Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection

Peter Horton* and Alexander Ruban

Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN, UK

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Abstract

The photosystem II (PSII) light-harvesting system carries out two essential functions, the efficient collection of light energy for photosynthesis, and the regulated dissipation of excitation energy in excess of that which can be used. This dual function requires structural and functional flexibility, in which light-harvesting proteins respond to an external signal, the thylakoid ΔpH, to induce feedback control. This process, referred to as non-photochemical quenching (NPQ) depends upon the xanthophyll cycle and the PsbS protein. In nature, NPQ is heterogeneous in terms of kinetics and capacity, and this adapts photosynthetic systems to the specific dynamic features of the light environment. The molecular features of the thylakoid membrane which may enable this flexibility and plasticity are discussed.

Key words: Light-harvesting complex, non-photochemical quenching, photoprotection, thylakoid membrane, xanthophyll cycle.

Introduction

During the evolution of oxygenic photosynthesis, there have been two conflicting demands placed upon the molecular machinery of photosystem II (PSII). On the one hand efficient collection of sunlight is needed to deliver excitation energy to the photosynthetic reaction centre at a rate sufficient to drive electron transport at the highest sustainable rate. On the other hand, excited states of chlorophyll and the presence of molecular oxygen provide a potentially lethal cocktail that can irreversibly damage the proteins, lipids, and pigments of the photosynthetic membrane. To combat this, constitutive features of the photosystem, such as the presence of carotenoids to scavenge singlet oxygen and trap triplet states of chlorophyll, are combined with other photoprotective mechanisms which are induced under high levels of illumination to bring about the dissipation of excess chlorophyll excited singlet states. In this paper those features of molecular design that promote efficient light harvesting will be reviewed, and then it will be shown how the dynamic nature of this system allows for an inducible enhancement of the rate of dissipation of excited states. It will be shown how these features of design differ in different classes of organism, and these differences will be rationalized in terms of evolution within particular ecological niches.

The molecular principles of light harvesting

The chlorophyll a and b molecules, bound to the pigment–protein complexes of the light-harvesting system, are arranged in three-dimensional space to serve as an antenna; gathering light over a large absorption cross-section, and ‘funnelling’ the absorbed energy to the reaction centres. Energy transfer between pigment molecules, both between and within individual complexes is highly efficient. Equilibration of energy within the antenna is rapid, and tends towards the red-shifted lower energy excited states of the reaction centre core complex (van Amerongen and van Grondelle, 2001). This highly efficient antenna depends entirely on the specific structures of the apo-proteins of the light-harvesting complexes: they must bind sufficient pigments to maximize absorption; they must provide for the optimal orientation and configuration of pigments for energy transfer; they must tune the excited state energy levels of the pigments to promote energy transfer; and they must permit protein–protein contacts to allow appropriate routes of energy transfer between complexes.

There are important constraints placed upon this design. Most notable is that the chlorophyll molecules must be...
arranged so as to prevent the tendency for excited states to dissipate by non-radiative decay. In the main light-harvesting complex, LHCII, the chlorophyll concentration is approximately 0.6 M: if chlorophyll was dissolved in an organic solvent at this concentration all absorbed energy would be dissipated in this way, a process described as concentration quenching (Beddard and Porter, 1976). This occurs via close chlorophyll–chlorophyll interactions, dimers or excimers. Clearly the apoprotein provides a specific 'solvent', an environment that allows a high chlorophyll concentration without the probability of quenching by such associations. This environment arises from the formation of hydrophobic binding pockets, liganding of the chlorophyll Mg atoms by amino acid residues of the alpha helices and hydrogen bonding to the porphyrin ring (Liu et al., 2004). It is useful to regard chlorophylls as the 'cofactors' of the light-harvesting ‘enzymes’, and many parallels exist between these and other proteins with essential cofactors, such as haem proteins and flavoproteins.

