Photoprotective conformational dynamics of photosynthetic light-harvesting proteins

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Abstract

Under high light conditions, excess energy can damage the machinery of oxygenic photosynthesis. Plants have evolved a series of photoprotective processes, including conformational changes of the light-harvesting complexes that activate dissipation of energy as heat. In this mini-review, we will summarize our recent work developing and applying single-molecule methods to investigate the conformational states of the light-harvesting complexes. Through these measurements, we identified dissipative conformations and how they depend on conditions that mimic high light. Our studies revealed an equilibrium between the light-harvesting and dissipative conformations, and that the nature of the equilibrium varies with cellular environment, between proteins, and between species. Finally, we conclude with an outlook on open questions and implications for photosynthetic yields.

Keywords: photosynthetic light harvesting, photoprotection, single-molecule spectroscopy, free-energy landscapes

1. Introduction

In the first steps of photosynthesis, chromophore-containing proteins known as light-harvesting complexes (LHCs) capture solar energy and transport it to the reaction center to power downstream biochemical reactions. Under high light (*i.e.*, sunny days), absorbed energy in excess of the capacity of the downstream molecular machinery can generate deleterious photoproducts. To prevent damage, oxygenic photosynthetic organisms have evolved a series of photoprotective processes, known as non-photochemical quenching (NPQ), which regulate the flow of absorbed energy [1, 2]. The crucial importance

of NPQ for plant fitness has been demonstrated, although inefficiencies in photoprotection limit biomass yields by up to 30% under natural conditions [3, 4]. In an initial effort to increase yields, the proteins involved in NPQ were upregulated, which led to a faster turn off of NPQ. However, one challenge to further optimization is that the mechanisms have not been resolved [3]. In particular, NPQ is known to involve the LHCs, but the specific changes and how they are induced have not yet been determined.

NPQ is activated when water splitting in excess of the capacity of the downstream molecular machinery leads to a pH drop in the lumen [5]. The decreased luminal pH directly triggers energy-dependent quenching in higher plants, known as qE, as well as the enzymatic conversion of the carotenoid violaxanthin to zeaxanthin, which activates zeaxanthin-dependent quenching, known as qZ [6]. Changes in the organization of the LHCs have also been observed under high light conditions [7]. This series of interrelated parameters is thought to induce conformational changes of the LHCs that enhance dissipative photophysical pathways, as the photophysics are highly sensitive to intermolecular interactions and thus to protein conformation. While the location and parameters responsible for photoprotection have been long established, the number of conformations, their structures, and the associated photophysical changes were much more challenging to identify.

In this mini-review, we discuss a series of single-molecule fluorescence measurements to identify the conformational states and dynamics of the LHCs, and how they depend on conditions that mimic the effect of high light [8, 9, 10, 11]. Because fluorescence intensity and lifetime generally decrease as the level of dissipation increases, fluorescence has been widely used to characterize photoprotective processes at length scales ranging from entire fields to proteins [12]. Here, we describe experiments that identified the level of dissipation in individual proteins through quenching of their fluorescence. These experiments revealed that the LHCs exist in a conformational equilibrium between dissipative and light-harvesting conformations. Depending on the light level, the primary role of the LHC, and the species, the level of dissipation increased by a shift in this equilibrium. Depending on the light level, the primary role of the LHC, and the species, the level of dissipation increased by a shift in this equilibrium rather than by a switch to a previously absent conformation. Thus, it is likely the conformational equilibrium of an LHC that changes under different conditions for a desired biological function.

