PHOTOSYNTHETICA 34 (3): 343-355, 1997

REVIEW

Mechanisms of non-photochemical chlorophyll fluorescence quenching in higher plants

P. POSPÍŠIL*

Department of Experimental Physics, Faculty of Sciences, Palacký University, tř. Svobody 26, CZ-771 46 Olomouc, Czech Republic

Abstract

The excitation energy of pigment molecules in photosynthetic antennae systems is utilised by photochemistry, partly it is thermally dissipated, and partly it is emitted as fluorescence. Changes in the quantum yield of chlorophyll (Chl) fluorescence reflect the changes in quantum yield of photochemical reaction and thermal dissipation of the excitation energy. Decrease of the Chl fluorescence quantum yield is called the Chl fluorescence quenching. The decrease of the quantum yield that is accompanied by photochemical reactions has been termed the photochemical quenching, and the decrease accompanied by thermal dissipation of the excitation energy is called the non-photochemical quenching. This review deals with mechanisms of the non-photochemical quenching.

Additional key words: antenna pigments; cytochrome; P680; photosystem 2; reaction centre; singlet state; triplet state; zeaxanthin.

Introduction

Regulation of non-photochemical Chl fluorescence quenching: The non-photochemical quenching is regulated by the concentration of protons [H^+] in thylakoid lumen (Briantais *et al.* 1979, Krause *et al.* 1988, Crofts and Horton 1991, Kuban and Horton 1995b). If Chls absorb more excitation energy than can be utilised by electron transport-dependent production of ATP and NADPH, protons accumulate in the lumen, and the [H^+] in the lumen increases. If pH in the lumen drops below 5.5, processes are switched on which in various ways protect photosystem 2 (PS2) against excessive radiant energy.

Localisation of non-photochemical Chl fluorescence quenching: Non-photochemical quenching occurs on the level of the reaction centre of photosystem 2 (RC2), and on

Received 29 January 1997, *accepted* 24 April 1997.
*Fax: (4268) 5225737; e-mail: pospip@risc.upol.cz

the level of the light-harvesting complex of photosystem 2 (LHC2) (Papageorgiou 1975). The non-photochemical quenching processes occurring in RC2 seem to be rather a protection through neutralisation of dangerous radicals arising during photoihibition than dissipation of the excessive excitation energy. The main part of the quenching processes occurs on the level of LHC2 (Horton and Ruban 1993,1994, Crofts and Yerkes 1994, Horton *et al.* 1994, Mullineaux *et al.* 1994, Ruban and Horton 1994). The LHC2 consists of four different Chl *a*/*b*-binding proteins (Peter and Thornber 1991). The bulk LHC2 which binds approximately 65 % of PS2 Chl is referred to as LHC2b, whereas the minor complexes, each accounting for only 5 % of Chl, are called LHC2a, LHC2c, and LHC2d (Peter and Thornber 1991). These minor complexes have also been named CP29, CP26, and CP24 (Jansson *et al.* 1992). Minor LHC2a,c,d play a key role in the quenching process, not only because these complexes are enriched in violaxanthin (Peter and Thornber 1991, Bassi *et al.* 1993), but also because they contain a site that binds protons upon their increasing concentration (Horton *et al.* 1992, Crofts and Yerkes 1994, Ruban *et al.* 1995). This is a strategic feature, because the minor LHC2a,c,d serve as bridges between the major LHC2 and RC2, and thus play a role in the regulation of excitation energy transfer from the main antennae complex LHC2b towards the RC2 (Bassi *et al.* 1993).

Mechanisms of non-photochemical quenching localised in RC2

increasing $[H^+]$ in the lumen → release of Ca^{2+} → inactivation of the oxygen evolving centre, OEC → accumulation of $P680^+$ → reduction of $P680^+$ by alternative ways → P680 is able to accept additional excitation energy from LHC2 and thus to quench Chl fluorescence

Increasing $[H^+]$ in the thylakoid lumen causes a release of Ca^{2+} cations from the binding site close to OEC (Homann 1988, Ono and Inoue 1988, Krieger and Weis 1992, 1993) changing the redox processes on the donor side of the RC2. The release of Ca^{2+} cations makes Mn-clusters unable to move into a higher S-state, and thus donates electrons to the secondary electron donor Z of RC2 (Boussac and Rutherford 1988, Ono and Inoue 1989, 1990, Yocum 1991). The secondary electron donor Z cannot under these conditions reduce the oxidized form of the primary electron donor $P680^+$ (Boussac and Rutherford 1992) which normally occurs in the time range of ns. Hence, conditions suitable for alternative ways of the reduction of $P680^+$ arise. The reduction occurs in a time range of ms (Schlodder and Meyer 1987). $P680^+$ can be reduced alternatively by Q_A^- or $Pheo^-$ (Schreiber and Neubauer 1987, 1990, Krieger and Weis 1992, 1993, Krieger *et al.* 1992) or by cytochrome b_{559} (cytb_{559}; Falkowski *et al.* 1986, Thompson and Brudvig 1988, Barber and De Las Rivas 1993).

Reduction of $P680^+$ by Q_A^- (PS2 back reaction): The release of Ca^{2+} cations influences redox processes on both the donor and acceptor sides of RC2 (Krieger and Weis 1992, 1993, Krieger *et al.* 1993, 1995, Andréasson *et al.* 1995, Johnson *et al.* 1995). The release of Ca^{2+} cations shifts the redox state of Q_A towards higher values

(from E_m -80 mV to E_m= +40 mV) (Krieger and Weis 1992, 1993, Krieger *et al.* 1993, 1995, Johnson *et al.* 1995) causing an inhibition of electron transfer from Q_A to Q_B (Andréasson *et al.* 1995). The effect of Ca^{2+} cation on the acceptor-side redox processes is probably mediated *via* a structural change of transmembrane helices acting as levers (Krieger and Weis 1992, Andréasson *et al.* 1995). The redox changes on the acceptor side of RC2 promote recombination of the radical pair $P680^+Q_A^-$ ($P680^+Pheo^-$) (Krieger and Weis 1992, 1993, Krieger *et al.* 1992, Andréasson *et al.* 1995). The $P680^+$ can be reduced by Q_A^- ($Pheo^-$) resulting in a singlet $^1P680^*$ or triplet $^3P680^*$ state of the primary electron acceptor (Booth *et al.* 1990, Schreiber and Neubauer 1990, Ramm and Hansen 1993) (see Fig. 1).

