From Disorder to Order: The Functional Flexibility of Single Plant Light-Harvesting Complexes

Kruger, T.P.J.

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SUMMARY

Regulation of every process in every cell depends upon dynamic changes in the structure and function of key proteins. In photosynthesis, changes in the function of the pigment-protein light-harvesting complexes (LHCs) regulate the efficiency of light collection and usage. One example of such regulation is the rapid switching of the main LHC of photosystem II (LHCII) between different functional states to protect the plant from the potentially damaging effects of intense sunlight. Another ingenious design is that of the photosystem I LHCs (lhca's), which are capable to absorb light at significantly lower energies than photosystem II LHCs (lhcb's), despite the fact that all of these complexes are structurally and compositionally very similar.

In order to gain new insight into the nature of the light regulation in LHCII and the spectroscopic differences between lhca's and lhcb's we have used single-molecule spectroscopy (SMS), an approach which uniquely allows exploration of the nature of the molecular processes that underlie these proposed functional changes in LHCs. The fluorescence experiments were performed on single LHCs near room temperature. The spectral fluctuations of lhca and lhcb complexes were resolved in real time, results which are described in Chapters 3 and 4. In Chapters 5 through 7, the interesting single-molecule phenomenon of fluorescence intermittency – switches between distinct intensity levels at irregular intervals – is explored for LHCII trimers. In Chapter 7, the relationship between the spectral and intensity fluctuations of this complex is assessed. A summary of the main findings is presented here.

In Chapter 3, the variety of fluorescence spectral states that can be accessed by LHCII is investigated. This study includes the spectral information of more than 2000 individually measured LHCII trimers as well as 2000 simulated spectral states as obtained by using a disordered exciton-Redfield model. It is found that most complexes remained in a stable spectral state during the course of a measurement, characterised by a spectral peak at ~682 nm. About 5% of the complexes exhibited spectral fluctuations of 2—15 nm, all of which can be explained by the intrinsic disorder of the proteins in the complex. Remarkably, the distribution of spectral peak positions matches perfectly with the model prediction, suggesting that the model describes very well the average effect of disorder in this system. However, another ~5% of the complexes exhibited a new spectral state, characterised by a second spectral peak beyond ~700 nm, reminiscent of emission from Lhca complexes. These spectral states could not be modelled in a disordered exciton-Redfield description and therefore strongly suggest the presence of special protein conformations. The spectral changes were fully reversible, suggesting that the new conformations are closely related to physiological states.

Chapter 4 extends the study to Lhca complexes and succinctly also to the minor antennas of photosystem II, thus enabling both a general and specific comparison of the spectral dynamics of Lhca and Lhcb complexes. It is shown that in time the typical red emission of an Lhca complex can be replaced reversibly by the characteristic emission of an Lhcb complex, and *vice versa*. Based on the high structural homology as well as the similar pigment content

and organisation of all these complexes, the results indicate that the specific functions related to Lhca and Lhcb complexes are realised by different stable conformations of a single generic protein structure. We propose that the protein environment modulates and controls this functionality by shifting the equilibrium between the two distinct conformational states, thereby exhibiting control over the intrinsic disorder. The ensemble spectra of the complexes are thus representative of the relative probability of each associated spectral state.

Chapter 5 presents a novel algorithm to analyse fluorescence intermittency data, in particular from multichromophore systems. Conventionally, the analysis of fluorescence intermittency involves binning the variety of intensity levels into two large bins. We demonstrate that by carefully resolving the different accessed intensity levels, a substantially larger amount of information is acquired and analysis artefacts are reduced. Both outcomes result from the fact that the obscuring effect of shot noise is reduced. Based on the data from LHCII trimers, we propose that the conventional method of data analysis can lead to a misleading interpretation of the physical mechanism behind fluorescence intermittency. Our results are relevant not only to LHCII but very likely also to various other systems that exhibit similar fluorescence fluctuations.

In Chapter 6, the algorithm of the previous chapter is used to investigate the environmental sensitivity of the fluorescence intermittency of LHCII trimers. In particular, the effect of different pH environments and varying light intensities is examined. It is shown that the efficiency of light harvesting, which is represented by complexes typically residing for long periods in strongly fluorescing states, is significantly reduced at a lower pH and a higher excitation power. The same environmental changes simultaneously increase the switching frequency between strongly and weakly fluorescing states. The strong environmental sensitivity suggests that the local environment of an LHCII complex can modulate the amount of energy dissipation. A simple model illustrates how this may be achieved: the dynamic equilibrium between the strongly and weakly fluorescing states can be shifted by environmentally controlling the conformational disorder of LHCII.

In the final chapter, the modulation of fluorescence intermittency by the local environment is investigated by considering three primary conditions known to be involved *in vivo* when qE takes place. Each of these conditions was found to stabilise the quenched states and destabilise the unquenched states, thereby decreasing the average intensity of large populations of LHCII trimers. The positive correlation between the amount of energy dissipation and the extent of qE strongly suggests that the principal mechanism underlying fluorescence intermittency and qE is related. This implies that the intrinsic disorder of LHCII, as represented by fluorescence intermittency, can be controlled by the system's local environment to serve a functional role. In other words, the protein is capable to exploit an intrinsic property – its disorder – to perform the vital regulatory function of qE.

The second part of Chapter 7 inspects the relationship between the extent of quenching and spectral shifts. The lack of correlation between these two properties strongly suggests that their underlying mechanisms have different origins. However, under conditions that mimic

qE, a second category of quenched states was observed, characterised by a 90−100-nm redshifted spectral profile. This state is likely connected to a secondary mechanism of qE in LHCII. The results thus reveal the presence of four distinct structural states of the LHCII protein, two of which are involved with quenching. We propose that the protein is in equilibrium between all four states and that its local environment shifts this equilibrium by inducing a subtle conformational change. This property would enable the protein to switch rapidly between different functional states according to the rapidly changing demands of the plant.

The key finding of this thesis can be stated as follows: Plants exploit and control the intrinsic disorder of their LHCs by creating specific local environments which stabilise different protein conformational substates to provide a highly sensitive and effective regulatory mechanism. Since such disorder is common in protein systems, it is highly likely that it is exploited in a similar way in other biologically important processes. The implications of our findings therefore extend beyond the elucidation of photosynthetic mechanisms to a profound increase in our understanding of the function of regulated proteins that is almost certainly universal.

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As they say, a Ph.D. project is never really finished: science frequently has the remarkable ability not only to answer some specified questions but also to create many more. However, the results presented in this thesis form a basis for many similar studies that can be performed on numerous other systems. The analysis methods developed during the course of the project enable fast and effective analysis of large SMS data sets. This will particularly facilitate enhancing experimental support for the relationship between fluorescence intermittency and photoprotection in LHCII and possibly in many other LHCs.

Further advances of the projected may be expected by extending the experimental setup to accommodate alternative applications. A few ideas include: time-correlated single photon counting (TCSCP) to enable measuring of fluorescence lifetimes; controlled variation of the excitation wavelength to perform excitation spectroscopy; polarisation studies by incorporating a rotating polariser in the detection branch; cryogenic SMS by immersing the objective and sample cell into a cryostat; combining SMS with atomic force microscopy to determine the size of the fluorescing system. Such a combination of experimental techniques is expected to become a powerful means to correlate optical properties with the structural and functional diversity of photosynthetic LHCs.