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Quenching of chlorophyll fluorescence in the major light-harvesting complex of photosystem II: A systematic study of the effect of carotenoid structure

(nonphotochemical quenching/photosynthesis)

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ABSTRACT **The role of carotenoids in quenching of chlorophyll fluorescence in the major light-harvesting complex of photosystem II has been studied with a view to understanding the molecular basis of the control of photoprotective nonradiative energy dissipation by the xanthophyll cycle *in vivo*. The control of chlorophyll fluorescence quenching in the isolated complex has been investigated in terms of the number of the conjugated double bonds for a series of carotenoids ranging from n = 5–19, giving an estimated first excited singlet state energy from 20,700 cm^{-1} to 10,120 cm^{-1}. At pH 7.8 the addition of exogenous carotenoids with ≥10 conjugated double bonds (including zeaxanthin) stimulated fluorescence quenching relative to the control with no added carotenoid, whereas those with $n \leq 9$ conjugated double bonds (e.g., violaxanthin) had no effect on fluorescence. When quenching in the light-harvesting complex of photosystem II was induced by a lowering of pH to 5.5, carotenoids with $n \leq 9$ conjugated double bonds (including violaxanthin) caused a noticeable inhibition of fluorescence quenching relative to the control. Of the 10 carotenoids tested, quenching induced by the addition of the tertiary amine compound, dibucaine, to isolated light-harvesting complex of photosystem II could only be reversed by violaxanthin. These results are discussed in terms of the two theories developed to explain the role of zeaxanthin and violaxanthin in nonphotochemical quenching of chlorophyll fluorescence.**

The operation of the xanthophyll cycle in higher plant photosynthetic tissues, involving the cyclic interconversion of three carotenoids—namely, violaxanthin, antheraxanthin, and zeaxanthin—has been reported for many years (for example, refs. 1 and 2). More recently the formation of zeaxanthin has been particularly well correlated with nonphotochemical quenching of chlorophyll fluorescence in plants and some algae (see refs. 3–5), a process that safely dissipates excess levels of excitation energy in the thylakoid membrane, preventing photodamage to the pigments and proteins of photosystem II. It is generally accepted that a synergistic effect exists on quenching between the conversion of violaxanthin into zeaxanthin and the formation of the transthylakoid pH gradient (6, 7). Two main ideas concerning the molecular mechanism of zeaxanthin-mediated quenching have been suggested: (*i*) singlet–singlet interaction between chlorophyll and carotenoid, resulting in the direct quenching of chlorophyll fluorescence in the presence of zeaxanthin but not of violaxanthin (8, 9), with the ΔpH being necessary to bring the pigments into close proximity or to otherwise alter the efficiency of quenching by direct effects on the pigments (8); (*ii*) an indirect role for zeaxanthin and violaxanthin involving control of the ΔpH-dependent structural reorganization of the light-harvesting complex of photosystem II (LHCII), the chlorophyll *a*/*b* complexes associated with photosystem II (PSII) (10) that leads to the formation of "quenchers"—chlorophyll–chlorophyll or chlorophyll–xanthophyll associates (11). Studies on the properties of purified xanthophylls have provided evidence for both these mechanisms (9, 12).

Only recently has the first experimental work been reported that establishes the feasibility of energy transfer from chlorophyll to zeaxanthin: Frank and colleagues (9, 13) determined the first excited singlet state (S_1) energy levels of purified xanthophyll cycle pigments by application of the energy-gap law to data obtained from femtosecond transient absorption spectroscopy. This work demonstrated that the relationship of these S_1 energy levels to that of chlorophyll *a* (14,700 cm^{-1}) was such that it would be possible for zeaxanthin (13,935 cm^{-1}) to effectively act as a quencher of chlorophyll fluorescence, but violaxanthin (15,120 cm^{-1}) would only act as a light-harvesting pigment. It has been suggested that the lifetimes of these excited states are also important in determining the role of these pigments *in situ* (8, 9). It should be noted that these values were determined for isolated pigments in organic solvent and not for pigments bound *in situ* in the pigment–protein complexes; moreover, because energy transfer requires orbital contact between carotenoid and chlorophyll, at this stage this model is hypothetical.

