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Spectral substructure and excitonic interactions in the plant antenna complexes LHC II and CP29 as revealed by non-linear laser spectroscopy.

H Lokstein[1], B Voigt[2], A Schubert[1,4], M Krikunova[2], W Beenken[2,4], K Teuchner[2], J Ehlert[2], K-D Irrgang[3], G Renger[3], D Leupold[2]

[1]*Institut für Biologie, Humboldt-Universität zu Berlin, D-10099 Berlin, Germany, Fax: +49-30-2093 6337, E-mail: lokstein@mbi-berlin.de*

[2]*Max-Born-Institut für Nichtlineare Optik und Kurzzeitspektroskopie, D-12489 Berlin*

[3]*Max-Volmer-Institut für Biophysikalische Chemie und Biochemie, TU Berlin, D-10623 Berlin, Germany*

[4]*Present address: Chemical Physics, Lund University, P.O. Box 124, S-22100 Lund, Sweden*

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Introduction

Extent of excitonic interactions and their physiological implications in plant light-harvesting complexes are a matter of controversial debate (Leupold et al., 1999). We have employed a variety of complementary nonlinear laser spectroscopic techniques (nonlinear absorption, NLA; nonlinear polarization spectroscopy in the frequency domain, NLPF; stepwise two-photon excited fluorescence spectroscopy) to investigate the origin of spectral substructure(s) and the extent of excitonic interactions in trimeric LHC II binding at least 7 chlorophylls (Chls) *a* and 5 Chls *b* per monomeric subunit (Kühlbrandt et al., 1994) and the structurally related - but simpler - monomeric minor complex CP29 (binding 6 Chls *a* and 2 Chls *b*). A comparison of the absorption spectra of both complexes is given in Fig. 1).

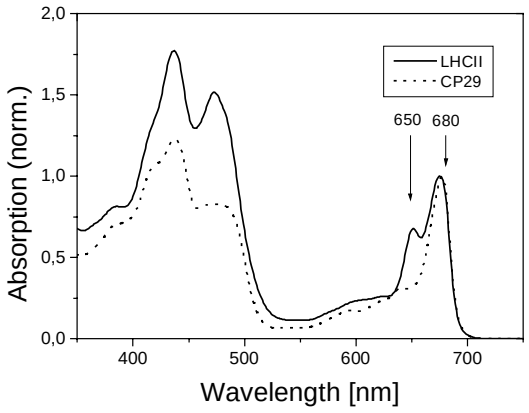

Fig. 1: Absorption spectra of LHC II (solid line) and CP29 (dotted line). Arrows indicate excitation wavelengths in the stepwise two-photon excited fluorescence experiment.

Materials and methods

LHC-II was isolated from pea leaves according to Krupa et al. (1987). Trimeric LHC-II was obtained in a buffer containing 10 mM Tricine (pH 7.8) and 1.2 % *n*-octyl β-D-glucopyranoside (or 0.06 % *n*-dodecyl β-D-maltoside) at 110 µg/ml Chl *a*+*b*. CP29 was isolated and purified as outline in Pieper et al. (2000). Absorption and fluorescence spectra were measured before and after the laser-spectroscopic experiments to monitor sample integrity. The NLA single beam set-up has been described previously (Stiel et al., 1991a). The fs-laser system (CPA 1000, CLARK-MXR Inc., Dexter, MI, USA) provides 120 fs-pulses with a spectral width of 6 nm FWHM (as monitored by a FROG, Clark-MXR Inc.). Intensity was varied using a neutral-density filter wheel. NLA data analysis was performed with the software package CALE (Stiel et al., 1991b). NLPF spectra were recorded applying a 90°-arrangement of pump and probe laser beams as introduced by Voigt et al. (1999). Both laser beams were obtained from dye lasers (DCM in DMSO) simultaneously pumped by excimer laser pulses. Stepwise two-photon excited fluorescence was measured as described in Teuchner et al. (2000).