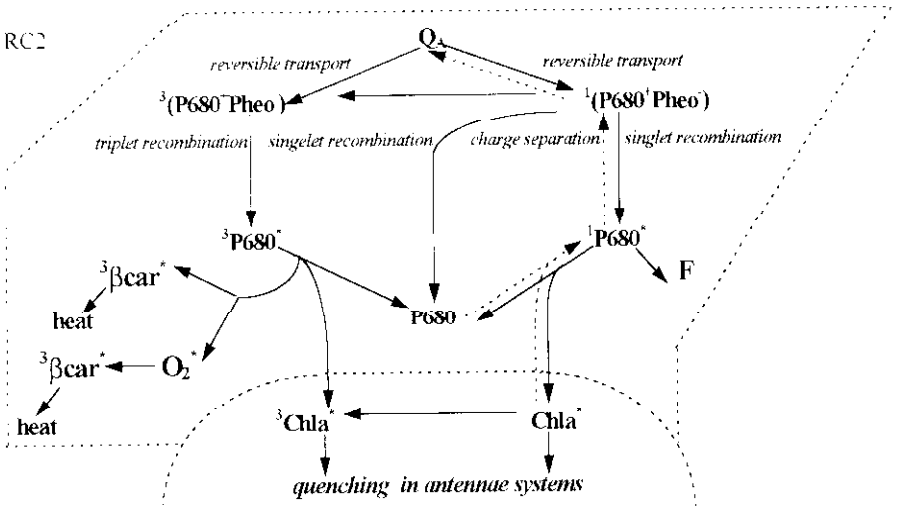

Fig. 1. A schematic model of processes associated with non-photochemical chlorophyll fluorescence quenching localised in RC2. The primary electron donor P680 accepts excitation energy from chlorophyll molecules of the antennae system and transforms it into the first singlet exited state $^1P680^*$. A charge separation occurs, resulting in radical pair $P680^+Pheo^-$. Electron from $Pheo^-$ is transported to Q_A. If the electron transport from OEC is inhibited, conditions favourable for charge recombination arise. The electron from Q_A^- is transported back to Pheo resulting in a singlet $^1(P680^+Pheo^-)$ or triplet $^3(P680^+Pheo^-)$ radical pair (reversible transport). The singlet radical pair $^1(P680^+Pheo^-)$ is unstable and recombines (singlet recombination). The singlet recombination results either in the ground state P680 or the first singlet exited state $^1P680^*$, which is emitted as 50 μs recombination chlorophyll fluorescence or transferred back to the antennae systems where the exitation is quenched. The triplet radical pair $^3(P680^+Pheo^-)$ is unstable and recombines (triplet recombination). The triplet recombination results in the triplet excited state $^3P680^*$, which is quenched by β-carotene directly or indirectly *via* singlet oxygen. Triplet exitation $^3P680^*$ can also be transferred to the antennae system where it is quenched (for further details see the text).

Singlet state: An electron from the reduced form of the primary electron acceptor Q_A^- reduces Pheo resulting in a singlet radical pair $^1(P680^+Pheo^-)$ (reversible transport). The singlet radical pair $^1(P680^+Pheo^-)$ is unstable and recombines (singlet recombination) into the ground state P680 or the first excited state $^1P680^*$ of the primary electron donor (Takahashi *et al.* 1987, Booth *et al.* 1990, Liu *et al.* 1993).

The ground state of the primary electron donor P680 is able to accept excitation energy from antenna systems and thus to quench Chl fluorescence. Excitation energy of the first excited state $^1P680^*$ is emitted as a 50 μs recombination fluorescence (Booth *et al.* 1990, Schreiber and Neubauer 1990) or may be transferred back to the antennae systems (Schatz *et al.* 1988), where the excitation is quenched (Schreiber and Neubauer 1989).

Triplet state: An electron from the reduced form of the primary electron acceptor Q_A^- can reduce Pheo resulting in a triplet radical pair $^3(P680^+Pheo^-)$ (reversible transport). The triplet radical pair $^3(P680^+Pheo^-)$ can also arise from the singlet radical pair $^1(P680^+Pheo^-)$ by dephasing of the pair electrons (Schreiber and Neubauer 1990, van Miegham *et al.* 1995). The $^3(P680^+Pheo^-)$ is unstable and recombines (triplet recombination) to give rise to the triplet excited state of the primary electron donor $^3P680^*$ (Takahashi *et al.* 1987, Booth *et al.* 1990, Miller and Brudvig 1991, Noguchi *et al.* 1993). The triplet excited state $^3P680^*$ is quenched by β-carotene or may be transferred to the antenna systems, where it is quenched (Schreiber and Neubauer 1990). The quenching of the triplet excited state $^3P680^*$ by β-carotene may occur directly or indirectly *via* singlet oxygen $^1O_2^*$ (Takahashi *et al.* 1987, Booth *et al.* 1990, Vass and Strying 1992, Telfer *et al.* 1994, Lambert and Redmond 1994) as is shown in the following scheme:

(*1*) directly:

$^3P680^* + \beta car \rightarrow P680 + {}^3\beta car^*$

$^3\beta car^* \rightarrow \beta car + heat$

(*2*) *via* singlet oxygen $^1O_2^*$:

$^3P680^* + {}^3O_2 \rightarrow P680 + {}^1O_2^*$

$^1O_2^* + \beta car \rightarrow {}^3O_2 + {}^3\beta car^*$

$^3\beta car^* \rightarrow \beta car + heat$

The triplet excitation $^3P680^*$ may be accepted by the β-carotene molecule (Jensen *et al.* 1982, Takahashi *et al.* 1987, Bialek-Bylka *et al.* 1995). The β-carotene is able to dissipate excited energy into heat. The $^3P680^*$ can also be accepted by the triplet oxygen in the ground state $^3O_2$ leading to the singlet oxygen $^1O_2^*$. The singlet excitation of $^1O_2^*$ may be accepted by the β-carotene, the result of which is the triplet state $^3\beta car^*$ (Telfer *et al.* 1994). The β-carotene dissipates the triplet energy to heat. Triplet-triplet energy transfer from Chl *a* to β-carotene is possible, because the triplet energy level of the β-carotene lies below the triplet energy level of Chl *a* (Lambert and Redmond 1994). Also the energy transfer from singlet oxygen $^1O_2^*$ to β-carotene is possible, because the triplet energy level of the β-carotene is below the energy level of the singlet oxygen $^1O_2^*$ (Lambert and Redmond 1994). Triplet-triplet energy transfer from Chl *a* to β-carotene occurs *via* an electron exchange mechanism (Farhoosh *et al.* 1994). The life time of the triplet state $^3P680^*$ is about 1 ms under anaerobic conditions, however, under aerobic conditions it is only 33 μs. Thus in the presence of oxygen, $^3P680^*$ is quenched predominantly *via* $^1O_2^*$ (Mathis *et al.* 1989, Durrant *et al.* 1990).

Reduction of $P680^+$ by cytb_{559} (cyclic electron transport around PS2): The release of Ca^{2+} cations decreases the stability of the high-potential form of cytb_{559} (HP cytb_{559}) and thus enables its conversion to the low-potential form of cytb_{559} (LP cytb_{559}) (McNamara and Gounaris 1995, Mizusawa *et al.* 1995). The conversion of the HP cytb_{559} (E_m = 380 mV) to LP cytb_{559} (E_m = 60 mV) occurs probably *via* a conformation change of the HP cytb_{559} molecule (Prasil *et al.* 1996). The LP cytb_{559} can accept an electron from $Pheo^-$ (Nedbal *et al.* 1992, Barber and De Las Rivas 1993) or plastoquinone (PQ) (Falkowski *et al.* 1986, Arnon and Tang 1988, Thompson and Brudvig 1988, Buser *et al.* 1992a,b). The HP cyt b_{559} can directly donate an electron to the oxidized form of the primary electron donor $P680^+$ (Arnon and Tang 1988, Satoh *et al.* 1990, Nedbal *et al.* 1992, Barber and De Las Rivas 1993), and indirectly *via* the secondary electron donor Z (Falkowski *et al.* 1986, Tamura *et al.* 1995) or *via* the accessory Chl *a* in the RC2 chl_z *a* (Thompson and Brudvig 1988, Buser *et al.* 1992a,b).

Mechanisms of non-photochemical quenching localised in antennae systems

increasing $[H^+]$ in lumen ⟨ creation of Chl *a*-Chl *a* dimers → quenching by Chl
conversion violaxanthin-zeaxanthin → quenching by zeaxanthin

Increasing $[H^+]$ in the lumen both causes a structural change of LHC2 leading to the creation of Chl *a*-Chl *a* dimers (aggregation of LHC2) and activates the enzyme violaxanthin deepoxidase leading to the conversion of violaxanthin, Vio, to zeaxanthin, Zea (xantophyll cycle). The Chl molecules in Chl *a*-Chl *a* dimers can quench the singlet excited state of antenna Chls. The Zea probably quenches both the singlet and triplet Chl-excited states or it may play a role of an amplifier of the Chl *a*-Chl *a* dimer quenching. All possible cases of the quenching in the antenna systems are described in detail below.

The quenching mediated by Chl

increasing $[H^+]$ in lumen → protonation of LHC2 proteins → structural change of LHC2 proteins → aggregation of LHC2 → association of Chl molecules in LHC2 → quenching:

The increasing $[H^+]$ in the lumen causes protonation of the LHC2 proteins. The H^+s bind to the carboxyamino acid residues of LHC2 proteins exposed to the lumen side of the thylakoid membrane (Horton *et al.* 1991). Another way of the protonation of LHC2 proteins represents a binding of H^+ to the carboxyamino acid residues of LHC2 proteins in the hydrophobic environment of thylakoid membrane (Noctor *et al.* 1991, 1993, Crofts and Yerkes 1994). In addition to the light-harvesting function, the LHC2 protein serves as a transport channel of protons released from the OEC into the lumen (Jahns and Junge 1990, 1993). Protons accumulate in the hydrophobic environment of the thylakoid membrane creating proton domains which act as a buffer of the protons. When this channel is blocked, protons leak to the stroma, but when it is absent (as in plants grown under intermittent irradiation), protons are

released directly into the lumen without the buffering. Protons in the buffering domains bind to the carboxyamino acid residues (aspartate and glutamate) of the minor LHC2a and LHC2c (Horton *et al.* 1992, Ruban *et al.* 1992b, Walters *et al.* 1994, Walters and Horton 1995). The protonation of the carboxyamino acid residues of LHC2 causes a structural change of this protein (Horton *et al.* 1991, 1994, Horton and Ruban 1993, 1994, Ruban and Horton 1995a). The carboxyamino acid residues are important for the structure of the transmembrane helix and co-ordination of pigment ligation to LHC2 proteins (Crofts and Yerkes 1994). Protonation of the carboxyamino acid residues evokes the charge neutralisation, which causes distortion of carboxyamino acid residues (Horton and Ruban 1994). The structural changes of LHC2 proteins allow these proteins to form aggregates (Ruban and Horton 1992, Pascal *et al.* 1995). The polarity of xanthophylls determined by the presence of terminal groups indicates the ability of xanthophylls to activate or inhibit aggregation of LHC2 (Ruban *et al.* 1993). Xanthophylls, such as Vio, neoxanthin and lutein, inhibit the aggregation of LHC2 and hence also the Chl *a*-Chl *a* quenching in LHC2 (Searle *et al.* 1990), whereas Zea promotes the aggregation of LHC2. The ability of xanthophylls to promote aggregation of LHC2 changes in the following order: neoxanthin < Vio < lutein < antheraxanthin < Zea (Ruban *et al.* 1993). Aggregation of LHC2 enables an association of Chl molecules in LHC2. Chl molecules come near together creating Chl-Chl dimers (Horton *et al.* 1991, Horton and Ruban 1994, Hagen *et al.* 1995). The physical mechanism of Chl fluorescence quenching in LHC aggregates is unknown. Aggregated Chl molecules may interact with one another influencing their electronic states and vibrational movements. The mechanism of Chl fluorescence quenching may be based upon strong interactions between electronic states and vibrational movements (Horton *et al.* 1991, Ruban and Horton 1992a).