Evidence has also been obtained to support the view that the xanthophyll-cycle carotenoids exert control over LHCII structure. These studies suggested that formation of zeaxanthin as a result of the operation of the xanthophyll cycle is an activator of quenching rather than being directly involved in the quenching mechanism. The evidence for this model has been recently reviewed (7, 11), and a principal observation is that quenching can be seen in the absence of zeaxanthin (14). It has been proposed that the events underlying quenching *in vivo* are essentially the same as those occurring when LHCIIb (the major form of LHCII) is converted into an aggregated quenched state, a process associated with clear changes in the properties of bound chlorophyll molecules (15–17). By using isolated LHCIIb it has been shown that the addition of carotenoids exerts strong control over quenching of chlorophyll fluorescence by affecting the aggregation state of the complexes (18); of particular importance was the observation that violaxanthin was an *inhibitor* of quenching produced by acidification of the complex, an observation not easily reconcilable with a direct role for zeaxanthin in quenching but consistent with the LHCII model in which the role of violaxanthin and zeaxanthin was described as inhibiting or stimulat-

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Abbreviations: CDB, conjugated double bonds; PSII, photosystem II; LHCII, light-harvesting complex of PSII; S_1, first excited singlet state.

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ing aggregation, respectively (6, 10, 11). In this model, the difference between these carotenoids would be physicochemical—e.g., a difference in conformation or the specific effect of the presence of the epoxide group. In line with this rationale a study of the six major xanthophylls found in the leaves of higher plants showed that there was a strong correlation between "apparent polarity", as determined by their solubility in ethanol/water mixtures (in effect, tendency to aggregate) and the length of the conjugated double-bond system (12). Thus, the available data does not allow discrimination between these two possible effects of converting violaxanthin into zeaxanthin because the low-lying energy levels and "apparent polarity" both depend on the extent of conjugation.

In the present study we have used the relatively simple and rapid assay of chlorophyll fluorescence quenching in isolated LHCIIb to systematically investigate which of these two properties of xanthophyll-cycle carotenoids may be most significant in the control of quenching; in particular, a range of carotenoids (with a number of structural features) for which the S_1 energy levels are either known or can be predicted have been used to examine their effect on fluorescence. It is shown that while some data can be explained by direct quenching of chlorophyll fluorescence by carotenoid, structural interaction between LHCIIb and carotenoid has to be invoked to fully explain the role of the xanthophyll cycle in the control of energy dissipation.

MATERIALS AND METHODS

LHCIIb Preparation. In this paper the term LHCII is used to describe all LHCII types found in the thylakoid membrane, both the minor complexes LHCIIa, LHCIIc, and LHCIId and

