Photoactivation dynamics in photosynthetic and signal transduction proteins studied by ultra-fast time-resolved spectroscopy

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Summary

The process of illumination is one, if not the most, important process that supports life on Earth. Living creatures absorb the energy that photons (particles of light) bring from the Sun or from an artificial source. Once a photon is absorbed, its energy is used by the organism as information carrier in the processes of vision, phototaxis, phototropism, etc.; it is also used in a process called photosynthesis to transform light energy in chemical energy (carbohydrates).

The photon absorption then, is the primary process that triggers photo-biological events; this is achieved by special pigments inside the cell, with the ability of trapping the photon and use its energy within a short period of time (normally in the order of femtosecond = one millionth of billionth of second). This thesis, with title: "*Photoactivation dynamics in photosynthetic and signal transduction proteins studied by ultra-fast time-resolved spectroscopy*", studies the primary light induced reactions in some photoactive proteins and isolated pigments and their role in the photoreaction, by using laser spectroscopy as main means of investigation.

The Chapters 2 titled: "*Identification of excited-state energy transfer and relaxation pathways in the peridinin-chlorophyll complex: an ultrafast mid-infrared study*", investigate the energy deactivation that takes place upon light excitation on Peridinin-Chlorophyll-*a*-Protein (from the marine alga *Amphidinium carterae*). The excited state process was addressed using time-resolved visible-pump mid/IR-probe spectroscopy, reveal the presence of two separate low-lying singlet excited states for the carotenoid, with charge transfer character (ICT) and to a slowly transferring (~30ps) vibrationally relaxed S_1 ($^{cold}S_1^*$). The analysis reveals that different peridinins are involved in the ICT and S_1 formation.

The excitation energy is transfered from peridinins to Chl-*a* which undergoes ISC to form triplet state, this exhibits a mixture of Chl-*a* and Per modes. Interesting is that the Per ICT and triplet states show lactone bleaches at different frequencies indicating that the two processes involve separate pathways.

In Chapter 3 (*Chl-a triplet quenching by peridinin in H-PCP and organic solvent revealed by step scan FTIR time-resolved spectroscopy*)

we identify a triplet component in H-PCP that closely match the fast triplet component previously observed in A-PCP. Further investigation were performed on isolated chlorophyll-*a* and peridinin, as function of the stoichiometry of the pigments and sample concentration. Using a target analysis procedure, triplet Chl-*a* and Per infrared spectra were obtained. The specific carbonyl frequencies of ^{3}Per and ^{3}Chl-*a* in THF confirm our assignment of their co-existence in the infrared spectra of H-PCP and A-PCP.

Chapter 4: *A photoactive carotenoid protein acting as light intensity sensor*, is dedicated to the study, by ultrafast visible pump and probe spectroscopy, the primary photo-physics of OCP. This Orange Carotenoid Protein (OCP) contains as chromophore a carotenoid, but plays the role of photoreceptor. Upon illumination with blue-green light, the OCP undergoes a reversible transformation from its dark stable orange form to a red "active" form. The illumination induces structural changes affecting both the carotenoid and the protein. Thus, the OCP is a photoactive protein which senses light intensity and triggers photoprotection.

Chapters 5: Hydrogen bond switching among flavin and amino acid side chains in BLUF photoreceptor observed by ultrafast infrared spectroscopy, explores the photochemistry of Slr1694 in its wild type form by time-resolved visible-pump mid/IR-probe spectroscopy. This techniques, together with the global analysis method allowed the estimation of the spectra belonging to transitional molecular species, proving that the activation pathways goes through the radical pair formation between flavin and tyrosine-8.

Finally, in Chapter 6 (*The role of key amino acids in the photoactivation pathway of the Synechocystis Slr1694 BLUF domain*) explores, by ultrafast visible pump and probe spectroscopy, the role of a small number of aminoacids sited close to the flavin in the light activation mechanism.