deactivation in nonexcessive light or darkness.

In order to test whether or not there is a causal relationship between zeaxanthin and energy dissipation in the antenna chlorophyll, two approaches have recently been taken. The first involves the use of dithiothreitol (DTT) which inhibits the formation of zeaxanthin from violaxanthin (Bilger and Björkman 1990, Bilger et al. 1989, Demmig-Adams et al. 1990c), and the second has relied on the existence of two closely related organisms, one of which possesses and one of which naturally lacks the xanthophyll cycle (Demmig-Adams et al. 1990a,b,e).

Comparison of chlorophyll fluorescence characteristics in zeaxanthin-containing and zeaxanthin-free leaves and lichen thalli

Figure 1A shows a typical time course of the induction and relaxation of the strong nonphotochemical fluorescence quenching which develops within a few minutes upon exposure of leaves to excess light and which is rapidly reversible upon return to darkness, i.e., the so-called 'high-energy-state quenching'. It was associated with a quenching of F_0' (see also Adams et al. 1989, Demmig-Adams et al. 1989b, Weis and Berry 1987). Nonphotochemical fluroescence quenching with the same characteristics as those observed in leaves also occurred in green algal lichens (Fig. 1C, Demmig-Adams et al. 1990a). We have compared the characteristics of chorophyll fluorescence in these zeaxanthin-forming organisms with those in two groups of zeaxanthin-free organisms. Those were, firstly, leaves and green algal lichens treated with DTT (Figs. 1B and 1D) which completely inhibited the formation of zeaxanthin (Bilger et al. 1989, Demmig-Adams et al. 1990c) by inhibiting the violaxanthin de-epoxidase (Yamamoto and Kamite 1972), and secondly, blue-green algal lichens (Figs. 1E and 1F) which do not possess the xanthophyll cycle, i.e., do not possess the xanthophyll cycle precursors of zeaxanthin, violaxanthin (di-epoxide) and antheraxanthin (mono-epoxide) (Stransky and Hager 1970). All of these, the DTT-treated leaves and thalli as well as blue-green algal lichens, did not contain any zeaxanthin prior to the treatment nor did they form zeaxanthin during the treatment. They also did not show any quenching of F_0' or any of the strong and rapidly reversible nonphotochemical quenching which is associated with F_0' quenching (Figs. 1B, D–F). Furthermore these zeaxanthin-free organisms all showed a very high level of steady-state fluorescence upon exposure to light, which indicates that they experienced a considerable overexcitation of the PS II reaction centers. In contrast to leaves and green algal lichens, the DTT-treatment did not alter the relationship between the levels of steady-state fluorescence and nonphotochemical quenching in blue-green algal lichens (Fig. 1F).

The rate of (light-saturated) photosynthetic O_2 evolution was around 300 μmol mg^{-1} Chl h^{-1} in both untreated and DTT-treated leaves, and around 63 μmol mg^{-1} Chl h^{-1} in both the green and the blue-green algal lichens. This indicates that a similar amount of excitation energy was consumed through photosynthesis in each set of leaves or thalli. This also means that the pH-

