Architecture of a Charge-Transfer State Regulating Light Harvesting in a Plant Antenna Protein

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Energy-dependent quenching of excess absorbed light energy (qE) is a vital mechanism for regulating photosynthetic light harvesting in higher plants. All of the physiological characteristics of qE have been positively correlated with charge transfer between coupled chlorophyll and zeaxanthin molecules in the light-harvesting antenna of photosystem II (PSII). We found evidence for charge-transfer quenching in all three of the individual minor antenna complexes of PSII (CP29, CP26, and CP24), and we conclude that charge-transfer quenching in CP29 involves a delocalized state of an excitedly coupled chlorophyll dimer. We propose that reversible conformational changes in CP29 can "tune" the electronic coupling between the chlorophylls in this dimer, thereby modulating the energy of the chlorophyll-zeaxanthin charge-transfer state and switching on and off the charge-transfer quenching during qE.

The photosynthetic apparatus in higher plants is designed to perform two seemingly opposing tasks: to efficiently harvest sunlight and transfer excitation energy to the reaction center (RC), but also to rapidly dissipate excessively absorbed light energy harmlessly as heat to avoid deleterious photodamage. Because highly reactive chemical species are inevitable by-products of photosynthesis, various regulatory processes are critical for the robustness of photosynthesis and for plant survival (J). Regulation of light harvesting is predominantly mediated by energy-dependent quenching (qE) (2, 3), a phenomenon that depends on the trans-thylakoid pH gradient (ApH) (4), zeaxanthin (Z) (5), and the photosystem II (PSII) antenna-associated protein PsbS (6). However, precisely how and where within PSII these components interact to mediate qE at the molecular level is still not well understood. Identification of the precise molecular architecture that is responsible for this vital regulatory process could provide insight into the design principles of photoprotection in natural photosynthesis and could inspire approaches to engineer more robust artificial systems for solar energy conversion (7–9).

Two different mechanisms, which are not mutually exclusive, have been proposed recently to explain qE (10, 11). According to Ruban et al. (11), qE occurs in the peripheral, trimeric antenna of PSII called LHClI (12), and its molecular mechanism involves energy transfer from chlorophyll a to a low-lying excited state of a carotenoid (lutein 1) in LHClIII (13). A change in the conformation of a different carotenoid (neo-
xanthin) was identified spectroscopically and correlated with LHCII quenching in vitro, and this conformational change was in turn correlated with qE in vivo (11). On the other hand, we have proposed a charge-transfer (CT) mechanism for qE on the basis of quantum chemical calculations (13) and ultrafast pump-probe experiments (10, 14). The CT mechanism involves energy transfer from the chlorophylls bound to the PSII-LHCII supercomplex to a chlorophyll-Z heterodimer. The chlorophyll-Z heterodimer then undergoes charge separation followed by recombination, thereby transiently producing a Z radical cation (Z+). Formation of Z+ in thylakoids depends on the three components that are necessary for qE in vivo (10, 14). In PSII, an inner layer of monomeric (minor) antenna proteins connects the peripheral LHCII trimers to the RC core complex (15, 16). Evidence for CT quenching was recently demonstrated in a composite mixture of isolated minor antenna components (CP29, CP26, and CP24), whereas no trace of Z+ formation signal could be found in isolated LHCII trimers (14). Thus, it is possible that different qE mechanisms are operating in different parts of the PSII antenna. Although each of these mechanisms has been proposed to account fully for qE (11, 14), their relative contributions to qE in vivo remain to be determined.

To investigate the molecular architecture of CT quenching and to identify precisely which of the minor complexes are capable of mediating CT quenching, we carried out ultrafast transient absorption experiments in the spectral region of Z+ absorption (14, 17) using all three of the individual, isolated minor complexes. We expressed recombinant apoproteins of CP29, CP26, and CP24 in bacteria and reconstituted them in vitro with chlorophylls (a and b), lutein, and either Z or violaxanthin (V). In plants and isolated thylakoids, the reversible enzymatic conversion of V to Z is correlated with qE (3), and previous work has shown that CT quenching is associated with the presence of Z in intact systems (10) and in isolated proteins (14), whereas V-bound complexes are inactive. Herein we refer to the CP29 complexes reconstituted with Z and V as CP29-Z and CP29-V, respectively (table S1 and fig. S1). We excited the samples at 650 nm and probed the near-infrared (NIR) region where carotenoid radical cation species (Car+) exhibited strong absorption (10, 14).

Figure 1A shows the transient absorption profile for CP29-V as compared to that of CP29-Z. Both exhibit rapid rise components followed by exponential decays. However, relative to the CP29-V kinetics, the CP29-Z kinetic profile shows a larger initial amplitude rise, the component dynamics of which are clearly evident in the NIR transient absorption difference trace (Fig. 1A).

