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

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2009

document version
Publisher's PDF, also known as Version of record

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.

Samenvatting

Lichtprocessen zijn een van de, zo niet de belangrijkste processen op aarde die leven mogelijk maken. Levende organismen absorberen de energie, die door fotonen (lichtdeeltjes) van de zon of een andere lichtbron naar het organisme overgebracht worden. Wanneer een foton is geabsorbeerd, wordt deze energie door het organisme gebruikt, als informatiedrager in processen zoals zien, het groeien van planten naar het licht, het richten van organismen naar het licht toe, enzovoort. De energie wordt ook gebruikt in een proces, dat men fotosynthese noemt, en waarbij de lichtenergie in chemische energie (koolhydraten) wordt omgezet.

Het absorberen van het foton, vormt dan het begin van het hoofdproces voor een bepaalde biologische activiteit; dit is mogelijk door speciale pigmenten binnen de cel, die in staat zijn om fotonen in te vangen, en de energie van deze fotonen te gebruiken. Deze gebeurtenissen vinden plaats op een zeer korte tijdschaal (normaal in de orde van femto-seconden = een miljoenste van een biljoenste van een seconde). Dit proefschrift, met de titel: "*Photoactivation dynamics in photosynthetic and signal transduction proteins studied by ultra-fast time-resolved spectroscopy*", is het resultaat van het bestuderen van de door het licht veroorzaakte reacties in enkele door licht geactiveerde eiwitten en geisoleerde pigmenten, alsmede de rol die zij spelen binnen de lichtreacties. Daartoe is laserspectroscopie als belangrijkste meettechniek voor dit onderzoek toegepast.

In hoofdstuk 2, getiteld: "*Identification of excited-state energy transfer and relaxation pathways in the peridinin-chlorophyll complex: an ultrafast mid-infrared study*", zijn de resultaten beschreven van de studie naar het deactiveren van energie, dat plaatsvindt onder lichtexcitatie van het peridinin-chlorofyl-*a*-eiwit, van de in zee levende algensoort *Amphidinium Carterae*. De processen van de aangeslagen toestanden, zijn met tijdsopgeloste zichtbare pomp en middeninfrarode probe spectroscopie aangetoond, waarbij het bestaan van twee afzonderlijke en lager gelegen aangeslagen toestanden van het caroteensingulet zichtbaar werden, met intermoluculaire ladingsoverdracht (ICT) en een langzame overdracht (~30ps) naar de

vibrationele gerelaxeerde toestand van S_1 ($^{cold}S_1$*). De analyse gaf aan, dat verschillende peridinin (Per) betrokken zijn bij de vorming van de ICT en S_1.

De energie van de aangeslagen toestand wordt overgedragen van de Per naar chlorofyl-a (Chl-*a*), die vervolgens een overgang ondergaat naar een triplet toestand (ISC), en zo een mengsel van Chl-a en Per modes laat zien. Interessant is dat de Per ICT en triplet toestanden een bleache van het lactone laten zien bij verschillende frequenties, wat aangeeft dat bij de twee processen verschillende routes betrokken zijn.

Hoofdstuk 3: "*Chl-a triplet quenching by peridinin in H-PCP and organic solvent revealed by step scan FTIR time-resolved spectroscopy*", geeft de beschrijving van de identificatie van een triplet component in H-PCP, dat nauw overeenkomt met de snelle triplet component, die eerder waargenomen is in A-PCP. Verder onderzoek is uitgevoerd aan geisoleerde Chl-a en Per, als functie van de stoichiometrie van de pigmenten en de concentraties van het preparaat. Door gebruik te maken van een target analysis, zijn infrarood spectra verkregen van de triplet Chl-a en Per. De specifieke carbonyl frequenties van ^{3}Per en ^{3}Chl-a in THF, bevestigen onze toewijzing van hun gelijktijdig bestaan in de infrarode spectra van H-PCP en A-PCP.

Hoofdstuk 4: "*A photoactive carotenoid protein acting as light intensity sensor*", is toegewijd aan het onderzoek, dat met behulp van ultrasnelle zichtbare pomp en probe spectroscopie is uitgevoerd, naar de fysica van de voornaamste lichtprocessen van OCP. Dit oranje caroteeneiwit (OCP) bevat als chromofoor een caroteen, echter deze vervult de rol van fotoreceptor. Onder belichting met blauw-groen licht ondergaat het OCP een omkeerbare transformatie van zijn donkere stabiele oranje vorm naar een rode "actieve" vorm. De belichting induceert een structuurverandering, die uitwerkt op zowel het caroteen als het eiwit. Het OCP is zodoende een door licht geactiveerd eiwit, dat de lichtintensiteit kan waarnemen en de lichtbescherming van het organisme initieert.

In hoofdstuk 5, dat de titel heeft: "*Hydrogen bond switching among flavin and amino acid side chains in BLUF photoreceptor observed by ultrafast*

infrared spectroscopy ", verkennen we de fotochemie van Slr1694 in zijn natuurlijke vorm, door middel van tijdsopgeloste zichtbare pomp en middeninfrarode probe spectroscopie. Deze techniek, samen met de global analysis methode, maakte een benadering mogelijk voor de spectra, die toebehoren aan overgangsvormen van moleculaire soorten, waardoor aangetoond kon worden, dat de activering verloopt via de vorming van een radicaal paar tussen flavine en tyrosine-8.

