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Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin

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Contents

Abbreviations: A, antheraxanthin; C, (α- and β-) carotene; Car, carotenoids; ^{3}Car*, triplet excited state of carotenoid; Chl, chlorophyll; ^{1}Chl*, singlet excited state of chlorophyll; D1/D2, heterodimer of the D1 and D2 proteins which comprises the reaction center of PS II; F_O, yield of instantaneous fluorescence at open PS II centers; F_M, maximum yield of fluorescence at closed PS II centers induced by pulses of saturating light; F'_M, F_M determined during actinic illumination; F_V ($= F_M - F_O$), yield of variable fluorescence at closed PS II centers induced by pulses of saturating light; F'_V, F_V determined during actinic illumination; ϕ_a, photon efficiency (= photon yield = quantum yield) of photosynthesis (determined at PFDs limiting to photosynthesis); ϕ_S ($= \phi'_a$), ratio of electron transport rate to photon flux density (P[ET]/PFD) or ratio of O_2 evolution rate to photon flux density (P[O_2]/PFD); ϕ_P, ϕ_S/q_P ($= \phi'_a/[1 - Q_r/Q_t]$), intrinsic photon efficiency of photosynthesis which has been corrected for the presence of closed Photosystem II centers; k_D, rate constant for radiationless energy dissipation in the chlorophyll pigment bed; k_F, rate constant for fluorescence; k_P, rate constant for photochemistry; SV, Stern-Volmer equation; k_T, rate constant for energy transfer into the PS II reaction center; LHC-II, light-harvesting chlorophyll *a*/*b*-protein complex of Photosystem II; P_{680}, reaction center chlorophyll of PS II; P(ET), rate of photosynthetic electron transport; $P(CO_2)$, rate of photosynthetic CO_2 uptake; PFD, photon flux density (400–700 nm); PS II_β, PS II lacking the peripheral light-harvesting complex; Q_A, primary quinone electron acceptor of PS II; Q_B, bound plastoquinone (secondary quinone electron acceptor of PS II); 'q_E', 'high-energy-state quenching' of chlorophyll fluorescence; 'q_I', 'photoinhibitory fluorescence quenching'; q_P, photochemical fluorescence quenching; Q_r/Q_t ($= 1 - q_P$), fraction of reduced or closed PS II centers; Tyr_Z, a tyrosine residue which reduces P^+_{680} and is in turn reduced by the water-splitting complex; V, violaxanthin; X, total xanthophylls (Z, A, V, lutein, neoxanthin); Z (= Zea), zeaxanthin

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I. Introduction

Photosynthetic organs such as green leaves are capable of operating a light-absorbing pigment system over a wide range of changes in photon flux density (PFD) including high PFDs which are potentially destructive to the system. With increasing PFD, the rate of photosynthesis initially increases linearly with PFD, i.e., most of the absorbed sunlight becomes converted into chemical energy leading to photosynthetic CO_2 fixation (Fig. 1). With further increases in PFD, however, the maximum photosynthetic capacity of the leaf is reached and a smaller and smaller fraction of the absorbed sunlight can become utilized through photosynthetic CO_2 fixation. There are recent reports showing that even crop plants with very high rates of photosynthesis cannot utilize all of full sunlight, and that the fraction of full sunlight which is in excess can be as large or larger than the fraction which is utilized in electron transport reactions (e.g. Ref. 28). As a consequence, this non-utilized excitation energy can accumulate within the photochemical system and potentially lead to a range of adverse and destructive reactions, including photooxidations. An accumulation of excitation energy will occur when the rate of absorption of photons exceeds the rate of utilization of excitation energy in photosynthetic electron transport. This can lead to a build-up of reduced electron acceptors, e.g., of Q_A in the reaction center of Photosystem II. This leads in turn to an accumulation of excited states of chlorophyll in the pigment bed and to the formation of a special excited state of chlorophyll (triplet state) which readily reacts with oxygen to give rise to singlet oxygen. Singlet oxygen is a destructive excited oxygen species causing photooxidations (Ref. 114; see also Fig. 6 below). Other reactions leading potentially to the formation of reactive oxygen species are reviewed, for example, in Ref. 14. The process of photosynthesis in our present atmosphere with 21% oxygen would not be possible without the presence of protective mechanisms which prevent such effects.

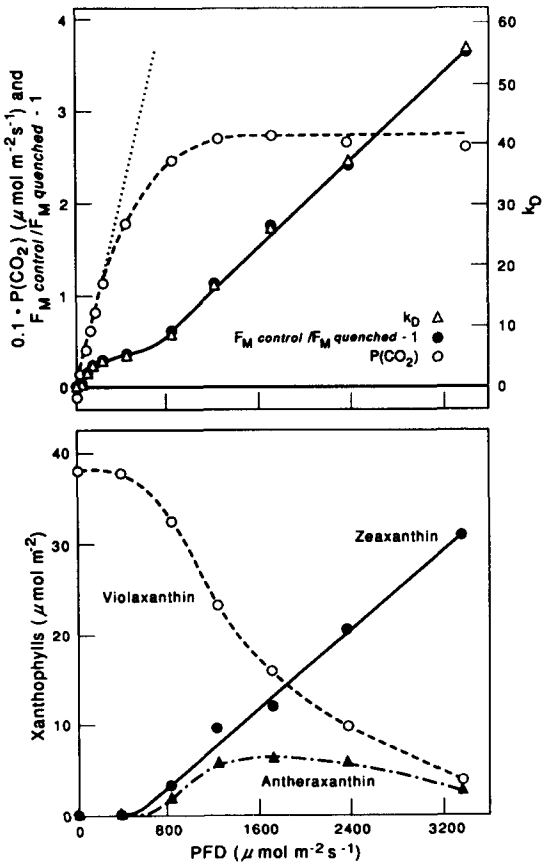

Fig. 1. Light response of (upper portion) photosynthesis [$P(CO_2)$], nonphotochemical quenching of chlorophyll fluorescence (expressed as $F_{Mcontrol}/F_{Mquenched} - 1$), and the rate constant for radiationless energy dissipation (k_D), and (lower portion) zeaxanthin, antheraxanthin, and violaxanthin levels for leaves of *Helianthus annuus*. Leaves were kept (sequentially) at each light level for 30–60 min and were maintained at 22.5–24°C. The level of k_D of 14.5 present in darkened leaves prior to illumination was subtracted from all values. The corresponding level of F_M in darkness was used as $F_{Mcontrol}$. Data from Ref. 60 and unpublished data.

In recent years, primary damage by intense light to the photochemical system itself has been claimed to occur at various sites associated with the Photosystem II reaction center including components of the primary reaction sequence between an electron donor (Tyr_Z) and the primary quinone acceptor Q_A [44,50,200], and the site of the second plastoquinone acceptor, Q_B [116,147,149]. The recent discovery that the same proteins (D1/D2) carry all of these functions (Ref. 141; for a review see Ref. 128) may explain why different authors have reported different sites to be affected. Furthermore, it has been recognized recently that damage to the reaction center of PS II and/or formation of destructive molecular oxygen species appears to be preceded by a whole suite of mechanisms which protect against such effects. These protective mechanisms seem to be rather more important than previously recognized, especially when plants become acclimated to conditions where they experience an excess of light. This review will focus on one such protective mechanism.

When light levels rise gradually over longer periods of time in the range of days, a gradual increase in the photosynthetic capacity (of electron transport and CO_2 fixation) of leaves may allow more of the additional sunlight to be utilized in photosynthetic reactions [9,23]. At the same time, the size of the light-harvesting chlorophyll pigment bed becomes smaller [9,23]. However, when light levels rise over shorter periods of time, or in cases where light levels exceed even the potential capacity of the photosynthetic reactions, a whole suite of other mechanisms, on all levels of organization, has been found to operate. Light can become excessive not only through an increase in incident PFD, but also at

constant incident PFD when plants become exposed to additional environmental stress factors which reduce their photosynthetic rates and thus their ability to utilize the absorbed sunlight.

The absorption of (excess) light can be decreased by movements occurring at different levels of organization, such as those of whole leaves [27,124,159] or chloroplasts [94]. Diversion of an excess of excitation from light which has already been absorbed is possible through electron transport leading to processes other than photosynthetic CO_2 fixation, such as a consumption of products of electron transport through photorespiration and reactions involving oxygen as an electron acceptor [109,137,142,151]. Recently, additional sites for the dissipation of an excess of excitation energy have been suggested to be located directly within the photochemical and pigment systems of Photosystem II and I [51,97,108,205]. Although PS II has been more widely studied, at least some of these processes appear to have an equivalent within PS I [51,92,145,206].

It has been proposed that dissipative processes may occur at several sites within or around the PS II reaction center. Dissipation of excitation energy has been suggested to occur via a back reaction within the reaction centers of PS II (and PS I) leading to a recombination after the initial charge separation [205,206]. This means that the photosynthetic reaction centers would be converted into dissipating traps that can accept and dissipate excitation energy as heat but have a low photochemical yield [205]. Furthermore a cyclic and thereby dissipative electron transfer process around PS II has been suggested to occur [48,72,99,100,106,152,165,175, 176] which may involve cytochrome *b*-559 [98,141,202, 207]. Alternatively or in addition, the chlorophyll pigment bed is thought to be a site where photoprotective responses can take place. As far as a decrease of the transfer of (excess) excitation energy from the chlorophyll pigment bed into the PS II reaction center is concerned, a decrease in the size of the light-harvesting pigment system of PS II is possible, even in the short-term, leading to a decrease in the absorptive cross-section of PS II [13,131,132,198]. While some conditions (elevated temperatures [197,198]) cause a spontaneous dissociation of the peripheral LHC-II, the changes in PS II absorptive cross-section under other conditions are related to a dissociation of peripheral LHC-II caused by phosphorylation [9,11,18,31,119]. It seems, however, that phosphorylation of LHC-II does not commonly occur when light is in excess [53,73,101,153]. Furthermore, a diversion of massive amounts of excitation energy away from the photochemical reaction centers has been suggested to occur within the pigment complexes associated with PS II and PS I, i.e., in the chlorophyll pigment bed in the form of a radiationless dissipation of excitation energy [24,51,97,165,166]. This dissipation process within the pigment bed is thought to involve the carotenoid zeaxanthin (e.g., Ref. 54, 56, 63) and will be the focus of this review.

Even if the above cascade of alternative pathways for the utilization of electron transport products and for the dissipation and diversion of excitation energy cannot fully prevent the formation of toxic excited molecular species of oxygen, such as singlet oxygen and others, further protective systems exist which can inactivate such species once formed. There are several systems present in the chloroplast which deactivate singlet oxygen or other reactive oxygen species. These antioxidants include enzymatic systems such as catalase, superoxide reductase, and glutathione reductase, and furthermore phenolic compounds, ascorbic acid, and carotenoids [14,114,118]. Carotenoids have been found to react with singlet oxygen (e.g., Ref. 74) and/or prevent its formation through reacting with excited triplet chlorophyll (Refs. 114, 127, 129; see also Fig. 6 below). Previously, the photoprotective function of carotenoids had been related entirely to these reactions (e.g., Ref. 114). In this article, I will arrive at the conclusion that the function of carotenoids is not restricted to these mechanisms, and that one particular carotenoid, zeaxanthin, has a different and specific function in the photoprotection of the photochemical and therefore of the photosynthetic system.

It should be pointed out that the discussion here centers on a direct involvement of the molecule zeaxanthin in energy dissipation, and not on the conversions between zeaxanthin and its precursors in the so-called xanthophyll cycle as such. It had previously been suggested that the operation of the xanthophyll cycle, which consumes, among other substrates, NADPH, might facilitate the induction of photosynthetic electron transport at the onset of illumination by decreasing the NADPH level [88]. However, the contribution of the xanthophyll cycle to 'energy dissipation' through linear electron transport is probably insignificant and is a very different mechanism from that suggested here, which is a massive radiationless dissipation of excess excitation energy within the chlorophyll pigment bed.

Evidence for an involvement of the xanthophyll zeaxanthin in radiationless energy dissipation in the chlorophyll pigment bed is summarized under II (A-E), and discussed together with possible reservations against such a conclusion. Evidence is presented to suggest that this zeaxanthin-associated energy dissipation process can relax with very different time constants, depending on plant species and the conditions to which leaves are exposed. Thus zeaxanthin-associated energy dissipation is suggested to be responsible for part of a slowly reversible type of chlorophyll fluorescence quenching as well as for part of a rapidly relaxing type of chlorophyll fluorescence quenching which is observed at a "high-energy state" of the photosynthetic membrane. An additional activation mechanism for the zeaxanthin-asso-

ciated energy dissipation process through this 'high-energy state' is discussed (subsection III-F, see also II-C and -D).

