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Hypothesis

A molecular mechanism for q_E-quenching

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Abstract We discuss energy-dependent fluorescence lowering (q_E-quenching), and suggest a model to explain the experimental data currently available. The main elements of the model are: (a) the q_E-quenching reflects a mechanism associated with a component of the light-harvesting antenna rather than the reaction center of photosystem (PS) II – we suggest that it occurs through formation of an efficient quencher in one of the minor chlorophyll protein (CP) complexes; (b) the minor CPs have glutamate residues instead of glutamines at positions shown in light-harvesting complex II (LHCII) to be ligands to chlorophylls near the lumenal interface. We suggest that the quenching reflects a change in ligation of chlorophyll on protonation of these glutamate residues leading to formation of an exciton coupled dimer with a neighboring pigment, in which additional energy levels allow vibrational relaxation of the excited singlet. The model accounts for the dependence on low lumenal pH, the ligand residue changes between LHCII and the minor CPs, the preferential distribution of components of the xanthophyll cycle in the minor CPs, the inhibition of q_E-quenching by DCCD, and the specific binding of DCCD to the minor CPs.

Key words: q_E-Quenching; Light-harvesting complex; Chlorophylls; Molecular mechanism; Photosynthesis

1. Introduction

Under high light intensities photosynthesis is regulated to match CO_2 availability by a set of physiological processes which channel excess excitation away from the photosynthetic apparatus, and give rise to a quenching of fluorescence. In general, fluorescence and non-radiative pathways compete with the photochemical reaction centers for the excited singlets formed in the light harvesting complexes; fluorescence yield has been much used as a 'reporter' of the condition of the photosynthetic apparatus, because the changes reflect changes in photochemical or dissipative pathways. The main dissipative mechanism involves formation of a state in which additional non-radiative pathway(s) are introduced which quench fluorescence. This state develops on formation of a low lumenal pH, and the process is called energy-dependent fluorescence lowering. Two related phenomena play major roles, q_E-quenching and zeaxanthin formation [1–7]. In this paper, we briefly review the field, and suggest a mechanism by which the quenching state may be formed.

2. Quenching of fluorescence associated with the proton gradient (q_E-quenching)

Fluorescence lowering was first reported by Murata and Sugahara [1], and the relation to the proton gradient was characterized by Wraight and Crofts [2], who showed that quenching depended on the pH gradient generated across the thylakoid membrane. Following the early work of Krause [3], a similar quenching has since been implicated in the physiological protective mechanism by which plants cope with high light [4–7]. Quenching can also be induced by the pH gradient generated by ATP hydrolysis [8]. It seems likely that all these ΔpH dependent quenching effects reflect the same quenching state, but the mechanism by which this is produced is still unclear. Two separate but interrelated phenomena are involved: q_E-quenching itself, and a modulation or amplification of the quenching by zeaxanthin and antheraxanthin formation [9–12]. These processes are distinguishable on the basis of differential effects of inhibitors, different light intensity dependencies, and an ascorbate requirement for zeaxanthin formation [9–15].

Three main classes of mechanism have been proposed to account for the q_E-quenching associated with the proton gradient.

(i) Donor-side mechanisms. The quenching is due to an inhibition of the oxygen-evolving reactions [5,16–19]. Such an inhibition would lead to formation of oxidized primary donor (oxidized P680, or P^+), which is a quencher. Accumulation of oxidized states in the donor-side complex would be consistent with the stimulation of delayed fluorescence at times <5 ms under similar conditions [16,17].

(ii) Acceptor-side mechanisms. Quenching is suggested to involve pheophytin reduction (Ph^-) and triplet formation, which occurs when the pool is fully reduced and Q_A becomes over-reduced [20], or a rapid dissipative cycle around PS II in which electrons from the reduced acceptor complex (Q_A^-, Q_A^{2-}, or Ph^-) are passed back to the donor side through a pathway involving cytochrome b_{559} [21]. Since a fraction of Q_A is always present in the oxidized form, even under intense illumination [4–6], it seems unlikely that the acceptor side normally achieves the degree of reduction required by these mechanisms, and a cycle round PS II does not seem to be a significant pathway [22].