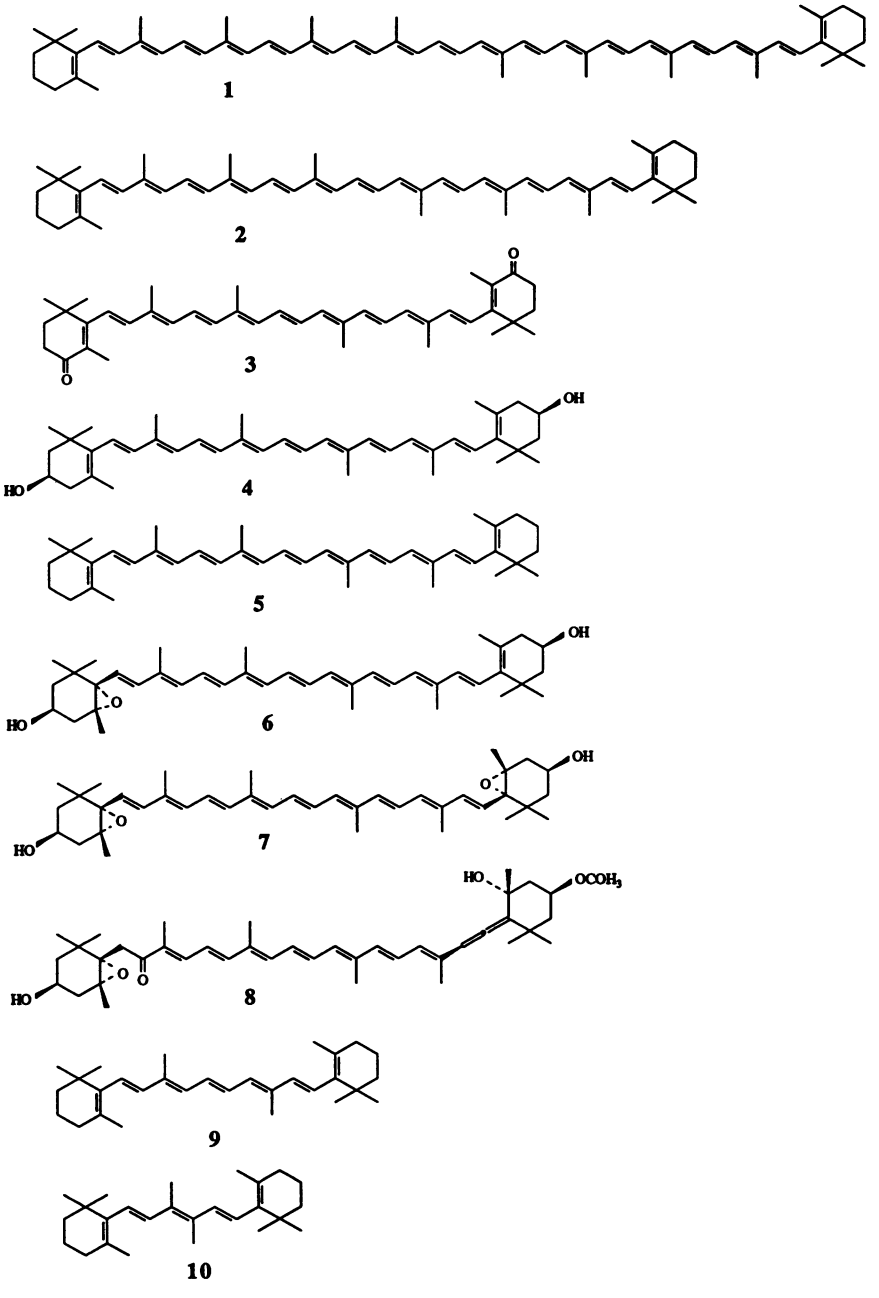

FIG. 1. Structure of the carotenoids used: **1**, Dodecapreno-β-carotene; **2**, decapreno-β-carotene; **3**, canthaxanthin; **4**, zeaxanthin; **5**, β-carotene; **6**, antheraxanthin; **7**, violaxanthin; **8**, fucoxanthin; **9**, mini-7-β-carotene; **10**, mini-5-β-carotene. Other compounds used in this study and for which no data on S_1 energies were available included astacene, echinenone, lactucaxanthin, lutein, mini-9-β-carotene, mini-3-β-carotene, and a series of canthaxanthin homologues.

Table 1. S_1 energies of carotenoids used in this study for which published values are available

Carotenoid	S_1 energy, cm^{-1}	Refs.
Dodecapreno-β-carotene	10,120	13, 24
Decapreno-β-carotene	10,880	13, 24
Canthaxanthin	13,300	13, 24
Zeaxanthin	13,935	9, 13
β-Carotene	14,100	13, 31
Antheraxanthin	14,510	9, 13
Violaxanthin	15,120	9, 13
Fucoxanthin	15,870	13, 32
Mini-7-β-carotene	20,000	13, 24, 25
Mini-5-β-carotene	22,700	13, 24, 25

The S_1 energy levels shown are all calculated using the revised energy gap law (13) from original data obtained in the references shown. See Fig. 1 for the structures.

the main complex LHCIIb. The latter, which has been the subject of many structural and spectroscopic investigations, is the experimental material used here, and it was prepared by isoelectric focusing from "BBY" particles obtained from dark-adapted leaves of both spinach and lettuce (19, 20). For each replicate analysis, LHCIIb from both species was obtained from a single isoelectric focusing run to minimize possible variations in preparation. The elution buffer was 20 mM Hepes, pH 7.8/200 μM *n*-dodecyl β-D-maltoside. The endogenous carotenoid content (mol/mol) of these complexes is ≈2 lutein, 1 neoxanthin, and 0.3 violaxanthin for spinach and 1.7 lutein, 1 neoxanthin, 1 lactucaxanthin, and 0.3 violaxanthin for lettuce; no detectable amounts of antheraxanthin or zeaxanthin are present in either complex (19, 20).