To further explore the molecular details of CT quenching in CP29, we studied a series of mutant CP29 complexes that each lacked a specific chlorophyll. According to the previously reported homology structural model (21, 22), CP29 contains eight chlorophyll-binding sites referred to as A1, A2, A3, A4, A5, B3, B5, and B6, along with two carotenoid-binding sites referred to as L1 and L2 (Fig. 2). Versions of CP29 with mutated ligands to each chlorophyll were reconstituted in vitro with chlorophylls (a and b), lutein, and either V or Z, and stable complexes each lacking specific chlorophylls were recovered except for the A1 mutant. These complexes are referred to as, for example, CP29–A2, where the subscript denotes the missing chlorophyll (table S2 and figs. S2 to S5). Note that it has been established experimentally that Z binds specifically to the L2 domain; therefore, we can exclude the likelihood that chlorophyll A1 is directly involved in CT quenching (23, 24).

The difference NIR transient absorption kinetic profile (blue trace) for CP29–B3 is characterized by both rise and decay components that are signatures of Z+ evolution during CT quenching (Fig. 3A), implying that CT quenching is active in the absence of chlorophyll B3. Likewise, the difference profiles for CP29–A2, CP29–A3, CP29–A4, and CP29–B6 also exhibit evidence of CT quenching irrespective of some variation in Z+ signal that might derive from pleiotropic effects on protein structure (fig. S6). These data show that CT quenching in CP29 is not eliminated by the removal of chlorophylls A2, A3, A4, B3, or B6, thus excluding the likelihood of their participation in CT quenching.
the kinetic profiles for CP29\textsubscript{AS}, whether reconstituted with Z or V, are characterized solely by decay features attributable to chlorophyll excited-state absorption, and the transient absorption difference profile reveals no amplitude change relative to the noise level (Fig. 3B). The interpretation of this result is complicated by the fact that mutation of the amino acid residue that coordinates chlorophyll A5 also results in the loss of chlorophyll B5, specifically in Z-bound CP29 complexes (table S2). However, the kinetic profiles for CP29\textsubscript{AS} (a complex lacking chlorophyll B5 only) are also characterized solely by chlorophyll excited-state absorption dynamics and no measurable Z\textsuperscript{+} formation signal (Fig. 3C), which clearly indicates that CT quenching in CP29 involves chlorophyll B5. According to the homology structural model (Fig. 4), chlorophyll B5 is placed farther away from Z (~13 Å) than is chlorophyll A5 (~6 Å). The chlorophyll A5-B5 pair is positioned within the L2 domain and chlorophyll A5 is oriented roughly cofacial to, and centered along the axis of, the Z-binding site, which is in good agreement with the requirements for CT quenching predicted by quantum chemistry calculations (13). Furthermore, chlorophylls A5 and B5 are reported to be strongly coupled (22, 25), and both are important for the regulation of chlorophyll excited states (21, 22). Therefore, we conclude that CT quenching in CP29 most likely depends on both chlorophylls A5 and B5, and that the coupling of chlorophyll-\textit{Z} heterodimer, the molecular site of CT quenching in CP29 comprises Z and a strongly coupled chlorophyll pair (A5 and B5).

What is the importance of this CT site architecture? Our finding indicates that the primary event of CT quenching in CP29 involves electron transfer from Z to a strongly coupled chlorophyll dimer in the A5-B5 pocket of CP29, rather than from Z to a monomeric chlorophyll molecule (26). Relative to a monomeric chlorophyll, a coupled chlorophyll dimer would be more favorable for CT quenching because the change delocalization over the two chlorophylls will make the product CT state more stable. As a result, controlling the coupling strength between chlorophylls A5 and B5 in CP29, either by changing the distance between them or by altering their orientations, would modulate the reduction potential of the chlorophyll dimer and therefore could be used to switch on and off the CT quenching. This CT switching mechanism provides a potential molecular basis for the regulation of CT quenching during qE in the higher plant antenna.

The rapid reversibility of qE at the physiological level is thought to result from changes in the trans-thylakoid \(\Delta \text{pH}\) that occur, for example, during fluctuations in incident solar flux density. It has been proposed that two thylakoid lumen-exposed glutamate residues of the PsbS protein sense these changes in pH (27) and induce protein conformational changes that control qE. Moreover, PsbS has recently been shown not to bind pigments (28) but to interact with CP29 (29). Therefore, we propose that pH-dependent protein conformational changes that are transduced from PsbS to CP29 (and possibly other minor antenna complexes) can alter the coupling strength between chlorophylls A5 and B5 in CP29 and induce CT quenching during qE. Experiments that can directly probe the efficiency of CT quenching while modulating the electronic coupling between chlorophylls A5 and B5 can test this proposal.