(iii) Antenna mechanisms. The quenching is due to a mechanism which operates at the level of the light-harvesting antenna. Horton and colleagues have been the main champions of this hypothesis, and have suggested various mechanisms by which quenching could occur in the light-harvesting complexes [4,24– 26]. They showed that aggregation of isolated LHCII in detergent solution led to a quenching of fluorescence, and proposed this as the most likely mechanism.

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3. Neither an active PS II, nor an oxygen-evolving complex is needed for q_E-quenching

Yerkes and Crofts showed that q_E-quenching does not require an active PS II [28]. In these experiments PS II was prevented from turning-over by DCMU inhibition, and a ΔpH was generated through donors diaminodurene (DAD) and acceptors (methylviologen (MV), or ferredoxin and NADP) using only PS I. Using a weak measuring flash to detect fluorescence yield, it was shown that the quenching on illumination was not significantly dependent on the state of the oxygen-evolving complex (OEC) at time of illumination (set by 0 or 2 flashes before addition of DCMU, [17]); and that quenching occurred with a normal rate and amplitude when the photochemistry of PS II was blocked by treatment with NH_2OH and DCMU, or when the extrinsic proteins of the OEC had been removed by Tris-treatment. With the latter treatments, the fluorescence yield returned to the maximal level after illumination, indicating that the quenching was not due to a dissipative cycle. Thus neither the S-state transitions, nor the turn-over of PS II, nor a cycle around PS II, were required for q_E-quenching, and no significant part of the quenching could be attributed to P^+ formation.

4. Where is the antenna quencher located?

If q_E-quenching reflects a mechanism for dumping excitation energy before it gets to the reaction center, then the location must be in the light-harvesting antenna. To be effective as a protective mechanism, the pathway must compete with the reaction center, with a rate in the low or sub-picosecond range. The antenna has three main components: the CP43 and CP47 subunits of the reaction center complex; the bulk antenna consisting of trimers of LHCII in up to 30-fold subunit excess over the PS II reaction center; and the minor chlorophyll–protein complexes, CP22, CP24 and CP29, (about 1 per reaction center), which can associate with either of the other complexes, and probably form an interface between them [29,30].

Most previous mechanisms have considered the quenching to resides in the bulk LHCII [24–26,32], but an efficient quencher would not need to be present at a concentration greater than one per PS II. If the mechanism involves a vibrational deactivation pathway (with sub-ps half-time), then we can be sure that the quencher is not present in all light-harvesting complexes, since quenching effects mainly the variable fluorescence (1–5 ns life-time), and components of F_o-fluorescence (with life-times in the 30–500 ps range) are not markedly quenched [27]. Either an efficient quencher is formed with weak statistical probability in the main complexes ([24–26], an inefficient quencher is former in most complexes [34], or an efficient quencher is formed at a specific site in some component of the antenna apparatus with a stoichiometry of ~1 per reaction center. We argue below that the minor chlorophyll–protein complexes (CP22, CP24, and CP29) are the most likely sites for the q_E-quenching mechanism (cf. [29]), and involve an efficient quencher.

5. Evidence for a mechanism involving LHCII

LHCII aggregation and light-scattering changes. Light-scattering changes in chloroplasts or leaves accompany formation of ΔpH [3,24,25,33,34], and Deamer et al. suggested that they must reflect conformational changes at the protein level [33]. Although light-scattering changes and q_E-quenching are poorly correlated [34], Horton and colleagues [24,25] suggested that light-scattering might reflect an aggregation of LHCII, leading to formation of new chlorophyll bands and a quenching state. Quenching occurred in isolated LHCII on aggregation at low detergent concentration [24,35], but Horton et al. [24] did not distinguish between these changes, and the well-characterized aggregation associated with interactions through stromal side residues, as reflected in the trypsin-sensitive, Mg^{2+}-dependent aggregation in chloroplasts and reconstituted LHCII [35]. The relation between these aggregation phenomena, and the Mg^{2+}-induced changes in chloroplasts is uncertain, not least because the fluorescence yield on aggregation increases in chloroplasts and decreases in isolated LHCII. Ruban and Horton [25] suggest that the quenching mechanism depends on formation of new chlorophyll absorbance bands near 660 nm and 690 nm on aggregation. However, Jennings et al. [49] have shown that the relative fluorescence yield is constant for chlorophyll forms absorbing between 650 nm and 690 nm, so that the minor chlorophyll bands formed on aggregation of LHCII are not quenching species.