Furthermore, suggestions are made as to how further phenomena related to chlorophyll fluorescence changes and changes in the photon efficiency of photosynthesis, which are currently discussed in the literature, may relate to the zeaxanthin-associated phenomena, and at which point 'photoinhibitory damage' might occur (subsection III-G).

Lastly, the acclimation of the photosynthetic apparatus to full sunlight is examined with respect to carotenoid composition (subsection IV-A). Futhermore, the apparent relative importance of regulatory versus damaging effects, and the relative importance of the zeaxanthin-associated versus other dissipation processes under natural conditions in the field, is discussed based on the data available to date (subsection IV-B).

II. High irradiance stress and the efficiency of photochemical energy conversion

At light levels which are limiting to the process of photosynthesis, the efficiency of photosynthetic energy conversion is remarkably high [3,26,29,71,133,204]. In a survey of C_3 plants from widely diverse taxa and life forms, the photon efficiency of O_2 evolution was found to be quite constant and close to the theoretical value (0.106 ± 0.001 mol O_2 per mol absorbed photons), and the photon efficiency of PS II (F_V/F_M) was 0.832 ± 0.004 [26]. These plants included individuals growing in the shade and in the sun. The latter were collected from sites where they experienced favorable conditions and were harvested in the morning prior to exposure to direct sunlight. Thus, sun and shade leaves can have the same high efficiency of photosynthetic energy conversion on the basis of absorbed light. However, upon exposure of photosynthetic systems to light levels exceeding those which can be utilized for photosynthesis, the intrinsic efficiency with which light becomes utilized for photosynthesis decreases. Any sustained decreases in this efficiency have been termed 'photoinhibition' of photosynthesis [10,105,158].

Whereas it had long been recognized that such a decreased photon efficiency may be the result of damaging effects to the photochemical apparatus, it has only recently been recognized that a decreased photon efficiency can also be the result of regulated processes leading to the dissipation of excess excitation energy within the photochemical apparatus (Refs. 51, 97, 205; see also Ref. 108). Consequently, the observed decrease in the efficiency of photosynthetic energy conversion caused by high light, often followed by recovery upon return to low light, is apparently caused by damaging processes followed by repair in some cases, and by increased energy dissipation followed by a reversal of this increase in other cases, or by a combination of the two. It is less clear how these two contrasting possibilities can be distinguished. The rapidity of the recovery process can be helpful for the interpretation of the underlying causes of depressions in photochemical efficiency in extreme cases. However, it must be noted that the rapidity of the reversal of an increase in energy dissipation has been shown to be highly variable, from as fast as minutes [111,113] to as long as days [1,4–6,24,30,55]. Similar differences may exist for the rapidity of repair processes.

Inhibition of the recovery process by protein synthesis inhibitors [83,123,148,170], indicating the involvement of repair processes, can assist in this distinction between 'photoinhibitory damage' and photoprotective dissipation processes. Another approach that has been employed is the interpretation of changes in the characteristics of chlorophyll fluorescence, the latter of which seem to be distinct for the different changes within the photochemical apparatus. In particular, the bipartite model of the photochemical apparatus, developed by Butler and co-workers [40,104], has been used for such analyses [24,25,51,146]. The yield of instantaneous chlorophyll fluorescence emission at open PS II centers, F_O, has been shown to exhibit changes in opposite directions, i.e., increases or decreases, which are associated with different processes. In the Butler model, a decrease in F_O results from an increase in the rate constant for radiationless energy dissipation in the chlorophyll pigment bed [40,104]. Therefore, net decreases in F_O [6, 24,25,30,51,60,205] have been associated with energy dissipation processes, particularly with a dissipation process within the chlorophyll pigment bed [6,24, 25,30,51,60]. An increase in F_O, according to the Butler model, can result, for example, from a decrease in the rate constant for photochemistry (k_P) or a decrease in the rate constant for energy transfer to the PS II center (k_T) [40,104]. Sustained increases in F_O [51,66] have been suggested to be associated with 'photoinhibitory damage'. Cases in which increases in F_O have been found to be reversed relatively rapidly (e.g., Ref. 190) still await interpretation. Cases in which no change in F_O is observed may result from a combination of the above dissipation process in the chlorophyll pigment bed and 'photoinhibitory damage'. However, processes (detectable primarily with ambient temperature chlorophyll fluorescence; [59]) which do decrease maximum fluorescence emission at closed PS II centers, F_M, but not F_O have also been inferred and related to a decrease in the activity of the water-splitting enzyme complex and perhaps a concomitant cyclic electron transfer within or around PS II (e.g., Ref. 175) (see subsection III-G).

It seems that the nature of the decreases in the efficiency of photosynthetic energy conversion which are caused by exposure to an excess of light is different

in organisms which have developed under high irradiances versus those which have developed under low irradiances (see Ref. 24, 51). Sun leaves not only possess a higher capacity for electron transport and CO_2 fixation than shade leaves, but also appear to possess a greater capacity for dissipative processes in the photochemical apparatus (Ref. 51; see also subsection IV-B) as well as, possibly, higher rates of repair of 'photoinhibitory damage' (cf. Ref. 169). In subsections III-E and -IV, the changes in chlorophyll fluorescence characteristics together with changes in the carotenoid composition will be used to distinguish between damage to and regulatory changes within the photochemical apparatus.

III. The relationship between zeaxanthin, the dissipation of excess energy, and photoprotection

III-A. Correlation between the activity of a radiationless energy dissipation process in leaves and their zeaxanthin content

Several studies aimed at identifying the underlying causes for decreases in the efficiency of photosynthetic energy conversion, which were reversible within hours to days in sun leaves, reported the quenching of chlorophyll fluorescence from PS II (and PS I) which is indicative of radiationless energy dissipation in the chlorophyll pigment bed [2,5,6,24,25,30,51,59]. This type of fluorescence quenching decreased both instantaneous fluorescence, F_O, and maximum fluorescence, F_M, to the extent [25,30,60] predicted from the Butler model for an increase in the rate constant k_D for energy dissipation in the chlorophyll pigment bed [104]. For an increase in k_D, a 50% decrease in F_M would be accompanied by an approx. 15% decrease in F_O. Such changes in fluorescence yield which were also accompanied by changes in the photon efficiency of photosynthesis were thus interpreted to be indicative of a regulatory process, i.e., the dissipation of an excess of excitation energy. Initially this process was thought to be responsible only for fluorescence quenching which relaxed within no less than 30 min and up to days [51,54,55].

After these initial studies, however, the same type of fluorescence quenching decreasing both F_O and F_M to the extent expected from an increase in k_D was also found to be responsible for a rapidly developing and relaxing quenching of chlorophyll fluorescence present in leaves during illumination with an excess of light [6,20,52,67,166,205]. The latter type of fluorescence quenching typically relaxes within a few minutes upon return of leaves to darkness or low light, and is currently referred to as 'pH-dependent', 'energy-dependent' or 'high-energy-state quenching', q_E, in analogy to a rapidly relaxing quenching process in chloroplasts [107,210]. It was proposed that the energy dissipation process in the chlorophyll pigment bed also causes this rapidly relaxing fluorescence quenching in leaves under certain conditions [20,52,60,67,76,97,144,165,166]. Thus, this type of chlorophyll fluorescence quenching in leaves (decreasing both F_M and F_O) reflects a dissipation process which can apparently persist for very different lengths of time subsequent to the return of leaves to limiting light, depending on the conditions prevailing during the high light exposure and possibly depending on the plant species involved.

For this type of chlorophyll fluorescence quenching a correlation was found between the calculated activity of a radiationless energy dissipation process and the content of the carotenoid zeaxanthin of these leaves (e.g., Ref. 54, 56, 63) (Fig. 1). Such a correlation was found to exist between these two parameters in cases where fluorescence quenching was reversible within minutes [60,68] (Fig. 1), in cases where fluorescence quenching relaxed within hours [54], as well as in cases where fluorescence quenching persisted for days [55]. The estimation of the dissipation activity was based either on the rate constant, k_D, for radiationless energy dissipation in the chlorophyll pigment bed after Kitajima and Butler (Ref. 104; see also Refs. 24, 25, 60) or on the Stern-Volmer equation, as suggested by W. Bilger (see Ref. 20), resulting in the same correlation with the zeaxanthin content of the leaves in both cases (Fig. 1). In fact, the Stern-Volmer equation is very similar to the form of the Butler model which was used.

Stern-Volmer equation: $SV = F_{Mcontrol}/F_{Mquenched} - 1$

Kitajima and Butler: $k_D = F_{Mcontrol}/(F_{Mquenched} \times 0.074) - 1$

From $F_M = k_F/(k_F + k_D)$;
with $k_F = 1$ and $k_D = 12.5$ in a control leaf
(with $F_V/F_M = 0.855$; [60]); therefore
the maximum possible absolute value
of F_M is $1/(1+12.5) = 0.074$.
Thus $k_D = 1/F_M - 1$
$= (1/[F_{Mquenched}/F_{Mcontrol}] \times 0.074) - 1$
or $k_D = F_{Mcontrol}/(F_{Mquenched} \times 0.074) - 1$

Therefore, the conclusion that the zeaxanthin content of leaves exhibits a correlation with the activity of this energy dissipation process is independent of the model by Kitajima and Butler [104] and any assumptions made within it. The observation of such a correlation led to the proposal that zeaxanthin may be causally involved in this dissipation process in the chlorophyll pigment bed (e.g., Ref. 54, 56, 63). Reservations against this suggestion, as well as recent evidence for a causal relationship, are discussed under subsections III-C–E.

III-B. Formation and removal of zeaxanthin via the xanthophyll cycle

Most photosynthetic organisms exhibit rapid, light-dependent, and reversible conversions of one

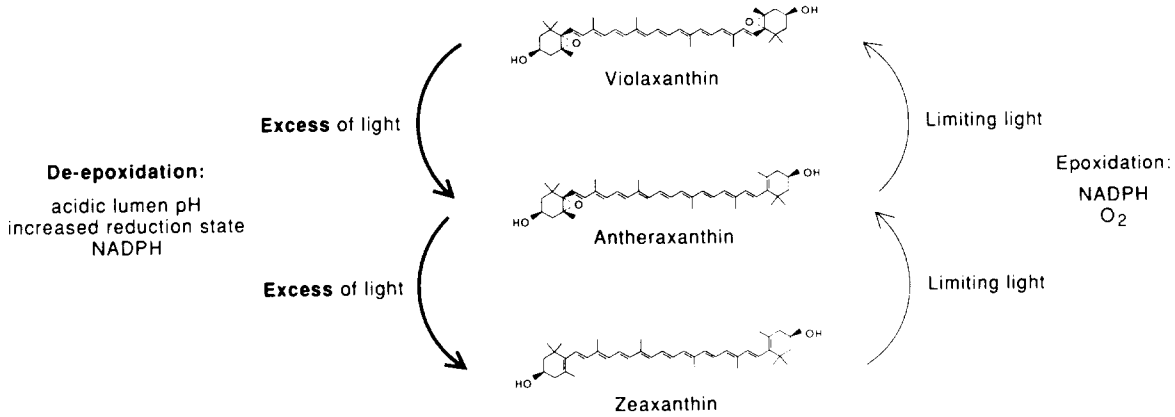

Fig. 2. The xanthophyll cycle involving the de-epoxidation of violaxanthin to zeaxanthin via antheraxanthin and the epoxidation of zeaxanthin to violaxanthin via antheraxanthin.

xanthophyll into another within the photosynthetic membrane. Those reactions are referred to as a 'xanthophyll cycle' (Fig. 2). However, even those few groups of photosynthetic algae which do not possess a xanthophyll cycle can still form zeaxanthin slowly. In this article it is proposed that zeaxanthin acts as a competitor for excitation energy. Since an increase in radiationless energy dissipation (which we suggest to be associated with zeaxanthin) results in a decrease in photochemical efficiency [24,25,51], the capacity for a rapid removal (or deactivation) of this competitor for excitation energy facilitates a rapid increase in the efficiency of photochemical energy conversion upon return to conditions under which light is no longer excessive. The potential presence of zeaxanthin in the more primitive organisms, which do not possess a xanthophyll cycle, suggests that the ability to form zeaxanthin evolved before an epoxide cycle was acquired. One may speculate that the epoxide cycle evolved subsequently to allow the rapid removal of zeaxanthin as well as its rapid re-accumulation.