**PSII macro-organization**

The basic structural unit of PSII is the so-called LHCII–PSII supercomplex (Hankamer et al., 1997). This is a macro-molecular dimer, consisting of the D1/D2/CP43/CP47 PSII core complex and several light-harvesting proteins, one copy of the minor monomeric complexes CP26 and CP29, and one of the major trimeric complex, LHCII. In the granal membrane of the chloroplast, the supercomplex is associated with further LHCII trimers and the monomeric CP24, to form 'megacomplexes', which frequently display a semi-crystalline order (Boekema et al., 2000).

The subunits of the PSII antenna are therefore associated together with increasing levels of complexity. For example, LHCII monomers form trimers, which are themselves associated, on the one hand, with the monomeric complexes and core complexes and on the other, with more LHCII trimers, to form a 2-D organization in the thylakoid membrane. Finally, the stromal-facing surfaces of membranes are appressed together to form the grana, with contacts between LHCII and between LHCII and PSII cores of the adjacent membranes. This three-dimensional macrostructure has been referred to as the macrodomain (Garab and Mustardy, 1999). The tendency for oligomerization is displayed by LHCII, which can *in vitro* self-assemble into 2-D and 3-D structures that have a strong resemblance to thylakoid membranes (Burke et al., 1987). Equally, oligomers of LHCII can be prepared following mild detergent treatment of thylakoid membranes (Ruban et al., 1999). The trimeric state of LHCII appears to be essential for this organization of PSII. When the main proteins that comprise LHCII are removed by expression of antisense genes, it was found that the amount of the normally monomeric CP26 dramatically increased in level and it became trimeric (Ruban et al., 2003). This change in expression of photosynthetic genes must be a response to the LHCII deficiency, as the plant attempts to compensate for the loss of an essential protein. Amazingly, this response allows the wild-type oligomeric PSII structure and the granal membrane to be maintained, emphasizing the importance and robustness of the thylakoid macrostructure.

**Molecular accessories for regulating light harvesting**

In addition to the main protein complexes, both the PSII core and the light-harvesting system contain a number of other proteins, pigments, and lipids (Hankamer et al., 1997). Whereas some of these may have essential roles in the structure and function of the light-harvesting systems (e.g. a specific phospholipid is required for LHCII trimerization; Remy et al., 1982) and DGDG may enable association between trimers (Liu et al., 2004), others appear to have another role, that of tuning the properties of light-harvesting proteins for regulation. Specific lipids appear to be necessary for certain dynamic events within the 3-D organization of LHCII (Simidjiev et al., 2000). Peripherally associated with LHCII are the carotenoids of the xanthophyll cycle (Ruban et al., 1999), which plays a vital role in photoprotective energy dissipation (Demmig-Adams, 1990), observed as the non-photochemical quenching of chlorophyll fluorescence (NPQ). This cycle is the reversible de-epoxidation of violaxanthin (found under light-limiting conditions) into zeaxanthin (found under light-saturating conditions) that is correlated with the induction of NPQ. Violaxanthin de-epoxidase catalyses this reaction, and this enzyme may have a specific docking site on LHCII (Liu et al., 2004). A group of LHC-related proteins encoded by the members of the *lhc* gene super family (Jansson, 1999), including the ELIPS, the Lil proteins, and PsbS are also involved in stress responses (Heddad and Adamska, 2002), with PsbS having a key role in NPQ. Protein kinases also interact with LHCII (Bennett, 1983) and CP29 (Croce et al., 1996), phosphorylating them. In the case of LHCII it is well-known that the redox regulation of kinase activity provides the basis for regulating the distribution of absorbed energy between PSII and PSI (Horton and Black, 1980), phosphorylation modulating the structure of the complex, and its interaction with other photosystem proteins (Allen and Forsberg, 2001). Phosphorylation of CP29 appears to be activated under cold stress, causing a structural change in the protein, which may have a photoprotective role (Croce et al., 1996).