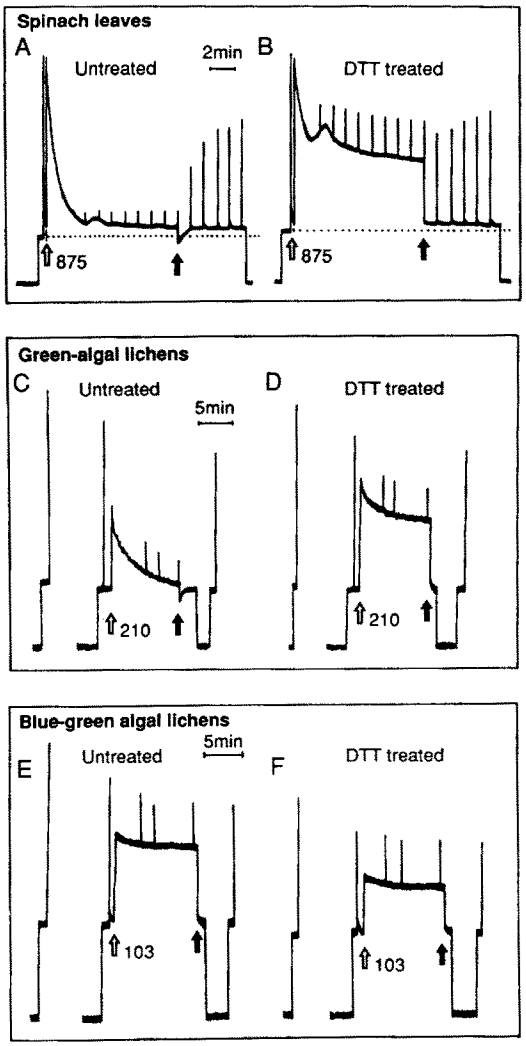

Fig. 1. Original traces of chlorophyll fluorescence from spinach leaves (A, B), thalli of the green algal lichen *Pseudocyphellaria rufovirescens* (C, D), and thalli of the blue-green algal lichen *Pseudocyphellaria dissimilis* (E, F). Chlorophyll *a* fluorescence was determined with a modulation fluorometer (Waltz, Effeltrich, FRG). Leaves and thalli were either untreated controls (A, C, E) or pretreated with DTT (B, D, F). Leaves were kept in darkness for 10 min, after which F_0 and F_M were ascertained, then exposed to a PFD of 875 μmol m^{-2} s^{-1} (red light) in 5% CO_2 at 25°C for 10 min. After 10 min of illumination the actinic light was switched off and fluorescence was recorded continuously in the presence of far-red light to obtain the F_0 level. For the pretreatment with DTT, leaves were harvested, their petioles rapidly placed in distilled water (control) or in a solution of 3 mM DTT, and the leaves maintained under 40 μmol photons m^2 s^{-1} at 25°C for 90 min prior to obtaining the above traces. Leaf data from Demmig-Adams et al. (1990c). Pretreatment of lichen thalli was with 3 mM DTT under 2 μmol photons m^{-2} s^{-1} for a minimum of 1 h. Thalli were subsequently transferred to darkness and F_0 and F_M were ascertained after 10 min in darkness (first signal shown). The thalli were then exposed for 10 min each to 5.7 and 38 (in case of the green algal lichens) or 5.7 and 56 (in the case of the blue-green algal lichens) μmol photons m^{-2} s^{-1}, followed by a 5 min dark period after each period of illumination (not shown). This was followed by a 10 min period at 103 (blue-green lichens) or 210 (green algal lichens) μmol photons m^{-2} s^{-1} and a 5 min period in darkness. Lichen data from Demmig-Adams et al. (1990a). Open arrows indicate the points at which the actinic light was switched on (numbers indicate the PFD, in μmol m^{-2} s^{-1}), and closed arrows indicate return to darkness. Saturating pulses of light were given at 1 min intervals in leaves and mostly after 3, 5 and 10 min of illumination, as well as after 5 min of darkness subsequent to each period of exposure to light in lichen thalli. With the lichen thalli, the measuring beam was switched off during part of the dark period to determine the baseline.

gradient across the photosynthetic membranes, which is one prerequisite for 'high-energy-state quenching' (Briantais et al. 1979), must have developed in the DTT-treated samples as well. Figure 2 shows that the relative magnitude between nonphotochemical (q_N) and photochemical (q_P, expressed as 1-q_P) quenching were reversed in DTT-treated versus untreated spinach leaves and in blue-green versus green algal lichens. Nonphotochemical fluorescence quenching, part of which is indicative of radiationless energy dissipation, was much greater in the zeaxanthin-containing than in the zeaxanthin-free organisms at all PFDs (Fig. 2). The expression 1-q_P is an approximate measure of the reduction state of PS II centers. A high reduction state indicates (potentially harmful) overexcitation of these centers. The fact that the reduction state of the PS II centers was higher in the zeaxanthin-free leaves and thalli than in the zeaxanthin-forming ones (Fig. 2), in spite of similar rates of O_2 evolution, also indicates that the radiationless dissipation of energy was not as great in the photochemical systems which did not contain zeaxanthin. The portion of nonphotochemical fluorescence quenching which occurred even in zeaxanthin-free leaves and thalli was not rapidly reversible and is therefore not considered to be 'high-energy-state quenching'. When all of the quenching of maximum fluorescence at closed PS II centers is expressed as an increase in the rate constant for radiationless energy dissipation in the antenna