We have observed that q_E-quenching occurs in chloroplasts in the absence of Mg^{2+} (in contrast to earlier results [34]), or after 'clipping' by trypsin treatment so as to eliminate the Mg^{2+} effect on fluorescence. The kinetics and amplitude of quenching (when normalized to the variable fluorescence) were not markedly different from those in chloroplasts with a full amplitude of variable fluorescence (C.T. Yerkes, and A.R. Crofts, manuscript in preparation). We concluded that q_E-quenching does not require the aggregated state induced by Mg^{2+}.

Light-scattering changes on illumination of chloroplasts were much more marked when the suspending medium contained the salts of weak acids [36]. Under these conditions, the grana stacks became strongly appressed, and the pH changes on illumination showed a transient uptake followed by efflux. The scattering changes were suggested to reflect both the volume changes on contraction due to loss of internal osmolyte, and a change in refractive index at the membrane–water interface due to 'precipitation' of membrane proteins as the internal pH falls [36]. In media containing 50–100 mM Na-acetate, a delayed q_E-quenching and enhanced light-scattering showed no kinetic correlation (C.T. Yerkes, and A.R. Crofts, manuscript in preparation).

Antimycin as a specific inhibitor of q_E-quenching. An important argument favoring the antenna hypothesis has been the specific inhibition of q_E-quenching by antimycin [13], and the demonstration that antimycin partly prevented the changes in fluorescence emission spectra attributed to aggregation of the isolated LHCII complexes [24,25]. We have re-examined the effects of antimycin on q_E-quenching under a variety of conditions, and demonstrated the following effects (C.T. Yerkes and A.R. Crofts, manuscript in preparation).

(a) The effects of antimycin on q_E-quenching can all be mimicked by classical uncoupling agents at low concentration. A substantial literature has previously demonstrated that antimycin is an uncoupler, with a concentration dependence which varies with the rate of electron transport [37,38]

(b) The concentration of antimycin required to inhibit q_E-quenching was variable over several orders of magnitude,

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LHCII 1          [--------C---------]    (stromal loop)          [--A-
                          131     139
pea         LVHAQSILAIWATQVILMGAVEGYRIAGGPLGEVVDPLYPGG SFDPLGLADDPEAFA
cucumb      LVHAQSILAIWACQVVLMGAVEGYRIAGGPLGEVTDPIYPGG SFDPLGLADDPEAFA
                          b6      b5 b5
CP29
Arabdopsis  SI---STLLIWI-EVLVIGYIEFQR--NAE-LDSEKRLYPGGKFFDPLGLAADPEKTA
Barley      PI---NLILAVVAEVVLVGGAEYYRITN--GLEFDDKLHPGG PFDPLGLATDPDQAA
Tomato      PI---NLILAVVAEVVLVGGAEYYRIIN--GLDLEDKLHPGG PFDPLGLAKDPDQAA
CP24
Tomato A    AIAPFSFGTLLGTQLILMGWVESKRWVDFFDPDSQSVEWATPWSKTAEN


