Towards in-cell structural study of light-harvesting complexes : an investigation with MAS-NMR

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APPENDICES

Summary

Light-Harvesting Complex II (LHCII) is responsible for light absorption and excitation energy transfer in plants and photosynthetic algae, while in high light it undergoes conformational changes by which it quenches excitations to prevent photodamage. The underlying molecular picture of these conformational changes has not yet been resolved. The main target of the research described in this thesis is to address the conformational dynamics of photosynthetic Light Harvesting Complex II and the role of the membrane environment. Hereto, I explored NMR-based methods that could eventually probe the molecular structure and dynamics of photosynthetic components *in-vivo* in functional membranes or cell systems.

In **Chapter 1,** a general introduction to photosynthetic antenna complexes and photoprotection mechanisms is presented. I further describe the methodological background of Nuclear Magnetic Resonance (NMR) spectroscopy and cross polarization (CP) and insensitive nuclei enhanced by polarization transfer (INEPT) based NMR dynamic spectral editing methods.

CP and INEPT polarization transfer solid-state NMR methods complemented with biosynthetic isotope labeling and NMR relaxation methods are successfully employed in **Chapter 2** for determining protein and lipid molecular dynamics in native thylakoid membranes. Our results provide a microscopic dynamic picture of thylakoid membranes of wild-type (WT) and the zeaxanthin (Zea)-accumulating *npq2* mutant of *Chlamydomonas reinhardtii* (*Cr.*). For both WT and *npq2* thylakoid membranes a larger fraction of ordered lipids than of mobile lipids is observed, which indicates that the majority of the lipids are immobilized within or between supercomplexes. In addition, lipid isomerization from all-trans to trans-gauche configurations is detected at higher temperatures for both WT and *npq2* thylakoid membranes. It is found that *npq2* membranes have more rigid xanthophylls and contain a fraction of rigid proteins and ordered lipids that are less sensitive to temperature changes than for the WT. This suggests an overall rigidity of the thylakoid membranes due to Zea accumulation, and the xanthophyll thus plays a role in membrane stabilization.

To investigate the plasticity of LHCs and the role of the thylakoid environment in controlling their light-harvesting function, it is essential to study their dynamic behavior in their native membrane environments. In **Chapter 3**, 2D CP and INEPT based experiments are employed to obtain detailed insight into conformational dynamics of LHCII in reconstituted membranes and in native thylakoid membranes. Interestingly, the NMR responses of LHCII can be detected within native thylakoid membranes and chemical shifts of selective spin systems can be partially assigned. Moreover, lipid signals and signals from

different Lhcbm polypeptides are identified by comparing the 2D ^{13}C NMR spectra of LHCII reconstituted in lipid bilayers and of whole thylakoid membranes. Different protein-associated glycolipids are distinguished based on their galactosyl head ^{13}C-^{13}C correlation signals. It is found that LHCII has significantly reduced flexibility in native thylakoid membranes compared to reconstituted membranes, emphasizing the importance of the native membrane environment. Membrane-reconstituted LHCII contains flexible sites located in the N- or C-terminus and, based on its flexibility, may undergo thermally-induced conformational transitions, allowing reversible switching between light-harvesting and quenched states. However, since LHCII has significantly reduced flexibility in native, stacked thylakoid membranes, the occurrence of spontaneous transitions *in vivo* is questionable. The detected dynamic sites in LHCII are in close proximity to the xanthophyll-cycle carotenoid and lutein 2 molecules, which have been proposed to be involved in excitation quenching in the photoprotective state.

In chapter 2, the effect of Zea accumulation on thylakoid membrane dynamics is described. The effect of Zea exchange on LHCII internal molecular dynamics is further explored in **Chapter 4** by comparing monomeric Zea-containing LHCII of *npq2* with WT trimeric LHCII. Interestingly, on a protein level, Zea exchange leads to an overall reduced dynamics of the protein and to binding of many lipids. Moreover, it was observed that *npq2* LHCII containing Zea adopts a different fold than WT LHCII in a lipid bilayer. Several Ser residues fold into strands and the NMR spectra of *npq2* LHCII lack three Ala signals that are attributed to the Ala in the N-terminus, suggesting that either the presence of Zea or the effect of monomerization changes the N-terminal fold. These results suggest that conformational changes of LHCII upon Zea binding may cause the overall rigidity of thylakoid membranes that is reported in chapter 2.

The polarization-transfer dynamic spectral editing NMR approaches are extended for screening of photosynthetic cell components in **Chapter 5**. We succeeded in distinguishing the signals from lipid head-groups, CH_2 and CH carbons of the lipid tails, protein backbone and side chain, and carbohydrates of cell wall components. Remarkable similarities are observed between the lipid NMR signals in ^{13}C spectra of thylakoid membranes and the lipid response in spectra of whole *Cr.* cells. This suggests that the majority of the cellular lipids are incorporated in the thylakoid membranes and that those can be detected against the background of other cellular components, which enabled us to perform an *in-vivo* analysis of the thylakoid lipid properties. Insights in membrane thermodynamics have been obtained by collecting spectra over a physiological temperature range. Quantitative analysis of the molecular dynamics of thylakoid components and cellular constituents are provided by

comparing the experimentally recorded NMR spectra of *Cr*. cells and thylakoid membranes with simulated INEPT and CP intensities as function of order parameter and rotational correlation times.

Finally, in **Chapter 6**, a general discussion on the results is presented in the context of existing literature, and perspectives for future research are presented.