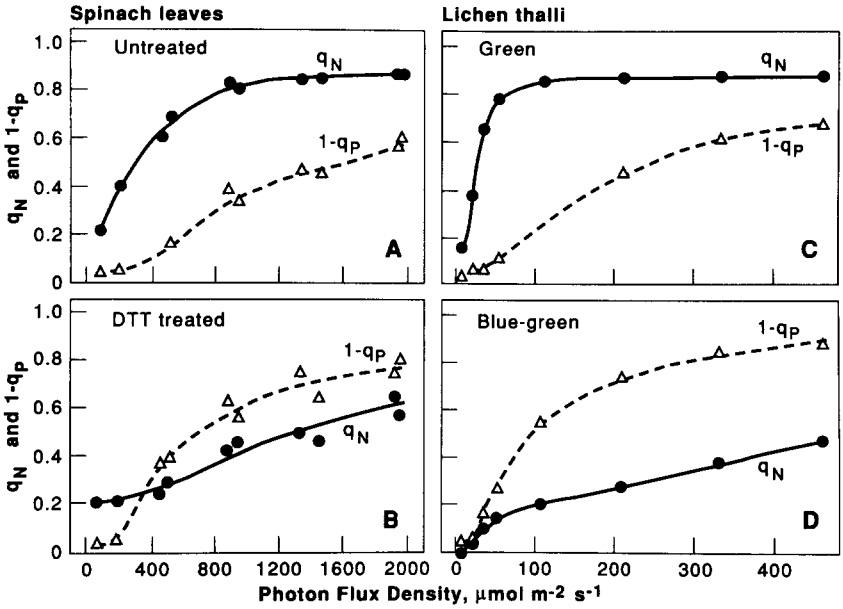

Fig. 2. Nonphotochemical fluorescence quenching, q_N, and the approximate reduction state of PS II centers, 1-q_P, in untreated (A) and DTT-treated (B) spinach leaves as well as in the phycosymbiodeme partners *Pseudocyphellaria rufovirescens* (with a green phycobiont; C) and *P. murrayi* (with a blue-green phycobiont; D) at various PFDs. Each point in A and B was obtained from a different leaf after 10 min of illumination. The expression 1-q_P (q_P being the coefficient of photochemical quenching; see Schreiber and Bilger 1987) was used an approximate measure of the reduction state of the acceptor Q_A of PS II. In *P. rufovirescens* nonphotochemical fluorescence quenching relaxed rapidly upon darkening, whereas in *P. murrayi* it did not. The variable fluorescence in control thalli (in darkness prior to exposure) was similarly high in both partners (coresponding to values of F_V/F_M of 0.70 to 0.72 in the partner with the green phycobiont and 0.67 to 0.69 in the partner with the blue-green phycobiont). Data for leaves from Demmig-Adams et al. (1990c) and for lichens from Demmig-Adams et al. (1990a).

chlorophyll (k_D) or an increase in $SV = F_M/F'_M - 1$ (Bilger and Björkman 1990), the difference between the untreated and DTT-treated leaves becomes even greater (Fig. 3A). The calculated difference between k_D in the untreated and the DTT-treated leaves exhibits a very close correlation with the zeaxanthin content of the untreated leaves as well as with the difference in F'_0 quenching between untreated and zeaxanthin-treated leaves (Fig. 3B, Demmig-Adams et al. 1990c). We therefore conclude that the zeaxanthin-correlated dissipation process in the antenna chlorophyll is absent in DTT-treated, and thus zeaxanthin-free, leaves. These findings argue for a causal relationship between zeaxanthin and this dissipation process in the antenna chlorophyll. One possible reservation against such a conclusion concerns the nature of DTT as a reductant, which might be able to reduce components associated with PS II and thereby interfere with a quenching process in or around the PS II center. However, DTT did not inhibit a second type of rapidly reversible fluorescence quenching which was also induced by an excess of light and had different characteristics from the DTT-sensitive quenching (see next section). The DTT-insensitive type of fluorescence quenching is likely to be the one related to processes which are associated with the PS II center. Furthermore, another reductant, ascorbate, was found to increase the magnitude of the DTT-sensitive portion of nonphotochemical fluorescence quenching in isolated chloroplasts at low PFD (Demmig-Adams et al. 1990c). Ascorbate stimulated zeaxanthin formation at low PFD (Demmig-Adams et al. 1990c), presumably by promoting the (reductive) formation of zeaxanthin from the di-epoxide violaxanthin at these PFDs (Siefermann and Yamamoto 1975).

The differences in the quenching of fluorescence between the green and blue-green algal lichens are not subject to any of the concerns

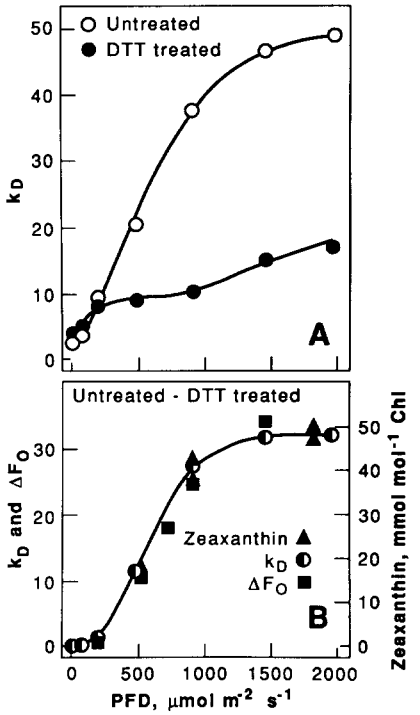

Fig. 3. The rate constant for radiationless energy dissipation in the antenna chlorophyll, k_D, in untreated control and DTT-treated leaves (A), and the zeaxanthin content of untreated control leaves as well as the DTT-sensitive portion of k_D and of F_0 quenching (B) at various PFDs. These DTT-sensitive portions, k_D (control) – k_D (DTT), and ΔF_0 (control) – ΔF_0 (DTT), were obtained by subtracting the values of k_D (from Fig. 3A), and the % changes in F_0 (F_0' relative to the pre-illumination value F_0) after a 10 min exposure to various PFDs in DTT-treated leaves from the values obtained from the untreated controls. The k_D values were calculated from the maximum yield of fluorescence, induced by a pulse of saturating light given during the illumination with actinic light after 10 min at each PFD. Data from Demmig-Adams et al. (1990c).