LHCII 1     -------A----------------]     {--D---}  (lumenal loop)
            180               197             212
pea         ELKVKELKNGRLAMFSMFGFFVQAIVTGKGPLENLADHLSDPVNNNAWSYATNFVPGK
cucumb      ELKVKELKNGRLAMFSMFGFFVQAIVTGKGPLENLADHLADPVNNNAWAYATNFVPGK
            a1 a2a4          a3              b3
CP29
Arabdopsis  QLQLAEIKHARLAMVAFLGFAVQAAATGKGPLNNWATHLSDPLHTTIIDTFSSS
Barley      LLKVKEIKNGRLAMFSMLGFFIQAYVTGEGPFENLCAHLSDPFGNNLLTVISGAAERVPSL
Tomato      ILKVKEIKNGRLAMFSMLGFFIQAYVTGQGPVENLAAHLSDPFGNNLLTVIGGASERVPTL
CP24
Tomato A    RLKVAEIKHARLAMLAMLIFYFEA GQGKTPLGALGL
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Fig. 1. Aligned sequences of LHCII, CP 29 and CP 24, showing changes in ligands. Bold residues are ligands in LHCII to the chlorophylls identified below. Bold residues in italics indicate residue substitutions which involve changes to dissociable groups. For the chlorophylls shown in italics below arginine residues, the ligation involves a glutamate–arginine pair. Helices are identified above, and lettered according to [43].

and depended markedly on the rate at which the onset of quenching occurred. This rate could be varied by light-intensity, by the electron transfer regime used, or by both. The rate seen in a non-cyclic system with water as donor and methyl viologen (MV) as acceptor was slower by a factor of > 10 than the rate when quenching was supported by electron transfer through PS I from DAD to MV in the presence of DCMU, and appeared to be much more sensitive to antimycin. However, when the light intensity was adjusted to allow similar rates with the DAD/MV/DCMU system, the titre for antimycin was more similar.

(c) The titre for antimycin could be lowered in all cases by addition of valinomycin and KCl. We suggest that the mechanism of enhancement of the effects of antimycin on q_E-quenching is through coupled ion flux leading to enhanced protonophoric activity (cf. [39]).

(d) Antimycin acts as a weak ADRY reagent [28]. The effects of antimycin on q_E-quenching are therefore similar to the effects of other protonophoric reagents, and suggest that there is no specific site for binding of antimycin which accounts for its inhibitory effect. However, when PS II was functional, or when formation of zeaxanthin by preillumination enhanced the level of quenching, antimycin inhibited the enhanced portion of q_E-quenching at much lower concentration than other protonophores, and its effects as a classical uncoupler require relatively higher concentrations. This suggests that the process affected by antimycin may not interact with the classical bulk-phase proton gradient in the same way as the phosphorylation reactions. It would be premature to speculate on the mechanism, but we note that PS II and the associated light harvesting antenna are sequestered in the grana stacks, which might impose a different physico-chemical environment allowing the lumenal phases in grana and stromal lamellae to be out of equilibrium (cf. [40]).

6. Evidence for a role for the minor light-harvesting complexes

Inhibition of q_E-quenching by DCCD. Incubation with DCCD leads to formation of covalent DCCD adducts which are located in the minor light-harvesting complexes (CP22, CP24, CP29) [40–42], and inhibition of q_E-quenching [42]. In our hands, inhibition was not apparent using the conditions of [42], but required preincubation under the conditions of Jahns and Junge [40]. Carbodiimides react preferentially with carboxylic acid groups, forming a covalent bond susceptible to hydrolysis in an aqueous environment. DCCD is a lipid soluble reagent, able to react with groups buried in the hydrophobic phase, where the covalent bond is protected from hydrolysis. This has directed attention to potentially reactive groups in the minor CPs, where several acidic residues have been identified on the lumenal side of folding models. From the structure of the LHCII complex [43], and homologous alignment [31], it is possible to identify some of these groups as probable chlorophyll ligands.

Quenching associated with formation of zeaxanthin. Early studies showed formation of zeaxanthin from violaxanthin under conditions similar to those leading to q_E quenching [9–11]. However, q_E-quenching can occur in the absence of zeaxanthin [14], and more recently, Horton and colleagues have shown that the presence of zeaxanthin in leaves preilluminated before rapid preparation of chloroplasts correlated strongly with an enhanced level of q_E-quenching [12], which otherwise shows properties similar to those in the absence of zeaxanthin. Gilmore and Yamamoto [45] showed that the amplitude of quenching was proportional to the sum of antheraxanthin and zeaxanthin, and suggested that the de-epoxidation products are necessary for quenching.

