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

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SUMMARY

Solar energy is an intermittent light-source, and is used by photosynthetic organisms to drive energy required cellular processes. Solar energy is primarily absorbed by two groups of pigments, chlorophylls and carotenoids, which are mainly located in the light harvesting complex proteins (LHCs). These proteins are essential for the performance of photosynthesis, not only because they are involved in harvesting the light, but also because they protect the photosynthetic system from excess of light that can cause photodamage.

In **Chapter 2**, I performed *in vitro* studies mimicking the two functions of LHCII by inserting the protein in nanodiscs and in liposomes to mimic respectively the light-harvesting and the photoprotector activity. I demonstrate that Chl excitation quenching is dependent on protein-protein interactions, and not on protein-lipid interactions, by performing fluorescence experiments on proteoliposomes with different PLRs and using thylakoids lipids or asolectin.
For mimicking native-like protein interactions, LHCII should be inserted unidirectionally in the membranes, like *in vivo*. Using enzymatic digestion, I show that there is a preferential insertion of the LHCII proteins in our model membranes.

Using asolectin model membranes, I investigated the specific interactions of LHCII with PsbS which are known to play a key role in quenching excitations under light-stress conditions *in vivo*. **Chapter 3** was dedicated to explore whether only the interaction between the two proteins, in acidified environments, is sufficient to promote LHCII fluorescence quenching. CD and TRF studies were performed on PsbS-LHCII proteoliposomes at different pH conditions. The fluorescence study of our minimal membrane models strongly suggests that the pH-dependent role of PsbS, during the fast qE response, lies in creating membrane rearrangements and supercomplex remodeling that could facilitate LHCII aggregation quenching, rather than in creating direct quencher states.

In **Chapters 4** and **5** the focus shifts from an intermolecular approach toward an intramolecular approach to understand how the protein in the quenched state changes the pigment conformations in comparison to the unquenched state. I successfully obtained ^{13}C lutein-rLhcb1 by refolding recombinant Lhcb1 from *Arabidopsis thaliana* in the presence of non-isotopically labelled pigments from fresh market spinach with excess of ^{13}C labelled lutein extracted from isotopically labelled *Chlamydomonas reinhardtii* and isolated via HPLC. The ^{13}C lutein-rLhcb1 protein in detergent, mimicking the unquenched state, and protein aggregates, mimicking the quenched state, were biochemically and spectroscopically characterized and further analysed with solid state NMR. The lutein ^{13}C chemical shifts could be assigned for LHCII in detergent. Ring current shifts of the lutein head signals indicate that the heads are in close proximity to specific Chls (Chl *a*610 and Chl *a*602), providing for the first-time structural information about lutein-Chl interactions in LHCII in its unquenched state.