Fluorescence quenching mediated by Zea: Its mechanism is still unclear. Zea may quench the singlet excited state ^{1}Chl a^* (Demmig-Adams 1990, Demmig-Adams and Adams 1992) by the following mechanism:

$$^1\text{Chl } a^* + \text{Zea} \rightarrow \text{Chl } a + {}^1\text{Zea}^*$$
$$^1\text{Zea}^* \rightarrow \text{Zea} + \text{heat}$$

The singlet excited ^{1}Chl a^* transfers excitation energy to Zea, whereby Chl goes to the ground state Chl *a*, and Zea is transferred to the first singlet excited state ^{1}Zea*. The singlet ^{1}Zea* is able to dissipate the excitation energy to heat.

Singlet-singlet energy transfer from Chl *a* to Zea is theoretically possible, because the first singlet excitation energy level of Zea lies below the first singlet excitation energy level of Chl *a* (Trash *et al.* 1979, Höfer *et al.* 1987, Owens *et al.* 1992, Frank *et al.* 1994). However, this energy transfer has so far not been shown *in vivo*. As the electron transition between the ground and first excitation energy levels in carotenoids' S_0-S_1 is formally dipole-forbidden, the singlet-singlet energy transfer from Chl to Zea cannot be *via* Foester's dipole-dipole resonance interaction, but it must involve Dexter's electron exchange mechanism (Naqvi 1980, Höfer *et al.* 1987, Owens *et al.* 1992, Owens 1994). Two mechanisms of singlet-singlet energy transfer between Chl and Zea occurring *via* electron exchange mechanism are possible:

(*1*) Effect of local fields (Cosgrove *et al.* 1990, Owens *et al.* 1992, Owens 1994)

increasing $[H^+]$ in lumen → protonation of LHC2 → local fields → energy overlap between S_1 level of Chl and S_1 level of Zea → singlet-singlet energy transfer from Chl *a* to Zea → quenching:

Increasing $[H^+]$ in the lumen cause protonation of LHC2 proteins. H^+ binds to carboxyamino acid residues of LHC2 protein exposed to the lumen (particularly to asparagin and glutamin) (Dilley *et al.* 1987). The protons bound to the LHC2 protein generate local fields, which interact with molecules of Chl bound to the LHC2 protein. The interaction between local fields of H^+ and molecules of Chl causes a shift of the first singlet excitation level S_1 of Chl towards the first singlet excitation level S_1 of Zea. The field-induced shift of the S_1 levels allows an overlap of both energy levels. The singlet-singlet energy transfer of excitation energy from Chl to Zea is possible.

(*2*) Aggregation of LHC2 (Adams *et al.* 1990, Ruban and Horton 1992, Ruban *et al.* 1992a)

increasing $[H^+]$ in lumen → protonation of LHC2 proteins → structural change of LHC2 proteins → aggregation of LHC2 → association of Chl *a* and Zea molecules → singlet-singlet energy transfer from Chl *a* to Zea → quenching:

Increasing $[H^+]$ in the lumen causes the protonation of LHC2 proteins. H^+ binds to carboxyamino acid residues of the protein LHC2 exposed to the lumen (particularly to asparagine and glutamine) (Dilley *et al.* 1987). Protonation of the LHC2 proteins results in a structural change of these proteins. This allows the aggregation of LHC2 and association of pigments bound to LHC2 proteins. Hence a singlet-singlet energy transfer of excitation energy from Chl to Zea is possible.

However, in a special case, when electron transition between the ground and first singlet excitation levels S_0-S_1 is allowed, the singlet-singlet energy transfer may occur *via* the Foester's dipole-dipole resonance interaction (Hudson *et al.* 1982, Shreve *et al.* 1991).

(*3*) Asymmetric perturbations of the pigment environment

increased $[H^+]$ in lumen → protonation of LHC2 proteins → asymmetric perturbations of the pigment environment → increase in the dipole strength of the Zea S_1-S_0 transition → transition S_1-S_0 is allowed → singlet-singlet energy transfer between Chl *a* and Zea → quenching:

Increasing $[H^+]$ in the lumen causes protonation of the LHC2 proteins. This protonation induces an asymmetric perturbation at the Zea-binding site, leading to an increase in the dipole strength of the Zea S_1-S_0 transition. The increase in dipole strength may allow the Zea S_1-S_0 transition and thus also the singlet-singlet energy transfer from Chl *a* to Zea. However, there is no evidence yet about the three main constraints limiting the rate constant of energy transfer by the dipole-dipole mechanism such as the proximity of interacting molecules (R^{-6} law), mutual orientation (Foester's k-factor), and energy gap.

The above noticed mechanism of direct quenching of Chl fluorescence by Zea has not yet been proved. Nevertheless, both the Chl fluorescence quenching and Zea accumulation are high irradiance-dependent processes, but their kinetics are independent (Lichtenthaler *et al.* 1992, Lichtenthaler and Schindler 1992, Schindler and Lichtenthaler 1994, 1996). In spite of this, there is no doubt that Zea photoprotects the photosynthetic pigment apparatus.