**Changes in structure as the basis for regulation of light harvesting**

Whereas the higher order structure of PSII was first thought to be important only in increasing the efficiency of light harvesting, more recently it has been suggested that it provides the essential dynamic properties involved in
regulation (Horton, 1999). When isolated light-harvesting complexes self-assemble into oligomers in vitro, there is a decrease in fluorescence yield (Mullet and Arntzen, 1980; Ruban et al., 1992), resulting from a decrease in excitation lifetime by non-radiative decay. Since, in vivo, the complexes also exist in an oligomeric state (Dekker et al., 1999; Ruban et al., 1999), it could be proposed that increased energy dissipation would result. Indeed, it has been recognized that the maximum fluorescence lifetime of chlorophyll in vivo is always less than free chlorophyll or chlorophyll in unaggregated LHCCI (Horton and Ruban, 1994). Perhaps the advantages of oligomeric organization outweigh the disadvantage of some waste of absorbed excitation energy.

More interesting, however, is the notion that structural changes within the oligomeric antenna could provide a physiological mechanism for regulating the partitioning of energy between utilization in photosynthesis and dissipation by NPQ (Horton et al., 1991, 1996). Indeed, there are many remarkable similarities between NPQ and in vitro fluorescence quenching in LHCCI and other antenna complexes (Horton et al., 1999). Whilst the exact mechanism of energy dissipation in these complexes remains to be determined, it almost certainly is equivalent to the process of ‘concentration’ quenching discussed above. Hence, given that the precise 3-D structure of the protein prevents such quenching, it is easy to imagine how (relatively subtle) changes in the precise 3-D structure of the protein prevent such ‘concentration’ quenching discussed above. Hence, given that the precise 3-D structure of the protein prevents such quenching, it is easy to imagine how (relatively subtle) changes in the precise 3-D structure of the protein prevent such ‘concentration’ quenching discussed above.

A conceptual model was formulated, comprising four conformational states of the light-harvesting system, with different fluorescence yields, differing in de-epoxidation and protonation state (Horton et al., 1991). The action of zeaxanthin was explained, it is an allosteric activator of quenching binding to a peripheral site on LHCCI or another light-harvesting antenna protein. There is considerable experimental support for this hypothesis, although direct participation of zeaxanthin in the quenching mechanism cannot be excluded (Frank et al., 1994; Ma et al., 2003). Paramount in this evidence is the startlingly different kinetic properties of qE observed in thylakoids isolated from leaves pretreated to have different de-epoxidation states (Ruban et al., 2001); qE forms more rapidly, at a lower ΔpH and with reduced co-operativity in the presence of zeaxanthin, all characteristics of it being an allosteric activator. Such properties also explain the kinetics of NPQ formation upon illumination of dark-adapted leaves, and the accelerated rate of formation upon a second illumination following a brief dark interval in which ΔpH, but not de-epoxidation is relaxed (Ruban and Horton, 1999). Recently it has been suggested that, rather than being a peripheral site on an antenna protein, the internal Lut 2 site discussed above provides the allosteric binding site for zeaxanthin (Formaggio et al., 2001). Indeed it has been shown that when the Lut 2 site in CP26 is occupied by zeaxanthin, there is a decrease in fluorescence lifetime, compared with when violaxanthin is bound (Moya et al., 2001; Crimi et al., 2001). In this model, reversible exchange of carotenoid binding to this site is proposed (Bassi and Caffarri, 2000), and in vitro this has been shown to be promoted at low pH (Morosinotto et al., 2002).

