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Fotokémiai reakciócentrumok szerkezeti és spektroszkópiai sajátságai különböző molekuláris környezetben
PDOTKA 121243

Type II photochemical reaction centers (RCs) have outstanding role in photosynthesis. In my project the RC of purple bacteria - as a primary model structure – and the related photosystem II (PSII) – which is a supercomplex capable of splitting water, and thus is responsible for the production of oxygen of the atmosphere – was in the focus of our investigations. The knowledge of the structure and functional states of these protein (super)complexes is of paramount importance since they play key roles in the photosynthetic electron transport processes and in different regulatory mechanisms. The fundamental objective of my project was to gain new knowledge on the structure and functions of the RCs – taking into account that these proteins are specifically sensitive to their physico-chemical and molecular environments.

During the project – which was 19 months – our measurements were performed on (i) photosystem II (PSII) core samples of Thermosynechococcus vulcanus, (ii) the reaction center (RC) complex of Rhodobacter sphaeroides, (iii) on Leptolyngbya ohadii cyanobacterial cells, (iv) the photosystem II (PSII) core samples of spinach, (v) PSI and lipid mutants of Synechocystis sp. PCC 6803 and (vi) light-harvesting complex II (LHCII) in oriented lipid bilayers

(i)

Our aim was to identify exciton interactions in the PSII core particles of T. vulcanus, using the recently elaborated technique in our laboratory, the anisotropic circular dichroisms (ACD); a novel method that has been successfully applied in the identification of bacteriochlorophyll-a dimers in the baseplate of carotenosomes (Nielsen et al. 2016). ACD is capable of revealing hidden or weak intensity circular dichroisms (CD) bands and carries unique structural information on pigment-pigment interactions.

CD spectra of solubilized PSII core particles were recorded at room temperature in the range of 350–750 nm using a JASCO 815 spectropolarimeter. PSII core of T. vulcanus samples in solution were diluted in storage buffer. Measurements were performed in a standard glass cell of 1-cm optical path length. Alternatively, gel strips of 1.5-mm thickness were sandwiched between glass plates.

The PSII core particles were embedded in a lipid membrane by following standard protocols (Moya et al 2001; Yang et al 2006; Zhou et al 2009). Large unilamellar lipid vesicles (liposomes) were prepared from egg phosphatidylcholine (PC). The lipids dissolved in chloroform:methanol were placed in a round-bottom flask and the solvent was dried in a rotary vacuum evaporator to form a thin film. The hydrated lipids were extruded through a 100-nm pore membrane (Mini-Extruder, Avanti Polar Lipids) to form unilamellar vesicles. Detergent was added to the preformed liposomes. PSII core detergent solution was added in drops to the liposome suspension to the desired lipid:protein ratio (in different range). The detergent was then removed by repeated incubation with absorbent beads (Akhtar et al 2015).

For linear dichroism (LD) and ACD measurements the proteoliposome samples were fixed in polyacrylamide gel. The orientation was then done by compressing the gel along one dimension while allowing it to expand along the other two dimensions.

CD is a sensitive tool of the molecular organization of the pigment-protein complexes and their macroassemblies (Garab and Amerongen 2009). The 675 nm LD band of the PSII core is clearly visible, which means the sample is in good orientation in the gel. We also verified that the gel environment had no effect on the CD spectra. In the ACD spectra some of the bands, which on the iso CD were hardly discernible, were amplified. The characteristic bands (+) 670 nm and (-) 675 nm inverted sign and a red shift was observed in the Qy region. Characteristic 'new' bands were observed in the Soret region, too, these studies will be extended to microcrystals, and will be published – after making further efforts to identify the origin of the newly observed ACD bands, using the atomic resolution structure of PSII core.

(ii)

The aim of this study was to elucidate the question whether or not the RC of R. sphaeroides exhibits exciton dimer bands – similar to the Bchl-a dimers in the carotenosome, or the special pair of the RC should be characterized by more complex model. To this end, we performed, CD, LD and ACD measurements.