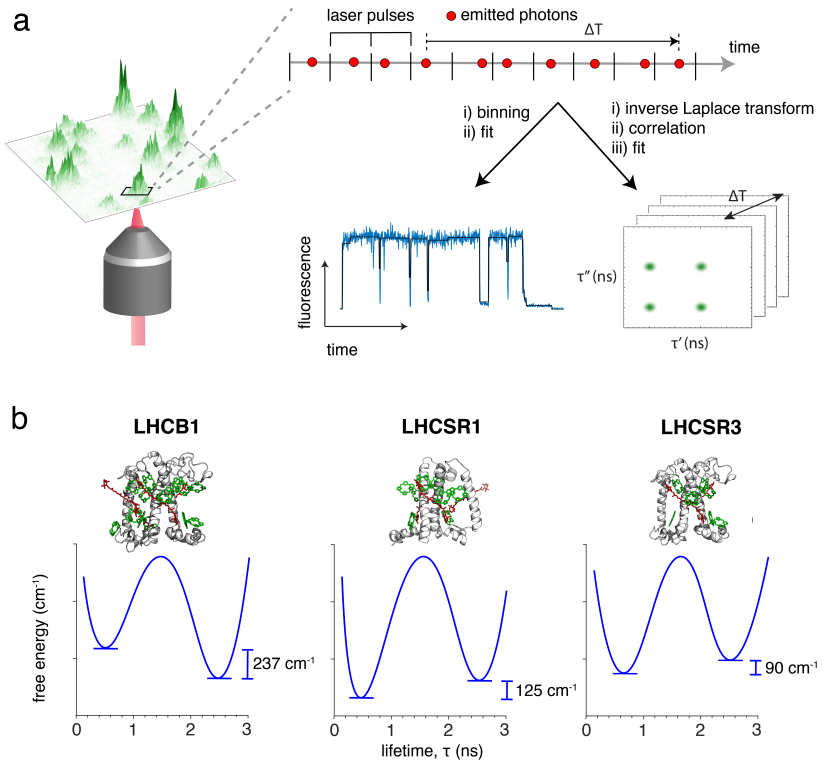

Figure 1: **Single-molecule measurements to probe conformational dynamics in LHC.** (a) Schematic of a single-molecule measurement in a confocal microscope of immobilized LHCs on a coverslip. The photon stream from the fluorescence emission is collected and the photons are binned over each millisecond or tens of millisecond period to construct fluorescence time traces. Through this analysis, heterogeneity between proteins can be identified and characterized. In the 2D fluorescence lifetime correlation analysis, the same photon stream is subjected to inverse Laplace transform analysis to identify lifetime states. Extraction of correlation at different lag times (ΔT) and global fits of the auto- and cross-correlations reveal the dynamics of inter-state transitions. Through this analysis, dynamics on timescales as fast as microseconds can be resolved. (b) Structural models (top) and corresponding free-energy diagrams (bottom) of LHCB1, LHCSR1 and LHCSR3. Protein backbones, chlorophylls and carotenoids are shown in grey, green and red respectively. The structural models of LHCB1 and LHCSR3 are constructed from LHCII crystal structure (PDB:1RWT) [13]. LHCSR1 structure is built from the crystal structure of CP29 (PDB:3PL9) [14, 15]. Free energy landscapes are constructed from the populations and transition rates extracted from 2D-FLC spectroscopy. Adapted from [9, 10].

2. Single-molecule fluorescence spectroscopy

In single-molecule fluorescence spectroscopy, the emission from individual proteins is detected, revealing heterogeneous and asynchronous behaviors [16, 17, 18, 19]. This is typically achieved by measuring dilute samples using a confocal microscope, which allows for multi-parametric detection of fluorescence brightness, lifetime, spectra, and/or polarization [20, 21]. For LHCs, the electronic transitions of the embedded chlorophyll are exquisitely sensitive to the surrounding protein conformation, and thus the fluorescence provides a reporter of the surrounding protein conformation [22, 17]. Changes in the fluorescence properties report on two types of dynamical processes: (i) conformational changes of the protein via their influence on the embedded chromophores and (ii) photodegradation, which ultimately culminates in photobleaching or an irreversible transition into a non-emissive state [23]. The fluorescence parameters are monitored over time, traditionally with tens to hundreds of millisecond resolution due to the temporal averaging required for accurate parameter estimation. This series of interrelated parameters is thought to induce conformational changes of the LHCs that enhance dissipative photophysical pathways, as the photophysics are highly sensitive to intermolecular interactions and thus to protein conformation [24, 25, 26].

