Final Report

Project: OTKA-PD 104530
Title: Photoprotective thermal dissipation and macroorganization changes in the photosynthetic light-harvesting antennae
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Summary

The topic and primary goal of the present project was to reveal details on the biophysical process of thermal dissipation (TD) of excitation energy in the photosynthetic light-harvesting antenna (LHA) in the context of its role in photoprotection and light adaptation and especially focusing on the intricate relationship between TD and changes in the macroorganization of the LHA in the photosynthetic membranes. More specifically, we aimed to clarify the relationship between LHCII macroorganization and light-induced TD, the physiological role of different non-photochemical quenching (NPQ) mechanisms and thylakoid membrane reorganizations and improve our understanding of the physical mechanisms of NPQ.

With these aims, we performed systematic studies on LHA complexes from plants (LHCII), diatom algae (FCP) and cyanobacteria, in their native membranes or in isolated states with or without inducing TD (by light or other means), and in reconstituted complexes and membranes of varying complexity. Structural and macrostructural aspects of the systems under study were monitored primarily by absorption, linear dichroism, and circular dichroism (CD) spectroscopy, and additionally by small-angle X-ray and neutron scattering, dynamic light scattering, atomic-force microscopy and electron microscopy. The method of anisotropic CD (ACD) was developed for use with LHA to further enrich the capabilities of CD spectroscopy as a probe for molecular structure. The functional properties of the systems under study, especially regarding the pathways and dynamics of energy transfer, photochemistry and TD, were examined by steady-state and time-resolved fluorescence spectroscopy (the latter performed with equipment planned and built within the project) as well as ultrafast transient absorption spectroscopy and multidimensional (2D and 3D) electronic spectroscopy.

By use of CD and ACD we discovered specific structural changes in LHCII that are sensitive to the molecular surrounding and to protein-protein interactions in the membrane, i.e. to the membrane macroorganization, that are indirectly related to changes in the rate of TD. Moreover, we demonstrated the inherent ability of LHCII to alter the membrane macroorganization and promote stacking or destacking of membrane sheets depending on light conditions. Experiments with diatom algae with reduced content of the NPQ-inducing LHA complex Fcp6 showed that regulation of TD by Fcp6 is achieved indirectly by controlling the membrane macroorganization and interactions between pigment-protein complexes. Induction of TD was correlated with changes in the repeat distances of the thylakoid membranes, detected by small-angle neutron scattering, in both plants and diatom algae. Studies with *in vitro* reconstituted membranes showed that the contacts between LHCII and Photosystem I (PSI) in the unstacked membrane regions can lead to efficient transfer of excitation energy to PSI and hence reduce the excitation pressure on PSII. These and other results of this project present

rich and diverse evidence for the two-way relationship between membrane macroorganization and NPQ.

We explored the physical mechanisms, pathways and dynamics of excitation energy transfer (EET) and dissipation in LHAs by ultrafast spectroscopy techniques including novel spectroscopy techniques, such as three-dimensional electronic spectroscopy, applied here for the first time. These studies reached new depths in resolving experimentally the complex EET dynamics. We found that aggregation of LHCII brings about not only induction of TD in the complexes but also acceleration of EET on the time scale of picoseconds. The dynamics of EET and TD were also examined in reconstituted LHA complexes (LH1) with artificial dissipating centres (Ni-BChl), demonstrating the potential use of this approach to study the mechanisms of TD in a model system.

The results of this project were reported in **10** peer-reviewed scientific journal articles and **13** international scientific conferences. All **10** articles were published in international journals ranked in the top quartile (**Q1**) in the respective field. The total impact factor is **59**.