Tot slot zijn in hoofdstuk 6, met de titel: "*The role of key amino acids in the photoactivation pathway of the Synechocystis Slr1694 BLUF domain* ", de resultaten beschreven van de verkenning naar de rol, die een klein aantal aminozuren spelen, welke op posities nabij de flavine gelegen zijn, in het door licht geactiveerde mechanisme. Ook hierbij werd gebruik gemaakt van ultrasnelle zichtbare pomp en probe spectroscopie.

Nawoord

Done! Well...almost! I should still defend my thesis, but at least now I can see the light at the end of the tunnel. If I look back, this was my longest and most incredible journey. I passed over subjects and projects like a biker swallows the streets. New people, new situations, moments of happiness and moments when I wished to pull over and get out. But I did not; honestly I do not know which component was predominant in this choice, determination or stupidity, for sure a bit of masochism. Now, at the end of it , I would like to tell you a bit about this period and I will thank the people that made it possible. Let us start from the beginning: *prima di tutto vorrei ringraziare I miei genitori, per essere stati presenti nei momenti difficili, e per avermi aiutato nel diventare me stesso. Andare via di casa e' stata una scelta necessaria, e io l'ho presa a cuor sereno sapendo che sarei sempre potuto tornare. Grazie.*

I wish to thank Clive and Sue, the first English people I met. I loved them from the first moment, and I still do. I like to think about them like if they are my English parents, because they treated me like a son. It is without exaggeration when I say that without the two of you I would not be here. Thanks.

The people in Leeds, that believed in me and pushed me in the right direction: Bob (my supervisor), Neil (my best English mate inside and outside the pubs!) and Nora (who tried to teach me English... without lots of results). Was a nice period there, and after almost two years of beer glasses big like buckets I moved across the British Channel and I arrived in Amsterdam. Here I started immediately my PhD – the VU looked amazing to me: long corridors with surreal colours, lots of corners to hide labs and Lithuanian people working in them.

Rienk, I got to know you bit by bit. Thanks for the support you gave to me. I really appreciated all the long conversations we had, your characteristic way to tell me things, both good and bad, pertinent and impertinent... and the impertinent things were the far most important and helpful.

John, you played an important role in all this, thanks to let me find my way.

Marloes, your enthusiasm for science is contagious. You were always there to give help, to discuss and correct my mistakes, and you always did that with a smile.

Jos, the first person to see in the morning was you, especially after a measuring night session. You are the one behind all the experiments, solving problems, kicking lasers and always up to explain and discuss any aspect of the lab-life. Thanks for your friendship and your help with the translation of the Summary.

Ivo, you helped me when I was struggling with the global analysis. Thanks for your help and for putting on some great pieces of music during onze samenwerking.

Luuk, you have been the first friend here in the lowlands. I immediately felt at home with you. Probably because you could speak an almost perfect Italian (sometimes better than mine). The best adjective to describe you is, I think, sharp. Sharp like your ability to observe and to understand. You have been a precious person in this journey, always present to help in the lab, to discuss scientific issues and not, and up to a good laugh. It is nice to think that the life does give back to people what people spread. Succes met jouw nieuwe leven.

Manolis, the only person I met that can complain more then a souther Italian! Great! It is difficult to explain but you have been a kind of emotional link between my previous experience in the UK and Amsterdam. In the Queen's island the best mates were Greek, and in Amsterdam I met you. We have been companions in a passionate battle to support the healthiness of the Olive Oil, we spoke for hours regarding the coffee (ristretto o cappuccino?), the tasteless tomatoes and oranges. The 3 days of trekking and rowing through one of the national parks of Canada was awesome. I still own you some pictures, right?

Maxime, my dearest colleague, collaborator, opponent, partner in crime but above all friend. We did it! We both managed to get out of here! We went out quite a lot to explore the city. Was funny, I remember that. What I forgot is how we always managed to get home! Mystery!
You helped me in the biochemistry lab, making that place less scary. I wish you would have taught me your ability to laugh and stay relaxed. I wish you all the best with your new life in Sacley .

And of course all the others: Mikas and Sofia (thanks guys for the FM files, it was very usefull to have them), Danielis, Natalia, Magda, Daniel (let's meet at 8:30 at Leidesplein?), Kinga, Rudi, Olga, Miguel,

Sandrine and Henny, Mariangela (in bocca al lupo per la VICI), Jason and Erica (also known as the Key-couple, since you left Amsterdam I never went again on Prinseneiland. To sad to be there.), Kate (you went to the Obama "coronation", right?), Eli (hola), Thomas, Toh, Andy, Stefan, Tjaart, Joris, Marian, Alessandro, Alisa, Stephane (we still have to cook together).

Marianne, probably I should give you half of my PhD. You beared all my stress and moody periods. It was not easy... but it is finished now.
And how to forget the Little Italy: Vito (e' bello averti qui con me ad Amsterdam), Giampiero, Jacopo (che si sposa con Maaike, AUGURIII!!), Vasco, Andrea, Valentina, Lorenzo, Andrea, Marianna, Giovanni, Diego, Valerio, Matteo ed Enrico.
Ciao Enrico.