Chlorophyll Fluorescence. For chlorophyll fluorescence measurements, the procedure of Ruban *et al.* (18) was followed. Aliquots of LHCIIb (from a stock preparation of 100 μM chlorophyll) were injected with continuous stirring into the 1.0-cm^3 cuvette containing 20 mM Hepes, pH 7.8, to give a final chlorophyll concentration of 3 μM and 6 μM *n*-dodecyl β-D-maltoside. The OD at 680 nm was ≈0.12. Chlorophyll fluorescence was measured at 20°C by using a Hansatech modulated fluorimeter; fluorescence was excited at 583 nm (36-nm bandwidth) and detected through a 700-nm interference filter (10-nm bandwidth). Purified carotenoids (see below) were added to this incubation mixture at a concentration of 5 μM. After ≈30 sec at pH 7.8, acidification was produced by adding sufficient acid to lower the pH to 5.5.

Pigment Purification and Analysis. Carotenoids were purified by a combination of normal-phase TLC/column chromatography (on silica followed by Brockman grade III alumina) and reversed-phase HPLC immediately before use to avoid possible sample degradation and to remove impurities (see ref. 21). The extinction coefficients used were those of Davies (22), but for the mini-β-carotene homologues values of ε_{mol} = 25,000 (312 nm in hexane) and 46,000 (371 nm) were used for the mini-5 and mini-7-β-carotenes, respectively. Some carotenoid standards were from George Britton (University of Liverpool). Decapreno-β-carotene and dodecapreno-β-carotene were from Hoffmann–La Roche. The structures of the various carotenoids used in the present study are shown in Fig. 1 together with their S_1 energy levels (Table 1). These compounds were selected for several reasons: (*i*) their S_1 energy levels have been determined directly either by experimental means or via extrapolation using the energy-gap law (13, 23–25), (*ii*) these S_1 energy levels lie either above or below those previously determined for the xanthophyll cycle pigments in the range 10,120–22,700 cm^{-1}, (*iii*) both hydrocarbons and xanthophylls are represented with a range of structural features (different number of conjugated double bonds and the presence/absence of different oxygen functions).

RESULTS AND DISCUSSION

The effects of exogenous carotenoid on fluorescence quenching in spinach LHCIIb have previously been demonstrated for both violaxanthin and zeaxanthin (18). Dilution of spinach LHCIIb into a solution containing only 6 μM dodecylmaltoside has been shown to create a condition in which chlorophyll fluorescence is quenched and aggregation occurs (18). This process could be accelerated or inhibited by agents or conditions known to control quenching *in vivo* and, in particular, contrasting effects of violaxanthin and zeaxanthin were demonstrated. Data obtained for addition of a range of carotenoids to LHCIIb prepared from spinach and lettuce are shown in Table 2. The values for the spontaneous quenching at pH 7.8 measured 30 sec after dilution in the absence of added carotenoid were ≈0.03 and 0.05 for lettuce and spinach, respectively. In the presence of zeaxanthin this value was increased by a factor of 4–5, whereas violaxanthin had only a small effect and antheraxanthin addition gave an intermediate quenching level. Particularly significant was that the effect observed with zeaxanthin could be replicated in the presence of other carotenoids with ≥11 conjugated double bonds (CDB), including hydrocarbons such as β-carotene. In contrast, those carotenoids with $n \leq 9$ CDB were without effect.

In Fig. 2 the extent of quenching is plotted against the S_1 energy level of the carotenoid; only carotenoids with S_1 energies lower than that of chlorophyll *a* (680 nm or 14,700 cm^{-1} —vertical dashed line) have the ability to increase the

Table 2. The effect of 5 μM carotenoid on chlorophyll fluorescence quenching in LHCIIb isolated from lettuce and spinach