associated with studies involving inhibitors, although it might be argued that additional differences between the photochemical systems of the two algal phycobionts, other than that of the presence or absence of zeaxanthin (such as the possession of phycobilisomes in blue-green algae), might complicate a direct comparison. However, both green and blue-green algal lichens were found to have very similar fluorescence characteristics (Demmig-Adams et al. 1990a,b,e) except for the difference in the ability for 'high-energy-state quenching'. This may be related to the fact that these blue-green algal lichens possessed an extremely low ratio of phycobilins to chlorophyll *a* relative to that normally found in algal suspensions, (unpublished data). Furthermore, in another blue-green algal lichen, *Peltigera polydactyla*, slow accumulation of zeaxanthin, presumably from β-carotene, took place in the field during long-term exposure to high PFD (Demmig-Adams et al. 1990a). This means that the blue-green algal lichens are unable to form zeaxanthin rapidly, due to the absence of the epoxides of the xanthophyll cycle, but are able to form zeaxanthin slowly in the normal biosynthetic pathway which does not *per se* involve the epoxides (see Demmig-Adams 1991). These blue-green algal lichen thalli contained approximately half the amount of zeaxanthin per unit of chlorophyll of that which the green algal lichens were capable of accumulating rapidly upon exposure to high light, and also exhibited an intermediate response to light between that of green algal lichens and zeaxanthin-free algal lichens with respect to nonphotochemical fluorescence quenching and the reduction state of the PS II centers (Demmig-Adams et al. 1990a). The zeaxanthin-containing blue-green algal lichens exhibited a high fluorescence yield in limiting light and a rapidly developing quenching of fluorescence upon exposure to an excess of light. This suggests that a similar activation in excess light/deactivation in limiting light to that proposed for higher plants (Demmig-Adams et al. 1989b) is also present in blue-green algal lichens. The different responses of zeaxanthin-containing versus zeaxanthin-free blue-green algal lichens suggest that it is indeed the presence or absence of zeaxanthin which determines whether or not the organism is capable of radiationless energy dissipation in the antenna chlorophyll.

Since 'high-energy-state quenching' can develop and relax more rapidly than the biochemical interconversions between violaxanthin and zeaxanthin in leaves, we suggest that there is an additional mechanism exerting (biophysical) control over the action of zeaxanthin as a quencher, probably through some factor associated with the 'high-energy state' of the thylakoid membrane which may facilitate the interaction between zeaxanthin and chlorophyll leading to radiationless energy dissipation.

Two kinds of 'high-energy-state quenching' with different properties

The use of DTT to inhibit chlorophyll fluorescence quenching also revealed the existence of two distinct types of rapidly reversible fluorescence quenching under an excess of light, i.e., at a 'high-energy state' of the thylakoid membrane. In the studies described in the previous section, leaves were kept under conditions favorable for high rates of photosynthesis (i.e., high rates of linear electron transport), and the majority of the rapidly reversible (Figs. 4A and 4B) 'high-energy-state quenching' under such conditions was DTT-sensitive, correlated with zeaxanthin, and associated with a quenching of F_0' (Fig. 3B). When leaves were exposed to conditions which were less than optimal for photosynthesis, or where linear electron flow was greatly suppressed, such as in the absence of CO_2 at greatly reduced partial pressures of O_2 (Figs. 4C and 4D), a considerable degree of the nonphotochemical fluorescence quenching which developed even in the leaves which had been treated with DTT was also rapidly reversible upon darkening (Adams et al. 1990). A rapidly reversible component of fluorescence quenching was furthermore observed in DTT-treated leaves at elevated temperatures as well as during the induction of photosynthesis, i.e., during the first few minutes following illumination (Adams et al. 1990). Intact chloroplasts isolated from (untreated) spinach leaves showed a very large component of rapidly reversible fluorescence quenching when treated with DTT under all circumstances (Demmig-Adams et al. 1990c, e.g., Figs. 4E and 4F). This rapidly reversible kind of fluorescence quenching which developed in the absence of zeaxanthin, i.e., in the presence of DTT, was not associated with F_0' quenching in either leaves or isolated chloroplasts. The zeaxanthin-correlated, DTT-sensitive component of