In the autumn semester of 2016, Dávid Allaga joined our group as a BSc student. I was his co-supervisor. In his thesis, we investigated the polarization spectroscopic properties of the isolated and proteoliposome-embedded PSII reaction center of R. sphaeroides, using our Jasco J-815 spectropolarimeter. Proteoliposomes were prepared with two different protocols: (i) with an extruder (see point 1.) and (ii) on a Sephadex G-75 column; then the RC was embedded and oriented in the gel. Calculated amount of phospholipid (PC) was solved in chloroform and dried on the wall of conical tube under nitrogen stream forming a thin film. The film was dissolved with 0.5 ml of Na-cholate (1.4% solution) in phosphate buffer. In order to form mixed phospholipid/detergent micelles the solution was sonicated. The RC solution was added and vigorously shaked to allow the formation of the phospholipid/protein/detergent micelles, then it was loaded on Sephadex G-75 column (previously equilibrated with phosphate buffer) (Trotta et al 2002).

The diameter of the proteoliposomes was around 100 nm, which was checked by microscope.

Main results:

- The 815 nm CD peak characteristic for the oxidized RC protein disappeared (Figure 1). This peak corresponds to the oxidation of the P band, in good agreement with literature data (Reed and Ke 1973).

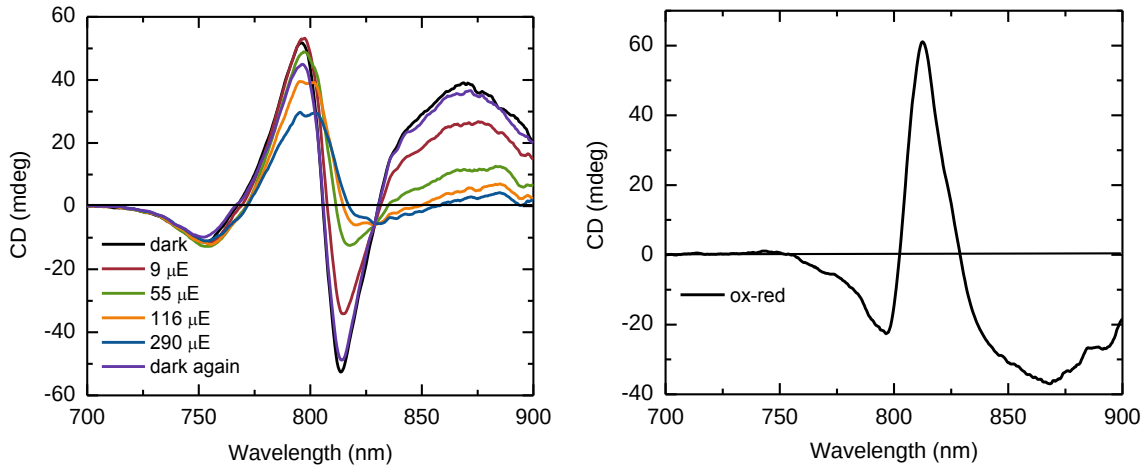

Figure 1. The CD spectra of isolated photosynthetic RC of *Rb. sphaeroides* R-26 in the dark (reduced state) and under different excitations of light intensity (oxidized state) and difference CD spectra (ox-red: difference between the illuminated (290 μmol photons m^{-2} s^{-1}) and the dark spectra).

- The RC embedded in liposome and fixed in gel becomes partially oxidized (Figure 2).