Anisotropic circular dichroism

The complexity of the CD spectra of LHA makes it extremely difficult to directly translate the spectral information into structural properties. The problem can be alleviated by measuring anisotropic CD (ACD) of macroscopically oriented samples. We applied this method for the first time on photosynthetic systems. The usefulness and applicability of ACD is demonstrated by the baseplate protein CsmA in chlorosomes of green sulfur bacteria, exhibiting CD spectrum in the near-IR region originating from two perpendicular excitonic transitions BChl (Figure 1). We could separate the two bands by ACD, thus providing direct information about the baseplate structure (1).

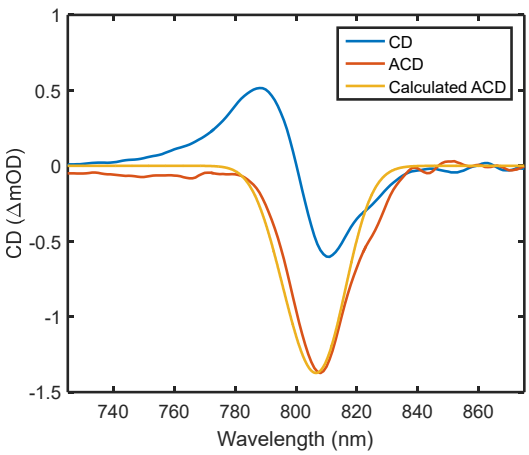

Figure 1. CD and ACD of BChl a in the chlorosome baseplate protein CsmA (1). ACD can separate the two oppositely signed excitonic CD bands.

To achieve macroscopic orientation of LHCII, the complexes were inserted into lipid membranes, which could be readily oriented by gel compression or (more recently) drying on a glass surface. The ACD spectra of LHCII membranes showed drastic differences compared to the isotropic CD (Figure 2). The bands enhanced in the ACD spectra are attributed to excitonic transitions oriented primarily in the plane of the membrane, whereas bands missing in the ACD spectra (e.g. 473 nm), are attributed to transitions oriented primarily perpendicularly to the membrane plane.

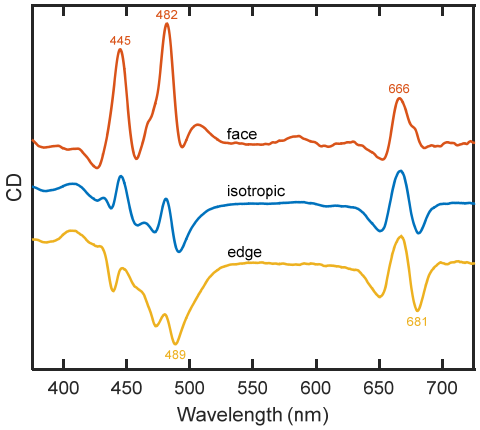

Figure 2. CD and ACD spectra in two orientations ("face", "edge") of LHCII proteoliposomes in polymer gel.

We recorded ACD spectra of a number of different types of photosynthetic pigment-protein complexes, including Photosystem I and II cores, bacterial RCs and LHAs and FCP from diatoms (2). Eventually these results will undoubtedly further our understanding of the exciton structure, optical spectra and light-harvesting mechanisms of these systems.

Membrane macroorganization and TD

Structural changes in LHCII in different molecular environments

LHCII is known to play a key role in the activation of photoprotective TD by several mechanisms that include different changes in the topology (macroorganization) of the membrane as well the structure of the protein itself. We examined the structural and functional behavior of LHCII in different molecular environments: native thylakoid membranes, thylakoid membranes enriched in LHCII, isolated LHCII complexes in different detergents, aggregates and macroaggregates in aqueous buffer, complexes in hydrated polymer gel, or reconstituted membranes with different lipids (3). We found that LHCII is sensitive to interaction with detergents replacing the native lipid environment in a way that affects both its structure and fluorescence yield, i.e. rate of TD. Aggregation of LHCII was accompanied by a different set of structural changes (detected by CD – cf. Figure 3) as well as by the induction of fast TD. Some of these structural changes could also be observed in reconstituted LHCII:lipid membranes with high protein content. We could separate spectral changes specific to protein-protein, lipid-protein and detergent-protein interactions. Time-resolved fluorescence spectroscopy of the reconstituted membranes showed that LHCII complexes in the membrane interact with each other so that excitation energy can hop between several complexes. The fluorescence lifetime of LHCII in the lipid membrane was reduced by 30–50% compared to detergent-solubilized complexes, meaning faster rate