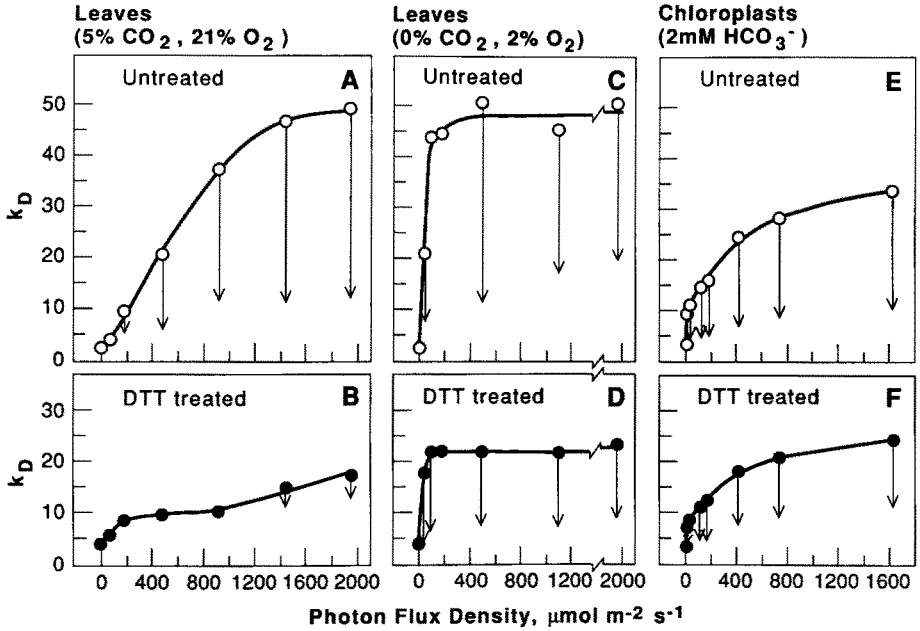

Fig. 4. The rate constant for radiationless energy dissipation in the antenna chlorophyll, k_D, during actinic illumination and 5 min after return to darkness (or rather far-red light) in untreated (A, C) and DTT-treated (B, D) spinach leaves and in untreated (E) and DTT-treated (F) spinach chloroplasts at various PFDs. The tips of the arrows depict the k_D values after 5 min in darkness (or rather far-red light). Leaves were illuminated for 10 min at 25°C in the presence of either 5% CO_2, 21% O_2 (A, B) or 0% CO_2, 2% O_2 (C, D). Chloroplasts were illuminated for 10 min at 20°C in the presence of 2 mM $NaHCO_3$. Intact chloroplasts were isolated from (untreated) leaves prior to the onset of artificial illumination, and did not receive the standard illumination preceding chloroplast isolation (Demmig-Adams et al. 1990c). DTT (final concentration of 1 mM) was added to chloroplast suspensions prior to measurements. Data from Adams et al. (1990) and Demmig-Adams et al. (1990c).

nonphotochemical fluorescence quenching in isolated chloroplasts, however, did exhibit the same correlation with F_0' quenching (Demmig-Adams et al. 1990c) as that observed in leaves (cf. Fig. 3B). At the same level of quenching of variable fluorescence (F_V') by 60–70%, the level of F_0' was quenched by 20–30% for the DTT-sensitive, zeaxanthin-correlated component of fluorescence quenching, and F_0' was not quenched at all for the DTT-insensitive component which occurred in the absence of zeaxanthin (see Adams et al. 1990; Demmig-Adams et al. 1990c).

Figure 5 is derived from the same set of data as that shown in Fig. 4; the levels of variable fluorescence at closed PS II centers correspond to the F_M' values which were used to calculate the k_D values depicted in Fig. 4. The portion of F_V quenching (in the leaves in 5% CO_2, 21% O_2) which was not reversible upon darkening and therefore did not represent 'high-energy-state quenching', represented as much as 50% of the reduction in F_V at the highest PFD. Whereas the patterns are similar, the difference between untreated and DTT-treated leaves, or particularly between untreated and DTT-treated chloroplasts, appears much smaller when fluorescence quenching is expressed as F_V' compared to k_D (compare Figs. 4 and 5). This is the expected response since F_V' is insensitive to changes at high degrees of quenching, whereas k_D, which is proportional to the reciprocal of F_M', is very sensitive to these changes. In order to estimate the dissipation activity of the zeaxanthin-associated dissipation process in the antenna chlorophyll, fluorescence quenching should preferably be expressed as an increase in k_D (Fig. 3, see also Demmig-Adams et al. 1989b, 1990c) or in SV (Bilger and Björkman 1990, Demmig-Adams 1991). Further differences between F_V' and F_M' (and thus k_D) can arise due to the fact that a reduction in F_V' can result not only from a decrease in F_M' (i.e., from an increase in the activity of the dissipation process) but also from an increase in F_0'. It should be emphasized, however, that these reservations against the use of F_V' or q_N apply mainly to estimates of the dissipa-