Results and discussion

NLA of 120 fs pulses indicates an about two-fold increase in absorption cross section in the red wing of the Q_y-band of LHC II as compared to monomeric Chl *a* in solution (Fig. 2). The pump intensity dependence of the NLPF signal (measured with the same sample) can be used to generate a spectrum of the saturation parameter ξ (a linear combination of the absorption and emission cross sections of the probed transition, see Beenken and May, 1997; a full account of these experiments is given in Schubert et al., *submitted*). The ξ-spectrum reveals that in LHC II a spectral form emitting at 682 nm is characterized by a 2.2(±0.8)-fold larger dipole strength than that of Chl *a* (Fig. 2). Low intensity NLPF experiments (with pump and

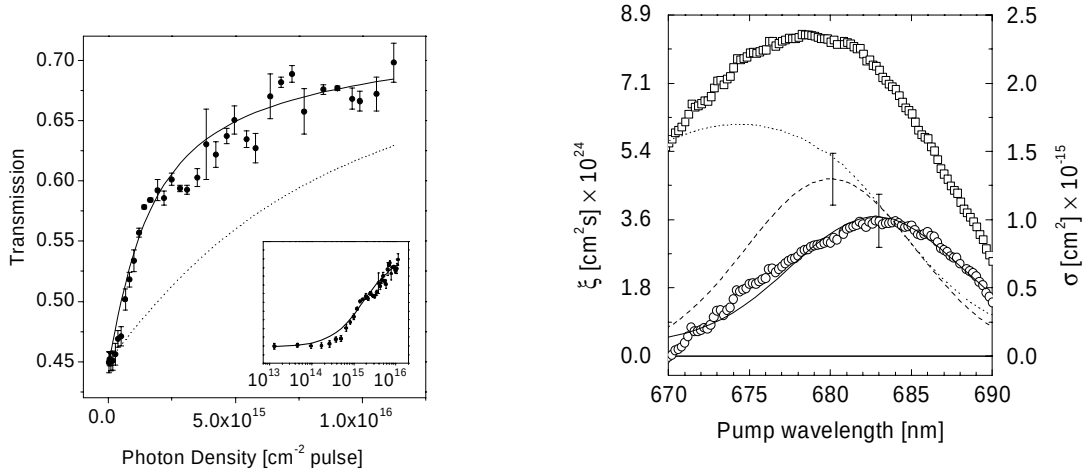

Fig. 2: Nonlinear Absorption of LHC II (left panel, insert represents the data on a logrithmic intensity scale; dotted line is a simulation for monomeric Chl *a* using 3.1×10^{-16} cm^{-2} for both, ground and excited state absorption cross sections). The ξ-spectrum (right panel, squares) was obtained from intensity dependent NLPF of LHC II. The right scale (σ) is calculated from ξ by multiplication with the fluorescence-decay time (3.6 ns). Subtracting σ^{abs} (dotted line) from the ξ-spectrum yields σ^{em} (circles) of the terminal acceptor; σ^{em} can be fitted by a Gaussian (solid line) centered at 683(±1) nm with a FWHM of 260(±30) cm^{-1}. For comparison to the NLA results an absorption band with the same FWHM (260 cm^{-1}) and a maximum absorption cross section of 1.3×10^{-15} centered at 680 nm was simulated (dashed line)

probe wavelengths located in well separated spectral regions, Fig. 3) indicate strong excitonic coupling between Chls *a* and *b* in LHC II and CP29. Namely, excitation in the Chl *a* Q_y region elicits also a NLPF response in the Chl *b* Soret region (arrows in Figs 3A,B). Moreover, the lowest Q_y-transition in CP29 (at 680 nm) can be assigned (in contrast to LHC II) to a non-excitonically coupled Chl *a* (Voigt et al., *submitted*; cp. also Pieper et al., 2000).