While straightforward analysis of the fluorescence parameters allows for characterization of the conformational ensemble and slow conformational changes, the dynamics have historically been temporally averaged and thus faster processes were inaccessible [27, 28, 8]. Recently, we extended a more sophisticated analysis method, two-dimensional fluorescence lifetime correlation (2D-FLC) spectroscopy, to the single-molecule level in order to resolve conformational dynamics of the LHCs on timescales from microseconds to seconds [9, 10]. In 2D-FLC spectroscopy, the fluorescence decay curves are subjected to an inverse Laplace transform to generate peaks at lifetime values, which indicate conformational states (Figure 1a) [29, 30]. The auto and cross correlations of these peaks contain information about the dynamics of the states and the transitions between them, respectively. To extract quantitative information about the states and dynamics, we also developed model functions to globally fit the correlations in order to obtain rate constants of interstate conversion [9]. Rate constants from these global fits were used to determine the free energy differences between the lifetime states. These differences describe the free energy landscapes, which can be used to visualize the conformational ensembles as illustrated in Figure 1b.

3. Single-molecule measurements of LHCSR

The key gene product for NPQ in green algae and mosses is light-harvesting complex stress-related (LHCSR) [31, 32, 33]. Direct protonation of the luminal acidic residues of LHCSR triggers photoprotective dissipation within LHCSR via interactions with the other LHCs. To characterize the conformational ensembles and investigate the role of conformational changes in this activation, a series of single-molecule experiments were performed on LHCSR and homologous proteins [8, 9, 10].

3.1. Conformational dynamics across species

Two isoforms of LHCSR, LHCSR1 and LHCSR3, have been implicated in NPQ. In *Chlamydomonas (C.) reinhardtii*, the model organism for green algae, both isoforms are present. However, LHCSR3 is accumulated at higher levels and exhibits a higher level of dissipation, indicating it has the primary role in NPQ [34, 35]. In contrast, in *Physcomitrella (P.) patens*, the model organism for moss, only LHCSR1 is present, indicating it has the primary role in this species [36, 37]. In single-molecule measurements of other LHCs, broad distributions of fluorescence properties were observed, which lacked well defined features. The single-molecule fluorescence lifetimes of both LHCSR isoforms exhibited two well-resolved peaks, one with a fluorescence lifetime of $\sim$2 ns and the other with a lifetime of $\sim$0.5 ns. These two populations also showed brighter and dimmer fluorescence, respectively. A shorter lifetime and lower fluorescence are canonical signatures of the presence of a dissipative pathway, and so these observations indicated that the two populations correspond to a light-harvesting and a dissipative conformation.

Analysis of the single-molecule fluorescence time traces (Figure 1a) showed transitions between the two populations [8], establishing that LHCSR can switch between light-harvesting and dissipative conformations. However, these traces are limited to dynamics on timescales of hundreds of milliseconds. To access transitions on timescales down to microseconds, 2D-FLC spectroscopy was also performed. Through this more sophisticated analysis, two distinct dynamical processes were resolved [9, 10]. The associated free energy landscapes for LHCSR3 are shown in Figure 2a and b. The chlorophyll are embedded within the LHCs throughout the protein structure, and serve as emissive sites due to their energetically overlapping excited states. As a result, quenching of fluorescence can occur at several locations within

the protein. The presence of two dynamical processes suggests that conformational changes enhance dissipative photophysics for the local chlorophyll at two different locations. Notably, the analysis also indicated that the two distinct processes occur in parallel within LHCSR3. Whether dynamics occur in parallel within a single protein or separately within different copies of the protein is hidden in ensemble measurements. Single-molecule measurements, and particularly 2D-FLC spectroscopy, are able to distinguish between these two cases, and thereby established the presence of these separate, yet simultaneous, conformational dynamics in LHCSR3.

Similar states and two dynamical processes were observed in both isoforms, although the timescales were different. In LHCSR1 from *P. patens*, the timescales were hundreds and tens of milliseconds. In LHCSR3 from *C. rheinhardtii*, the timescales were tens of milliseconds and hundreds of microseconds. Due to their ecological niche, green algae are generally more exposed to sunlight, experiencing faster and more frequent fluctuations than mosses experience in their shady environment. Consistently, the faster dynamics of LHCRS3 enables faster equilibration between light-harvesting and dissipative conformations, which may minimize bottlenecks in acclimation to varying light intensities.