A number of laboratories have looked at the distribution of carotenoid pigments among the different light-harvesting chlo-

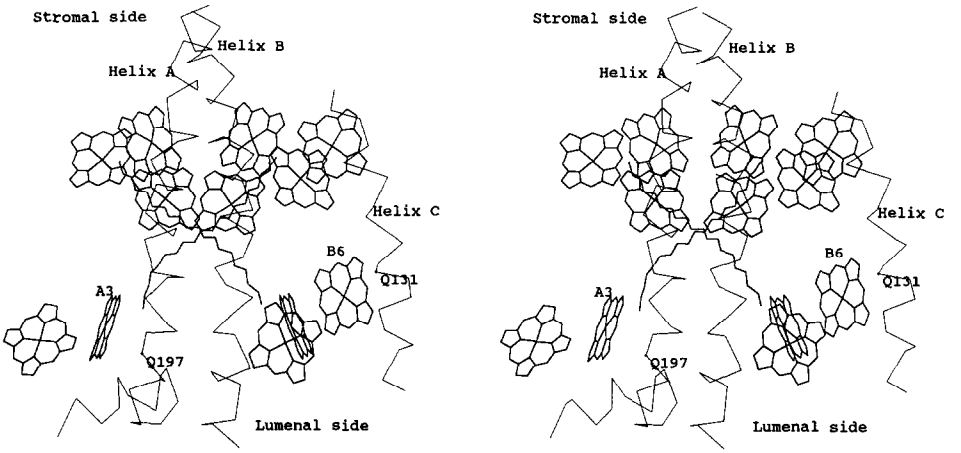

Fig. 2. Structure of LHCII, showing features discussed in the text. Stereo-pair for crossed-eye viewing (pdVWIN software). Coordinates kindly provided by Dr. W. Kühlbrandt (see [43]).

rophyll protein complexes [29,44,45]. Violaxanthin, the precursor of antheraxanthin and zeaxanthin in the xanthophyll cycle, partitioned predominantly into the minor complexes, CP29, CP26 and CP24. Little or no violaxanthin was found in LHCII or in the PS II reaction center complexes CP43, CP47, or the D1/D2 Cyt b_{559} core. From sequence studies [31], the minor complexes are clearly closely related to the major LHCII components, but are more closely associated with PS II, and can co-isolate with either PS II or LHCII on biochemical separation [29].

A mechanism for q_E-quenching has been proposed by Owens et al. based on the change in singlet energy levels on de-epoxidation of violaxanthin to zeaxanthin, relative to the chlorophyll lowest singlet level, and modulation of the energy levels by low lumenal pH [32]. They suggest that this would be a relatively inefficient pathway occurring in a main fraction of light-harvesting complexes. In support of this idea, they indicated that maximal non-photochemical quenching involves accumulation of 75–100 zeaxanthins per PS II reaction center. This is greatly in excess of the stoichiometry of the minor CPs, and would require that a large fraction of zeaxanthin must reside elsewhere, possibly in LHCII complexes. However, although zeaxanthin formation can occur in excess of the binding sites available in the minor CPs [14], under conditions leading to maximal q_E-quenching, the formation of zeaxanthin is much less than the maximum, and is preferentially associated with the minor CPs [44].

7. Hypothetical mechanism for fluorescence-lowering

General conclusions. Stimulation of delayed fluorescence by ΔpH shows that the oxidation potential in the donor-side reactant pool is substantially increased as the lumenal pH falls. Lowering the lumenal pH will likely change the equilibrium constant between the S-state reactants and the P/P^+ couple [16,17]. Experiments with NH_2OH-treated [46] or Tris-treated [47] chloroplasts, in which the Mn-center has been disrupted and the extrinsic proteins of the oxygen-evolving complex have been removed, are photoinhibited even in relatively weak light, indicating that centers in which P^+ is stabilized are especially susceptible to photo-oxidative damage. Although formation of P^+ by a donor-side inhibition is not the mechanism of quenching, it seems likely that plants will have evolved strategies to avoid this potentially hazardous state by preventing the generation of a low pH local to the donor-side of PS II.