It is interesting, and rather unexpected, that there is no evidence for any one light-harvesting complex having an obligatory role in NPQ, at least in higher plants; wild-type levels of NPQ are found in Arabidopsis plants expressing antisense genes for CP29, CP26, and LHCCI (Andersson et al., 2001, 2003). Similarly, chl b less mutants, which have only low levels of all Lhcb proteins show NPQ (Lokstein et al., 1993). This indicates that no single Lhcb protein can be the unique site of either energy dissipation or zeaxanthin activation. Furthermore, it suggests that NPQ could be ‘shared’ by all antenna proteins, including those of the PSII core, since the above principle of control of concentration quenching can apply to them all. It remains to be shown what the minimum structural unit for NPQ is. In vitro studies also show how each antenna subunit has only a small capacity for zeaxanthin-induced quenching (Wentworth et al., 2000; Crimi et al., 2001), but that this is amplified when subunit interactions are enabled (Ruban and Horton, 1992; Moya et al., 2001). Indeed, this suggests that the intra- and inter-subunit conformational changes act in a concerted manner, for example, a quenching locus in LHCCI or CP26 could be controlled by the Lut 2 domain, which is in turn affected by the xanthophyll bound there.
the binding of xanthophylls at the more peripheral neoxanthin (N1) and violaxanthin (V1) sites, and interactions with neighbouring protein subunits.

The role of PsbS and zeaxanthin activation

*Arabidopsis* plants with mutation in the PsbS gene (*npq4* mutants) show none of the rapidly reversible type of NPQ known at qE, despite having wild-type levels of zeaxanthin and ΔpH, and unaltered levels of light-harvesting and reaction centre complexes (Li *et al*., 2000). It was suggested that PsbS provides the site of NPQ, by binding both protons and zeaxanthin, and interacting with a chlorophyll-containing complex to introduce a new quenching pathway. This hypothesis for the role of PsbS has been subject to intensive investigation. Evidence for proton binding has come from the identification of binding sites for DCCD (Dominici *et al*., 2002), and from the inhibition of qE by site-directed mutagenesis of potential proton binding residues (Li *et al*., 2002a). The pigment-binding properties of PsbS are unclear; purified PsbS appears to contain no chlorophyll or carotenoid.

Finding the location of zeaxanthin binding sites has been an important goal in understanding NPQ. It has been shown that isolated LHCII and the minor complexes CP26 and CP29 contain violaxanthin (Ruban *et al*., 1994, 1999; Bassi and Caffarri, 2000). Only some of this is available for de-epoxidation, shown to include that loosely bound to the complex (Ruban *et al*., 1999; Caffarri *et al*., 2001), and there is evidence that violaxanthin bound to the Lut 2 site on CP26 and CP24 could exchange for zeaxanthin (Morosinotto *et al*., 2002). However, such studies of zeaxanthin binding sites could be misleading, since it is likely that only a small proportion of the zeaxanthin pool is involved in NPQ (Gilmore, 2001). This subpopulation of zeaxanthin has been studied using a spectroscopic approach. It is well known that qE is very strongly correlated with an absorption change at 535 nm (Bilger *et al*., 1988; Ruban *et al*., 1993), whilst it was considered to arise from selective light scattering, its proximity to the carotenoid absorption bands, suggested it may be electronic in origin. Indeed, the ΔA535 spectrum could be modelled as a red shift in 1–2 carotenoid molecules (Ruban *et al*., 2002). Resonance Raman spectroscopy has been used to identify the absorption bands from each of the xanthophylls (Ruban *et al*., 2000). In leaves, induction of qE was associated with the appearance of a zeaxanthin species selectively excited at 528 nm. This signal was absent in the *npq4* mutant. These data not only proved the electronic nature of ΔA535, but also showed that it most likely arose from a small population of red-shifted zeaxanthin molecules (Ruban *et al*., 2002). It is suggested that this was indicating the activation of zeaxanthin, required to fulfil its role in qE. The Raman signal also indicated that this species was found in a rather specific configuration, indicating binding to a protein component of the membrane. In order to test whether this site could be on PsbS, zeaxanthin was reconstituted with a purified sample of this protein; a red-shifted absorption band was created with an identical Raman signature to ΔA535 (Aspinall-O’Dea *et al*., 2002).