Carotenoid	$\Delta F/F$ at pH 7.8		$\Delta F/F$ at pH 5.5	
	Lettuce LHCIIb	Spinach LHCIIb	Lettuce LHCIIb	Spinach LHCIIb
Control (no carotenoid)	0.028	0.050	0.795	2.180
Dodecapreno-β-carotene	0.147 (0.004)	0.279 (0.009)	1.350 (0.040)	3.440 (0.110)
Decapreno-β-carotene	0.136 (0.004)	0.274 (0.008)	1.300 (0.035)	3.320 (0.090)
Canthaxanthin	0.121 (0.003)	0.266 (0.007)	0.910 (0.026)	2.800 (0.084)
Zeaxanthin	0.114 (0.003)	0.258 (0.006)	0.880 (0.026)	2.270 (0.080)
β-Carotene	0.112 (0.002)	0.253 (0.006)	0.860 (0.016)	2.180 (0.066)
Antheraxanthin	0.070 (0.002)	0.152 (0.004)	0.560 (0.018)	1.340 (0.061)
Violaxanthin	0.038 (0.001)	0.075 (0.004)	0.410 (0.012)	0.710 (0.042)
Fucoxanthin	0.031 (0.001)	0.066 (0.003)	0.290 (0.010)	0.630 (0.039)
Mini-7-β-carotene	0.028 (0.001)	0.062 (0.002)	0.190 (0.009)	0.600 (0.032)
Mini-5-β-carotene	0.022 (0.001)	0.056 (0.002)	0.160 (0.008)	0.580 (0.028)

Fluorescence was measured 30 sec after dilution at pH 7.8 and after acidification to pH 5.5. Quenching was calculated as $\Delta F/F$, where ΔF is $F_m - F$, F_m being the maximum level of fluorescence of LHCIIb in the unquenched state and F the quenched fluorescence level. The control refers to experiments in which only ethanol was added. Results shown are the means (±SE) of three to six replicates with LHCIIb obtained from at least three separate isoelectric focusing runs.

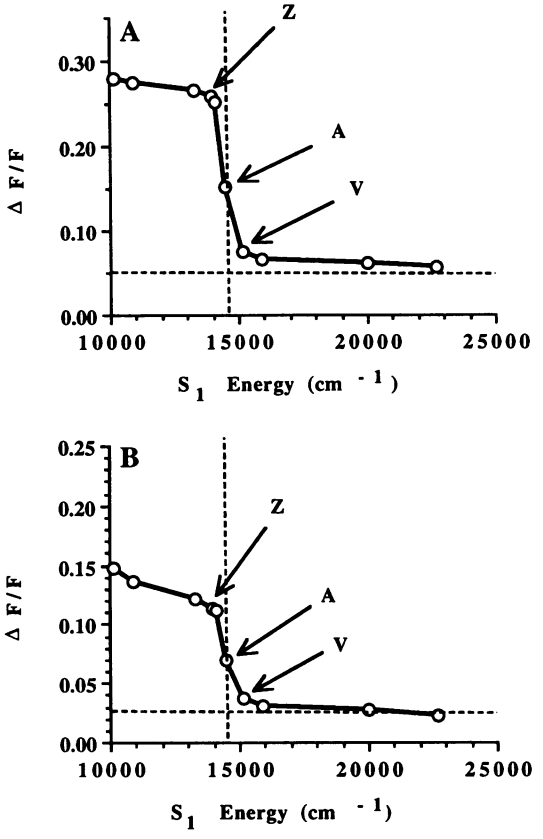

FIG. 2. Relationship between carotenoid S_1 energy and the extent of initial quenching in LHCIIb isolated from spinach (*A*) and lettuce (*B*) at pH 7.8. Quenching data are taken from Table 2, and the carotenoid S_1 energy levels are taken from Table 1. The vertical line indicates the position of the S_1 (Q_y) band of chlorophyll *a*, and the horizontal line indicates the control level of quenching in the absence of added carotenoid. Z, A, and V show the positions of the xanthophyll cycle carotenoids—namely, zeaxanthin, antheraxanthin, and violaxanthin.