Zea may also quench the triplet excited state ^{3}Chl a^* (Schreiber and Neubauer 1990, Lidon and Henriques 1993, Crofts and Yerkes 1994). The first singlet excited state ^{1}Chl a^* can change into the triplet excited state ^{3}Chl a^* by an intersystem crossing. This means a reorientation in which the magnetic moments of both electrons are orientated in parallel, and the molecule acquires an overall spin equal to 1. An alternative way of generating the triplet state is the back energy transfer of the triplet energy from RC2 into antenna systems (Schreiber and Neubauer 1990). Quenching of the first triplet excitation state ^{3}Chl a^* may occur directly or indirectly *via* singlet oxygen $^1O_2{}^*$ (Lidon and Henriques 1993) according to the following formulas:

(*1*) directly

$^3\text{Chl}^* + \text{Zea} \rightarrow \text{Chl} + {}^3\text{Zea}^*$

$^3\text{Zea}^* \rightarrow \text{Zea} + \text{heat}$

(*2*) *via* singlet oxygen $^1O_2{}^*$

$^3\text{Chl}^* + {}^3O_2 \rightarrow \text{Chl} + {}^1O_2{}^*$

$^1O_2{}^* + \text{Zea} \rightarrow {}^3O_2 + {}^3\text{Zea}^*$

$^3\text{Zea}^* \rightarrow \text{Zea} + \text{heat}$

The triplet, ^{3}Chl a^*, transfers excitation energy to Zea which is transformed into the first triplet excited state ^{3}Zea*. The latter may dissipate excitation energy to heat. ^{3}Chl a^* can also transfer excitation energy to oxygen in its ground triplet state $^3O_2$, whereby Chl changes to the ground state Chl *a* and triplet oxygen $^3O_2$ into the singlet excited state $^1O_2{}^*$. The latter transfers excitation energy to Zea and transforms into the triplet ground state, whereas Zea changes into ^{3}Zea*. The latter might dissipate excitation energy to heat. Triplet-triplet energy transfer from Chl *a* to Zea may be possible, because the first triplet energy level of Zea lies below the first triplet excitation level of Chl (Frank *et al.* 1994). Triplet-triplet energy transfer between Chl and Zea may also occur *via* the electron exchange mechanism (Höfer *et al.* 1987).

Lichtenthaler and Schindler (1992) propose a different way of Zea-mediated Chl fluorescence quenching: Zea may respond directly with highly reactive oxygen species and become epoxidized in a non-enzymatic way to Vio, which is then reduced back to Zea *via* the xanthophyll cycle (Lichtenthaler and Schindler 1992).

If carotenoid quenching is saturated, the triplet excited states can be annihilated (Breton *et al.* 1979, Nechushtai *et al.* 1988):

$^3\text{Chl } a^* + {}^3\text{Chl } a^* \rightarrow {}^1\text{Chl } a^* + \text{Chl } a$

References

Adams, W.W., III, Demmig-Adams, B., Winter, K.: Relative contributions of zeaxanthin-related and zeaxanthin-unrelated type of "high-energy-state" quenching of chlorophyll fluorescence in spinach leaves exposed to various environmental conditions. - Plant Physiol. **92**: 302-309, 1990.

Andréasson, L-E., Vass, I., Styring, S.: Ca^{2+} depletion modifies the electron on both donor and acceptor side in Photosystem 2 from spinach. - Biochim. biophys. Acta **1230**: 155-164, 1995.

Arnon, D.I., Tang, G.M.S.: Cytochrome *b*-559 and proton conductance in oxygenic photosynthesis. - Proc. nat. Acad. Sci. USA **85**: 9524-9528, 1988.

Barber, J., De Las Rivas, J.: A functional model for the role of cytochrome b_{559} in the protection against donor and acceptor side photoinhibition. - Proc. nat. Acad. Sci. USA **90**: 10942-10946, 1993.

Bassi, R., Pineau, B., Dainese, P., Marquardt, J.: Carotenoid-binding proteins of photosystem II. Eur. J. Biochem. **212**: 297-303, 1993.

Bialek-Bylka, G.E., Tomo, T., Satoh, K., Koyama, Y.: 15-*cis*-β-carotene found in the reaction centre of spinach photosystem II. - FEBS Lett. **363**: 137-140, 1995.

Booth, P.J., Crystall, B., Giorgi, L.B., Barber, J., Klug, D.R., Porter, G.: Thermodynamic properties of D1/D2/cytochrome *b*-559 reaction centres investigated by time-resolved fluorescence measurements. - Biochim. biophys. Acta **1016**: 141-152, 1990.

Boussac, A., Rutherford, A.W.: Ca^{2+} binding to the oxygen evolving enzyme varies with the redox state of the Mn cluster. - FEBS Lett. **236**: 432-436, 1988.

Boussac, A., Rutherford, A.W.: The origin of the split S_3 EPR signal in Ca^{2+} depleted photosystem II: Histidine versus tyrosine. - Biochemistry **31**: 7441-7445, 1992.

Breton, J., Geacintov, N.E., Swenberg, C.E.: Quenching of fluorescence by triplet excited states in chloroplasts. - Biochim. biophys. Acta **548**: 616-635, 1979.

Briantais, J.-M., Vernotte, C., Picaud, M., Krause, G.H.: A quantitative study of the slow decline of chlorophyll *a* fluorescence in isolated chloroplasts. - Biochim. biophys. Acta **548**: 128-138, 1979.

Buser, C.A., Diner, B.A., Brudvig, G.W.: Reevalution of the stoichiometry of cytochrome b_{559} in photosystem II and thylakoid membranes. - Biochemistry **31**: 11441-11448, 1992a.

Buser, C.A., Diner, B.A., Brudvig, G.W.: Photooxidation of cytochrome b_{559} in oxygen-evolving photosystem II. - Biochemistry **31**: 11449-11459, 1992b.

Cosgrove, S.A., Guite, M.A., Burnell, T.B., Christensen, R.L.: Electronic relaxation in long polyenes. - J. phys. Chem. **94**: 8118-8124, 1990.

Crofts, A.R., Yerkes, C.T.: A molecular mechanism for qE-quenching. - FEBS Lett. **352**: 265-270, 1994.

Crofts, J., Horton, P.: Dissipation of exitation energy by Photosystem II particles at low pH. - Biochim. biophys. Acta **1058**: 187-193, 1991.

Demmig-Adams, B.: Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. - Biochim. biophys. Acta **1020**: 1-24, 1990.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. - Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Dilley, R.A., Theg, S.M., Beard, W.A.: Membrane-proton interactions in chloroplast bioenergetics: localised proton domains. - Annu. Rev. Plant Physiol. **38**: 347-389, 1987.

Durrant, J.R., Giorgi, L.B., Barber, J., Klug, D.R., Porter, G.: Characterisation of triplet state in isolated photosystem II reaction centres: oxygen quenching as a mechanism for photodamage. - Biochim. biophys. Acta **1017**: 167-175, 1990.