Although specific binding to another protein site is not excluded, these data suggest that the activator role of zeaxanthin in qE requires that it be bound to PsbS. This binding, *in vivo*, requires ΔpH, most likely because of proton binding to PsbS, and confers upon zeaxanthin very new configurational and excited state features. How these properties are related to its mechanism in qE is currently unknown. On the one hand, these features may be needed for zeaxanthin to quench chlorophyll excited states on a neighbouring antenna complex directly (Ma *et al*., 2003). On the other hand, protonated PsbS may ‘deliver’ zeaxanthin to its active site in the antenna: in this case it again may be a direct quencher, or as suggested above, it may fulfil an allosteric activating role. Finally, a protonated zea/PsbS complex may have an allosteric role. In the latter two cases, PsbS would be regarded as the regulatory subunit of the light-harvesting antenna. Further work is needed to distinguish these possibilities.

Comparative biochemistry and physiology of NPQ

NPQ is observed in all higher plants; it has been detected in a wide range of species in many different plant types, in a wide range of habitats (Demmig-Adams, 1998; Johnson *et al*., 1993). It has also been found in lower plants (Campbell *et al*., 1998; El Bissati *et al*., 2000), green algae (Niyogi *et al*., 1997), and diatoms (Casper-Lindley and Björkman, 1998; Arsalane *et al*., 1994). It seems reasonable to assume that the underlying molecular mechanisms are the same in all cases. Nevertheless a comparative study of NPQ in different species has proved to be rewarding, different aspects of NPQ appear to be enhanced in certain species, making them good models for further study. Moreover, in such cases, the knowledge obtained, can give new approaches to gaining fundamental information on the mechanism of NPQ.

NPQ has been described as being the sum of two different processes (Horton *et al*., 1996). In addition to the rapidly relaxing qE type discussed above, there is further NPQ that relaxes very slowly when excess light is removed. This latter type, called qI, is usually much smaller than qE, and is attributed to a more sustained effect of excess light. Therefore qI has been associated with ‘photo-inhibition’ rather than ‘regulation’, and was thought to arise from quenching by photodamaged PSII reaction centres. However, it was suggested that qI, like qE, may not be an effect of excess light stress, but an adaptive response to it (Horton *et al*., 1988, 1996; Ruban *et al*., 1993). Thus, perhaps quenching by a photoinhibited reaction centre is
a useful response that provides photoprotection of the antenna and thylakoid membrane (Chow et al., 2002). Since strong evidence of the accumulation of damaged PSII under photoinhibitory conditions was absent, it was further suggested that qI occurs in the antenna, perhaps even by the same or similar mechanism to qE (Demmig and Winter, 1988; Ruban et al., 1993; Ruban and Horton, 1995b).

In order to determine the relationship between qE and qI, species that show particular NPQ characteristics have been sought. The epitrophic plant, Guzmania monostachia, exhibits a large NPQ capacity (Ruban et al., 1993). Both qE and qI are induced in excess light: successive periods of illumination progressively ‘pump’ qE to its maximum value, and this is activation is correlated with an increase in qI. Thus, qI is the signature of the light ‘activation’ described above, and is therefore attributed to the de-epoxidation of violaxanthin. The large extent of activation and qI in this species is also associated with a large xanthophyll cycle pool and a high de-epoxidation state. Spectroscopy carried out on Guzmania leaves upon the induction of qI showed the formation of red-shifted chlorophyll emission previously identified in aggregated LHCII. A very stable type of NPQ found in plants acclimated to low temperature also has this feature, the ‘cold hard band’, suggesting that evergreen plants which can withstand freezing conditions over winter exist in a permanently photoprotected qI state of aggregated LHCII. The slow relaxation could arise from extreme stability of the de-epoxidized states, the strength and number of xanthophyll cycle binding sites, the xanthophyll cycle pool size, the level of PsbS and the pH of the thylakoid lumen would determine the amounts of qE and qI observed in different plants. Extending this rationale to the green algae, perhaps the ΔpH is not large enough, and the zeaxanthin concentration not high enough to cause protonation of the qE active sites in the absence of PsbS.