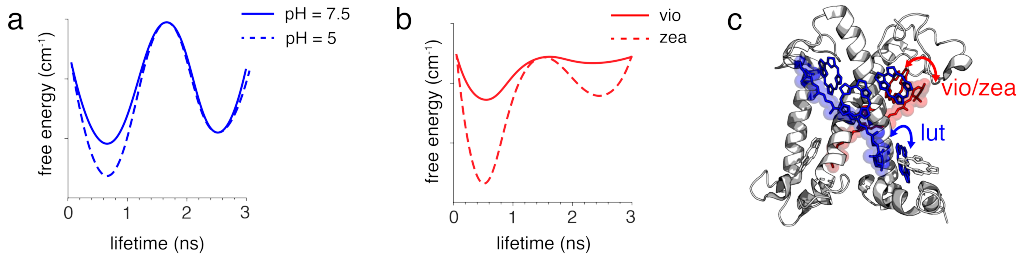

Figure 2: **pH- and carotenoid-dependent quenching in LHCSR3.** (a) Free-energy landscape of the slower process constructed from the populations and transition rates between short- and long-lifetime states of LHCSR3 from the 2D-FLC analysis. Upon a pH drop, the short-lifetime state (dissipative conformation) is stabilized. (b) Free-energy landscape of the faster process of LHCSR3. Upon carotenoid conversion from violaxanthin (vio) to zeaxanthin (zea), the short-lifetime state (dissipative conformation) is stabilized. (c) The likely structural origins of the two conformational changes that lead to switches between lifetime states is shown in corresponding colors. Adapted from Ref. [10].

3.2. Conformational dynamics with cellular parameters

In order to investigate the role of the identified conformational dynamics in NPQ, single-molecule measurements were performed on LHCSR as a function of pH and carotenoid composition. While both LHCSR1 and LHCSR3 were studied, here we focus on LHCSR3 as similar behaviors were observed in both proteins. Because of the complexity of the activation of NPQ and the large number of homologous light-harvesting complexes, the individual contributions from LHCSR3, pH, and zeaxanthin have been challenging to disentangle. In higher plants it has been long established that both a luminal pH drop and zeaxanthin induce NPQ, but the role of zeaxanthin in *C. reinhardtii* was less clear. By measuring individual proteins, the conformations of LHCSR3 could be characterized as a function of both molecular parameters, pH, and zeaxanthin.

The two dynamical processes in LHCSR3 each responded to one of the two parameters. Upon a decrease in pH from 7.5 to 5.0, the free-energy difference between the long-lifetime and short-lifetime states shifted toward the short lifetime state by over 200 cm^{-1} for the slower process, and so the dissipative (short lifetime) conformation became dominant. The relative rates, and thus the free energy differences, of the faster process were nearly unaffected. In contrast, upon a change in the carotenoid composition from violaxanthin to zeaxanthin, the free-energy difference for the faster process shifted towards the short lifetime state by 550 cm^{-1} , and so again the dissipative conformation became dominant. The relative rates of the slower process were only minorly affected upon the change in carotenoid composition. Thus, the two dynamical processes had opposite dependencies on pH and zeaxanthin; whereas one favored dissipative conformations exclusively upon a pH drop, the other favored dissipative conformations exclusively in the presence of zeaxanthin.

From these results, along with other biochemical and spectroscopic studies of LHCSR3 and homologous proteins, the dynamical processes were assigned to potential sites within the protein. The primary proposals for the photophysical mechanism of dissipation involve carotenoid-chlorophyll interactions, which can either lead to charge transfer/recombination or energy transfer to carotenoids followed by rapid non-radiative decay [38, 39, 40, 41]. The likely sites for dissipation, therefore, contain neighboring chlorophyll and carotenoids. Given that there are two carotenoids bound at the internal positions within LHCSR3, the two dynamical processes are assigned to the two carotenoids and their neighboring chlorophyll as illustrated in Figure 2c

[10]. While many factors supported this assignment [10], the pH-dependent process likely involves the carotenoid that sits above the luminal helix with protonable residues and the zeaxanthin-dependent process likely involves the carotenoid that converts from violaxanthin to zeaxanthin. Thus, the single-molecule data suggest that pH and carotenoid composition regulate two conformational changes at distinct sites within LHCSR3.