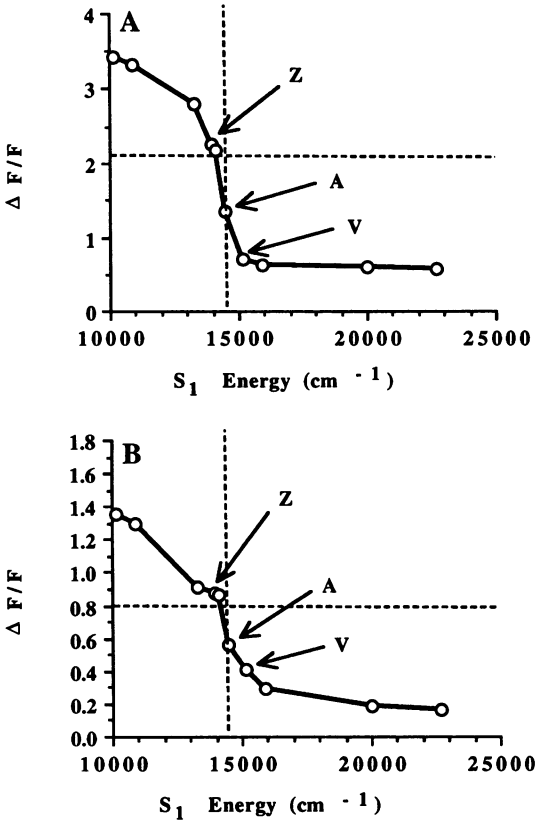

FIG. 3. Relationship between carotenoid S_1 energy and the final extent of quenching in LHCIIb isolated from spinach (*A*) and lettuce (*B*) at pH 5.5. See legend to Fig. 2 for full details.

quenching of fluorescence in this *in vitro* system relative to the control with no added carotenoid (horizontal dashed line), whereas carotenoids with S_1 energies above that of chlorophyll *a* have little, if any, effect on fluorescence yield of LHCIIb. An important observation from this study is that there is no difference in the response resulting from the addition of either xanthophylls or carotenes that have similar S_1 energies (e.g., fucoxanthin and mini-9-β-carotene; β-carotene and zeaxanthin) showing that "structural" features of the carotenoid other than the extent of conjugation are apparently unimportant in this effect. Despite different extents of quenching, the same trend was seen for both spinach and lettuce LHCIIb.

It is important to note that the transition from a "nonquencher" to a "quencher" for a carotenoid molecule in Fig. 2 is only related to whether the S_1 energy is above or below an energy level threshold that corresponds to that of the Q_y of chlorophyll *a* (vertical dashed line). Little or no additional benefit is obtained from possessing a lower S_1 energy than that of zeaxanthin (13,935 cm^{-1}), but, given the predicted large bandwidth of the carotenoid absorption, this may not be unexpected (H. A. Frank, personal communication).

It has been shown that strong quenching in isolated LHCIIb could be induced by lowering the pH of the incubation medium to pH 5.5 (18), the estimated pH of the thylakoid lumen *in vivo*. In this study, the quenching observed in the absence of added carotenoid was 30–40 times greater at pH 5.5 than at pH 7.8 (Table 2). The overall trend observed for the effect of adding specific carotenoids is the same at pH 5.5 as that described above for pH 7.8, except that the presence of carotenoids with chain lengths <10 resulted in a strong (≈70%) *inhibition* of quenching compared with the control value obtained without added carotenoid (Table 2). Although this time zeaxanthin had negligible effect, those carotenoids with chain lengths longer than 11 resulted in a 50% increase in the level of quenching, to 3.4 for spinach and 1.4 for lettuce. When these data are plotted against the carotenoid S_1 energy level, a sigmoidal relationship to Fig. 2 is again revealed (Fig. 3), this time with the energy threshold below which inhibition of quenching occurs corresponding to the energy level of the chlorophyll Q_y transition; thus, this trend is seen despite the fact that *inhibition* of quenching accounts for all the data for carotenoids with CDB < 11.

Comparison of Figs. 2 and 3 shows that the quantitative effect (i.e., the relative position of the control observed in the absence of carotenoid addition) depends on the pH. Thus, when measurements are made at pH 6.0, the inhibition of quenching by short-chain carotenoids is less dominant and the stimulatory effect of the long-chain carotenoids is more clearly revealed. Such effects reveal a synergism between pH and carotenoid very similar to that seen for nonphotochemical quenching *in vivo*.

A number of other carotenoids were used in this study but for which no data on S_1 energies were available. These carotenoids included astacene, echinenone, lactucaxanthin, lutein, mini-9-β-carotene, mini-3-β-carotene, and a series of canthaxanthin homologues. Due to the lack of any verification of the S_1 energies of these compounds by experimental means, data obtained by using these carotenoids have not been included in this paper. However, it is clear that when calculations (based on extrapolation using the structural similarities between these and the compounds shown in Fig. 1) are made giving the approximate S_1 energies of this second set of carotenoids, the

same trends are observed for both stimulation and inhibition of fluorescence quenching as shown in Figs. 2 and 3 (data not shown).

