Molecular Architecture of the Photoprotective Switches of Plants and Algae

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Summary

For plants and algae, fluctuations in sunlight intensity are a daily challenge. In their natural habitat, exposure to strong irradiance may occur over both slow and fast timescales and has required these organisms to develop strategies to protect themselves from the danger of photooxidation. Therefore, beside the efficient mechanisms of light harvesting and excitation energy transfer to fuel the photosynthetic machinery, both plants and algae evolved a series of complex feedback mechanisms aimed at dissipating excess absorbed sunlight energy. As widely reported in recent years, these mechanisms act on a variety of timescales and at different levels of organization within the photosynthetic membrane, known as the thylakoid. However, the precise molecular-scale mechanisms that modulate photosynthesis within a few minutes of exposure to high light intensities remain unknown.
This question was the motivation for the research reported in this thesis. This research has utilized a variety of experimental and theoretical methods and in particular combined mutational analysis, ultrafast spectroscopy and molecular dynamics (MD) simulations.

It has been proposed that the smallest units active in photoprotection in plants and algae are the same ones active in the first steps of photosynthesis, specifically, the light-harvesting complexes (LHCs). Several different LHCs are present in the thylakoid: all of them are membrane proteins most of which bind a variety of chlorophyll (Chl) and carotenoid (Car) pigments (up to 18 per single protein). Single-molecule fluorescence spectroscopy has previously shown that all LHCs have the intrinsic ability to reversibly switch between different emissive states, suggesting that all LHCs can potentially be stabilized in selected energy states functional for light harvesting or photoprotection. However, it has not previously been determined which domains are responsible for the conformational flexibility of LHCs and, in particular, for varying the energetics of these systems. To investigate these open questions we ran long MD simulations of a monomer of LHCII, the most abundant LHC of plants, in a model thylakoid membrane. The results are reported in Chapter 2. By characterizing the dynamics of each single protein domain and of each pigment, we showed that in general the most flexible regions of the protein are the ones exposed to the solvent. In particular, we showed that, at least in the case of LHCII, the N-terminus is a highly disordered domain and conformational changes in this domain may strongly affect the interaction strength between the two Chls responsible for the lowest-energy state of the system, thereby varying the energetics of LHCII.

Chapter 2 shows that LHCs are indeed potential sites for light-harvesting regulation, thanks to their ability to undergo conformational changes between different energy states. However, from previous *in vivo* studies, it is known that despite the highly similar structure and functional pigment organization shared by most LHCs, only two of these complexes are strictly necessary to trigger photoprotective mechanisms. In particular, one of these triggers is found in plants (PsbS), while the other is specific to algae (LHCSR). If these complexes are missing, photoprotection is drastically affected in these organisms. The fact that PSBS and LHCSR function differently from other LHCs, despite their high homology, led us to try to identify structural elements that are functional for photoprotection in LHCSR and PsbS, and also missing from most LHCs. Further, we aimed to identify the environmental stimuli to which these elements respond. We investigated these problems, in the case of LHCSR, via an *in vitro* study that combined site-directed mutagenesis and time-resolved fluorescence. Our results are reported in Chapter 3. LHCSR was found to be responsive to changes in pH, consistent with prior knowledge that low pH is known to be the major high-light stress indicator in the thylakoid. In particular, low-pH was found responsible for activating a quenching mechanism in LHCSR and not in other LHCs. The structural element of LHCSR involved in pH-sensing was found to be its C-terminus, characterized by a high concentration of acidic residues at variance with all other LHCs. To probe this, we had constructed a mutant of LHCSR missing all the acidic residues on the C-terminal sequence and we found that, indeed, pH-sensing ability and the switch to a quenched state were absent from this mutant.

Our results in Chapter 3, strongly suggest that LHCSR is able to undergo functional conformational changes by sensing variations in its environment under stress conditions. A reason why LHCSR is the only known pigment-binding LHC able to trigger photoprotection might lie in its structure and pigment organization, which may differ from other LHCs. Moreover, a recent crystal structure of the trigger of photoprotection in plants, PsbS, has revealed that its structure differs significantly from most LHCs (additionally, PsbS does not bind pigments), suggesting that the triggers of photoprotection might not have the same organization as the antennae. To address this point, we have combined ultrafast spectroscopy with theoretical modeling to build a minimal model of the pigment organization in LHCSR. These results are reported in Chapter 4. By simultaneously fitting a large set of linear spectra with an exciton model based on the pigment organization of LHCII, we found that LHCSR can be expected to have the structure of an antenna protein and, in particular, it is highly similar to LHCII (but with fewer pigments). Moreover, as suggested also by the results in Chapter 3, the fact that at high pH values (compatible with non-stress conditions) LHCSR shows a fluorescence lifetime similar to other LHCs suggests that this complex can work as an antenna or as a quencher in different conditions.

The evidence that LHCSR shares a similar pigment organization with LHCII (Chapter 4) and that LHCII is able to switch between different energetic states (Chapter 2), suggests that a pH-sensitive switch, similar to that of LHCSR, might be engineered into LHCII. In Chapter 5 we tested this possibility *in vitro* by engineering an LHCII in which the original C-terminus (not sensitive to pH) was replaced with that from

LHCSR. The result is a stable antenna that can be triggered to a quenched state by low pH. The implementation of such a reversible switch directly in LHCII, is not only interesting from the perspective of fundamental science, but it might also be of significant help in increasing crop yields. Indeed, it has recently been proven that by speeding up the relaxation kinetics from photoprotective states it is possible to increase plant biomass production by about 15% [Kromdijk, J. et al (2016) *Science*, *354*(6314), 857-861].

Overall, we have seen that the protein matrix of LHCs plays an important role in light-harvesting regulation. However, it has never previously been shown whether the pigments themselves possess conformational flexibility and whether this might be functional in activating photoprotection. In Chapter 6, we report the analysis of a model LHC system, where all the native Cars of LHCs are missing and are replaced by a single type of Car, astaxanthin. This LHCII, although preserving the same protein structure and pigment functional organization of the native one, is stabilized in a highly quenched state. Via ultrafast spectroscopy combined with global and target analysis, we were able to disentangle different excited states in the energy landscape of the Car bound to the quenched LHCII. Some of these states act as energy donors and some as acceptors of Chl excitations, overall suggesting that the Car is bound in different conformations to the protein and that different conformations have different functions in light harvesting. To understand where conformational switches of Cars are favored in LHCII (and most likely in LHCs in general) we then analyzed a large set of MD simulations. We found that functional distortions of Cars might be expected most likely at one of the internal Car binding sites, known as L2, and in particular in the lumen region, where acidification is expected under stress conditions. These results show for the first time that structural changes of the Cars are at the basis of the on/off switch controlling photoprotection.

Together, the investigations reported in this thesis have demonstrated that LHCs are dynamic systems whose protein and pigments are able to undergo conformational changes that can regulate the level of the excited states in the membrane. For the first time, we reported in detail the structure-function relationship for both the protein domains and the pigments involved in the photoprotective switch, linking the effect of selected conformational changes to the energetics of LHCs.