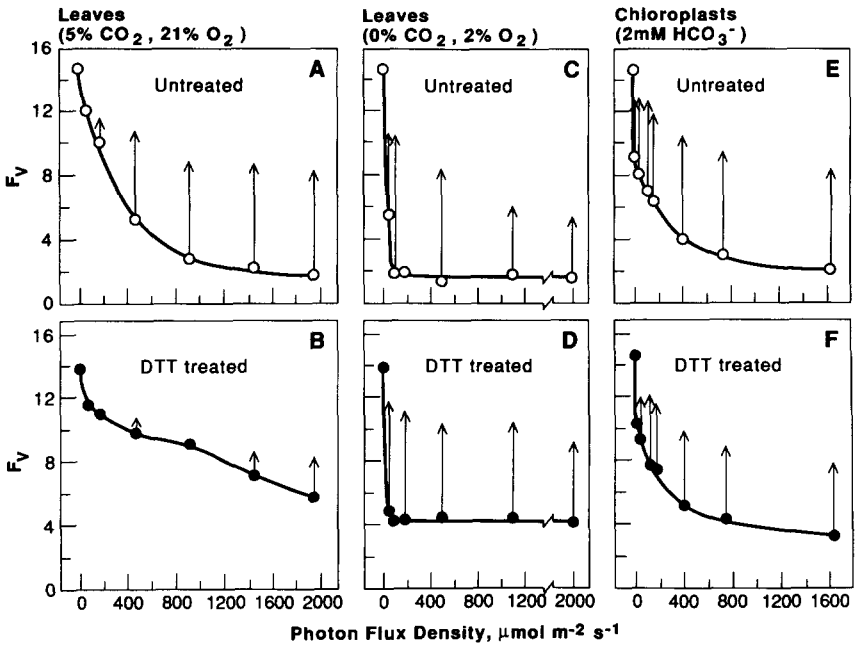

Fig. 5. Decrease in the yield of variable fluorescence at closed PS II centers during and subsequent to actinic illumination in untreated (A, C) and DTT-treated (B, D) spinach leaves and in untreated (E) and DTT-treated (F) spinach chloroplasts at various PFDs. The open and filled circles represent the variable fluorescence during actinic illumination, as calculated from maximum fluorescence (F_M') induced by a saturating pulse of light during actinic illumination minus F_0' obtained upon darkening of the leaves. The F_M' values are those which were used to calculate k_D in Fig. 4. The tips of the arrows depict the values of variable fluorescence after 5 min in far-red light. See legend of Fig. 4 for further details.

tion activity. The percentage of dissipated excitation energy appears to be described very closely by these parameters (compare e.g., Schäfer and Björkman 1989). On the other hand, it is probably just as inappropriate to express the second rapidly reversible and zeaxanthin-independent (i.e., that which is DTT-insensitive) quenching process as k_D.

In spite of these expected differences between the use of variable fluorescence and k_D to estimate the degree of radiationless energy dissipation in the photochemical apparatus, the main results are the same independent of which parameter is used: A large fraction of what has been considered 'high-energy-state quenching' is inhibited by DTT in photosynthetically active spinach leaves. In contrast, a large fraction of what has been considered 'high-energy-state quenching' in isolated spinach chloroplasts is insensitive to DTT and occurs in the absence of zeaxanthin. This, together with the fact that the DTT-sensitive fluorescence quenching is associated with F_0' quenching whereas the DTT-insensitive type is not, suggests that there are two different processes which can cause rapidly reversible fluorescence quenching under an excess of light, i.e., that 'high-energy-state quenching' as it has been defined and observed previously is likely to be composed of variable degrees of two components with a different nature.

It is noteworthy that the DTT-sensitive quenching relaxes less rapidly in 2% O_2 than in air (with 5% CO_2), whereas the reverse seems to be true for the DTT-insensitive quenching (this is most apparent from Fig. 5). We have indeed reported widely different relaxation kinetics for zeaxanthin-associated fluorescence quenching (Demmig et al. 1988, Demmig-Adams et al. 1989c). As far as the DTT-insensitive portion of fluorescence quenching is concerned, there is a rather slowly reversible zeaxanthin-independent quenching present in all systems (untreated and DTT-treated; least of all in DTT-treated leaves in 2% O_2). The rapidly reversible zeaxanthin-independent quenching occurs to a small extent at the two highest PFDs in air and to a considerable extent in 2% O_2 (Fig. 5). It remains to be seen whether a single type of zeaxanthin-independent (DTT-insensitive) quenching may also exhibit different relaxation kinetics.

We conclude that there are probably two dissipation processes within the photochemical system which are both induced by the 'high-energy state' of the thylakoid membrane, and therewith by an excess of light. One of these (presumably) occurs in the antenna chlorophyll, is associated with zeaxanthin and F_0' quenching, and is most appropriately described by an increase in k_D (Kitajima and Butler 1975) or in SV (Bilger and Björkman 1990). This is the major component of 'high-energy-state quenching' in leaves experiencing an excess of light under otherwise favorable conditions for photosynthesis or linear electron flow. Secondly, under conditions which are less favorable for photosynthesis, i.e., when there is presumably a considerable degree of cyclic electron flow, a second type of rapidly reversible 'high-energy-state quenching' develops which is not associated with zeaxanthin, is not inhibited by DTT, is not accompanied by F_0' quenching, and may reflect the dissipation process within or around the reaction center of PS II that has been suggested by several authors (Butler and Kitajima 1975, Horton and Lee 1983, Schreiber and Neubauer 1987, Weis and Berry 1987). This latter type was the predominant one present under all conditions examined by us in chloroplasts isolated from zeaxanthin-free leaves (Demmig-Adams et al. 1990c).