Converging results based on theoretical calculations, molecular genetic analysis, and ultrafast spectroscopy have shown that the mechanism involving CT quenching in minor complexes is emerging as a key component of qE. Our results show that CT quenching can occur in all three minor antenna complexes, which are positioned between LHClII and the RC, a perfect setting for regulating excitation energy transfer to the RC.

Fig. 3. NIR transient absorption kinetics for CP29 mutants lacking the ligands binding to chlorophylls B3, A5, and B5. Transient absorption profiles were measured using CP29\textsubscript{AS} (A), CP29\textsubscript{AV} (B), and CP29\textsubscript{BV} (C). Other conditions are the same as Fig. 1A.

Fig. 4. Molecular details of the CT quenching site in CP29. Molecular sites responsible for CT quenching in CP29, including the Z bound in the L2 site and chlorophylls A5 and B5, are shown. On the basis of the homology model, the center-center distances for chlorophyll A5 to Z, chlorophyll A5 to chlorophyll B5, and chlorophyll B5 to Z are estimated to be ~6 Å, ~10 Å, and ~13 Å, respectively. The dihedral angle between chlorophylls A5 and B5 is about 41°. The structure indicates that chlorophylls A5 and B5 are strongly coupled to each other.
Phosphorylation of Retinoblastoma Protein by Viral Protein with Cyclin-Dependent Kinase Function

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As obligate intracellular parasites, viruses expertly modify cellular processes to facilitate their replication and spread, often by encoding genes that mimic the functions of cellular proteins while lacking regulatory features that modify their activity. We show that the human cytomegalovirus UL97 protein has activities similar to cellular cyclin-dependent kinase (CDK) complexes. UL97 phosphorylated and inactivated the retinoblastoma tumor suppressor, stimulated cell cycle progression in mamalian cells, and rescued proliferation of Saccharomyces cerevisiae lacking CDK activity. UL97 is not inhibited by the CDK inhibitor p21 and lacks amino acid residues conserved in the CDKs that permit the attenuation of kinase activity. Thus, UL97 represents a functional ortholog of cellular CDKs that is immune from normal CDK control mechanisms.

Because the large spectral absorbance gap (~700 cm⁻¹) between chlorophyll a and b prohibits delocalization over a chlorophyll heterodimer, no CT state can be produced in the L2 site of LHCl. One additional factor of specificity is that the capacity of LHCl to undergo exchange of V to Z in site L2 in vivo is very low relative to minor complexes (T.2).

Three small-molecule inhibitors of CDK activity, flavopiridol, BI2536, and PD0332991, potently inhibit UL97 kinase activity of UL97-null virus, and the growth defect was rescued in the revertant virus (fig. S4). Thus, UL97 kinase activity is necessary for UL97-null virus inactivation of three potential Rb-binding motifs in UL97 (fig. S2). We suspected that UL97 was required for Rb phosphorylation during HCMV infection. Phosphorylation of Rb on Ser²⁷⁸, Ser²⁸⁷, and Thr³²⁵ inactivates the cell cycle—tumor-suppressor pathways, and loss of these residues prevents growth of HCMV-infected cells (Fig. 2A). Residues not modified by CDKs in HCMV-infected cells but were phosphorylated in serum-stimulated cells (Fig. 2A). A recombinant HCMV in which the wild-type (WT) UL97 gene was replaced with an allele encoding a null UL97 protein substituted at the active site Lys²⁴⁸ → Gin²⁴⁸; K₃₅₅Q failed to induce phosphorylation of Rb, but a WT revertant virus derived from the K₃₅₅Q mutant did induce the phosphorylation of Rb (Fig. 2B). The UL97-K₃₅₅Q mutant virus exhibited a growth defect similar to that of the UL97-null virus, and the growth defect was rescued in the revertant virus (fig. S3). The CDK inhibitor flavopiridol again failed to prevent HCMV-induced phosphorylation of Rb in HCMV-infected cells, but two drugs that inhibit UL97 kinase activity (Gö6976 and maribavir) did inhibit such phosphorylation (fig. S4). Thus, in HCMV-infected cells, kinase activity of UL97 is necessary for Rb phosphorylation on residues that inactivate its function. Rb degradation and phosphorylation in HCMV-infected cells are independent events (fig. S1).

We also tested whether UL97 alone is sufficient to induce Rb phosphorylation. Transfection of expression plasmids for epitope-tagged wild type [but not a catalytically inactive (Ly₂⁴⁸ → Met²⁴⁸; K₃₅₅M) mutant (I)] induced the phosphorylation of cotransfected Rb on inactivating residues (Fig. 3A) in Saos-2 cells that are intrinsically unable to phosphorylate Rb. Drugs that inhibit UL97 partially suppressed Rb phosphorylation when added to UL97-expressing