In vitro investigation of the photoprotection mechanism of Light Harvesting Complex II

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CHAPTER 6

CONCLUSIONS and OUTLOOK

CONCLUSIONS

The Light-Harvesting Complex II is the most abundant Light-Harvesting Complex in nature and is involved in both photoprotection and light harvesting processes.
By comparing the fluorescence characteristics of LHCII in liposomes and in lipid nanodiscs the effects of protein and lipid interactions have been discriminated in the presented approach.
LHCII fluorescence quenching is not the result of a specific thylakoid lipid microenvironment but is driven by LHCII protein-protein interactions. We also demonstrated that LHCII is unidirectional inserted in proteoliposomes, according to the analysis of enzyme cleavage products, which is a significant achievement in membrane protein reconstitution that has importance for mimicking protein functions *in vitro*.
The transition from a light-harvesting to a quenching state is associated with LHCII pigment-protein conformational changes. To understand the role of the lutein in this process, recombinant Lhcb1 from *A. thaliana* with ^{13}C lutein was successfully refolded and investigated by solid state NMR. By this approach, we could assign the lutein heads of ^{13}C lutein-rLhcb1 and for the first time obtain structural information of lutein in LHCII in the active, fluorescent state.
In vivo Chl excited-state quenching of the photosynthetic antenna associated with photoprotection is the result of a series of events and has been proposed to involve the interaction between LHCII and PsbS. The time-resolved fluorescence study of our minimal PsbS-LHCII membrane model do not point toward functional quenching interactions between LHCII and PsbS, neither show a significant effect after lowering the pH. The results strongly suggest that the pH-dependent role of PsbS during the fast qE response lies in creating membrane rearrangements and super complex remodelling that could facilitate LHCII quenching, rather than in creating direct quencher states.

OUTLOOK

By complementing the solid-state NMR experiments on ^{13}C lutein-rLhcb1 in unquenched state with NMR experiments on quenched ^{13}C lutein-rLhcb1 aggregates, it will be possible to reveal if the photoprotective switch of LHCII involves a change in lutein interactions. Further studies can be performed on the protein inserted in liposomes or nanodiscs to mimic the *in vivo* environment and reproduce *in vitro* different protein functional states.
To address the role of lutein-Chl *a* interactions in forming a quencher state, it would be of interest to refold Lhcb1 in presence of ^{13}C labelled Chl *a* and compare NMR spectra of ^{13}C Chl *a*-rLhcb1 in quenched and unquenched state. With this experiment will be possible to specifically investigate if any changes are involved in the surrounding of the Chl *a,* which pigments so far have only been detected in NMR spectra of uniformly labelled LHCII sample.
The experiments presented in Chapter 3 show that interaction between PsbS and LHCII is not sufficient to induce the photoprotective switch of LHCII. It would be of interest to

test the interaction between PsbS and zeaxanthin-binding LHCII, which is naturally present in the protein under stress conditions due to the depoxidation of violaxanthin as the presence of Zea seems to be indispensable to induce qE quenching *in vivo*.