All photosynthetic organisms from green and brown algae to higher plants possess the 'xanthophyll cycle' or 'violaxanthin cycle' involving the di-epoxide violaxanthin [a], a monoepoxide antheraxanthin [b], and the epoxide-free form zeaxanthin [c] (Figs. 1 and 2) [88,89,171,184,212]. Six other classes of algae possess another xanthophyll cycle, consisting of two components, the monoepoxide diadinoxanthin and an epoxide-free form diatoxanthin, in which one half of the molecule has the same structure as (half of) the zeaxanthin molecule [89,193]. There are only few algal classes in which no xanthophyll cycle is present, the blue-green algae, the red algae, and the Cryptophyceae [89,194]. In red algae some authors find no epoxides [194], whereas one survey reports the consistent presence of epoxides in one genus of red algae [39]. However, even in these groups which do not possess a xanthophyll cycle, zeaxanthin is frequently present and can be formed slowly, as will be discussed later (see subsection III-D). In both blue-green [95] and red algae [39,194] zeaxanthin contents range between zero to a major fraction of the total carotenoid content. Thus the ability to synthesize zeaxanthin (or diatoxanthin) is rather ubiquitous among aerobic photosynthetic organisms.

In point of fact, we do not know whether only zeaxanthin, or perhaps also antheraxanthin, is involved in energy dissipation. If diatoxanthin has a function similar to that of the suggested function of zeaxanthin, one might conclude that 'half of a zeaxanthin molecule' was sufficient to comply with this function. And antheraxanthin, of course, contains 'half of a zeaxanthin molecule'. Whether or not antheraxanthin has a function similar to that of zeaxanthin cannot clearly be determined from the data accumulated to date; in most instances where correlations between zeaxanthin and chlorophyll fluorescence quenching have been reported, antheraxanthin levels were rather low (e.g., Ref. 54), and the zeaxanthin level alone gave a good correlation. However, in cotton plants in the field, higher antheraxanthin (and violaxanthin) levels were observed and a contribution of antheraxanthin to energy dissipation cannot be ruled out [68].

In the xanthophyll cycle, zeaxanthin is formed through de-epoxidation of violaxanthin via antheraxanthin in an enzymatic reaction catalyzed by a de-epoxidase [89,212] (Fig. 2). There is also a second enzyme, an epoxidase, which reconverts zeaxanthin to antheraxanthin and violaxanthin. The de-epoxidase has a pH-optimum at pH 5.2 [87,90], whereas the epoxidase exhibits maximum activity at pH 7.5 [88,181]. Since

[a] [(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5,6,5′,6′-Diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol].
[b] [(3*S*,5*R*,6*S*,3′*R*)-5,6-Epoxy-5,6-dihydro-β,β-carotene-3,3′-diol].
[c] [(3*R*,3′*R*)-β,β-Carotene-3,3′-diol].

both enzymes are active in the light in vivo it was suggested that the de-epoxidase is located at the inner side of the thylakoid membrane facing the (acidic) lumen and the epoxidase at the outer side facing the (alkaline) stroma [89,184,212]. This model still awaits experimental confirmation. Factors involved in the regulation of zeaxanthin formation in addition to pH are (a) the availability of violaxanthin for the de-epoxidase [185], which seems to depend on the redox reactions between Photosystem I and II [180], and (b) ascorbate which appears to act as an endogenous reductant for the de-epoxidation if it is reduced by PS I in the sequence NADPH-glutathione-ascorbate [178,179], although another electron carrier may be involved after ascorbate [212]. Furthermore, the epoxidase requires O_2 and NADPH as co-substrates [181,182].

These regulating parameters are mostly measures of the balance between the rate of photon absorption and the rate of photochemistry or electron transport, i.e., they are indicators of whether or not light is 'in excess'. Thus the control of the xanthophyll cycle, and therefore of zeaxanthin accumulation, exhibits all of the features that are required for the regulation of a process which diverts excitation energy away from the photosynthetic reaction centers. At low PFD in the range limiting for photosynthesis, no zeaxanthin is accumulated [60,184] (Fig. 1), whereas at PFDs beyond the limiting range, a linear increase in the zeaxanthin content of leaves with increasing PFD is observed over a wide range of PFDs [60,184] (Fig. 1).

Concerning the kinetics involved, half-times of zeaxanthin formation of 5–10 min had initially been reported for spinach leaves [86,89]. Recently, half-times of 1.5 min were reported for zeaxanthin formation in leaves of various species [20,22], including spinach [67]. The epoxidation process takes longer than the de-epoxidation [22,86,89]. Removal of zeaxanthin can actually occur extremely slowly such as in water-stressed *Nerium oleander* leaves [55]. Furthermore, subsequent to repeated exposures to a mild excess of light, zeaxanthin apparently also becomes removed increasingly more slowly [61]. It is unknown whether the rate of epoxidation per se is low under these various conditions, or whether zeaxanthin may become inaccessible to the epoxidase.

The localization of the three xanthophylls of the xanthophyll cycle within the thylakoid membrane is also uncertain. With the isolation methods available to date, a considerable portion of these xanthophylls is found in the free pigment band [e.g. 186], which is probably due to a release of the pigments during preparation. In some studies, the view is presented that xanthophylls are generally found preferentially in the light-harvesting chlorophyll *a/b* complexes [186]. However, violaxanthin and zeaxanthin were also reported to be present in all chlorophyll-protein complexes to some degree [161,185]. Furthermore, it has been reported that the xanthophyll cycle components were present in both grana and stroma thylakoids [183]. The fact that sun leaves have much higher total pools of the xanthophyll cycle components than shade leaves (Ref. 59,199; see subsection IV-A) also suggests that the components of the xanthophyll cycle may be located in all chlorophyll-binding complexes, i.e., also in the chlorophyll *a* 'core' antenna complexes and not only in the light-harvesting chlorophyll *a/b* complexes, since the size of the latter ones decreases strongly with increasing growth PFD [9,23]. In this review the whole of these chlorophyll-binding complexes are referred to as the chlorophyll pigment bed. It is noteworthy that the characteristics of the zeaxanthin-associated type of chlorophyll fluorescence quenching also suggest that radiationless energy dissipation occurs in both the PS II and the PS I chlorophyll-binding complexes [51].

There are indications that zeaxanthin can also be epoxidized to violaxanthin in the chloroplast envelope [46], followed by a transfer of violaxanthin to the thylakoid membrane [187]. This second zeaxanthin epoxidase in the chloroplast envelope apparently possesses different co-substrate requirements from that in the thylakoid membranes [47]. The violaxanthin de-epoxidase, however, has been found exclusively in the thylakoid membranes [187]. It is controversial whether the amounts of carotenoid present in the envelope are significant [78,85,92,162,187,191]. Nevertheless, it seems that in the biosynthetic pathway leading to the accumulation of the xanthophyll cycle components, zeaxanthin, and not violaxanthin, is formed first [192] (see also subsection III-D).

III-C. The effect of an inhibitor of zeaxanthin formation on radiationless energy dissipation

In this and the following sections comparative studies between zeaxanthin-free and zeaxanthin-forming or -containing photosynthetic organisms are discussed (see Table I). These studies use either an inhibitor of zeaxanthin formation in the xanthophyll cycle, low temperatures to slow down zeaxanthin formation, or organisms which naturally lack the xanthophyll cycle. In spite of the fact that the formation of zeaxanthin was prevented by these very different means, the response of all zeaxanthin-free organisms was similar and disparate from that of the zeaxanthin-containing organisms (Table I).

The correlations between the zeaxanthin content of leaves and radiationless energy dissipation were described under III-A, and led to the suggestion of an involvement of zeaxanthin in the radiationless dissipation process within the chlorophyll pigment bed. One reservation against this suggestion is that the presence of zeaxanthin alone in limiting light or darkness did not

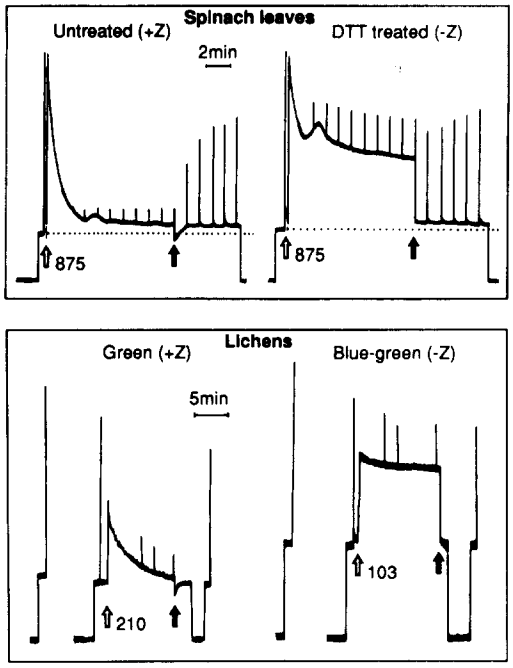

Fig. 3. Original traces of chlorophyll fluorescence from spinach leaves (upper portion) and thalli of the zeaxanthin-forming green algal lichen *Pseudocyphellaria rufovirescens* and the zeaxanthin-free blue-green algal lichen *Pseudocyphellaria dissimilis* (lower portion). Leaves were either untreated, zeaxanthin-forming or DTT-pretreated and zeaxanthin-free. Leaves or thalli were exposed to the PFDs which are indicated in the graphs. After 10 min the actinic light was switched off and chlorophyll fluorescence recorded in the presence of far-red light. Leaves or thalli were kept at 5% CO_2 at 25°C or 15°C, respectively. Leaf data from Ref. 67 and lichen data from Ref. 65.

necessarily result in fluorescence quenching [22,61,63,157]. Both the presence of zeaxanthin and the presence of a 'high-energy state' of the photosynthetic membrane, induced by an excess of light, appeared to be required in the case of the rapidly relaxing fluorescence quenching [60,61]. Furthermore, the chlorophyll fluorescence quenching present in leaves under illumination with an excess of light had also been thought to be the same as that characterized in isolated chloroplasts under such conditions, the so-called 'pH-dependent quenching' or 'high-energy-state quenching' [36,113,210]. In addition, this latter type of fluorescence quenching had been related to processes in the PS II reaction center [205,206] and on the PS II donor side [15,34,175], but has also been suggested to occur in the chlorophyll-binding complexes [97,165,166].

Bilger and Björkman [20,22] reported that an inhibitor of the violaxanthin de-epoxidase, the sulfhydryl reagent dithiothreitol [156,214], could be administered through the cut petiole of leaves. In DTT-treated leaves, zeaxanthin formation was completely inhibited and the treated leaves showed the same rates of O_2 evolution as control leaves [22]. DTT inhibited a large portion (*Hedera canariensis* [20]) to virtually all (spinach [67]) of the rapidly reversible component of (nonphotochemical) chlorophyll fluorescence quenching in leaves kept at saturating CO_2, and completely abolished the quenching of F_O in all leaves. An example of the original traces of chlorophyll fluorescence for untreated and DTT-treated leaves is shown in Fig. 3. The corresponding calculated values of $F_{M control}/F_{M quenched} - 1$ for a range of PFDs for these two groups of leaves are shown in Fig. 4. When this DTT-sensitive component of chlorophyll fluorescence quenching at various PFDs was plotted against the zeaxanthin content of untreated control leaves at these PFDs, a very close correlation between these two parameters was obtained [67]. Furthermore, the zeaxanthin-containing leaves exhibited a much lower reduction state of the PS II centers at a given degree of excessive light than the zeaxanthin-free DTT-treated leaves [20,22,67] (see also Fig. 3). The reduction state of Q_A, the primary quinone electron

TABLE I

Ability of various organisms to form zeaxanthin rapidly and to exhibit quenching of F_M and F_O (indicative of radiationless energy dissipation in the pigment bed) upon exposure to an excess of light, and their tolerance to high light stress

A high tolerance to light stress is assigned to organisms which do not experience sustained depressions in the efficiency of photochemical/photosynthetic energy conversion of the type which are indicative of 'photoinhibitory damage'. In the cases marked with * zeaxanthin was present prior to the treatment with an excess of light. n.d., not determined.