Falkowski, P.G., Fujita, Y., Ley, A., Mauzerall, D.: Evidence for cyclic electron flow around photosystem II in *Chlorella pyrenoidosa*. - Plant Physiol. **81**: 310-312, 1986.

Farhoosh, R., Chynwat, V., Gebhard, R., Lugtenburg, J., Frank, H.A.: Triplet energy transfer between bacteriochlorophyll and carotenoids in B850 light-harvesting complexes of *Rhodobacter sphaeroides* R-26.1. - Photosynth. Res. **42**: 157-166, 1994.

Frank, H.A., Cua, A., Chynwat, V., Young, A., Gosztola, D., Wasielewski, M.R.: Photophysics of the carotenoids associated with the xanthophyll cycle in photosynthesis. - Photosynth. Res. **41**: 389-395, 1994.

Hagen, C., Pascal, A.A., Horton, P., Inoue, Y.: Thermoluminescence studies on the mechanism of photon protection. - In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. I. Pp. 359-362. Kluwer Academic Publishers, Dordrecht - Boston - London 1995.

Höfer, M., Walter, G., Meister, A., Hoffmann, P.: Pigment accumulation and energy flow between carotenoids and chlorophyll during greening of etiolated wheat seedlings under continuous and intermittent irradiation. 2. Accessory and dissipative energy migration. - Photosynthetica **21**: 131-140, 1987.

Homann, P.H.: The chloride and calcium requirement of photosynthetic water oxidation: effects of pH. - Biochim. biophys. Acta **934**: 1-13, 1988.

Horton, P., Ruban, A.V.: ΔpH-dependent quenching of the F_0 level of chlorophyll fluorescence in spinach leaves. - Biochim. biophys. Acta **1142**: 203-206, 1992.

Horton, P., Ruban, A.V.: The role of light-harvesting complex II in energy quenching. - In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis - from Molecular Mechanisms to the Field. Pp. 111-128. Bios Scientific Publ., Oxford 1994.

Horton, P., Ruban, A.V., Rees, D., Pascal, A.A., Noctor, G., Young, A.J.: Control of the light-harvesting function of chloroplast membranes by aggregation of the LHCII chlorophyll-protein complex. - FEBS Lett. **292**: 1-4, 1991.

Horton, P., Ruban, A.V., Walters, R.G.: ΔpH-dependent control of chloroplast light harvesting by binding of DCCD to LHCII. - In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. I. Pp. 311-314. Kluwer Academic Publishers, Dordrecht - Boston - London 1992.

Horton, P., Ruban, A.V., Walters, R.G.: Regulation of light harvesting in green plants. Indication by nonphotochhemical quenching of chlorophyll fluorescence. - Plant Physiol. **106**: 415-420, 1994.

Hudson, B.S., Kohler, B.E., Schulten, K.: Linear polyene electronic structure and potential surfaces. - In: Lim, E.C. (ed.): Excited States. Vol. 6. Pp. 1-95. Academic Press, New York 1982.

Jahns, P., Junge, W.: Dicyclohexylcarbodiimide-binding proteins related to the short circuit of the proton-pumping activity of photosystem II. Identified as light-harvesting chlorophyll *a/b*-binding proteins. - Eur. J. Biochem. **193**: 731-736, 1990.

Jahns, P., Junge, W.: Another role of chlorophyll *a/b* binding proteins of higher plants: They modulate protolytic reactions associated with photosystem II. - Photochem. Photobiol. **57**: 120-124, 1993.

Jansson, S., Pichersky, E., Bassi, R., Green, B.R., Ikeuchi, M., Melis, A., Simpson, D.J., Spangfort, M., Staehelin, L.A., Thornber, J.P.: A nomenclature for the genes encoding the chlorophyll *a/b*-binding proteins of higher plants. - Plant mol. Biol. Rep. **10**: 242-253, 1992.

Jensen, N.-H., Nielsen, A.B., Wilbrandt, R.: Chlorophyll *a* sensitised *trans-cis* photoimerization of *all-trans* β-carotene. - J. amer. chem. Soc. **104**: 6117-6119, 1982.

Johnson, G.N., Rutherford, A.W., Krieger, A.: A change in the midpoint potential of quinone Q_A in Photosystem II associated with photoactivation of oxygen evolution. - Biochim. biophys. Acta **1229**: 202-207, 1995.

Krause, H.G., Laasch, H., Weis, E.: Regulation of thermal dissipation of absorbed light energy in chloroplasts indicated by energy-dependent fluorescence quenching. - Plant Physiol. Biochem. **26**: 445-452, 1988.

Krieger, A., Moya, I., Weis, E.: Energy-dependent quenching of chlorophyll *a* fluorescence: effect of pH on stationary fluorescence and picosecond-relaxation kinetics in thylakoid membranes and Photosystem II preparations. - Biochim. biophys. Acta **1102**: 167-176, 1992.

Krieger, A., Rutherford, A.W., Johnson, G.N.: On the determination of redox midpoint potential of the primary quinone electron acceptor, Q_A, in Photosystem II. - Biochim. biophys. Acta **1229**: 193-201, 1995.

Krieger, A., Weis, E.: Energy-dependent quenching of chlorophyll-*a*-fluorescence: The involvement of proton-calcium exchange at photosystem 2. - Photosynthetica **27**: 89-98, 1992.

Krieger, A., Weis, E.: The role of calcium in the pH-dependent control of Photosystem II. - Photosynth. Res. **37**: 117-130, 1993.

Krieger, A., Weis, E., Demeter, S.: Low-pH-induced Ca^{2+} ion release in the water-splitting system is accompanied by a shift in the midpoint redox potential of the primary quinone acceptor Q_A. - Biochim. biophys. Acta **1144**: 411-418, 1993.

Lambert, C., Redmond, R.W.: Triplet energy level of β-carotene. - Chem. Phys. Lett. **228**: 495-498, 1994.

Lichtenthaler, H.K., Burkart, S., Schindler, C., Stober, F.: Changes in photosynthetic pigments and *in vivo* chlorophyll fluorescence parameters under photoinhibitory growth conditions. - Photosynthetica **27**: 343-353, 1992.

