The heart of oxygenic photosynthesis illuminated

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SUMMARY

Photosynthesis is the process by which photosynthetic organisms convert solar energy into chemical energy. Photosynthesis in plants, algae and cyanobacteria releases oxygen and is referred to as oxygenic photosynthesis. The primary photosynthetic processes of energy conversion occur inside the reaction center (RC), a pigment-protein complex located at the heart of the photosynthetic complexes. Among all the photosynthetic RCs only PSII provides a redox reaction that is sufficiently strong to oxidize water. PSII consists of a protein matrix with highly tuned cofactors facilitating electron transfer (ET) with quantum efficiency close to 100%. The cofactor arrangement in the PSII RC shows a strong similarity to that found in bacterial RCs of purple bacteria, yet the oxidation potentials differ greatly. Despite extensive research, the exact nature of the exceptionally high redox potential of PSII and the role of the protein matrix in establishing this redox potential are still unknown. The electrons flow from PSII to PSI, a complex optimized to provide a strong reductant which is used for the formation of NADPH. While the electron flow in PSII is strictly asymmetric, in PSI different levels of bidirectionality indicate functional flexibility of this complex. This raises the question whether the electronic architecture of PSI varies across different plant sources and influences the electronic ground state of P700.

Photo-CIDNP (chemically induced nuclear polarization) is non-Boltzmann nuclear magnetization caused by photochemical reactions and can be observed by NMR spectroscopy as strongly enhanced absorptive or emissive signals. For RC studies photo-CIDNP has proven to be a powerful tool. It provides selective excitation of cofactors, electron density profiles in the ground state from the chemical shifts, excited state profiles from the signal intensities and gives insight into the kinetic stabilization of photosynthetic intermediates and other transient species. When used in a time-resolved manner, photo-CIDNP provides access to molecular dynamics from correlation signals in two-dimensional dipolar spectroscopy.

Photo-CIDNP studies on PSI and PSII have been limited to the isolated complexes due to difficulties with incorporating selective isotope labels into plant RCs. In this thesis photo-CIDNP solid state NMR with selective isotope labeling has been applied to get direct access to the heart of large PSII and PSI photosynthetic complexes in intact and isolated systems of *Spirodela oligorrhiza* and *Synechocystis* sp. PCC 6803. For the first time the direct observation of selective atoms within the heart of the PSII and PSI complexes by

experiments on entire plants and whole cells is reported. In this way the conservation of the electronic structure of the PSII electron donor at various levels of biological preparations have been addressed and the electron spin density (ρ_i) in the active cofactors of PSI has been constructed. In addition the functional heterogeneity of the PSI electron donor among different plant species was probed.

In **chapter 2** experiments have been performed on selective ^{13}C- isotope labeled whole cells of *Synechocystis* sp. PCC 6803 cyanobacteria. The results demonstrate for the first time the occurrence of the solid-state photo-CIDNP effect in cyanobacteria. In addition, the observed photo-CIDNP features suggested a remarkable conservation of the electronic properties of the photochemical machineries from plants and cyanobacteria. The observation of the photo-CIDNP effect directly in whole cells implies that photo-CIDNP MAS NMR studies on oxygenic photosystems are not limited to isolated plant photosystems.

Chapter 3 reports experiments that have been performed on ^{15}N- isotope labeled PSI-110 particles from spinach and duckweed. The results demonstrate a strict conservation of the electronic ground state of the PSI electron donor among different plant systems, while simultaneously a large flexibility of the PSI was observed in terms of its electronic architecture. Despite the highly optimized redox properties of P700, PSI shows functional heterogeneity in terms of the relative participation of the two branches in ET. The results discussed in this chapter reveal that these variations do not affect P700 but remain in the periphery.

In **chapter 4** experiments have been performed on D1D2 and PSII core complexes from spinach and duckweed and on selectively ^{13}C- labeled duckweed. The solid-state photo-CIDNP effect in conjunction with selective isotope incorporation allows for the observation of NMR signals from the primary radical pair of PSII, directly from thylakoid membranes, as well as from entire plants. The data presented here demonstrate that the photochemical machinery is remarkably robust and remains essentially unaffected by the various preparation procedures, this is important for the validation of results obtained from the extensively studied D1D2 complex. In addition the results presented in this chapter show that the electronic structures of the PSII electron donor in spinach and duckweed are identical. Analysis of the spectra obtained from selectively ^{13}C- labeled thylakoid membranes showed clearly that the radical pair in PSII is formed by a single Chl *a* and a single Phe *a*. In addition the involvement of the protein matrix and the mechanism of photo-CIDNP buildup in larger PSII systems have been discussed in this chapter.

In **chapter 5** time-resolved ns-flash photo-CIDNP MAS NMR experiments on PSI and PSII have been discussed. The results are in agreement with bidirectional electron transfer in PSI and the photo-CIDNP buildup is PSI is discussed. As expected, a significant contribution of the DR mechanism in the photo-CIDNP buildup is observed in PSII, which is not detected for PSI. The results show that the photo-CIDNP buildup in PSII resembles the buildup observed for *Rb. sphaeroides* R26, due to the long triplet lifetime.

Finally, **chapter 6** contains an outlook for applications of photo-CIDNP to selectively labeled PSII and PSI and 2- and 3-dimensional MAS NMR experiments are proposed. The observed field effect and light induced changes in various experiments on PSI particles are discussed and the first data obtained from selectively ^{15}N-ALA labeled PSII is presented. Based on the preliminary data presented in Chapter 6, the relative participation of the two cofactor branches in ET in PSI and the involvement of a histidine with spin density ρ_i in ET in PSII is discussed.