Despite the clarity of the general relationship between quenching and carotenoid energy level revealed above, the inhibitory effect of carotenoid on quenching seen at pH 5.5 cannot be simply explained on the basis of the energetics of the pigment molecules involved. Two explanations need to be discussed: (*i*) that the interactions giving rise to quenching may be more complex than previously suggested (9); (*ii*) that the relationship shown in Fig. 2 may not mean that zeaxanthin–chlorophyll energy transfer is involved and that the effects have a purely structural basis.

In terms of the first idea, it is proposed that the addition of any carotenoid to isolated LHCIIb may disrupt chlorophyll–chlorophyll interactions responsible for quenching at low pH, resulting in a rise in chlorophyll fluorescence. Such "anti-quenching" effects of carotenoids, including xanthophylls, have been observed with purified pigments in liposomes (26). However, for carotenoids with low S_1 energies, this anti-quenching effect would compete with the direct quenching of chlorophyll excited states by the carotenoid itself. Hence, whether quenching was inhibited or stimulated would depend on the balance between these opposing effects. In fact, such behavior has also recently been demonstrated *in vitro* in organic solvents for β-carotene-mediated quenching of chlorophyll fluorescence when the addition of carotenoid initially de-aggregates chlorophyll, resulting in a rise in fluorescence (33). It is therefore proposed that one explanation of the data observed here is that the addition of carotenoids disrupts chlorophyll aggregates formed as a result of a lowering of pH, effectively increasing fluorescence and thereby giving an inhibition of quenching by those carotenoids whose energy levels are not low enough to quench chlorophyll fluorescence directly. Complex interaction between pH and specific carotenoids would ensue because of various strengths of chlorophyll/chlorophyll interaction, chlorophyll/carotenoid interaction, and quenching efficiency. Although this rationale provides an explanation of the data obtained in an *in vitro* system, similar effects *in vivo*, in which the quencher may also be a chlorophyll/chlorophyll/xanthophyll associate, as suggested by Horton and Ruban (11), may provide an explanation of the apparently contradictory results in the literature for nonphotochemical quenching and reconcile the direct and indirect role of the xanthophyll cycle.

The exact nature of the site of interaction between exogenous carotenoid and chlorophyll molecules bound to the isolated LHCIIb is not known. Given the similarity in the response seen for the molecules used in this study with a wide range of polarities (ranging from carotenes such as β-carotene through to a xanthophyll such as fucoxanthin), it is likely that any interaction takes place with the peripheral chlorophylls of the complex only. This is the most likely site of the binding of violaxanthin and zeaxanthin *in vivo* given their accessibility to the enzymes of the xanthophyll cycle and the tendency for these carotenoids (but not lutein) to be released from the complex (19). Because only two carotenoid molecules (probably luteins) are shown in the structural model of LHCIIb (27), this again indicates a location at the periphery of the complex.

Quenching in isolated LHCIIb due to low pH is associated with aggregation (18), suggesting that the influence of carotenoids has a structural basis and, therefore, that the appearance of the plots shown in Figs. 2 and 3 need not indicate a key role for energy transfer from chlorophyll to zeaxanthin. This seems unlikely, however, and instead it is possible that LHCIIb aggregation is driven by chlorophyll/chlorophyll association and that violaxanthin is an inhibitor of this mechanism; to fulfill such a role of controlling LHCII organization and maintain a high quantum efficiency for LHCII function, the energy level of violaxanthin would need to be higher than