	Green leaves				Lichens			
	Control	+ Inhibitor (DTT)	Chilling	Chilling	Green	Green (DTT)	Blue-green	Blue-green
Zeaxanthin present * or formed rapidly	yes	no	no	yes *	yes	no	no	yes
Radiationless energy dissipation (F_M and F_O quenching)	yes	no	n.d.	n.d.	yes	no	no	yes
Reduction state of PS II	low	high	n.d.	n.d.	low	high	high	low
Tolerance to high light stress	high	low	low	high	high	low	low	high
References	7,20,22,57,58,67		57,62		57,58,65,66,69			57,69

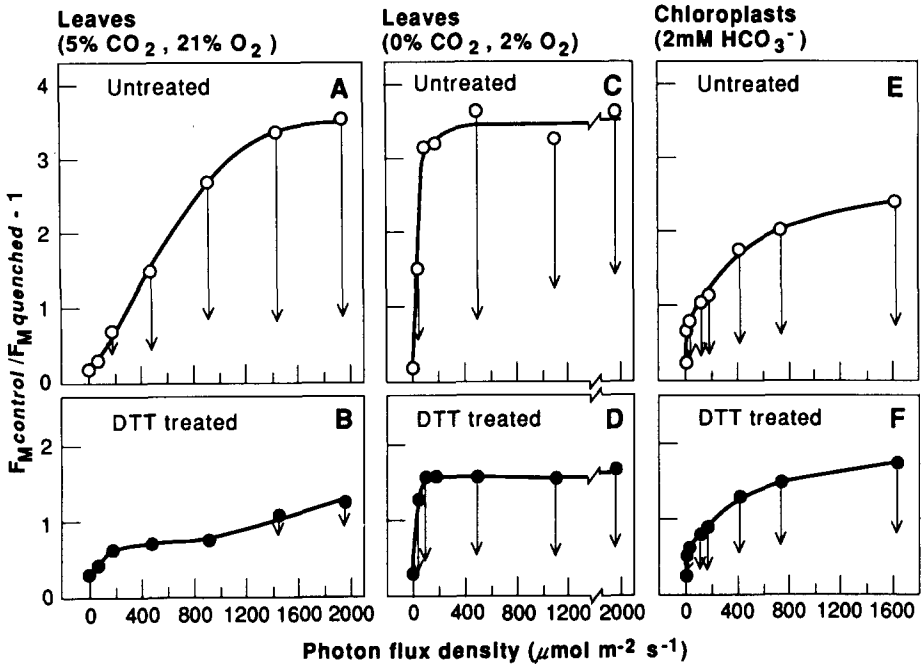

Fig. 4. Nonphotochemical quenching of chlorophyll fluorescence (expressed as $F_{Mcontrol}/F_{Mquenched} - 1$) for untreated and DTT-treated spinach leaves and intact chloroplasts isolated from these leaves. The tips of the arrows depict values of $F_{Mcontrol}/F_{Mquenched} - 1$ after return to darkness (or rather far-red light) for 5 min. $F_{Mcontrol}$ in this case is a calculated value extrapolated for a value of $F_V/F_M = 0.855$ prior to illumination. Leaves were illuminated for 10 min at 25°C and in the presence of either 5% CO_2 and 21% O_2 (A,B) or 2% O_2 without CO_2 (C,D). Intact 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 illumination and did not receive the standard illumination preceding chloroplast isolation [67]. Data points represent different individual leaves. Modified after Ref. 58; data from Refs. 8 and 67.

acceptor of PS II, reflects the balance between the rate of excitation energy transfer into PS II centers and the rate of electron transport beyond PS II. Healthy plants in the field have been found to operate at a reduction state of PS II as low as 20–30% (Ref. 6; see also Ref. 205). A high reduction state of PS II centers is indicative of overexcitation of these centers and is thought to result in 'photoinhibitory damage' over longer periods of time. The fact that DTT-treated, zeaxanthin-free leaves had an approx. 30% higher reduction state of PS II centers than untreated zeaxanthin-containing leaves at various PFDs beyond the limiting range indicates that a considerably higher amount of excessive and potentially destructive excitation energy reaches the PS II centers in the DTT-treated zeaxanthin-free leaves. Furthermore, there was a difference in the tolerance to high light stress between zeaxanthin-containing and DTT-treated, zeaxanthin-free leaves. The zeaxanthin-free leaves were more susceptible to sustained decreases in the efficiency of photosynthetic energy conversion (see subsection III-E). All of these results are consistent with an involvement of zeaxanthin in energy dissipation.

Another reservation against such a conclusion is that the reductant DTT may inhibit another type of chlorophyll fluorescence quenching, particularly one related to dissipation processes in the PS II centers and involving redox reactions. However, this does not seem to be the case. The presence of DTT did not inhibit a large component of the rapidly relaxing chlorophyll fluorescence quenching in isolated spinach chloroplasts (Fig. 4), which could in turn be abolished through the addition of an uncoupler. These chloroplasts were isolated from zeaxanthin-free leaves (with the exclusion of any pre-illumination directly before isolation). Adams et al. [7] reported that DTT also did not inhibit a component of rapid chlorophyll fluorescence quenching in spinach leaves kept in a gas stream with no CO_2 and 2% O_2 (Fig. 4); conditions under which these leaves probably have high ratios of cyclic to linear electron transport. Under these conditions, spinach leaves therefore exhibited one DTT-sensitive (with F_O quenching) and one DTT-insensitive (no F_O quenching) component of rapidly relaxing fluorescence quenching. That one component of rapid fluorescence quenching in leaves was associated with F_O quenching and a second one was not was also concluded by Bilger and Schreiber [21] in a study on *Arbutus unedo*. In all of these studies, F_O quenching occurred as a net decrease in the F_O value determined immediately after illumination relative to the pre-illumination level.

However, Rees et al. [166] report some type of F_O quenching to be associated with a zeaxanthin-unrelated component of fluorescence quenching as well. In this

case, however, there was no net decrease in F_O below the pre-illumination level. Instead, an increase in F_O subsequent to the transfer from light to dark was used to estimate the magnitude of an assumed decrease in F_O. In the study by Demmig-Adams et al. [67] on isolated spinach chloroplasts, DTT did inhibit a small component of rapidly reversible fluorescence quenching which was that component associated with net F_O quenching (as was also the case for spinach leaves). Thus, these isolated spinach chloroplasts also exhibited one DTT-sensitive (with net F_O quenching) and one DTT-insensitive component (no F_O quenching). It is noteworthy that, at low PFD, the addition of another reductant, ascorbate, to chloroplast suspensions increased their zeaxanthin content (Ref. 67; cf. also Refs. 179, 184) as well as the magnitude of the type of fluorescence quenching which was associated with F_O quenching and inhibited by DTT [67]. It is important to mention that a decrease in F_O is only detectable when the quenching of F_M (or F_V) is pronounced (see Ref. 104). At levels of quenching of variable fluorescence of 60–70% for the DTT-sensitive component, the quenching of F_O was 20–30% [67], as expected from Ref. 104. At the same level of quenching of variable fluorescence for the DTT-insensitive component there was no quenching of F_O subsequent to illumination relative to the pre-illumination level [7,67].

I conclude that there are indeed two presumably dissipative processes which can lead to rapidly relaxing chlorophyll fluorescence quenching under illumination with an excess of light. One which was not related to zeaxanthin, not accompanied by a net F_O quenching, and DTT-insensitive was responsible for a large part of the rapidly reversible fluorescence quenching in isolated spinach chloroplasts but contributed only little to the rapidly relaxing fluorescence quenching in photosynthetically active spinach leaves [7] (Fig. 4 and Table II). The other dissipation process exhibited the correlation with zeaxanthin content, was accompanied by F_O quenching (below the pre-illumination level), was inhibited by DTT, and was responsible for the majority of chlorophyll fluorescence quenching in spinach leaves which showed high rates of photosynthesis (and presumably largely linear electron transport) in saturating CO_2 and 21% O_2 [58,67] (Figs. 3 and 4 and Table II). The inhibition of both zeaxanthin formation and the type of chlorophyll fluorescence quenching associated with F_O quenching – but not other types of fluorescence

TABLE II

Characteristics of the presumed dissipation process in the pigment bed (A) versus other dissipative processes (B) in the photochemical apparatus

Type of fluorescence quenching	Quenching of F_O below pre-illumination value	ϕ_P or ϕ_a decreased in proportion or more than F_V/F_M	Particularly pronounced under (conditions)	References
(A) Energy dissipation in the chlorophyll pigment bed				
Fluorescence quenching expressed proportionally at 77 K and ambient temperature	yes	in proportion		8,51
Slowly reversible zeaxanthin-associated fluorescence quenching (Dissipation activity correlated with the zeaxanthin content)	yes	in proportion	water stress	24,55
Rapidly reversible zeaxanthin-associated fluorescence quenching; part of 'q_E' (Dissipation activity correlated with the zeaxanthin content)	yes	in proportion	steady-state photosynthesis (5% CO_2, 21% O_2)	60,67 Fig. 7
(B) Presumed energy dissipation associated with PS II center possibly involving the donor side				
Rapidly reversible zeaxanthin-unrelated fluorescence quenching (in DTT-treated leaves); part of 'q_E'	no	more	induction of photosynthesis elevated temperatures no CO_2, 2% O_2	7 Fig. 7
Fluorescence quenching detectable only at ambient temperature	no	more		8,59
Putative PS II cycle/back reaction	no	more or ϕ_P exclusively	induction of photosynthesis i.e., at lower lumen pH than dissipation within the chlorophyll pigment bed	97,143, 144,166
Putative inactivation of donor side	no	more	low lumen pH elevated temperature	45,173, 175,211,216

quenching – supports the proposal that this dissipation process is causally related to zeaxanthin and occurs in the chlorophyll pigment bed.

Because the type of chlorophyll fluorescence quenching which is associated with F_O quenching and correlated with the zeaxanthin content can exhibit widely different relaxation kinetics in leaves (see above), the distinction between 'q_E' and the so-called 'photoinhibitory quenching (q_I)', which relaxes more slowly (cf. Ref. 108), is misleading if these terms are understood to represent distinct mechanisms. It seems that part of 'q_E' and part of 'q_I' have the same underlying mechanism, and that there is a second component to 'q_E', as there are likely to be other components to 'q_I'. Until the processes leading to rapidy relaxing fluorescence quenching have been further characterized it is advisable to use a more general description for this type of fluorescence quenching in leaves such as 'rapidly relaxing chlorophyll fluorescence quenching induced by an excess of light'.

III-D. Radiationless energy dissipation in organisms that naturally lack the xanthophyll cycle

Although the above conclusions appear to be fairly straightforward, the use of an inhibitor in vivo is always subject to some doubt. Whereas there are at present no known mutants which specifically lack the xanthophyll cycle, there are, as mentioned above, several algal classes which naturally do not possess this cycle. We have compared the responses of the blue-green algae, which lack the xanthophyll cycle [89,194], with those of green algae, which do possess the xanthophyll cycle [91]. Blue-green algal lichens were found to be unable to form zeaxanthin rapidly. But after several days under an excess of light they did contain zeaxanthin which had been formed in a pathway not involving the xanthophyll cycle (Fig. 5).

In order to test the hypothesis that zeaxanthin is involved in energy dissipation in the chlorophyll pigment bed, a comparative study of chlorophyll fluorescence characteristics as well as the tolerance of photosynthesis to high irradiance (see subsection III-E) in untreated, zeaxanthin-forming and DTT-treated, zeaxanthin-free green algal lichens, and in zeaxanthin-free and zeaxanthin-containing blue-green algal lichens was undertaken. As will be described in detail below, rapidly developing chlorophyll fluorescence quenching, as well as a high tolerance to high light stress was observed exclusively in zeaxanthin-containing organisms (Table I), i.e., green algal lichens and those blue-green algal thalli which had had an opportunity to accumulate zeaxanthin slowly. We suggest that a similar activation of an interaction between zeaxanthin and chlorophyll complexes in excess light (through a 'high-energy state') as was postulated for leaves is also required in zeaxanthin-containing lichens.

The symbiotic association of an alga with a fungus, i.e., a lichen, was used to facilitate the comparison between blue-green and green algal systems. Leaflike lobate lichen thalli allowed measurements to be made in a similar configuration as those in leaves. Furthermore, repeated measurements could be made from the same thallus for days, allowing a characterization of the recovery process. A number of lichen species, in which blue-green and green algal lichens occur in the same genus, were used for this comparison [65,69]. Furthermore, the two partners of a *Pseudocyphellaria* phyco-

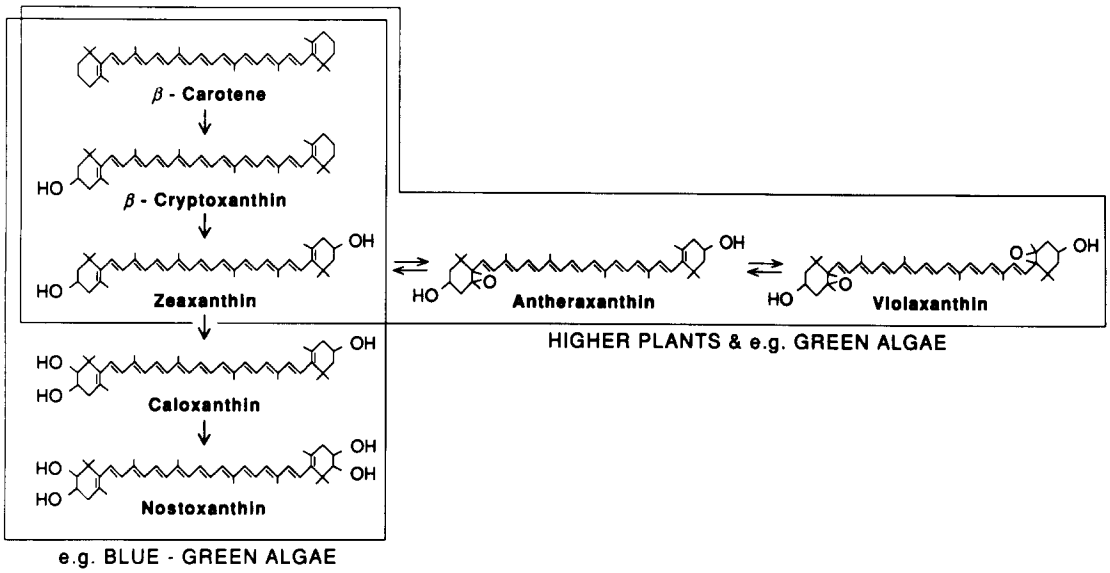

Fig. 5. Possible biosynthetic pathway for the synthesis of zeaxanthin (cf. Ref. 192) and related xanthophylls in higher plants and green and blue-green algae.

symbiodeme [168] were also compared [66]. In a phycosymbiodeme blue-green and green algal partners grow beside each other on separate lobes of the same thallus (they share a common mycobiont) and have therefore developed in an identical light environment. These two partners exhibited very similar rates of photosynthesis (on a chlorophyll or a thallus area basis at a given PFD). Unlike some free-living blue-green algae, the blue-green algal lichens which were used for this study contained extremely low levels of phycobilins and contained amounts of chlorophyll *a* which were, on either a thallus area or thallus dry weight basis, similar to those of Chl $a + b$ in the green algal lichens [66,69]. This indicates that the photochemical systems of these green and blue-green algal lichens were even more similar than those of free-living blue-green and green algae, apart from the difference with respect to the xanthophyll cycle.