In diatoms, the large NPQ can be explained by either a stronger quencher, or by a larger number of quenching sites. There is currently insufficient data to distinguish these. The kinetic features indicate a qI-type process, but one which is nevertheless dependent upon protonation, and formed rapidly. The fact that formation of diatoxanthin only requires one de-epoxidation, could explain the rapid activation of quenching. Since, PsbS is absent from diatoms, NPQ must occur by direct effects on the light-harvesting complexes. The slow relaxation could arise from extreme stability of the protonated state of these complexes when diatoxanthin is present, so that reversal of quenching absolutely depends upon the rather slow kinetics of de-epoxidation. This stability may arise from particularly strong protein interactions within this antenna that ‘locks’ the quenched state. This blurring of the distinction between qE and qI leads to a new suggestion about the role of the PsbS protein in plants: perhaps it provides control over NPQ, enabling not only induction of NPQ, but also its rapid reversibility.

**Comparative ecology of NPQ**

The variation in character of NPQ in different organisms suggests that facets of this process have undergone evolutionary adaptation to particular ecological niches. In land plants, a major requirement for the regulation of light harvesting is a rapid, dynamic response to changes in light levels. The activation by de-epoxidation, and triggering by protonation, is adapted to the more rapid changes associated with leaf movements and sunflecks, against a background of diurnal and daily changes. The relatively slow kinetics of de-epoxidation act as a molecular memory of ‘average’ light conditions, whereas protonation provides an
Light-harvesting proteins can then be considered to have evolved from these stress proteins, by binding chlorophylls, and excluding many carotenoids (Fig. 1). The trimeric state of plant LHCII is probably the most evolved complex for light harvesting (Wentworth et al., 2003). It is interesting to consider the LHCF protein of diatoms; this complex binds chlorophyll, but has retained a high amount of carotenoid binding. The level of this protein increases under conditions of high NPQ, and hence may be considered as truly bifunctional—operating equally in both photoprotection and light harvesting, its two modes depending upon de-epoxidation. By contrast, in plants, photoprotection requires PsbS, a protein not involved in light harvesting. However, this protein is able to confer upon LHCII (or any PSII antenna complex) a photoprotective function, perhaps by providing delivery and removal of the ‘active’ zeaxanthin.

The presence of PsbS, therefore, allows the light-harvesting complex to function as an efficient light harvester, with a high absorption cross-section, its chlorophyll pigments not needing to be diluted by binding high levels of carotenoid. The extra benefit from this mode of control is rapidity as reversal does not depend upon epoxidation of zeaxanthin. Moreover, once the appropriate de-epoxidation state has been reached, NPQ can be turned on and off rapidly. In this context it is interesting to consider the effect of PsbS concentration on NPQ. Manipulation of the amount of PsbS in Arabidopsis shows that its stoichiometry correlates with changes in the amplitude of the qE component of NPQ (Li et al., 2002b). Mutants which lack PsbS altogether have only a slowly relaxing NPQ; in the absence of PsbS the unassisted process is revealed, one which relies upon the passive mass action effect of the xanthophyll cycle and in which qE and qI are indistinguishable. The evolution of qE in plants, driven by the need to respond rapidly to changing light intensity, resulted from the specification of an ancestral carotenoid–protein complex to a new role reversibly binding xanthophyll cycle carotenoids and protons, and interacting with the PSII light-harvesting antenna proteins.

**Conclusions**

The principles that have guided the molecular design of the PSII light harvesting have been elucidated. Modifications to this design are found in different organisms’ adaptations to
specific environments. An essential feature is the structural flexibility of the light-harvesting proteins, which allow control over the transfer of excitation energy to particular quenching species, associates of chlorophyll or chlorophyll and xanthophyll. The recent elucidation of the three-dimensional structure of LHCII has revealed the possible molecular mechanisms by which this may occur (Liu et al., 2004). High resolution electron microscopy and image analysis of wild-type and genetically altered plants are showing how these structures are organized within the intact membrane (Yakushevskaya et al., 2003; Ruban et al., 2003) and new spectroscopic approaches to the investigation of intact systems are beginning to reveal details of how such mechanisms operate (Robert et al., 2004).

References