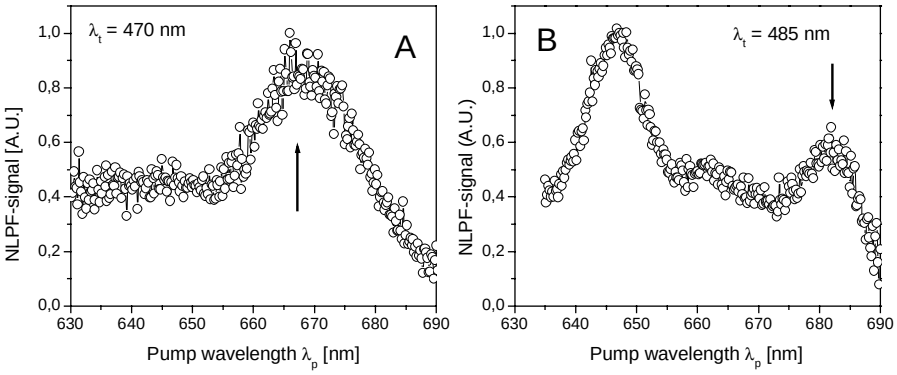

Fig. 3: NLPF spectra of CP29 (A) and LHC II (B) pumped in the Q_y band and probed in the Soret region (λ_t is indicated, arrows point at features thet can be assigned to Chl *a* forms)

Stepwise two-photon excitation of Chls *a* and *b* in LHC II and CP29 with 100 fs pulses in the (red) Q_y-region results in a weak "blue" fluorescence (quantum yield less than 10^{-4}). The dependence of the spectral shape of the blue fluorescence on excitation wavelength (Chl *a vs.* Chl *b*) together with a comparison to properties of Chls in solution are consistent with the existence of (a) strongly excitonically coupled Chl *a/b*-heterodimer(s) in LHC II. The results obtained with CP29 are consistent with the above mentioned observation that the (low energy) 680 nm state in CP29 can be assigned to an uncoupled Chl *a* (Leupold et al., *submitted*; cp. also Pieper et al., 2000).

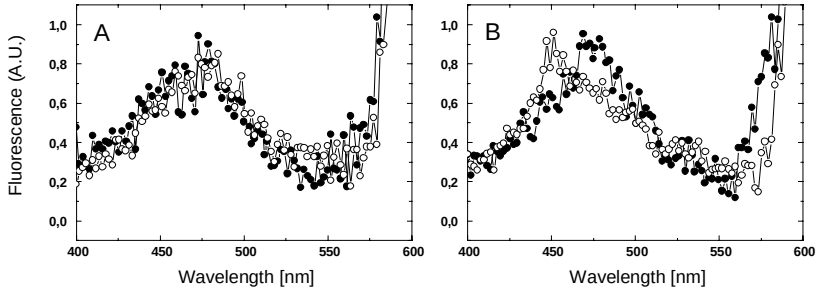

Fig. 4: Stepwise two-photon excited fluorescence spectra of LHC II (A) and CP29 (B). Excitation was at 680 nm (open symbols) or at 650 nm (closed symbols).

Different lines of evidence for excitonic coupling of Chls in CP29 and LHC II are provided. A proposed Chl dimer can be assigned to the Chl binding sites **a2** and **b2** in the LHC II structure

(Kühlbrandt et al., 1994). Site **b2** is apparently lacking in CP29 - consistent with the above laser-spectroscopic results. The Chl **a2**/**b2** pair appears to be located on the outer surface of the LHC II trimer. Its absorption is shifted to the red edge of the Q_y-band by excitonic interaction. The pair appears to function as a "trapping state" and starting point of excitation energy transfer to neighboring trimers. Enhancement of the transition dipole can increase the connectivity of adjacent trimers - thus forming an extended PS II-network. Additionally, the enhanced dipole moment may attract excitations from inside to the surface of LHC II. Thus, the fluorescent dimer at the end of the excitation energy transfer chain is expected to play a crucial role for the overall efficiency of plant photosynthetic energy conversion.

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