Upon exposure to an excess of light, the green algal lichens formed zeaxanthin rapidly from violaxanthin and also exhibited the same type of rapidly reversible fluorescence quenching, associated with a quenching of F_O, as leaves [65] (Fig. 3). The reduction state of PS II centers was maintained low up to high degrees of (nonphotochemical) fluorescence quenching. In contrast, the blue-green algal lichens which did not contain any zeaxanthin nor any epoxides, did not form any zeaxanthin over periods of several hours and also did not exhibit this type of rapidly developing and relaxing fluorescence quenching indicative of radiationless energy dissipation (Fig. 3). There was also no quenching of F_O detectable in the blue-green algal lichens and the reduction state of PS II centers was high at all PFDs beyond the limiting range, indicating that an excess of excitation energy reaches the PS II centers of the zeaxanthin-free blue-green algal lichens, even at slight degrees of excessive light. At the same time the tolerance to longer exposures to high PFDs was considerably greater in zeaxanthin-containing green algal lichens versus zeaxanthin-free blue-green algal lichens (see subsection III-E). Thus the response pattern of the zeaxanthin-forming green-algal lichens resembled that of control leaves and the response of zeaxanthin-free blue-green algal lichens resembled that of DTT-treated leaves [57,58] (Fig. 3).

The difference between zeaxanthin-forming green algal lichens and zeaxanthin-free blue-green algal lichens could, when considered in isolation, have additional or other causes than the absence or presence of zeaxanthin. The finding, however, that the responses of green and blue-green algal lichens can be reversed by treatments affecting the zeaxanthin metabolism, does argue for a causal relationship between zeaxanthin and photoprotective energy dissipation (Table I). Treatment with the inhibitor (DTT) of zeaxanthin formation from violaxanthin caused the chlorophyll fluorescence characteristics of green algal lichens to become very similar to those of the zeaxanthin-free blue-green algal lichens [65]. Conversely, slow zeaxanthin accumulation in the blue-green algal lichens led to a response similar to that of zeaxanthin-forming green-algal lichens [57,65]. Blue-green algal *Peltigera* species were found to contain considerable amounts of zeaxanthin, but no epoxides, when collected directly from the field after a number of sunny days [57,65]. It is likely that this zeaxanthin was formed slowly from β-carotene in its presumed biosynthetic pathway [37,38,42,79,134,192] (Fig. 5). These thalli also contained β-cryptoxanthin, a monohydroxy-intermediate between β-carotene and zeaxanthin, as well as products of further oxygenation of β-carotene which are typical compounds found in blue-green algae [65] (Fig. 5). In many zeaxanthin-containing thalli of *Peltigera* species, all four of the hydroxylated derivates of β-carotene were present, with one (β-cryptoxanthin), two (zeaxanthin), three (presumably caloxanthin), and four (presumably nostoxanthin) hydroxy groups [65,80] (Fig. 5).

These zeaxanthin-containing blue-green algal lichen thalli showed a different response pattern of chlorophyll fluorescence quenching than zeaxanthin-free thalli of the same species [57,65] (Table I). The zeaxanthin-containing thalli exhibited considerable degrees of rapidly developing quenching of F_M and F_O and maintained a lower reduction state of PS II at a given degree of quenching than zeaxanthin-free thalli. Furthermore, the zeaxanthin-containing blue-green thalli showed an increased tolerance to high light stress similar to that of green algal lichens [57] (see next subsection, III-E). These differences between the zeaxanthin-free and the zeaxanthin-containing blue-green algal lichens, in particular, suggest that it is indeed the absence or presence of zeaxanthin which is related to the difference in the ability for radiationless energy dissipation.

III-E. Zeaxanthin and protection from adverse effects of high irradiance

Whereas in the previous section the association of a presence of zeaxanthin with chlorophyll fluorescence characteristics indicative of the energy dissipation process in the chlorophyll pigment bed was discussed, this section is concerned with the resulting tolerance of the different systems considered above to high light stress, i.e., longer exposures to very high light levels.

Subsequent to an exposure to light levels equivalent to full sunlight, control leaves of spinach grown at less than 10% of full sunlight showed a depression of PS II photochemical efficiency (F_V/F_M) associated with strong (nonphotochemical) fluorescence quenching, both of which were relatively rapidly reversible (Table I). In contrast, zeaxanthin-free, DTT-treated leaves, in which (nonphotochemical) fluorescence quenching was in-

hibited, exhibited smaller decreases of F_V/F_M which were associated with increases in F_O and were very slowly reversible [7]. Similar differences in the recovery of F_V/F_M were found by Bilger and Björkman [20], as well as differences in the recovery of corresponding depressions in the photon efficiency of photosynthesis. In this latter study, a high light treatment for 3 h plus a subsequent recovery period of 45 min under low light resulted in almost no depression of the photon efficiency of photosynthesis in control sun leaves of *Hedera canariensis*. In contrast, zeaxanthin-free DTT-treated leaves showed a significant and sustained depression of the photon efficiency of photosynthesis of an average of 35% [20].

In another study two sets of *Rhizophora mangle* leaves, one pre-illuminated (in low light with 2% O_2) and thus containing zeaxanthin and the other pre-darkened and thus zeaxanthin-free, were subjected to a high light treatment at chilling temperatures during which no zeaxanthin was formed in either set of leaves [57,62] (Table I). The subsequent recovery at low PFD and room temperature revealed that rapid recovery of PS II photochemical efficiency took place in the pre-illuminated, zeaxanthin-containing leaves but that sustained depressions of F_V/F_M were experienced by the pre-darkened, zeaxanthin-free leaves [62] (Table I).

Furthermore, similar differences also existed between zeaxanthin-free blue-green and zeaxanthin-containing green algal lichens as well as between the zeaxanthin-containing and the zeaxanthin-free thalli of blue-green algal lichens of the genus *Peltigera* [57,66,69] (Table I). In a survey involving 11 species of green and 10 species of blue-green algal lichens, the photosynthetic capacities varied within each group but did not vary significantly between the two groups [69]. Nevertheless, the blue-green algal lichens generally exhibited a much slower recovery from high light exposure than did the green-algal lichens [69]. Similar results were obtained when the two partners of the *Pseudocyphellaria* phycosymbiodeme were compared with respect to their capacity for rapid recovery from high light stress. While the photon efficiency of photosynthesis as well as F_V/F_M were severely depressed subsequent to a high light treatment in both partners, recovery took place within 1 day in the green algal partner, but was still incomplete even after several days in the blue-green algal partner [66]. Furthermore, the depression in F_V/F_M was due mainly to a strong decrease in F_M in the green algal partner and due mainly to a strong increase in F_O in the blue-green algal partner [66]. These chlorophyll fluorescence characteristics were indicative of increased energy dissipation being the cause for the depression in photon efficiency in the green (zeaxanthin-containing) partner, and with 'photoinhibitory damage' being the cause in the blue-green (zeaxanthin-free) partner. Lastly, the zeaxanthin-free thalli of the blue-green *Peltigera* species showed recovery rates which were low and typical for blue-green algal lichens [57,69]. In contrast, the zeaxanthin-containing thalli of the same species showed recovery rates similarly high to those of green algal lichens of the same genus [57] (Table I).

For all of these vastly different systems, those photosynthetic organisms which contained zeaxanthin prior to or formed zeaxanthin during the exposure to high light exhibited a rapid recovery subsequent to these treatments which showed the characteristics of a reversal of an increase in radiationless energy dissipation (Table I). In contrast, those organisms which did not contain or form zeaxanthin were incapable of rapid recovery and showed sustained depressions in the photon efficiency of photosynthesis and photochemistry which were indicative of some form of 'damage' through high light. Thus, high zeaxanthin contents during an exposure to an excess of light were associated with a high resistance to light stress. When all of these observations are considered together, it seems likely that it is indeed the presence or absence of zeaxanthin which is related to the ability or inability to recover rapidly from high light stress.

In the context of possible photoprotective effects of carotenoids, it is interesting to note that a fraction with a demonstrated photoprotective function isolated from insect eyes was reported to contain zeaxanthin and lutein but no β-carotene [103]. Zeaxanthin is also present in the eyes of vertebrates such as birds [77]. The eyes of animals with the photoreceptors present therein are probably the closest analogue to the photosynthetic membranes of plants. In both systems the exposure to light is necessary for the functioning of the systems. Avoiding the absorption of a large excess of light through pupil narrowing is analogous to, for example, leaf movements to avoid the absorption of light. However, in both systems additional means of photoprotection through dissipation of an excess of absorbed light are also required. The mechanism of the function of carotenoids in eyes of animals is unknown.

III-F. Possible mechanisms for zeaxanthin-associated energy dissipation

Carotenoids have previously been thought to act as photoprotective pigments through one of two reactions (Fig. 6). Firstly, through a direct quenching/deactivation of singlet oxygen [74,114] and, secondly, through a prevention of singlet oxygen formation via triplet-triplet energy transfer from the excited triplet state of chlorophyll to carotenoid [127,129,209]. The close correlation between the quenching of chlorophyll *a* fluorescence, which arises from the singlet excited state of chlorophyll, and the zeaxanthin content of leaves suggests a quenching/deactivation of the singlet excited state of chlorophyll by zeaxanthin (Fig. 6). In

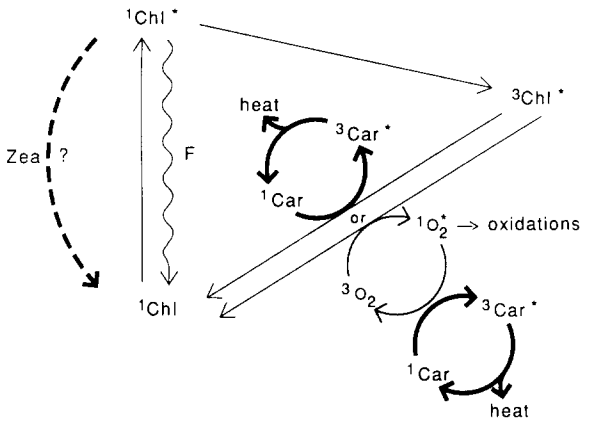

Fig. 6. Schematic diagram of possible sites for an interaction of carotenoids with excited states of chlorophyll or oxygen; through quenching/deactivation of singlet oxygen [cf. 74], through triplet energy transfer from triplet chlorophyll to carotenoid [114,127,129], and through the proposed interaction between zeaxanthin and a singlet excited state of chlorophyll.

fact, for synthetic carotenoporphyrins, in which a porphyrin and a carotenoid are linked together and are therefore held together closely, strong quenching of up to 75% of the porphyrin fluorescence through the carotenoid moiety has been consistently observed [19,122, 140].