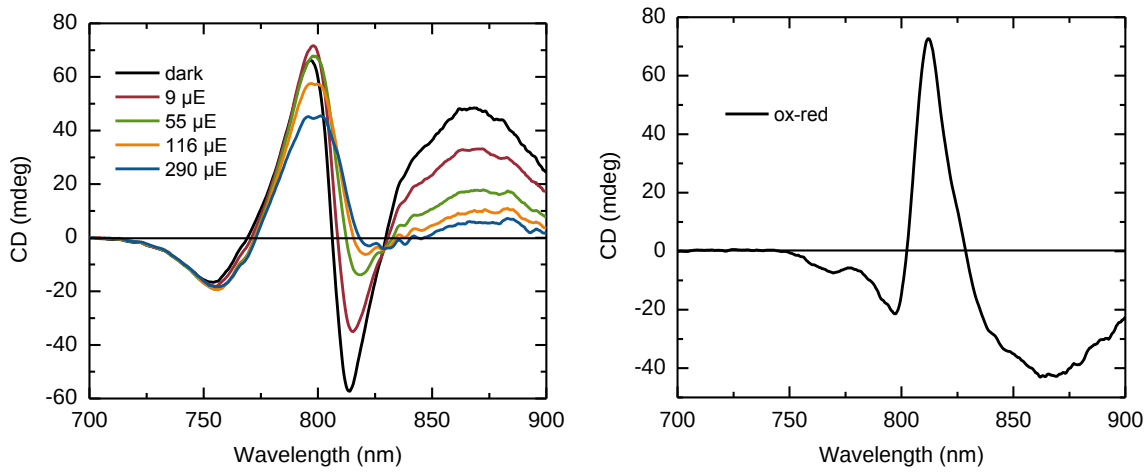

Figure 2. The CD spectra of isolated photosynthetic RC of *Rb. sphaeroides* R-26 in the dark (reduced state) and under different excitations of light intensity (oxidized state) and difference CD spectra (ox-red: difference between the illuminated (290 μmol photons m^{-2} s^{-1}) and the dark spectra).

- Our ACD and LD measurements, in good agreement with crystallographic data, indicate that the pigment dipoles belonging to the B and P transitions involved in the redox transients preferentially lie in the plane of the membrane.

- The characteristics of the ACD spectra (Figure 3) does not indicate the presence of attributable dimer structure (belonging to the special pair). Instead, our data can be explained with those models which hypothesize complex exciton interactions within the reaction center complex (Strümpfer and Schulten 2012)

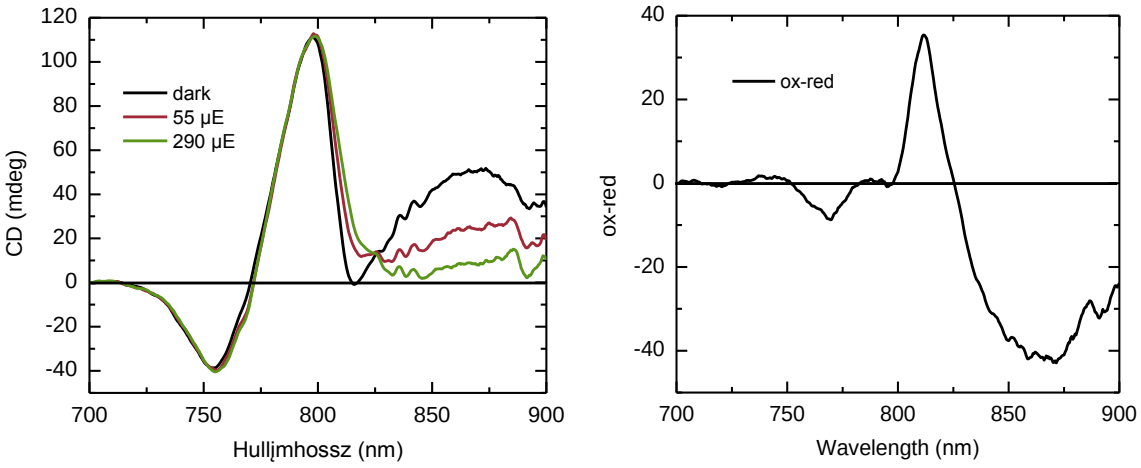

Figure 3. The ACD spectra of isolated photosynthetic RC of *Rb. sphaeroides* R-26 in the dark (reduced state) and under different excitations of light intensity (oxidized state) and the difference CD spectrum (ox-red:difference between the illuminated (290 µmol photons m^{-2} s^{-1}) and the dark spectra).