Lichtenthaler, H.K., Schindler, C.: Studies on the photoprotective function of zeaxanthin at high-light conditions. - In: Murata, N. (ed.): Research in Photosynthesis. Vol. IV. Pp. 517-520. Kluwer Academic Publishers, Dordrecht - Boston - London 1992.

Lidon, F.C., Henriques, F.S.: Oxygen metabolism in higher plant chloroplasts. - Photosynthetica **29**: 249-279, 1993.

Liu, B., Napiwotzki, A., Eckert, H.-J., Eichler, H.J., Renger, G.: Studies on the recombination kinetics of the radical pair $P680^+Pheo^-$ in isolated PS II core complexes from spinach. - Biochim. biophys. Acta **1142**: 129-138, 1993.

Mathis, P., Satoh, K., Hasson, Ö.: Kinetic evidence for the function of Z in isolated photosystem II reaction centers. - FEBS Lett. **251**: 241-244, 1989.

McNamara, V.P., Gounaris, K.: Granal photosystem II complex contains only the high redox potential form of cytochrome *b*-559 which is stabilised by ligation of calcium. - Biochim. biophys. Acta **1231**: 289-296, 1995.

Miller, A.-F., Brudvig, G.W.: A guide to electron paramagnetic resonance spectroscopy of Photosystem II membranes. - Biochim. biophys. Acta **1056**: 1-18, 1991.

Mizusawa, N., Ebina, M., Yamashita, T.: Restoration of high potential form of cytochrome *b*-559 through the photoreactivation of Tris-inactivated oxygen-evolving centre. - Photosynth. Res. **45**: 71-77, 1995.

Mullineaux, C.W., Ruban, A.V., Horton, P.: Prompt heat release associated with ΔpH-dependent quenching in spinach thylakoid membranes. - Biochim. biophys. Acta **1185**: 119-123, 1994.

Naqvi, K.R.: The mechanism of singlet-singlet excitation energy transfer from carotenoid to chlorophyll. - Photochem. Photobiol. **31**: 523-524, 1980.

Nechushtai, R., Thornber, J.P., Patterson, L.K., Fessenden, R.W., Levanon, H.: Photosensitization of triplet carotenoid in photosynthetic light-harvesting complex of photosystem II. - J. phys. Chem. **92**: 1165-1168, 1988.

Nedbal, L., Samson, G., Whitmarsh, J.: Redox state of a one-electron component controls the rate of photoinhibition of photosystems II. - Proc. nat. Acad. Sci. USA **89**: 7929-7933, 1992.

Noctor, G., Rees, D., Young, A., Horton, P.: The relationship between zeaxanthin, energy-dependent quenching of chlorophyll fluorescence, and trans-thylakoid pH gradient in isolated chloroplasts. - Biochim. biophys. Acta **1057**: 320-330, 1991.

Noctor, G., Ruban, A.V., Horton, P.: Modulation of ΔpH-dependent nonphotochemical quenching of chlorophyll fluorescence in spinach chloroplasts. - Biochim. biophys. Acta **1183**: 339-344, 1993.

Noguchi, T., Inoue, Y., Satoh, K.: FT-IR studies on the triplet state of P_{680} in the photosystem II reaction center: Triplet equilibrium within a chlorophyll dimer. - Biochemistry **32**: 7186-7195, 1993.

Ono, T., Inoue, Y.: Discrete extraction of the Ca atom functional for O_2 evolution in higher plant photosystem II by a simple low pH treatment. - FEBS Lett. **227**: 147-152, 1988.

Ono, T., Inoue, Y.: Removal of Ca by pH 3.0 treatment inhibits S_2 to S_3 transition in photosynthetic oxygen evolution system. - Biochim. biophys. Acta **973**: 443-449, 1989.

Ono, T., Inoue, Y.: Abnormal redox reactions in photosynthetic O_2-evolving centres in NaCl/EDTA-washed PSII. A dark stable EPR multiline signal and an unknown positive charge accumulator. - Biochim. biophys. Acta **1020**: 269-277, 1990.

Owens, T.G.: Excitation energy transfer between chlorophylls and carotenoids. A proposed molecular mechanism for non-photochemical quenching. - In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis - from Molecular Mechanisms to the Field. Pp. 111-128. Bios Scientific Publishers, Oxford 1994.

Owens, T.G., Shreve, A.P., Albrecht, A.C.: Dynamics and mechanism of singlet energy transfer between carotenoids and chlorophylls: Light harvesting and non-photochemical fluorescence quenching. - In: Murata, N. (ed.): Research in Photosynthesis. Vol. I. Pp. 179-186. Kluwer Academic Publ., Dordrecht - Boston - London 1992.

Papageorgiou, G.: Chlorophyll fluorescence: An intrinstic probe of photosynthesis. - In: Govindjee (ed.): Bioenergetics of Photosynthesis. Pp. 319-375. Academic Press, New York 1975.

Pascal, A.A., Ruban, A.V., Young, A.J., Horton, P.: The effect of pH on LHCII. - In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. I. Pp. 247-250. Kluwer Academic Publishers. Dordrecht - Boston - London 1995.

Peter, G.F., Thornber, J.P.: Biochemical composition and organization of higher plant photosystem II light-harvesting pigment proteins. - J. biol. Chem. **266**: 16745-16754, 1991.

Prasil, O., Kolber, Z., Berry, J.A., Falkowski, P.G.: Cyclic electron flow around Photosystem II *in vivo*. - Photosynth. Res. **48**: 395-410, 1996.

Ramm, D., Hansen, U.P.: Can charge recombination as caused by pH-dependent donor-side limitation in PS 2 account for high-energy state quenching? - Photosynth. Res. **35**: 97-100, 1993.

Ruban, A.V., Horton, P.: Mechanism of ΔpH-dependent dissipation of absorbed excitation energy by photosynthetic membranes. I. Spectroscopic analysis of isolated light-harvesting complexes. - Biochim. biophys. Acta **1102**: 30-38, 1992.

Ruban, A.V., Horton, P.: Spectroscopy of non-photochemical and photochemical quenching of chlorophyll fluorescence in leaves: evidence for role of the light harvesting complex of Photosystem II in the regulation of energy dissipation. - Photosynth. Res. **40**: 181-190, 1994.