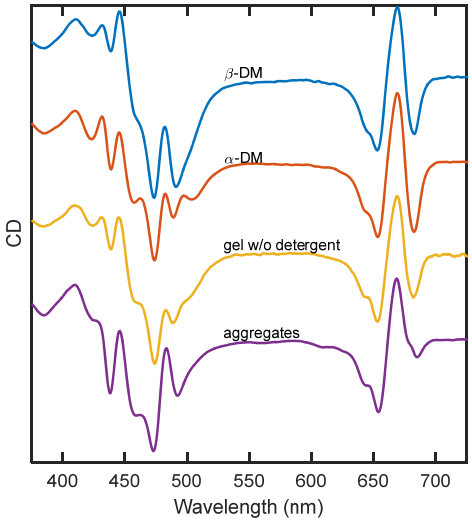

Figure 3. CD spectra of LHCII in the presence and absence of detergent: LHCII trimers solubilized in β-DM, α-DM, trimers trapped in polyacrylamide gel without detergent, and aggregates in detergent-free buffer solution.

of TD; moreover, the fluorescence decays were markedly multiexponential, indicating structural and functional heterogeneity in the membranes.

Light-induced changes in the membrane macroorganization

In comparison with the CD data, scanning transmission electron microscopy images from the Brookhaven National Lab, Long Island, USA were analyzed. Using the mass-measuring capability of scanning electron microscopy we demonstrated that LHCII possesses the ability to undergo light-induced dark-reversible reorganizations, independently of the photochemical apparatus (4). Unstacking of the membrane layers was observed after brief intense irradiation. LHCII is known to promote stacking of adjacent membrane sheets assisted by cations screening the surface-exposed negative charges. We propose that reversible stacking and unstacking involves cation movement driven by protein conformational changes. Similar reversible changes (membrane unstacking) could also be induced by cation depletion.

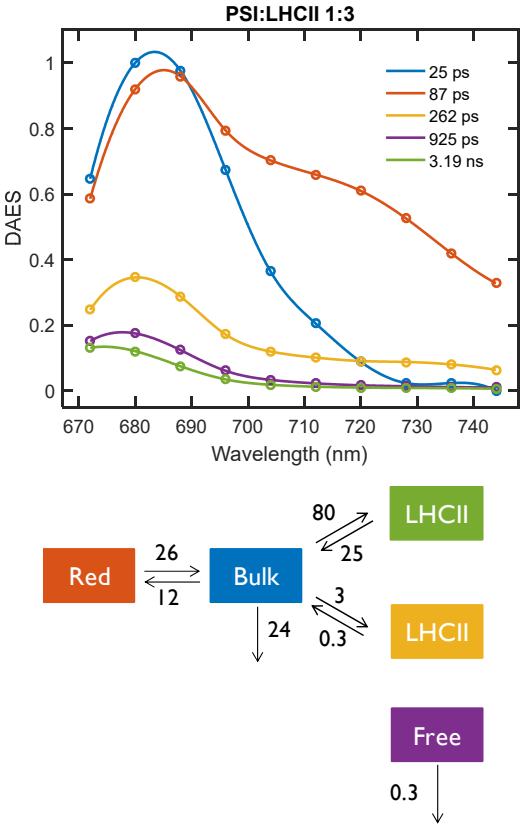

Figure 4. Time-resolved fluorescence of PSI-LHCII membranes. Top – decay-associated fluorescence emission spectra, bottom – kinetic model of energy transfer (PSI is represented by bulk and "red" Chl compartments, rate constants in ps^{-1}).