(iii)

Our part in this study was to investigate – using polarization spectroscopy – the mechanism of desiccation in the desert crust cyanobacterium Leptolyngbya ohadii. We propose an energy dissipation mechanism that is completely reliant on changes in the aggregation state of the phycobilisome light-harvesting antenna components (Eyal et al 2017).

This cyanobacteria was isolated in Israel, from the Nizzana region of the NW Negev desert, like a keystone bacteria in this environment. When the bacteria is rehydrated after full drying, the photosynthetic activity of the cells becomes perfect again. The drying does not result the degradation of the photosynthetic protein complexes, so even morning mist can prevent damage of the drying day (Ohad et al 2010). These organisms spend most of the daytime in the desiccated state hence the absorbed energy is completely quenched (Eyal et al 2017).

I used our Jasco J-815 spectropolarimeter for measuring circular dichroism (CD), linear dichroism (LD), and anisotropic circular dichroism (ACD), using procedures similar to those described earlier (Miloslavina et al 2012; Nielsen et al 2016). In order to study the small dry droplets, we equipped the dichrograph with specially designed, home-built sample holders for LD and ACD measurements.

CD and ACD signals were measured on dried and rehydrated samples. Only minor changes were found in the spectra. We could clearly demonstrate that the photosynthetic pigment-protein complexes do not change sizeably either due to drying or rewetting.

In the desiccated state (i) reduced excitation energy transfer between phycobilisome components, (ii) extensive heat dissipation, and (iii) fluorescence red shift were observed. These changes coincided with a loss of the organized phycobilisome (PBS) structure in the interthylakoidal space. Our CD data indicated no or only very small detectable difference in the optical properties of the phycocyanin units themselves – consistent with the fast recovery of photosysthetic functions during re-wetting (Figure 4).

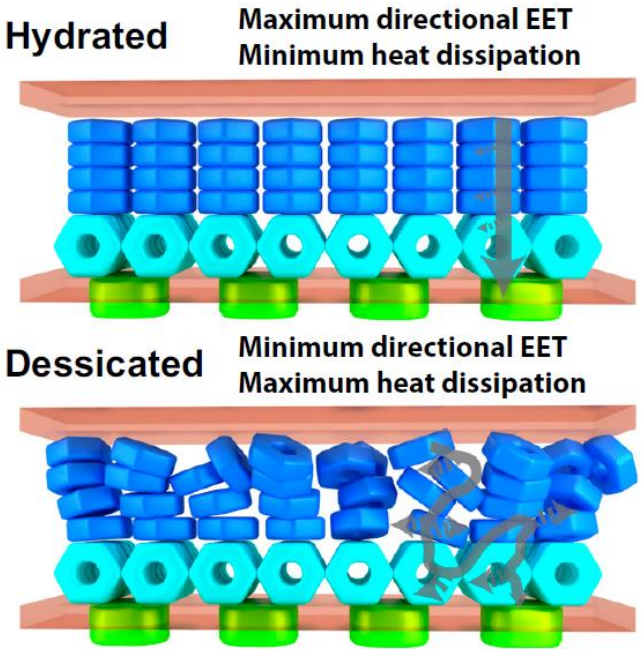

Figure 4. Model for excitation energy transfer (EET) processes in the hydrated and desiccated states. A schematic representation of the aggregation state of the PBS antenna in both states. The components are drawn roughly to scale. Green, photosystems; cyan, allophycocyanin (APC); blue, PC; gray arrows, directional EET; dashed arrows, heat dissipation. In the crowded interthylakoidal space, only small changes in aggregation state are possible; these are exaggerated in the figure for demonstration purposes.