The mechanism of this fluorescence quenching is unknown. For very high pulse intensities a quenching of chlorophyll fluorescence through an interaction between ^{1}Chl* and ^{3}Car* once formed has been suggested [35,126,130,217]. But this mechanism is unlikely to account for the type of strong fluorescence quenching in the steady state reported here (or for the carotenoporphyrins). It has been proposed that the quenching of fluorescence of chlorophyll or porphyrins through carotenoids involves a rapidly reversible electron transfer reaction, e.g., after $^1\text{Chl}^* + \text{Car} \rightarrow [\text{Chl}^- \cdot \text{Car}^+] \rightarrow \text{Chl} + \text{Car}$ [17,19,117,122,136,140]. However, there also appears to be some uncertainty with regard to the energy levels of excited singlet states of carotenoids relative to that of chlorophyll. There appears to be a low-lying symmetry-forbidden energy level ($^1A_g^*$) for carotenoids which has been postulated to mediate energy transfer *from* carotenoid *to* chlorophyll [203]. In contrast, according to Snyder et al. [188] the energy level of $^1A_g^*$ might be significantly lower than suggested by Thrash et al. [203]. If this was the case, energy transfer in the opposite direction, from a singlet excited state of chlorophyll to zeaxanthin, could not be a priori excluded.

The fact that zeaxanthin is the only carotenoid which is formed exclusively under an excess of light and removed under limiting light is also consistent with the asssumption that the singlet excited state of chlorophyll, which is normally used for photochemistry, may interact with zeaxanthin under an excess of light in a process leading to de-excitation.

Any speculation as to the mechanism of an involvement of zeaxanthin in radiationless energy dissipation has to take into account the fact that the presence of zeaxanthin per se may not result in chlorophyll fluorescence quenching [22,61,63,65]. The presence of an excess of light, i.e., a 'high-energy state' of the photosynthetic membrane, is also required for zeaxanthin-associated fluorescence quenching present during illumination. It is possible that an altered 'state' of the photosynthetic membrane, initially induced by the 'high-energy state' of the membrane, leads to a closer physical association between chlorophyll and zeaxanthin molecules which are present within the chlorophyll-binding protein complexes. A closer physical arrangement might favor an interaction which would otherwise not be possible. On the other hand, long-term exposure to excessive light has been found to result in a sustained kind of zeaxanthin-associated fluorescence quenching where fluorescence quenching relaxes slowly and zeaxanthin is removed at a corresponding (slow) rate [55]. In this case the altered state of the chlorophyll-binding complexes is apparently preserved after the direct effects of excessive light on the thylakoid membrane have subsided.

At this point it is equally feasible to assume that de-excitation occurs through pathways involving zeaxanthin more indirectly, e.g., through structural alterations. Horton and co-workers [97,144,167] have suggested that, in the presence of zeaxanthin, 'q_E' can occur at a much less acidic lumen pH than in the absence of zeaxanthin. This would mean that zeaxanthin modulates the sensitivity of the energy dissipation process within the chlorophyll-binding complexes to the ΔpH, i.e., that zeaxanthin can raise the pK for 'q_E formation'. However, this argument rests on their assumption, which differs from ours, that zeaxanthin-associated and zeaxanthin-unrelated types of quenching have the same mechanistic basis.

Concerning an association between zeaxanthin and structural changes in the photosynthetic membrane, a study by Bilger et al. [22] on the magnitude of light-induced absorbance changes in zeaxanthin-containing versus DTT-treated, zeaxanthin-free leaves is also of interest. In addition to the expected absence of an absorbance change at 505 nm (which is related to the difference in the absorption spectrum of zeaxanthin versus violaxanthin) in DTT-treated leaves (Ref. 22; see also 189, 215), the magnitude of the absorbance change at 535 nm was also considerably reduced in these leaves [22]. This 'light scattering signal' at 535 nm is caused by a change in the light-scattering properties of the thylakoid membranes in response to conformational changes [96,154], which in turn are thought to be induced by the 'high-energy state' of the thylakoid mem-

brane [107]. Bilger et al. [22] interpreted this observation to indicate that a scattering pigment, in this case apparently zeaxanthin, was required for the scattering signal to become detectable. One might, however, also speculate that the reduction in the magnitude of the 535 nm absorbance change may indicate that a conformational change, induced by, for example, the transthylakoid pH gradient, fails to develop in the absence of zeaxanthin. It is unlikely that the pH gradient itself was diminished by DTT, since there was no effect on the rate of O_2 evolution [22]. It has previously been speculated that carotenoids, and perhaps particularly those of the xanthophyll cycle, may alter membrane properties [125,182,185,212,213]. In the study by Bilger et al. [22] it was also reported that another light-induced absorbance change, the 'electrochromic shift' ($\Delta A_{515-518}$; [70,208]), was not affected by the DTT treatment.

III-G. Additional regulatory changes in the photochemical system in response to an excess of light

It has previously been suggested in some studies that the dissipation of excess excitation energy occurs within/around the reaction center of PS II (e.g., Refs. 175, 205; see also Ref. 108) whereas others have suggested the chlorophyll pigment bed as the major site of radiationless energy dissipation [24,25,51,76,97,165,166]. The indirect evidence which has been used to indicate the site of energy dissipation is based on several arguments (see also Table II). Firstly, the quantitative relationship between decreases in chlorophyll fluorescence (F_V/F_M) and photosynthesis has been used to make this distinction [8,26,59,76,97,110,112,155,163,172,177, 205] as will be discussed in detail below. Secondly, a net quenching of F_O has been used to indicate that energy dissipation occurs in the chlorophyll pigment bed [6,24,25,30,51,60]. Thirdly, the expression of fluorescence quenching at ambient temperature versus 77 K has been used for this distinction. It has been shown that the type of chlorophyll fluorescence quenching associated with F_O quenching was expressed to the same extent at ambient temperature and at 77 K [59], as would be expected from energy dissipation in the chlorophyll pigment bed. In contrast, a second type of fluorescence quenching which is not associated with F_O quenching was expressed predominantly at ambient temperature [8,59]. Furthermore, the differential effect of DTT on fluorescence quenching can also be used to distinguish two components of fluorescence quenching (Table II).

In the model of the photochemical apparatus by Butler and co-workers [104], the photon efficiency of PS II as judged from chlorophyll fluorescence (F_V/F_M) and the photon efficiency of electron transport decrease in proportion when k_D increases, i.e., energy dissipation in the chlorophyll pigment bed. The same proportional decrease would result from decreases in k_T or k_P (see section II). Several studies have reported such proportional decreases for F_V/F_M and the photon efficiency of photosynthesis [8,26,51,59,76,97,155]. In some of these studies sustained decreases in these two parameters were compared at limiting PFD [8,26,51,59]. In other studies, the decreases in these two parameters were determined directly during the exposure to various PFDs representing an excess of light [76,97,110,112, 155,163,172,177,205]. In these latter cases F_V/F_M was determined during actinic illumination (currently referred to as F'_V/F'_M), and the intrinsic photon efficiency of photosynthesis, ϕ_P, is obtained by correction for those PS II centers which are closed at this PFD, as was first suggested by Weis and Berry [205,206]. The parameter ϕ_P is defined as the steady-state photon efficiency of photosynthesis, $\phi_S = P(ET)/PFD$, corrected for the presence of closed PS II centers at high PFD, $\phi_P = \phi_S/q_P$, where q_P is the coefficient for photochemical fluorescence quenching. The expression $1 - q_P$ (or Q_r/Q_t [172]) is a measure of the reduction state of PS II, i.e., the proportion of Q_A which is reduced.

Two different groups of data have been obtained, particularly in those studies performed during high light exposure. Some studies report a proportional decrease in F_V/F_M (F'_V/F'_M) and in the intrinsic photon efficiency of photosynthesis [76,97,155]. These authors consequently argue that radiationless energy dissipation as indicated by chlorophyll fluorescence quenching occurs in the chlorophyll pigment bed. In contrast, a number of other studies report relatively greater decreases in the intrinsic photon efficiency of photosynthesis than in F'_V/F'_M [97,110,112,163,172,177,205]. In these cases, a different parameter derived from chlorophyll fluorescence, the coefficient of nonphotochemical quenching $q_N = 1 - F'_V$(in the light)$/F_V$(in the dark-adapted state), is found to give a more linear relationship with ϕ_P (cf. also Fig. 7). In those studies it has been argued that energy dissipation does not occur in the chlorophyll pigment bed. Alternative suggestions for the site of energy dissipation include a cyclic electron transfer around PS II or a back reaction within PS II [97,175,205,206] and a decrease in the activity of the water splitting system, i.e., a decreased electron donor activity [175].

However, there are also some studies which propose how this discrepancy may be resolved. They suggest that the different relationships reported are caused by different combinations of two dissipation processes. We have recently investigated the relationship between the photon efficiency of photosynthesis (ϕ_a) and F_V/F_M at limiting PFD, subsequent to an exposure to high PFD. Initially, a type of chlorophyll fluorescence quenching was present which was detectable at ambient temperature but not at 77 K and the photon efficiency was depressed more strongly than F_V/F_M [8,59]. This effect

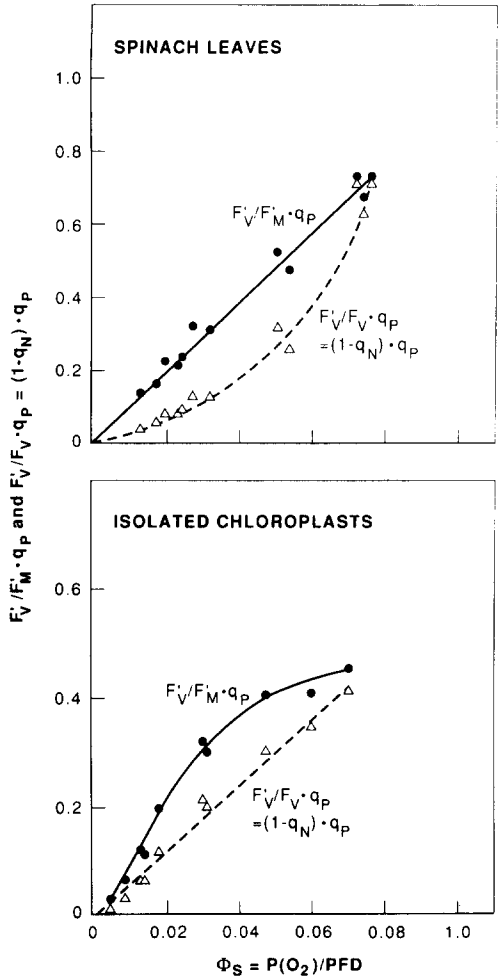

Fig. 7. Relationship between the steady state photon efficiency of photosynthesis ϕ_S ($= P[CO_2]/PFD$) and the product of the ratio of variable to maximum chlorophyll fluorescence in the light and the coefficient of photochemical fluorescence quenching ($F_V'/F_M' \cdot q_P$) or the product of the coefficients for nonphotochemical and photochemical fluorescence quenching ($1-q_N) \cdot q_P$ ($= F_V'/F_V \cdot q_P$) during actinic illumination with PFDs between 70 and 1950 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in spinach leaves and 15 and 1650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in isolated spinach chloroplasts (data from [67]).

is not caused by energy dissipation in the chlorophyll pigment bed. After the relaxation of this component of fluorescence quenching, the remaining strong quenching of fluorescence showed a linear relationship between F_V/F_M and the photon efficiency of photosynthesis (Ref. 8; see also Refs. 26, 51). This latter component of fluorescence quenching can therefore be attributed to energy dissipation in the chlorophyll pigment bed, i.e., an increase in k_D, and/or decreases in k_T or k_P.

Furthermore, the relationship between F_V'/F_M' and ϕ_P (or between $F_V'/F_M' \cdot q_P$ and ϕ_S) differs also between the spinach leaves versus spinach chloroplasts examined by Demmig-Adams et al. [67] (Fig. 7) which showed very different contributions of a DTT-sensitive and a DTT-insensitive component of rapidly relaxing fluorescence quenching under an excess of light [67]. A linear relationship between $F_V'/F_M' \cdot q_P$ and ϕ_S is equivalent to a linear relationship between F_V'/F_M' and ϕ_P. In leaves, which showed a strong contribution of the zeaxanthin-associated, DTT-sensitive fluorescence quenching, ϕ_S was depressed in a fashion proportional to $F_V'/F_M' \cdot q_P$. In contrast, chloroplasts with a strong contribution of DTT-insensitive fluorescence quenching exhibited a depression of ϕ_S which was greater than that of $F_V'/F_M' \cdot q_P$ (Fig. 7). These results further suggest that the DTT-sensitive, zeaxanthin-associated and the DTT-insensitive, zeaxanthin-unrelated type of chlorophyll fluorescence quenching do reflect two different processes and that the zeaxanthin-associated process occurs in the chlorophyll pigment bed.