Fluorescence lowering is a physiological device to dump exciton energy as heat before it reaches the reaction center of PS II. The mechanism must be poised so that quenching comes in before the lumenal pH falls low enough to inhibit the donor-side reactions. The mechanism involves a change in state of the antenna complex (with a pK in the range 5.5 in higher plant chloroplasts), leading to dissipation of the singlet state, and hence quenching of fluorescence. The formation of zeaxanthin is enhanced at low lumenal pH, but has a slower onset and longer decay, and thus represents a secondary process to cope with more extended exposure. Association of zeaxanthin (and possibly antheraxanthin, [45]) with CP29 (or the other minor LHCs) [12,29,44], amplifies (or is required for) the effect of low lumenal pH in quenching the fluorescence. Structural models [29] of the interface between PS II and the antenna, and fluorescence emission spectra [30], suggest that the minor complexes serve as 'bridges' between the major LHCIIs and the reaction center antenna proteins. It thus seems likely, as suggested by Bassi and colleagues [29], that the main action in q_E-quenching is at this interface, and involves CP29 (or the other minor LHCs), and not aggregation of LHCII as suggested by Horton and colleagues [24–26].

Molecular mechanism. The mechanism of quenching could in principle be quite simple. Physico-chemical studies show that chlorophylls in solution at concentrations comparable to those in the leaf show no fluorescence, and this is attributed to an interaction between 'statistical dimers' which introduces additional energy levels allowing thermal pathways for de-excitation [48]. The chlorophylls in LHCII are held apart by ligation, with a variety of groups providing ligands [43]. We assume a similar structure for CP24 and CP29 based on sequence homology. If an acidic chlorophyll ligand were accessible to H^+ from the lumenal phase, the liganding properties would change on acidification. We suggest that such a change might allow the effected chlorophyll to interact at short enough range to form exciton-coupled bands with a neighboring chlorophyll or carotenoid, and thus form a quencher of fluorescence. A change in ligand properties could either lead to release of a chlorophyll

previously bound, specific ligation of a previously loosely bound chlorophyll, or an exchange of ligands.

Sequence comparison and alignment can be used to identify the ligands in CP24 and CP29 by homology with the ligands identified in LHCII [43] (Fig. 1). Residue changes with appropriate properties are Q131E in CP29 (numbering as in [43]), and Q197E in CP24. Glutamines at both positions are fully conserved in LHCII. Other known ligands are conserved between the LHCII, CP29 and CP24 sequences, except for N183H in the *Arabidopsis* CP29, and in CP24s. Other residue changes which might affect ligation are the substitutions of P82V (*Arabidopsis* CP29) or P82G (CP24) (not shown). In the LHCII structure, the proline in helix B liberates the peptide >C=O group of G78 so that it can act as a ligand [43]. None of these other ligand changes would be expected to lead to pK changes in the acid range. It should be noted that the Q131E change is also seen in LHCI sequences [31].

The structure for LHCII [43] is shown in Fig. 2, with the location of the two glutamines and the chlorophylls they ligate labeled. Both residues are on the lumenal side of the structure, but in the hydrophobic domain; Q131 in particular is quite well buried. We assume similar locations for the glutamates identified as substitutes in the alignment above for CP24 or CP29. To account for the dependence of quenching on low lumenal pH, we would have to suggest that a channel exists to allow access of H^+ to one or both of these glutamates from the lumen. In contrast to the glutamate ligands in LHCII, which form charge compensating pairs with arginine residues from elsewhere in the sequence (chlorophylls in italics in Fig. 1) [43], there are no obvious conserved changes in the lumenal loops which might provide a similar compensating group for E197 (in CP24) or E131 (in CP29). The buried glutamates identified here might therefore be the sites at which DCCD reacts to block q_E-quenching [42] and might account for the preferential labeling by DCCD of the minor chlorophyll binding proteins [40–42], and also explain the effects of DCCD on protolytic processes associated with the donor-side reactions [40]. In order for these residues to form stable adducts with DCCD, the H^+-channel postulated above would have to allow access of H^+, but not of H_2O at high activity. This seems to be the case with the DCCD binding proteolipid (subunit c in *E. coli*) which is thought to contribute to a H^+-channel through the F_0-part of the ATP-synthase. If biochemical evidence confirms our suggestion, the LHCII molecular structure might provide important clues as to how a relatively bulky hydrophobic residue gains access to sites from which water is restricted.