(iv)

Our aim was to identify CD, LD and ACD spectra of PSII core complexes, in comparison with its biochemically separable parts – the D1/D2 RC, and the core antenna complexes CP43 and CP47 to test whether the whole structure is well represented by the sum of its constituents and detect possible changes occurring upon assembly of the complex.

First, we had to elaborate a protocol for the PSII preparation. PSII membrane fragments were prepared according to the method of Berthold et al. (1981) with minor modifications on P.J. van Leeuwen et al. (1991). Initial test preparations were conducted on a small scale using a 5 ml ion-exchange chromatography column. The separation through the column depends on the concentration of $MgSO_4$ and Triton X-100 in the mobile phase. A 10 % (w/v) Triton X-100 solution in BTS200 (20 mM Bis-Tris, 20 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mM MgSO4, 0.4 M sucrose pH 6.5) was allowed to enter the column until all of the PS II core bands were exposed to the detergent and then the flow was stopped for 20 min. This incubation step was repeated once with a fresh detergent solution. The column was washed with BTS200 until the eluate became colorless and the Triton X-100 absorption at 280 nm disappeared ($A_{280} < 0.2$), which took about 40 min. The PS II RC particles were eluted from the column with BTS200 +$MgSO_4$ (75 mM final). The critical point of the protocol is the length of the washing time, the refining of the Triton X-100 and $MgSO_4$ concentrations, so to ensure that the PSII core received is of the highest quality. The spectroscopic analysis of PSII, D1/D2 RC, CP43 and CP47 will be performed following this preparation method. However, due to the low purification yield, the preparation protocol must be scaled up and a larger column must be purchased.

(v)

We have recorded fluorescence induction transients of PSII isolated from Synechocystis PCC 6803 cells. Cyanobacteria, the ancestors of chloroplasts, are ideal model organism to study photosynthetic processes in vivo. The fundamental objective of the project is to clarify open questions on the structure and functions of PSII. We examined the nature of the fluorescence yield rise of PSII elicited by trains of single-turnover saturating flashes (STSFs) in the presence of PSII inhibitor, permitting only one stable charge separation. We show that a substantial part of the rise originates from light-induced processes that occur after the stabilisation of charge separation, induced by the first STSF; the temperature-dependent relaxation characteristics suggest the involvement of conformational changes in the additional rise (Magyar et al. 2018). I measured the relative fluorescence yields with PAM 101 fluorometer (Walz, Effeltrich, Germany). The cyanobacterial cells were placed in a cuvette of the temperature controlling part of a thermoluminescence apparatus, which enabled the precise control of the temperature during measurements. The fluorescence yield was measured as a function of temperature. The timing of the flashes and the temperature was controlled by using a home-designed programmable digital pulse generator and temperature controller. We used intact Synechocystis PCC 6803 cells in BG11 medium for the measurements. The chlorophyll concentration of each sample was 50 μg/ml. The samples were supplemented with 40 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) dissolved in dimethyl sulfoxide (DMSO) and dark adapted for 5 minutes at room temperature before the measurements.

In the thylakoid membrane the lipid dynamics is important for the low- and high temperature adaptation (Laczkó-Dobos and Szalontai 2009). Previous research has shown that the extent of lipid disorder is usually higher in the thylakoid membranes than in other membranes of the cell (Szalontai et al. 2000). The Synechocystis PCC 6803 wild-type and the mutant cell membranes desA-ldesD- are different in their lipid composition. The mutant cells contain only monounsaturated fatty acyl chains in their membrane lipids (Kiss et al. 1998). These mutants were applied in order to investigate the possible role of lipids in the characteristic temperature dependence of the chlorophyll a fluorescence increment at low temperature (Magyar et al. 2018) Our measurements showed, however, that the background fluorescence of whole cyanobacterial cells was too high, not allowing the detection of chlorophyll a fluorescence increment. To this end, we shall design and construct suitable STSF source for the excitation of the sample and a system for observing chlorophyll a fluorescence without the contribution of phycobilisome and PSI emission.