Under field conditions we have found the quenching of chlorophyll fluorescence during periods of high light to be largely associated with zeaxanthin in a crop plant which exhibited high rates of net CO_2 uptake (Demmig-Adams et al. 1990d). In *Arbutus unedo* in the Portuguese macchia, under conditions under which the plants experienced a combination of excessive light, high temperatures, and water stress, a combination of a massive increase in radiationless energy dissipation in the antenna complexes, associated with a massive increase in the zeaxanthin content was accompanied by one or two other quenching processes (Demmig-Adams et al. 1989a). Firstly, a quenching process which affected ambient temperature and not low temperature (77 K) chlorophyll fluorescence also developed during peak irradiation, which has been speculated to be associated e.g., with a PS II cycle and/or a decrease in water splitting activity (Demmig-Adams et al. 1989a, see also Schreiber

and Neubauer 1987). In addition, changes in the absorptive cross section of PS II and PS I (as judged from 77 K fluorescence) occurred which were detectable during the low light periods and appeared to persist (and possibly became stronger) during exposure to high light (see also Adams et al. 1989).

The suggested sites of energy dissipation within the photochemical system can be summarized as follows: when photosynthesis, and linear electron transport rates, are high and light is excessive, the zeaxanthin-associated dissipation process seems to be the predominant one (left panel of Fig. 6), at least in spinach leaves (Figs. 3–5). From the changes in fluorescence characteristics determined from leaf discs frozen to 77 K we concluded that there were increases in k_D in the antenna chlorophyll of both PS II and PS I under such conditions (Demmig and Björkman 1987). The right panel of Fig. 6 is a much more speculative attempt to summarize other reported phenomena; conditions leading to a presumed increase in cyclic electron flow also promote an additional dissipation process in spinach leaves which may occur in or around PS II. A decreased rate of electron donation from water (Schreiber and Neubauer 1987) would be compatible with cyclic electron flow and could help maintain such cyclic flow by preventing overreduction of the electron transport chain. We should like to stress that under both sets of conditions representing excessive light, the zeaxanthin-associated energy dissipation process in the antenna chlorophyll was strongly expressed.

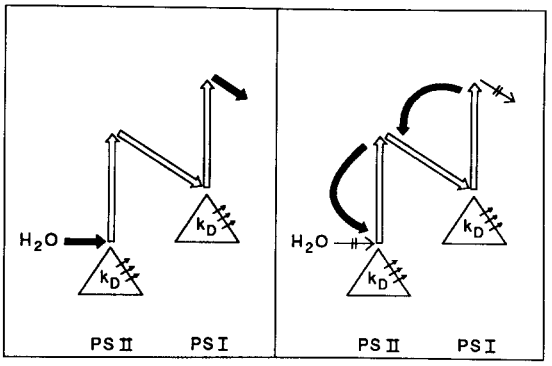

Fig. 6. Schematic diagram to illustrate potential sites for energy dissipation (radiationless energy dissipation and/or dissipative cyclic electron flow) within the photosynthetic apparatus under conditions of high rates of linear electron flow (left part) or low rates of linear electron flow, i.e., presumably largely cyclic electron flow (right part).

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References

Adams WW III, Díaz M and Winter K (1989) Diurnal changes in photochemical efficiency, the reduction state of Q, radiationless energy dissipation, and non-photochemical fluorescence quenching in cacti exposed to natural sunlight in northern Venezuela. Oecologia 80: 553–561

Adams WW III, Demmig-Adams B and Winter K (1990) Relative contributions of zeaxanthin-related and zeaxanthin-unrelated types of 'high-energy-state' quenching of chlorophyll fluorescence in spinach leaves exposed to various environmental conditions. Plant Physiol 92: 302–309

Bilger W and Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth Res 25: 173–185

Bilger W, Björkman O and Thayer SS (1989) Light-induced spectral absorbance changes in relation to photosynthesis and the epoxidation state of xanthophyll cycle components in cotton leaves. Plant Physiol 91: 542–551

Björkman O and Powles SB (1984) Inhibition of photosynthetic reactions under water stress: interaction with light level. Planta 161: 490–504

Briantais JM, Vernotte C, Picaud M and Krause GH (1979) A quantitative study of the slow decline of chlorophyll *a* fluorescence in isolated chloroplasts. Biochim Biophys Acta 548: 128–138

Butler WL (1978) Energy distribution in the photochemical apparatus of photosynthesis. Ann Rev Plant Physiol 29: 345–378

Butler WL and Kitajima M (1975) Fluorescence quenching in photosystem II of chloroplasts. Biochim Biophys Acta 376: 116–125

Demmig B and Björkman O (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77 K) and photon yield of O_2 evolution in leaves of higher plants. Planta 171: 171–184

Demmig B, Winter K, Krüger A and Czygan F-C (1987) Photoinhibition and zeaxanthin formation in intact leaves. A possible role of the xanthophyll cycle in the dissipation of excess light energy. Plant Physiol 84: 218–224

Demmig B, Winter K, Krüger A and Czygan F-C (1988) Zeaxanthin and the heat dissipation of excess light energy in *Nerium oleander* exposed to a combination of high light and water stress. Plant Physiol 87: 17–24

Demmig-Adams B (1991) Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. Biochim Biophys Acta, Reviews on Bioenergetics, in press

Demmig-Adams B, Adams WW III, Winter K, Meyer A, Schreiber U, Pereira JS, Krüger A, Czygan F-C and Lange OL (1989a) Photochemical efficiency of photosystem II, photon yield of O_2 evolution, photosynthetic capacity, and carotenoid composition during the 'midday depression' of net CO_2 uptake in *Arbutus unedo* growing in Portugal. Planta 177: 377–387