Small-angle neutron scattering (SANS) carries accurate information on the membrane ultrastructure. In a series of SANS experiments on isolated thylakoids and algal cells repeat distances (RDs) of membranes were determined, and small (<20 Å) but well-discernible light-induced reversible RD changes were revealed on time scales of several minutes (5). The data showed that these reorganizations can be linked to different regulatory mechanisms, including TD-assisted ultrastructural changes and NPQ.

Interaction of LHCII with Photosystem I in reconstituted membranes

One role of LHCII is balancing the excitation energy between the two photosystems by the process of state transitions, which shuttles mobile LHCII between PSII and PSI. This process is considered an NPQ mechanism as it quenches PSII fluorescence independently from PSII photochemistry. There is substantial recent evidence that LHCII and PSI can interact in the non-appressed thylakoid membrane regions (stromal thylakoids and grana margins) even if state transitions are not activated. Temporary unstacking of the membranes can increase the contacts between LHCII and PSI. We created model reconstituted membranes with PSI and LHCII to study the energy exchange between the two. Employing picosecond time-resolved fluorescence and kinetic modeling (Figure 4), we were able to estimate the rate constant and efficiency of EET from LHCII to PSI and the photochemical quantum yield, showing that LHCII can be an efficient antenna for PSI *in vitro* (6).

Control of NPQ by membrane macroorganization in a diatom alga

Diatoms possess special LHA proteins involved in NPQ that are part of the fucoxanthin-chlorophyll protein (FCP) antenna. We investigated the influence of Fcp6 in *Cyclotella meneghiniana* (2). In cells with antisense-reduced Fcp6, CD spectra did not show any structural alterations compared to WT on the level of individual FCP complexes but changes could be observed on the level of thylakoid membranes and intact cells and their responses to high-light treatment. The results support a model of NPQ in diatoms, wherein FCP aggregation is a major feature. Thus, Fcp6 appears to induce TD by way of altering pigment interactions between complexes (macroorganization changes) but not within the FCP complexes.

Mechanisms and pathways of energy transfer and TD

To gain insight into the pathways, dynamics and mechanisms of excitation transfer and dissipation in the LHA, we performed time-resolved optical spectroscopy experiments with various systems using several techniques: time-resolved fluorescence spectroscopy by single-photon counting, femtosecond transient absorption, two-dimensional coherent electronic spectroscopy (2DES) and fifth-order three-dimensional spectroscopy (3DES).

TD in bacterial LH1 antenna reconstituted with Ni-BChl

The LHA complex LH1 of purple bacteria is a membrane-integral pigment-protein complex binding BChl and carotenoids that normally exists as a ring-like structure surrounding the reaction center. LH1 can be reversibly dissociated into its building subunits, enabling chemical exchange of the bound pigments and modification of its properties. We reconstituted LH1 with partial exchange of the native BChl to its Ni-derivative (Ni-BChl), which acts as a fast and efficient trap for excitations. These complexes serve as a model for studying TD – its mechanisms, dynamics, kinetic limitations, etc. Femtosecond transient absorption measurements were performed on LH1 reconstituted with or without carotenoids and Ni-BChl (7). A key result of these experiments was the observed quenching of excitations by Ni-BChl in the LH1 complexes which occurs on a time scale of about 1–2 ps and is independent from the presence of carotenoid. This is about an order of magnitude slower than the excited-state lifetime of Ni-BChl in solution but nearly two orders of magnitude faster than the decay of BChl in native LH1. The effective deactivation rate constant was apparently limited by exciton relaxation in the LH1 ring. The results also revealed details on the pathways of EET from the carotenoids to BChl.