We have started chl-a fluorescence transient measurements at different temperatures on two other Synechocystis mutant strains, in which, with combinatorial mutagenesis, multiple amino acid changes had been generated (Vavilin et al 1999). The CD loop mutations were transferred into a PSI-less Synechocystis strain to facilitate characterization of PSII properties in the mutants. Most of the combinatorial PSI-less mutants obtained had a high yield of Chl-a fluorescence. However, in the mutants, which shared a replacement of Phe181 by Trp, the variable fluorescence yield was dramatically reduced although a high rate of oxygen evolution was maintained. Our results revealed that the Fv/Fm ratio is close to zero also at cryogenic temperatures - the underlying physical mechanism will have to be investigated.

(vi)

Our aim was to identify CD, LD and ACD spectra of light-harvesting complex II (LHCII) – the main peripheral antenna of photosystem II in plants – in oriented lipid bilayers were recorded in the visible wavelength region. In this publication (Akhtar et al 2019), our measurements prove ACD spectroscopy to be a valuable tool linking the three-dimensional structure and the photophysical properties of pigment−protein complexes.

The ACD spectra in the visible spectral region are determined by the optical properties of the protein-bound pigments and their excitonic interactions. The nonconservative nature of the experimental isotropic CD spectrum suggests that there is an influence of higher excited Chl and carotenoid states that could explain the remaining deviations between calculated and measured ACD spectra, in particular, on the high-energy side of the visible spectrum. Inclusion of carotenoid states will help to better understand the higher energy spectral regions, where the ACD spectra possess the most characteristic and striking features. ACD will serve as an important complement to CD for the characterization of carotenoid−Chl interactions.

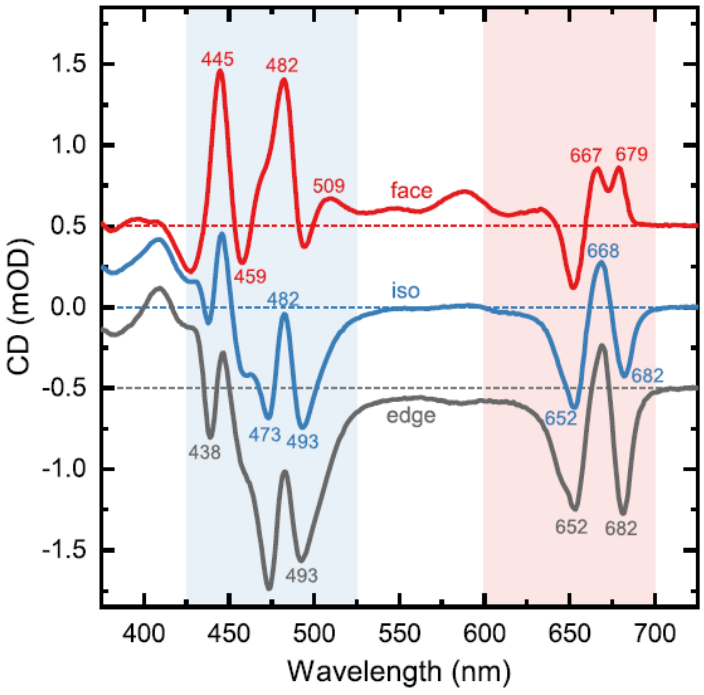

Figure 5. CD and ACD spectra of reconstituted LHCII membranes. Red curve – face-aligned ACD recorded from dehydrated membrane patches; blue curve – isotropic CD measured in buffer medium; gray curve – edge-aligned ACD calculated as (3CDiso − ACDface)/2. (Miloslavina et al 2012, Lindorfer and Renger 2018) The spectra are plotted in units of $\Delta A \times 10^{-3}$ (mOD) and normalized to unity isotropic absorbance at 675 nm. For clarity, the ACD spectra are shifted vertically by 0.5 units. For each spectrum the horizontal dotted line is the corresponding zero line. Numbers indicate peak wavelengths.

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