Ruban, A.V., Horton, P.: An investigation of the sustained component of nonphotochemical quenching of chlorophyll fluorescence in isolated chloroplast and leaves of spinach. - Plant Physiol. **108**: 721-726, 1995a.

Ruban, A.V., Horton, P.: Regulation of non-photochemical quenching of chlorophyll fluorescence in plants. - Aust. J. Plant Physiol. **22**: 221-30, 1995b.

Ruban, A.V., Horton, P., Young, A.J.: Aggregation of higher plant xanthophylls: differences in absorption spectra and in the dependency on solvent polarity. - J. Photochem. Photobiol. **B 21**: 229-234, 1993.

Ruban, A.V., Rees, D., Pascal, A.A., Horton, P.: Mechanism of ΔpH-dependent dissipation of absorbed excitation energy by photosynthetic membranes. II. The relationship between LHCII aggregation *in vitro* and qE in isolated thylakoids. - Biochim. biophys. Acta **1102**: 39-44, 1992a.

Ruban, A.V., Walters, R.G., Horton, P.: The molecular mechanism of the control of excitation energy dissipation in chloroplast membranes. Inhibition of ΔpH-dependent quenching of chlorophyll fluorescence by dicyclohexylcarbodiimide. - FEBS Lett. **39**: 175-179, 1992b.

Ruban, A.V., Young, A.J., Horton, P.: Quenching of chlorophyll fluorescence in the minor chlorophyll *a-b* binding protein of photosystem II. - In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. I. Pp. 295-298. Kluwer Academic Publ., Dordrecht - Boston - London 1995.

Satoh, K., Hansson, Ö., Mathis, P.: Charge recombination between stabilized P-680$^+$ and reduced cytochrome *b*-559 in quinone-reconstituted PS II reaction center. - Biochim. biophys. Acta **1016**: 121-126, 1990.

Schatz, G.H., Brock, H., Holtzwarth, A.R.: Kinetic and energetic model for the primary processes in photosystem II. - Biophys. J. **54**: 397-405, 1988.

Schindler, C., Lichtenthaler, H.K.: Is there correlation between light-induceed zeaxanthin accumulation and quenching of variable chlorophyll *a* fluorescence? - Plant Physiol. Biochem. **32**: 813-823, 1994.

Schindler, C., Lichtenthaler, H.K.: Photosynthetic CO_2 assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field-grown maple trees in the course of a sunny and a cloudy day. - J. Plant Physiol. **148**: 399-412, 1996.

Schlodder, E., Meyer, B.: pH dependence of oxygen evolution and reduction kinetics of photooxidized chlorophyll *a* II (P-680) in Photosystem II particles from *Synechococcus* sp. - Biochim. biophys. Acta **890**: 23-31, 1987.

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

Schreiber, U., Neubauer, C.: Correlation between dissipative fluorescence quenching at photosystem II and 50 ms recombination luminescence. - FEBS Lett. **258:** 339-342, 1989.

Schreiber, U., Neubauer, C.: O_2-dependent electron flow, membrane energization and the mechanism of non-photochemical quenching of chlorophyll fluorescence. - Photosynth. Res. **25**: 279-293, 1990.

Searle, G., Brody, S.S., van Hoek, A.: Evidence for the formation of a chlorophyll *a*/zeaxanthin complex in lecithin liposomes from fluorescence decay kinetics. - Photochem. Photobiol. **52**: 401-407, 1990.

Shreve, A.P., Trautman, J.K., Owens, T.G., Albrecht, A.C.: A femtosecond study of electronic state dynamics of fucoxanthin and implication for photosynthetic carotenoid-to-chlorophyll energy transfer mechanisms. - Chem. Phys. **154**: 171-178, 1991.

Takahashi, Y., Hansson, Ö., Mathis, P., Satoh, K.: Primary radical pair in the photosystem II reaction centre. - Biochim. biophys. Acta **893**: 49-59, 1987.

Takahashi, Y., Satoh, K., Itoh, S.: Silicomolybdate substitutes for the function of a primary electron acceptor and stabilizes charge separation in the photosystem II reaction center complex. - FEBS Lett. **255:** 133-138, 1989.

Tamura, N., Iwasaki, I., Shibano, S., Oka, I., Okayama, S.: Evidence on the specific interaction between manganese and cyt *b*-559 on the PS II oxidising side. - In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. II. Pp. 337-340. Kluwer Academic Publishers, Dordrecht - Boston - London 1995.

Telfer, A., Dhami, S., Bishop, S.M., Phillips, D., Barber, J.: β-Carotene quenches singlet oxygen formed by isolated Photosystem II reaction centres. - Biochemistry **33**: 14469-14474, 1994.

Thompson, L.K., Brudvig, G.W.: Cytochrome *b*-559 may function to protect photosystem II from photoinhibition. - Biochemistry **27**: 6653-6658, 1988.

Trash, R.J., Fang, H.L.B., Leroi, G.E.: On the role of forbidden low-lying excited state of light-harvesting carotenoids in energy transfer in photosynthesis. - Photochem. Photobiol. **29**: 1049-1050, 1979.

van Mieghem, F., Brettel, K., Hillmann, B., Kamlowski, A., Rutherford, W.A., Schlodder, E.: Charge recombination reactions in Photosystem II. 1. Yields, recombination pathways and kinetics of primary pair. - Biochemistry **34:** 4798-4813, 1995.

Vass, I., Styring, S.: Spectroscopic characterization of triplet forming states in photosystem II. - Biochemistry **31**: 5957-5963, 1992.

Walters, R.G., Horton, P.: DCCD binds to lumen-exposed glutamate residues in LHCIIc. Implications for the mechanism of photoprotective energy dissipation. - In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. I. Pp. 299-302. Kluwer Academic Publishers, Dordrecht - Boston - London 1995.

Walters, R.G., Ruban, A.V., Horton, P.: Higher plant light-harvesting complexes LHCIIa and LHCIIc are bound by dicyclohexylcarbodiimide during inhibition of energy dissipation. - Eur. J. Biochem. **226**: 1063-1069, 1994.

Yocum, C.F.: Calcium activation of photosynthetic water oxidation. - Biochim. biophys. Acta **1059**: 1-15, 1991.