A novel suggestion concerning the other process has been made by Horton and co-workers (Ref. 97; see also Refs. 144, 165). It was suggested that the intrinsic photon efficiency of photosynthesis, ϕ_P, can be lowered by a dissipative process associated with the PS II center without causing any fluorescence quenching per se. Horton also suggests that this dissipative process involves either cyclic electron transfer around PS II or a back reaction within the PS II center. Horton and co-workers furthermore summarize the conditions leading to this decrease in ϕ_P versus the conditions leading to the energy dissipation process in the chlorophyll pigment bed which results in quenching of both F_O and F_M. Both processes require a low pH in the thylakoid lumen. However, the decrease in ϕ_P requires an even lower lumen pH than the dissipation process in the chlorophyll pigment bed [143,144]. Furthermore, the decrease in ϕ_P develops more rapidly than the other process and is particularly pronounced during the induction phase of photosynthesis [97,143,144]. The decrease in ϕ_P can be pronounced at relatively low PFD as well as at extremely high PFDs. These characteristics are indeed all consistent with those of the DTT-insensitive, zeaxanthin-unrelated type of rapidly relaxing fluorescence quenching in spinach leaves and chloroplasts [7,67]. It remains to be resolved whether or not (some) fluorescence quenching is per se associated with the strong decrease in ϕ_P described by Horton and co-workers.

There seem to be two different possibilities. We cannot at this point exclude the possibility that the fluorescence quenching in DTT-treated leaves may simply be caused by a failure to overcome a futile dissipative cycle within/around PS II and to fully reduce Q_A and obtain the maximum fluorescence yield under these conditions. However, since the DTT-insensitive quenching of fluorescence can be quite strong it is more likely that it is caused by some further alteration in the photochemical apparatus. A process, which causes chlorophyll fluorescence quenching (of F_M and not F_O) and may occur in concert with a dissipative process within PS II, is a decrease in water-splitting activity. If elec-

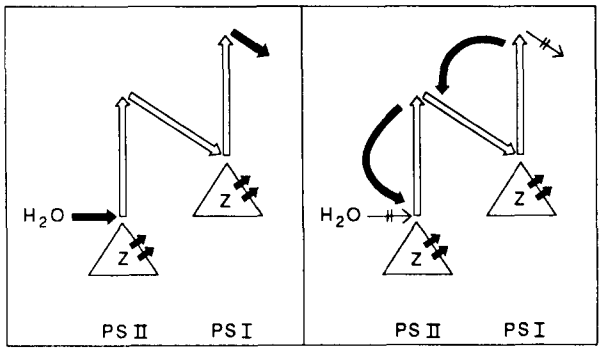

Fig. 8. Schematic diagram to illustrate possible sites for energy dissipation (radiationless energy dissipation in the chlorophyll pigment bed and/or dissipative processes associated with the PS II center) within the photochemical apparatus under various conditions. Modified after Ref. 58.

trons are to return from Q_A^- to P_{680}^+, a decreased supply of electrons from water might be an expected consequence of such a process. A decrease in water-splitting activity can also be induced by a low pH of the thylakoid lumen [45,173,211] and possibly by elevated temperatures [216]. We have observed an increased expression of the DTT-insensitive, zeaxanthin-unrelated component of fluorescence quenching at elevated leaf temperatures [7]. A decreased electron donor activity could also explain the expression of one type of fluorescence quenching at ambient temperature but not at 77 K. At 77 K one electron is sufficient to reduce Q_A and obtain the maximum fluorescence yield, whereas at ambient temperature considerably more electrons are required.

These different findings are summarized tentatively in Fig. 8, where a zeaxanthin-associated dissipation process is depicted to occur in the chlorophyll pigment bed, since all of the above-listed observations are consistent with such an assumption. A second complex of changes in the photochemical apparatus is suggested to involve a cyclic electron transfer around PS II (or a back reaction within PS II), together with a decreased water-splitting activity under those conditions where the DTT-insensitive type of fluorescence quenching was observed in spinach leaves, e.g., at a (presumably) high ratio of cyclic to linear electron transfer. Such a combination of a dissipative process in the PS II centers with a decreased donor side activity would result in PS II centers which exhibit little or no O_2 evolution and no reduction of Q_B, the second quinone electron acceptor after Q_A. These are, however, the characteristics of the so-called 'non-functional' or 'non Q_B-reducing' PS II centers (cf. Ref. 150). Photosystem II centers which do not function in the normal linear electron flow may be centers in which a dissipative cycle, as that depicted in Fig. 8, is operating. 'Non-functional' PS II centers can also have a smaller absorptive cross-section, i.e., can lack the peripheral LHC-II and thereby correspond to PS II_β centers [138,139,201]. A smaller pigment system and thus decreased excitation energy transfer into the PS II reaction center can also be considered advantageous under conditions when light is excessive. Detachment of the peripheral LHC-II can either occur spontaneously [197,198] or as a consequence of phosphorylation of LHC-II [9,11,18,31,119]. However, the involvement of a phosphorylation of LHC-II in the complex of changes depicted in Fig. 8 is unclear at this point. Recent studies show that phosphorylation of LHC-II is inhibited under conditions representing an excess of light in several species [53,73,101,153] but not in all [43].

A third effect of high light on the photochemical apparatus is, potentially, 'photoinhibitory damage' to PS II centers, which has been associated with sustained decreases in the photon efficiency of photosynthesis and (proportional) decreases in F_V/F_M from PS II as well as sustained increases in F_O from PS II [8,51,66]. I should like to stress the fact that correlations between decreases in the photon efficiency of photosynthesis and of PS II and increases in the calculated rate constant for radiationless energy dissipation in the chlorophyll pigment bed [55,61] and concomitant increases in the zeaxanthin content in some plants under specific conditions should not be taken to suggest that all changes in F_V/F_M are associated with zeaxanthin [75]. Any dissipative reactions within or around PS II as well as other means of inactivation of reaction center function (decreases in k_P or k_T in the Butler model) would lead to decreases in F_V/F_M unrelated to zeaxanthin. In fact, sustained decreases in F_V/F_M have been consistently observed in zeaxanthin-free leaves [7,20,62] and organisms [66] (see Table I).

'Photoinhibitory damage' is likely to involve sustained effects on the oxidizing side of PS II [15,16,32,41,102,195,196]. It is possible that continued exposure to large degrees of excessive light, exceeding the capacity of (all) dissipative processes, leads to a transition from rapidly reversible effects involving the donor side of PS II to rather irreversible effects. Concerning the sequence of events in time during 'photoinhibition', recent studies suggest that removal of the D1 protein (or previously also termed 'Q_B protein') [115,148] occurs only after such sustained effects on the oxidizing side of PS II have been noted [102,195,196]. It is therefore possible that 'nonfunctional' PS II centers may also include those in which the D1 protein is ultimately being removed.

IV. Acclimation of the photosynthetic apparatus to high irradiance

IV-A. Increase in size of the total pool of xanthophyll cycle components in response to an excess of light

In plants grown at rather low irradiance levels as are often used in artificially lit growth chambers, the total

pool of the xanthophyll cycle components represents only a small fraction of the total carotenoid content of the photosynthetic membrane. Typical values range from 5 to 15% of the total carotenoid content for the sum of zeaxanthin + antheraxanthin + violaxanthin, 40–45% for lutein, and 25–40% for β-carotene. However, acclimation to higher irradiances or to full natural sunlight leads to a preferential increase in the contribution of the three components of the xanthophyll cycle to the total carotenoid fraction ([199] and Table III). In some species, the percent contribution of the components of the xanthophyll cycle can rise to 40%, with lutein and β-carotene dropping to, for example, 30 and 20%, respectively (unpublished data). This change in the size of the xanthophyll cycle pool is the most pronounced relative change during shade-sun acclimation within the photosynthetic pigments. The increase in the xanthophyll cycle pool size in response to increasing degrees of excessive light further supports a special photoprotective role for these xanthophylls, and in particular for zeaxanthin.

Upon a strong increase in growth PFD, i.e., a transfer of plants to a large excess of light, leaves of species from widely different habitats all showed a strong increase in the sum of the three xanthophylls of the xanthophyll cycle, zeaxanthin, antheraxanthin, and violaxanthin (Fig. 9) [64]. In all species the ratio of carotenes to total xanthophylls (lutein, neoxanthin, zeaxanthin, antheraxanthin, and violaxanthin) decreased during a 7–10 day period of acclimation to high light [64]. This is opposite to what has been reported in previous comparisons of the pigment composition of 'low-light' versus 'high-light' grown leaves [9,12,120, 121,186]. However, in these previous studies, even the high-light level was typically equivalent to only 10–25% of full sunlight and the conclusion was based mainly on the ratio of carotenes to the xanthophylls lutein and neoxanthin. The latter two appear to be located mainly in the LHC-II [9]. To date there has only been one very extensive survey of plants growing in the field (in which, however, the identification of one xanthophyll as lutein-epoxide seems questionable) that suggests generally lower ratios of carotenes to xanthophylls in sun versus shade leaves, as well as decreased amounts of β-carotene, in 73 species of higher plants [49]. When Thayer and Björkman [199] compared the pigment com-

TABLE III

Levels of zeaxanthin, the xanthophyll cycle pool (Z + A + V), and carotenes, as well as the ratio of carotenes to total xanthophylls (including Z, A, V, lutein, neoxanthin) in various sun and shade leaves

Leaves developed either in natural sun or shade or in shaded locations in greenhouses (g.h.). S = South exposure. *Gossypium hirsutum* and several other C_3 species were collected in California in the summer, *Arbutus unedo* in Portugal in August, *Euonymus kiautschovicus* in Colorado in the winter with night temperatures of around 0°C and day temperatures of around 10°C, *Pinus ponderosa* in Colorado during the winter on a cloudy day with only 200 μmol photons·m^{-2}·s^{-1} and 0°C at noon. All samples were immersed in liquid nitrogen within 2–5 s upon removal from the leaves.

Species	Exposure	Time of day	Pigments and pigment ratios							Reference
			μmol·m^{-2}			mmol·mol^{-1} Chl *a* + *b*			C/X	
			Z	Z+A+V	C	Z	Z+A+V	C		
4 species of C_3 plants [a]	shade			30 ± 4			42 ±11			199
	sun			100 ±24			163 ±68			199
Monstera deliciosa [b]	deep shade, g.h.	noon	0	6.5± 4.5	126± 5	0	7.8± 3.0	150± 7	1.04±0.18	65
Nerium oleander [b]	shade, g.h.	noon	5.6±4.9	16 ± 5.2	101±16	4.3±5.9	21.3± 8.7	137±21	1.08±0.16	unpubl.
Arbutus unedo [b]	shade, g.h.	noon	0	10	84	0	14	114	0.90	unpubl.
	sun, S	predawn	13	32	63	36	88	173	0.53	59
	sun, S	noon	44	51	46	130	151	137	0.38	59
Gossypium hirsutum [a]	sun	predawn	4	115		6.3	185			68
	sun	noon	33	118		53	190			68
Euonymus kiautschovicus [c]	shade, winter	predawn	0	17	45	0	37	97	0.46	unpubl.
	sun, winter	predawn	21	69	41	43	142	86	0.23	unpubl.
	sun, winter	noon	40	65	41	75	122	77	0.21	unpubl.
Pinus ponderosa [c]	(open) cloudy, winter	noon				131	153	159	0.31	unpubl.

[a] Determined by HPLC from acetone extracts.
[b] Determined by TLC from petroleum ether extracts, which may give an underestimation of the neoxanthin content.
[c] Determined by TLC directly from acetone extracts.

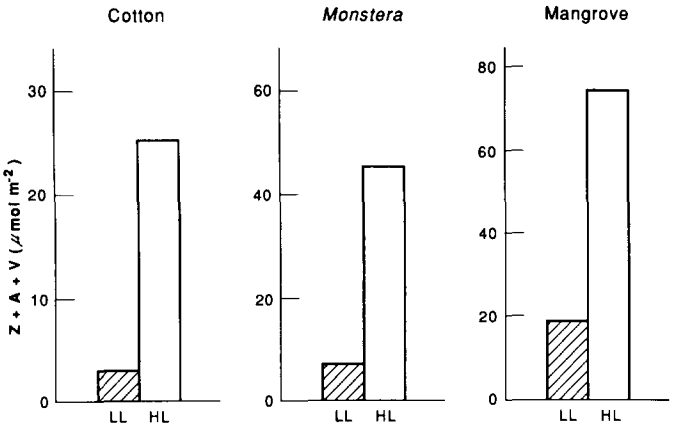

Fig. 9. Xanthophyll cycle pool (Z+A+V) in leaves of cotton, *Monstera deliciosa*, and the mangrove *Rhizophora mangle* before and 4–7 days after transfer from low light (LL) to high light (HL). Plants were transferred to 1100 μmol photons·m^{-2}·s^{-1} provided by a metal halide lamp in a growth cabinet (12 h photoperiod). Pigment determinations were made on samples collected at the end of the 12 h light period. The low light regimes were 30 (*Monstera*) or 200 μmol photons·m^{-2}·s^{-1}. Data represent means of two adjacent samples from each leaf. Data from Ref. 64.

position of shade and sun leaves for a range of species growing in natural shade or full sun, they consistently found very large pool sizes of the xanthophyll cycle in the sun-grown leaves, typically greater than 4-times higher xanthophyll cycle pools in sun versus shade leaves (cf. Table III). A similar trend had been observed for leaves of *Arbutus unedo* growing in Portugal [59] (Table III). Generally, sun leaves possessed larger xanthophyll cycle pools (Z + A + V) and rather low ratios of carotenes to xanthophylls, particularly in comparison with leaves that had developed in shaded locations in greenhouses (Table III). Furthermore, higher xanthophyll cycle pools were found in cotton leaves growing in natural versus artificial light even at the same daily integrated PFD [199].