Role of zeaxanthin. The conversion of violaxanthin to antheraxanthin and zeaxanthin in the minor CPs could enhance the quenching process either directly, by contributing to the quenching through triplet formation or through singlet mechanisms involving the energy level changes suggested by Owens et al. [32], or indirectly through structural changes. In the Kühlbrandt structure [43], the luteins are in close proximity to both the chlorophylls ligated by the pertinent glutamines, or to potential dimer partners; if the components of the xanthophyll cycle occupy the same relative positions, interactions could readily occur, but it is premature to guess at what these might be.

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References

[1] Murata, N. and Sugahara, K. (1969) Biochim. Biophys. Acta 189, 182–189.
[2] Wraight, C.A. and Crofts, A.R. (1970) Eur. J. Biochem. 17, 319–323.
[3] Krause, G.H. (1973) Biochim. Biophys. Acta 292, 715–728.
[4] Foyer, C., Furbank, R., Harbinson, J. and Horton, P. (1990) Photosynth. Res. 25, 83–100.
[5] Weis, E. and Berry, J. (1987) Biochim. Biophys. Acta 894, 198–208.
[6] Genty, B., Briantais, J-M., and Baker, N.R. (1989) Biochim. Biophys. Acta 990, 87–92.
[7] Krause, G.H. and Weis, E. (1991) Annu. Rev. Plant Physiol. Plant Mol. Biol. 42, 313–349.
[8] Gilmore, A.M. and Yamamoto, H.Y. (1993) Proc. Natl. Acad. Sci. USA 89, 1899–1903.
[9] Hager, A. (1969) Planta 89, 224–243.
[10] Yamamoto, H.Y. and Kamite, L. (1972) Biochim. Biophys. Acta 267, 538–543.
[11] Demmig, B. and Björkman, O. (1987) Planta 171, 171–184.
[12] Ruban, A.V., Young, A.J., Pascal, A.A. and Horton, P. (1994) Plant Physiol. 104, 227–234.
[13] Oxborough, K. and Horton, P. (1987) Photosynth. Res. 12, 119–128.
[14] Demmig-Adams, B. (1990) in: Curr. Res. Photosynth., (Baltscheffsky, M. ed.) vol. II, pp. 357–364, Kluwer Academic Press, The Netherlands.
[15] Yamamoto, H.Y. (1979) Pure Appl. Chem. 51, 639–648.
[16] Crofts, A.R., Wraight, C.A. and Fleischman, D. (1971) FEBS Lett. 15, 89–100.
[17] Bowes, J.M. and Crofts, A.R. (1981) Biochim. Biophys. Acta 637, 464–472.
[18] Schreiber, U. and Neubauer, C. (1990) Photosynth. Res. 25, 279–293.
[19] Krieger, A., Moya, I. and Weis, E. (1992) Biochim. Biophys. Acta 1102, 167–176.
[20] Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E.M. and Andersson, B. (1992) Proc. Natl. Acad. Sci. USA 89, 1408–1412.
[21] Nedbal, L., Samson, G. and Whitmarsh, J. (1992) Proc. Natl. Acad. Sci, USA 89, 7929–7933.
[22] Meunier, P.C. and Bendall, D.S. (1993) Photosynth. Res. 37, 147–158.
[23] Rees, D., Young, A., Noctor, G., Britton, G. and Horton, P. (1989) FEBS Lett. 256, 85–90.
[24] Horton, P., Ruban, A.V., Rees, D., Pascal, A.A., Noctor, G. and Young, A.J. (1991) FEBS Lett. 292, 1–4.
[25] Ruban, A.V. and Horton, P. (1992) Biochim. Biophys. Acta 1102, 30–38.
[26] Mullineaux, C.W., Pascal, A.A., Horton, P. and Holzwarth, A.R. (1993) Biochim. Biophys. Acta 1141, 23–28.
[27] Krieger, A., Moya, I. and Weis, E. (1992) Biochim. Biophys. Acta 1102, 167–176
[28] Yerkes, C.T. and Crofts, A.R. (1992) in: Research on Photosynthesis (Murata, N. ed.) vol. II, pp. 635–638. Kluwer Academic Press, Dordrecht.
[29] Dainese, P., Santini, C., Ghiretti-Magaldi, A. Marquardt, J., Tidu, V., Mauro, S., Bergantino, E. and Bassi, R. (1992) in: Research on Photosynthesis (Murata, N. ed.) vol. II, pp. 13–20. Kluwer Academic Press, Dordrecht.
[30] Bratt, C.E. and Åkerlund, H.-E. (1992) in: Research on Photosynthesis (Murata, N. ed.) vol. I. pp. 231–234. Kluwer Academic Press, Dordrecht.
[31] Green, B,R., Durnford, D. and Pichersky, E. (1992) in: Research on Photosynthesis (Murata, N. ed.) vol. I, pp. 195–202, Kluwer Academic Press, Dordrecht.
[32] Owens, T.G., Shreve, A.P. and Albrecht, A.C. (1992) in: Research on Photosynthesis (Murata, N. ed.) vol. I, pp. 179–186. Kluwer Academic Press, Dordrecht.