4. Single-molecule measurements of LHCII

4.1. Conformational dynamics across proteins

The major light-harvesting complex in plants, LHCII, is a trimer comprised of monomers homologous to LHCSR. LHCII has been implicated in qE [42, 43, 44], and the formation of LHCII arrays has been observed in vivo under high light conditions and shown to enhance dissipative photophysics [7, 45]. Single-molecule fluorescence traces of trimeric LHCII from *Arabidopsis thaliana* and the monomeric unit, LHCB1, from *P. patens* revealed broad distributions of fluorescence brightness and lifetime, indicating the presence of multiple conformations with different levels of dissipation [8, 11, 46]. The distributions were broader and more heterogeneous for LHCB1 than for LHCII [9, 11]. The average transition rates for the intensity traces, which reflect slow dynamics and photodegradation events, were faster for LHCB1 than LHCII with values of 0.8 s^{-1} and 0.1-0.5 s^{-1}, respectively [8, 11, 46]. To more fully characterize the dynamics, 2D-FLC spectroscopy was also employed. Consistently, millisecond dynamics were identified for LHCB1 whereas no dynamics were resolved on this timescale for LHCII. Collectively, these results show that LHCB1 is more heterogeneous and dynamic than LHCII, which suggests that trimerization enhances the rigidity of the protein.

Despite their homologous structures, differences between LHCB1 and LHCSR were observed in the conformations and dynamics. The free energy landscapes from 2D-FLC spectroscopy are shown in Figure 1b for the dynamical process that corresponds to most of the fluorescence emission. As illustrated, LHCSR1 and LHCSR3 have near-equal populations of light-harvesting and dissipative conformations, whereas LHCB1 is dominated by the light-harvesting conformation, consistent with its primarily biological role. Furthermore, the average transition rates for the intensity traces were 3.4 s^{-1} in LHCSR1, which is approximately an order of magnitude faster than the rates for LHCB1 and LHCII. This difference in rates, along with

the absence of dynamics in the 2D-FLC spectroscopy of LHCII, indicates that overall LHCSR exhibits more dynamics, enabling faster re-equilibration. As LHCSR has been implicated in NPQ, its facile and controllable conformational dynamics may have a functional role. The land area is associated with more stress, and higher plants have evolved a dedicated regulatory protein, the non-pigment binding homologue of LHCSR, PsbS, [47, 48, 49]. PsbS may be designed exclusively for photoprotective conformational dynamics, alleviating the need for such behaviors in LHCII.