Demmig-Adams B, Winter K, Krüger A and Czygan F-C (1989b) Light response of CO_2 assimilation, dissipation of excess excitation energy, and zeaxanthin content of sun and shade leaves. Plant Physiol 90: 881–886

Demmig-Adams B, Winter K, Krüger A and Czygan F-C (1989c) Zeaxanthin and the induction and relaxation kinetics of the dissipation of excess excitation energy in leaves in 2% O_2, 0% CO_2. Plant Physiol 90: 887–893

Demmig-Adams B, Adams WW III, Czygan F-C, Schreiber U and Lange OL (1990a) Differences in the capacity for radiationless energy dissipation in green and blue-green algal lichens associated with differences in carotenoid composition. Planta 180: 582–589

Demmig-Adams B, Adams WW III, Green TGA, Czygan F-C and Lange OL (1990b) Differences in the susceptibility to light stress in two closely related organisms, one possessing and one lacking the xanthophyll cycle. Oecologia, in press

Demmig-Adams B, Adams WW III, Heber U, Neimanis S, Winter K, Krüger A, Czygan F-C, Bilger W and Björkman O (1990c) Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. Plant Physiol 92: 293–301

Demmig-Adams B, Björkman O, Bilger W, Adams WW III, Thayer SS and Shih C (1990d) Diurnal changes in zeaxanthin content and radiationless energy dissipation in leaves of field-grown cotton (*Gossypium hirsutum* L.). Physiol Plant, in press

Demmig-Adams B, Máguas C, Adams WW III, Meyer A, Kilian E and Lange OL (1990e) Effect of high light on the efficiency of photochemical energy conversion in a variety of lichen species with green and blue-green phycobionts. Planta 180: 400–409

Hager A (1980) The reversible, light-induced conversions of xanthophylls in the chloroplast. In: Czygan F-C (ed) Pigments in Plants, pp 57–79. Fischer, Stuttgart

Horton P and Hague A (1988) Studies on the induction of chlorophyll fluorescence quenching in isolated barley chloroplasts. IV. Resolution of nonphotochemical quenching. Biochim Biophys Acta 932: 107–115

Horton P and Lee P (1983) Stimulation of cyclic electron-transfer pathway around photosystem II by phosphorylation of chloroplast thylakoid proteins. FEBS Lett 162: 81–84

Kitajima M and Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim Biophys Acta 376: 105–115

Krause GH, Briantais JM and Vernotte C (1983) Characterization of chlorophyll fluorescence quenching in chloroplasts by fluorescence spectroscopy at 77 K. I. ΔpH-dependent quenching. Biochim Biophys Acta 723: 169–175

Krause GH, Vernotte C and Briantais JM (1982) Photoinduced quenching of chlorophyll fluorescence in intact chloroplasts and algae. Resolution into two components. Biochim Biophys Acta 679: 116–124

Oxborough K and Horton P (1987) Characterisation of the effects of antimycin A upon high energy state quenching of chlorophyll fluorescence (q_E) in spinach and pea chloroplasts. Photosynth Res 12: 119–128

Schäfer C and Björkman O (1989) Relationship between efficiency of photosynthetic energy conversion and chlorophyll fluorescence quenching in Upland cotton (*Gossypium hirsutum* L.). Planta 178: 367–376

Schreiber U and Bilger W (1987) Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. In: Tenhunen JD, Catarino FM, Lange OL and Oechel WC (eds) Plant Response to Stress – Functional Analysis in Mediterranean Ecosystems, pp 27–53. Berlin: Springer-Verlag

Schreiber U and Neubauer C (1987) The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination: II. Partial control by the photosystem II donor side and possible ways of interpretation. Z Naturforsch 42c: 1255–1264

Siefermann-Harms D (1977) The xanthophyll cycle in higher plants. In: Tevini M and Lichtenthaler HK (eds) Lipids and Lipid Polymers in Higher Plants, pp 218–230. Berlin: Springer-Verlag

Siefermann D and Yamamoto HY (1975) Light-induced de-epoxidation of violaxanthin in lettuce chloroplasts. IV. The effect of electron transport conditions on violaxanthin availability. Biochim Biophys Acta 387: 149–158

Stransky H and Hager A (1970) Das Carotinoidmuster und die Verbreitung des lichtinduzierten Xanthophyllcyclus in verschiedenen Algenklassen. IV. Cyanophyceae und Rhodophyceae. Arch Mikrobiol 72: 84–96

Weis E and Berry JA (1987) Quantum efficiency of photosystem II in relation to 'energy'-dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta 894: 198–208

Yamamoto HY (1979) Biochemistry of the violaxanthin cycle in higher plants. Pure Appl Chem 51: 639–648

Yamamoto HY and Kamite L (1972) The effects of dithiothreitol on violaxanthin de-epoxidation and absorbance changes in the 500-nm region. Biochim Biophys Acta 267: 538–543