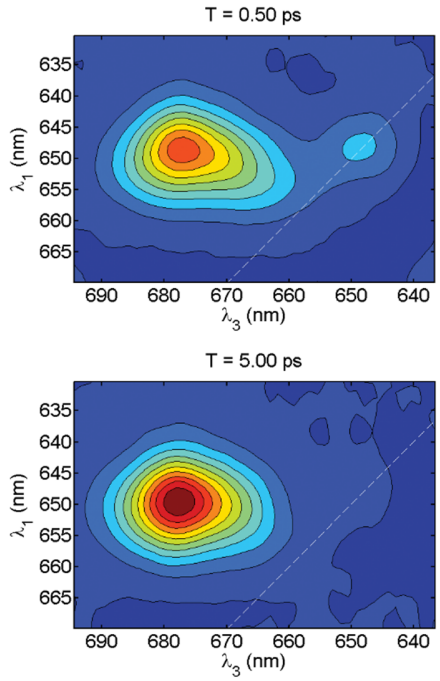

Figure 5. 2D spectra of LHCII trimers at waiting times of 0.5 and 5 ps after initial excitation in the Chl b region. At 0.5 ps a diagonal peak at 650 nm signifies remaining population of Chl b, whereas at 5 ps all excitations are confined to Chl a.

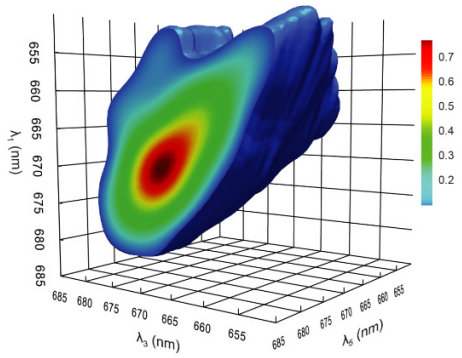

Figure 6. 3D spectrum of LHCII obtained after excitation at 670 nm and waiting times t_2 = 300 fs (first EET step) and t_4 = 800 fs (second EET step). A cross-peak in the upper part of the figure shows EET from Chl b (655) through an intermediate state (670 nm) to a final state (680 nm).

2D spectroscopy of LHCII

The pathways and dynamics of EET between Chls in LHCII were studied by 2DES. The two frequency axes in a 2D spectrum enable direct correlation of the energies of coupled donor-acceptor pairs, thus revealing excited state population transfer from one state to another. By excitation in the Chl *b* Q_y band, EET from Chl *b* to Chl *a* states was monitored on time scale from ~0.2 ps to tens of ps (8).

Although individual Chl states could not be resolved in the 2D spectra (Figure 5), different pools of Chl *b* and Chl *a* were distinguished coupled by EET occurring with different time constants. The exciton relaxation dynamics in the Chl *a* manifold was further investigating by using excitation pulses tuned to the absorption of Chl *a*. Overall we could resolve an impressive amount of EET pathways occurring on different time scales, to our knowledge giving the most detailed experimentally obtained picture of EET to date.

EET dynamics in aggregated LHCII

Using the same approach – 2DES with excitation in the Chl *b* and Chl *a* regions – we studied quenched LHCII aggregates (9) and compared the results with unquenched solubilized LHCII. The results showed an apparent acceleration of the EET, particularly the slow, picoseconds transfer from Chl *a* on the lumenal side of the complex to the lowest-energy Chls on the stromal side. These results corroborate the notion that the structure of LHCII is sensitive to the molecular environment and to protein-protein interactions in the aggregated state.

3D spectroscopy of LHCII

EET in pigment-protein complexes like LHCII occurs through a sequence of steps between different pigments until the excitation reaches a trap where it is dissipated or utilized. Using a novel experimental technique – fifth-order 3DES, we directly observed multistep EET in LHA for the first time (10). The cross-peaks in the 3D spectra correlate three frequencies (wavelengths) – of an initial, intermediate and final state – and two time scales of EET (Figure 6). Thus, the technique gives unprecedented power to resolve individual EET pathways and is a step forward to a complete experimental mapping of EET and TD processes in photosynthetic systems.

Publications

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