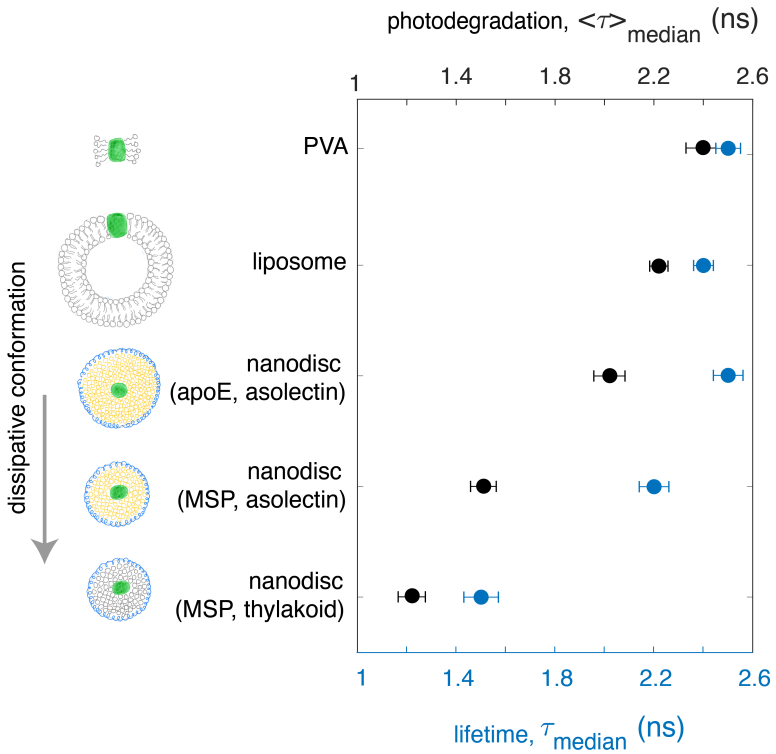

Figure 3: **Dissipative conformations of LHCII depend on the local membrane environment.** Median excited state lifetime (blue circle) and median photodegradation (black circle) obtained from single-molecule measurements of LHCII in detergent embedded in a PVA matrix, in liposomes and in nanodiscs with various size and lipid compositions. Median lifetimes were obtained from the first levels of single-molecule traces. Photodegradation is quantified as the dwell-time weighted lifetimes. Error bars are the standard deviations. Fluorescence quenching and photodegradation increased in smaller membrane areas and with charged lipids. Figure adapted from Ref. [11].

4.2. Conformational dynamics across membrane environments

As discussed above, dissipative conformations of LHCII have been observed in single-molecule measurements, consistent with the role of LHCs in NPQ. While pH, zeaxanthin, and aggregation were shown to enhance dissipation [27, 28, 46, 50], how other aspects of the local environment influenced the conformations, and thus photophysics, was less well characterized. To investigate the effect of the membrane environment, single-molecule measurements were performed on LHCII in model membranes with different size, shape and lipid compositions, revealing membrane-dependent conformations and photodegradation [11].

From the single-molecule measurements, an increase in dissipative conformations and in photodegradation was observed for LHCII in membrane environments as compared to in detergent (Figure 3), consistent with previous work [23]. Within the membrane environments, a further increase in dissipative conformations was observed for LHCII confined in a discoidal lipid bilayer, known as a nanodisc, as compared to in a spherical bilayer of a liposome. The increase in dissipation may be due to the increased lateral membrane pressure of nanodiscs [51], indicating dissipative conformations can be enhanced via this effect. Within the nanodiscs, smaller membrane areas also gave rise to more dissipation and faster photodegradation than the bigger areas, potentially due to interactions between LHCII and the scaffolding proteins and/or disordered lipids around the edges of the discs. Finally, increased dissipation and photodegradation was observed for thylakoid lipids as opposed to asolectin. The main difference between the lipids is the presence of negatively charged lipids in the thylakoid, which may stabilize charge transfer or charge separated states. Collectively, these results indicated that the nature of the surrounding lipid bilayer plays an active, and previously underappreciated, role in controlling LHC conformation and thus function.

5. Outlook

Single-molecule measurements have been used to reveal conformational dynamics of LHCs on timescales from microseconds to seconds. Changing the timescales of these dynamics, and thus the equilibrium, under conditions that mimic high light provides a mechanism to regulate absorbed energy using associated changes in photophysical pathways. Recent advances in ultrafast spectroscopy, such as two-photon excitation [52, 53], multidimensional spectroscopy [26], and snapshot transient absorption spectroscopy

[54], have provided insight into these pathways. Investigating the femtosecond to nanosecond photophysics present in each conformation requires new tools to measure these ultrafast processes at the single-molecule level. Such experiments are just becoming possible with the advent of single-molecule pump-probe spectroscopy [55, 56], and will undoubtedly provide new information. At the same time, understanding how the multi-timescale dynamics of NPQ occur in the organism requires spectroscopic experiments that extend beyond individual LHCs to membrane environments and eventually in vivo [57]. Single-molecule measurements in tandem with other spectroscopic, biological, and computational tools can elucidate the behavior of LHCs and, in particular, how they balance light harvesting and photoprotection to power most life on Earth.

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