At this point it is not clear which step(s) within the biosynthetic pathway is/are responsible for this response. During the transfer from low to high growth PFD, considerably more zeaxanthin became accumulated than could have been formed from the violaxanthin and antheraxanthin present prior to the transfer [64]. In some species, the size of the xanthophyll cycle pool increased together with the amount of carotene present [64,199] whereas in others the increase in the xanthophyll cycle pool size was accompanied by transient or permanent decreases in the carotene content (Ref. 64; unpublished data). During the first 12 h period at increased PFD, the carotene content showed a decrease of a similar magnitude to that of the increase in the sum of the three xanthophylls of the xanthophyll cycle in all species (Ref. 64; see also Ref. 59). Smaller antiparallel changes in the carotene content versus the sum of the three components of the xanthophyll cycle were also found upon transfer of a leaf from air to 2% O_2, no CO_2 (no endogenous electron acceptor) without any change in PFD [54]. This further indicates that an excess of light and not high light per se induces the increased accumulation of xanthophyll cycle components [cf. 54, 199]. One important step for the increased zeaxanthin accumulation may be a synthesis of zeaxanthin either directly from β-carotene or from a common precursor. There is evidence that one major pathway for zeaxanthin synthesis is through hydroxylation of β-carotene via β-cryptoxanthin [37,38,42,134, 192] (cf. Fig. 5). But the existence of alternative routes of zeaxanthin biosynthesis cannot be ruled out, such as potentially one via rubixanthin and β-cryptoxanthin [134,135,160,192]. Such a possibility could also explain a decrease in β-carotene content, if the turn-over rates were high enough, since zeaxanthin would be formed instead of β-carotene.

The underlying mechanisms responsible for increased carotenoid synthesis in excessive light are poorly understood. However, it is interesting that there is circumstantial evidence for an involvement of singlet oxygen as a signal for increased carotenoid synthesis [164].

IV-B. Plants in the field: carotenoid content and capacity for energy dissipation

From the relationship between zeaxanthin and the activity of the energy dissipation process in the chlorophyll pigment bed, one might expect sun leaves with large xanthophyll cycle pools [59,199] to possess a high capacity for energy dissipation in the pigment bed. This was indeed found to be the case as evidenced from massive and reversible chlorophyll fluorescence quenching indicative of energy dissipation in the chlorophyll pigment bed in sun leaves in the field at noon or at peak irradiance (e.g., Refs. 6, 59). This type of very strong quenching of chlorophyll fluorescence was accompanied by a pronounced net quenching of F_O in many cases where F_O determinations were made in the field [1,4–6,30,59]. Even though chlorophyll fluorescence characteristics (magnitude of quenching/effect on F_O) were similar in cases such as *Arbutus unedo* [59], *Nerium oleander* [24], the mangrove *Rhizophora stylosa* [30], *Hoya australis* [5], and the cactus species *Nopalea cochenillifera* [6] and *Opuntia basilaris* [4], the time it took for these pronounced decreases in chlorophyll fluorescence to relax were extremely variable. In mangrove species or *Nerium oleander*, full recovery took more than a week whereas in cases such as in *Nopalea cochenillifera* [6], *Arbutus unedo* [59] or cotton [68], recovery was completed by the end of the same day or at least the next morning. The mangroves and *Nerium oleander* were exposed to a combination of high light and salinity or water stress, respectively. Extensive field

studies by Adams and co-workers [4,6] on various cactus species showed that, whereas in well-watered individuals of *Nopalea cochenillifera* recovery was rapid, water-stressed individuals of other cactus species exhibited a considerably slower recovery. Furthermore, during the course of a year recovery also became slower during periods of very limited water availabitity in *Opuntia basilaris* in Death Valley [4].

In those cases where recovery of the efficiency of photochemical energy conversion took days, these decreases in photochemical efficiency have been referred to as 'photoinhibition'. It is likely, however, that in all of the above-mentioned cases regulatory and photoprotective energy dissipation contributed largely to these decreases. The field data available to date all suggest that the dissipation process in the chlorophyll pigment bed is rapidly reversible in well-watered plants and becomes increasingly more sustained when photosynthesis rates decrease due to long-term water stress. A sustained diversion of excitation energy from the photosynthetic reaction centers, however, may rightfully be addressed as 'photoinhibition' of photosynthesis and may limit productivity for some period upon return to more favorable conditions.

Whereas it seems quite clear that the energy dissipation process which quenches F_M and F_O and presumably occurs in the chlorophyll pigment bed (and is associated with zeaxanthin Ref. 24,59; cf. also Ref. 68), is strongly expressed in all of the above cases, the presence of additional dissipative processes in the photochemical apparatus cannot be excluded, particularly in those cases where only 77 K fluorescence was determined. Studies with *Arbutus unedo* [59] and *Hoya australis* [5] are the only instances to date where both 77 K and ambient temperature fluorescence were determined simultaneously in the field, and this approach revealed the presence of an additional fluorescence quenching process, of the type which is expressed exclusively at ambient temperature. *Arbutus unedo* transiently closes stomates completely at peak irradiance during the 'midday depression' of CO_2 exchange in the hot and dry summer period and exhibited relatively high leaf temperatures [59], and *Hoya australis*, a CAM plant, was also found to experience very high leaf temperatures during peak irradiance [5].

Whereas water stress and salinity seem to promote the development of radiationless energy dissipation (e.g., in the chlorophyll pigment bed), other environmental factors, such as low temperatures, remain to be examined in terms of their effect on energy dissipation processes and, particularly, the levels of carotenoids. In leaves which were not acclimated to low temperatures we found a pronounced decrease in the rapidity of the increase in radiationless energy dissipation, as indicated by a very slow development of chlorophyll fluorescence quenching, as well as very slow zeaxanthin formation under an excess of light at chilling temperatures. Thorough studies by Greer and co-workers of the response of *Actinidia deliciosa* (kiwi-fruit), which was not acclimated to chilling conditions, to a combination of high light and low temperatures [81,82,84] have revealed that decreases in photochemical efficiency of PS II upon exposure to high light result from different combinations of processes at low versus moderate temperatures. At low temperatures the decrease in photochemical efficiency was concluded to result largely from 'photoinhibitory damage' to PS II (a decrease in the rate constant for photochemistry, k_P, or energy transfer, k_T), whereas at higher, more moderate temperatures such decreases in photochemical efficiency were due to a relatively greater increase in radiationless energy dissipation (increase in k_D). This may, however, be quite different in low temperature-acclimated plants (cf. Refs. 174, 190) (see also Table III; *Euonymus kiautschovicus* and *Pinus ponderosa*).

Plants growing in natural sunlight in the field which have been examined so far contained considerable amounts of zeaxanthin at noon (Table III), even those with high photosynthetic capacities such as cotton [68]. Zeaxanthin levels showed pronounced diurnal changes with typically little zeaxanthin present in the morning when leaves received low light levels, increasing accumulation of zeaxanthin with increasing PFD, and maximum zeaxanthin levels at peak irradiance [59,68] (Table III). One might therefore expect that all plants growing in natural sunlight will contain zeaxanthin at noon. One might ask why these species growing in full sunlight, such as cotton, do not increase their photosynthetic capacity further to allow complete utilization of all of the light absorbed at peak irradiance at noon. The fact that the light levels are lower during a considerable portion of the rest of the day, however, probably means that a very high investment of energy and nitrogen into an extremely high photosynthetic capacity would not be matched by a sufficiently high return in terms of carbon gain when the latter is integrated over the whole day with low light and high light periods.

There seems to be a relationship between the photosynthetic capacity, the 'epoxidation state' $(V + 0.5A)/(Z + A + V)$, and the total pool size of the xanthophyll cycle as was recently reported by Thayer and Björkman [199]. These authors suggest that one might be able to use this ratio in conjunction with the xanthophyll cycle pool size to predict the photosynthetic capacities of plants. There were, for example, pronounced differences in the percent of the xanthophyll cycle pool which was present as zeaxanthin at noon in leaves receiving full sunlight, from only 20–30% in cotton [68] to 86% in *Arbutus unedo* [59]. Whereas *Arbutus* exhibited no net CO_2 uptake at midday cotton had a CO_2 uptake rate of 35–40 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Furthermore, cotton had very large pools of violaxanthin

such that only a relatively small percentage of violaxanthin had to be converted to zeaxanthin. In contrast, *Arbutus* converted all of the violaxanthin present to zeaxanthin and showed a further small increase in zeaxanthin content accompanied by a small decrease in β-carotene at noon during the study period.

Judging from the relatively rapid reversal of chlorophyll fluorescence quenching subsequent to peak irradiance and from its characteristics in plants like cotton [68] or *Nopalea* [6], it seems that these plants in the field are not in danger of exceeding their capacity for energy dissipation in the photochemical apparatus. In contrast, those leaves or photosynthetic systems which have been used in many laboratory studies are typically grown at considerably lower light and therefore presumably have much smaller xanthophyll cycle pools (cf. Ref. 199) (Table III). They are therefore likely to possess, and have in fact been shown to possess, lower capacities for energy dissipation within the photochemical apparatus and to be prone to damaging effects [24,51] which may be considerably less common in the field. The relevance of the occurrence of damaging and repair processes versus increased energy dissipation and its reversal therefore needs to be investigated in the field.

V. Summary and conclusions

Exposure of the photosynthetic apparatus to an excess of light induces a more or less rapidly reversible decrease in the intrinsic efficiency of photosynthetic energy conversion; this can be due either to 'photoinhibitory damage' or to a regulated dissipation of the excess of excitation energy within the photochemical system.

Changes in chlorophyll fluorescence characteristics during exposure to an excess of light under a wide range of conditions and in a variety of species suggest that a major and generally employed photoprotective dissipation process within the photochemical apparatus occurs in the chlorophyll pigment bed via radiationless dissipation of excitation energy. The association between this radiationless dissipation process in the pigment bed and the zeaxanthin content, again under a wide range of different conditions and in many species, suggests that zeaxanthin is involved in the de-excitation of the excited singlet state of chlorophyll in the chlorophyll pigment bed under an excess of light. This interaction seems to depend on a special state of the chlorophyll-binding complexes. The exact mechanism of this interaction remains to be determined.

Evidence for a causal relationship between zeaxanthin and radiationless energy dissipation in the chlorophyll pigment bed is summarized in Table I. The main lines of evidence are (1) the inhibition of the dissipation process in the chlorophyll pigment bed in organisms which are treated with an inhibitor of the formation of zeaxanthin from violaxanthin which does not affect the rate of photosynthesis nor other dissipation processes and (2) the inability for radiationless energy dissipation in the chlorophyll pigment bed in organisms which lack the xanthophyll cycle, as long as they have not synthesized zeaxanthin otherwise.

The zeaxanthin-associated energy dissipation process in the chlorophyll pigment bed can occur alone or in combination with other dissipation mechanisms such as, apparently, energy dissipation within or around the PS II centers.

A photoprotective function for zeaxanthin and for the radiationless energy dissipation process in the chlorophyll pigment bed is supported by the finding that a variety of zeaxanthin-containing organisms (1) exhibit the ability to prevent overexcitation of the PS II centers more efficiently than the corresponding zeaxanthin-free organisms and (2) possess the capacity for a rapid and complete recovery from high light stress whereas the corresponding zeaxanthin-free organisms suffer sustained depressions in the efficiency of photochemical and photosynthetic energy conversion. Thus the zeaxanthin-containing organisms possess a greater resistance against adverse effects of high light stress than the zeaxanthin-free organisms.

Furthermore, during the acclimation of leaves to full sunlight or other conditions representing an excess of light, the major change in the carotenoid composition of the photochemical apparatus is a strong increase in the xanthophyll cycle pool size.

The ecological relevance of zeaxanthin is indicated by the fact that leaves growing in full sunlight under favorable conditions undergo a daily synthesis and removal of large amounts of zeaxanthin. In plants experiencing a combination of high light and water stress or high salinity, the reversal of an increase in radiationless energy dissipation in the chlorophyll pigment bed becomes increasingly slower. In these cases 'photoinhibition' of photosynthesis in the field may be attributed to this slow reversal of the photoprotective process. It remains to be determined whether or not 'damage' to the photosynthetic system ever occurs as a result of an insuffient capacity of photoprotective processes in the field.

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