[33] Deamer, D.W., Crofts, A.R. and Packer, L. (1967) Biochim. Biophys. Acta 131, 81–96.
[34] Krause, G.H. (1974) Biochim. Biophys. Acta 333, 301–313.
[35] Mullet, J.E. and Arntzen, C.J. (1980) Biochim. Biophys. Acta 589, 100–117
[36] Crofts, A.R., Deamer, D.W. and Packer, L. (1967) Biochem. Biophys. Acta 131, 97–118.
[37] Izawa, S., Connolly, T.N., Winget, G.D. and Good, N.E. (1967) Brookhaven. Symp. Biol. 19, 169–187.
[38] Drechsler, Z., Nelson, N. and Neumann, J. (1969) Biochim. Biophys. Acta 189, 65–73.
[39] Karlish, S.J.D. and Avron, M. (1968) FEBS Lett. 1, 21–24.
[40] Jahns, P. and Junge, W. (1988) Eur. J. Biochem. 193, 731–736.
[41] Webber, A. and Gray, J.C. (1989) FEBS Lett. 249, 79–82.
[42] Ruban, A.V., Walters, R.G. and Horton, P. (1992) FEBS Lett. 309, 175–179.
[43] Kühlbrandt, W., Wang, D.N. and Fujiyoshi, Y. (1984) Nature 367, 614–621.
[44] Bassi, R., Pineau, B., Dianese, P. and Marquardt, J. (1993) Eur. J. Biochem. 212, 297–303.
[45] Gilmore, A.M. and Yamamoto, H.Y. (1993) Photosynth. Res. 35, 67–78.
[46] Chen, G.X., Kazimir, J. and Cheniae, G.M. (1992) Biochemistry 31, 11072–11083.
[47] Yerkes, C.T. and Crofts, A.R. (1992) EBEC Short Reports, 7, 5.
[48] Beddard, G.S., Carlin, S.E. and Porter, G. (1976) Chem. Phys. Lett. 43, 27–32.
[49] Jennings, R.C., Zucchelli, G., Bassi, R., Vianelli, A. and Garlaschi, F. (1994) Biochim. Biophys. Acta 1184, 279–283.