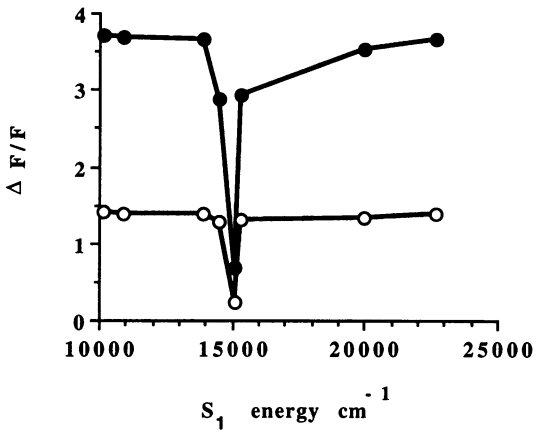

FIG. 4. Recovery of chlorophyll fluorescence (expressed as fluorescence quenching: $\Delta F/F$) in dibucaine-treated LHCIIb brought about by the addition of 5 μM carotenoid (●, spinach; ○, lettuce). Points correspond to individual carotenoids of Table 1.

chlorophyll. This model predicts that there may be a specific interaction between violaxanthin and a binding site on LHCII. Evidence for such a site is provided by the data shown in Fig. 4. In addition to pH reduction, other factors have been identified as inducing quenching in isolated LHCIIb (18). The addition of the tertiary amine compound, dibucaine, to isolated LHCIIb induces rapid quenching similar to pH reduction. The extent of quenching can be greater than that obtained by a reduction in pH to 5.5 and the addition of carotenoids to this system does not result in any further decrease in fluorescence (data not shown). In a previous report it was demonstrated that the recovery of fluorescence could be observed in dibucaine-treated LHCIIb with violaxanthin but not with zeaxanthin (18). In the present study we have taken the opportunity to extend these experiments by using the series of carotenoids listed in Table 1 (Fig. 4) and other selected xanthophylls (data not shown). Fig. 4 shows the effect on fluorescence quenching when purified carotenoids are added to the isolated complex. Only one compound—namely, violaxanthin—shows any effect on fluorescence in this system, and the addition of this particular xanthophyll results in an actual reversal of fluorescence quenching. Other carotenoids with similar or higher S_1 energies have no effect. Both dibucaine and a reduction in pH can control quenching in isolated thylakoids (28), and, although the mechanism whereby dibucaine mediates such quenching is still unclear, both have been shown to be linked to LHCII aggregation (18). It is possible that dibucaine competes for a violaxanthin-binding site on LHCII, and it is interesting in this regard to note the recent observation that dibucaine inhibits violaxanthin de-epoxidase activity in a membrane-free partially purified enzyme (29). The data reveal a specific role for violaxanthin that is not directly related to its S_1 energy level but to a specific binding site in LHCIIb; it is suggested that this binding site has a significant role to play in the control of LHCII structure/organization and, hence, quenching of chlorophyll fluorescence *in vivo*. It should be added that although the data shown here and in a previous report (18) have been obtained with isolated LHCIIb, the effects of low pH and violaxanthin and zeaxanthin on quenching in the minor LHCII components LHCIIa and LHCIIc are even larger (30), consistent with suggestions that these complexes play a key role in nonphotochemical quenching *in vivo* (6, 7).

In conclusion these results show that the effects of interaction between carotenoids and LHCII are complex and cannot be completely explained in terms of direct energy transfer and quenching of chlorophyll excited states by carotenoids with low S_1 energies. The switch between carotenoids with conjugated

chain lengths of <9 and >11 specifies the change from quenching inhibition and stimulation, respectively. Although this change corresponds to the S_1 energy level of chlorophyll, suggesting a role for direct quenching by longer-chain carotenoids, close examination of the data suggests that features of the xanthophyll-cycle carotenoids other than their S_1 energy levels may also be important in the control of nonphotochemical quenching *in vivo*. However, the present observations do not reveal the mechanism of any energy transfer between chlorophyll and carotenoid in the isolated LHCIIb or *in vivo*. At pH 7.8, without protonation, the effects of added carotenoid are relatively weak, and it is only at low pH that any significant transfer can be invoked, exactly as for nonphotochemical quenching *in vivo*. It has been assumed that such transfer requires orbital contact between molecules for electron exchange and that protonation allows this close contact to occur (7). However, the oscillator strength of the carotenoid S_0–S_1, which may be increased upon interaction with amino acid side chains and further elevated upon protonation, may allow dipole coupling at distances of 5–10 Å (8, 23). Nevertheless, it is important to point out that, irrespective of the exact molecular mechanism(s) involved, the data reveal the elegance of the biological control of light-harvesting efficiency in green plants, in which a simple reversible chemical change in the xanthophyll molecule elicits a profound change in LHCII function.

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