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Studying Spatio-Energetic Dynamics in Light Harvesting Complex II using Two-Dimensional Electronic-Vibrational Spectroscopy

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Abstract: We present two-dimensional electronic-vibrational spectra of the chlorophyll (Chl) binding protein light harvesting complex II (LHCII) from spinach, and of isolated Chl *a* and Chl *b*. We show how energy transfer from Chl *b* to Chl *a* and subsequent relaxation can be directly observed in LHCII using this multidimensional technique.

OCIS codes: 300.0300, 300.6530.

1. Introduction

Light harvesting complex II (LHCII) serves as the primary antenna pigment-protein complex for photosystem II in higher plants. Each monomer binds eight chlorophyll (Chl) *a*, and six Chl *b*, in addition to several carotenoids. These Chl *a* and Chl *b* are the primary light absorbers in LHCII. A complete understanding of the energetic structure and excitation dynamics in LHCII is crucial for an accurate model of the photosynthetic apparatus as a whole. Numerous studies have been performed to understand the energetic structure of LHCII, [1–3] but these rely on purely electronic transitions and cannot provide direct information about the transfer of excitation energy through space. Here we present two-dimensional electronic-vibrational (2DEV) spectra of LHCII. This technique has the potential to provide a direct experimental connection between the electronic states and the spatial location of the excitation within the complex. [4] We observe clear signatures of the energy transfer from Chl *b* to Chl *a*, as well as indications of the subsequent relaxation within the Chl *a* manifold.

2. Methods

The details of the 2DEV technique have been detailed previously. [5] The visible pump laser (~ 325 nJ, ~ 15 fs, ~ 250 μm focal spot) was tuned to span the Q_y bands of the Chl *a* and Chl *b* absorption spectra ($\sim 14250 - 16700$ cm^{-1}). The infrared laser (~ 100 nJ, ~ 80 fs, ~ 200 μm focal spot) was centered at 1620 cm^{-1} , in resonance with the high frequency carbonyl and chlorin ring C=C stretching vibrational modes. The visible pump-pulse pair (time delay t_1) was generated and controlled using a pulse shaper (Dazzler, Fastlite). To detect the changes in the infrared absorption (ω_3), the infrared probe laser was dispersed on a spectrometer, imaged onto a dual-array 64 element HgCdTe detector (Infrared Systems Development), and normalized against a reference beam. The waiting time t_2 between the visible pump pair and the infrared probe was controlled with a motorized delay stage. The relative polarization of the pump and probe lasers was set to parallel. The desired signal was isolated by phase cycling the pump pulse pair in a 4×1 scheme, and a Fourier transform was performed along t_1 to produce the final ω_1 vs. ω_3 correlation spectra, parametrized by t_2 . The Chl *a* and Chl *b* samples were dissolved in ethanol- d_6 to an optical density of 2 at the absorption maximum, in a 100 μm path-length cell, and placed in a cryostat (OptistatDN2, Oxford Instruments) at 77 K. The LHCII sample

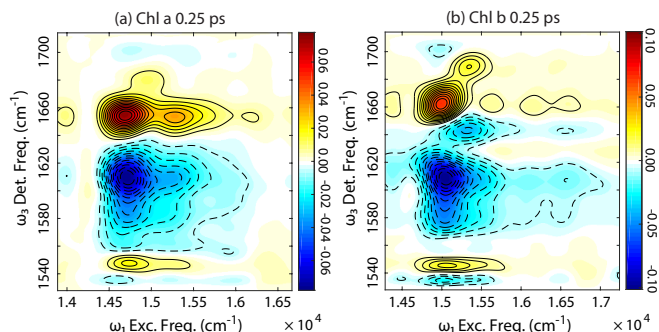


Fig. 1. 2DEV spectra of (a) Chl *a* and (b) Chl *b* in ethanol- d_6 at waiting time $t_2 = 0.25$ ps and 77 K.

was isolated from spinach, and kept in a buffer of 50 mM Tris-HCl (pH 7.8), 0.12 M NaCl, 0.3 M sucrose, and 0.03% *n*-dodecyl β -D-maltoside in D_2O . This solution was mixed with glycerol- d_8 in a 70:30 (vol/vol) glycerol:LHCII ratio to a maximum optical density of 0.8, in a 100 μ m path-length cell, and held at 77 K.

3. Discussion and Results

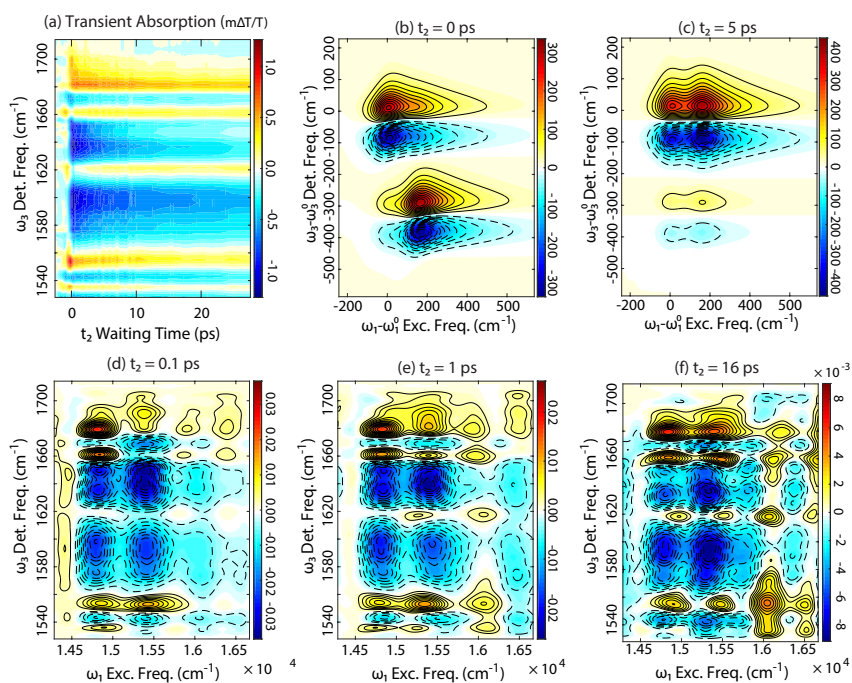


Fig. 2. (a) TIR spectrum of LHCII at 77 K illustrating the overall dynamics of the spectrum. (b) and (c) show simulated 2DEV for a model coupled dimer at $t_2 = 0$ ps and $t_2 = 5$ ps, demonstrating the effect of energy transfer on the 2DEV spectrum. Experimental 2DEV spectra of LHCII at 77 K are shown in (d-f) at $t_2 = 0.1$ ps (b), 1 ps (c) and 16 ps (d).

2DEV spectra of Chl *a* and Chl *b* at waiting time $t_2 = 0.25$ ps are shown in Fig. 1. The major features (at $\omega_3 \simeq 1660$ cm^{-1} and $\omega_3 \simeq 1610$ cm^{-1}) correspond to the C=O stretching mode of the 13^1 ketone group, and high energy stretching modes on the macrocycles. The positive features arise from bleaching the vibrational mode on the ground electronic state, while the negative signals are due to absorption by the vibrations on the excited state. The negative band at 1640 cm^{-1} in the Chl *b* spectrum is due to the formyl group in this species. The Chl *a* spectrum shows signals at $\omega_1 = 15400$ cm^{-1} that arise from the initial excitation of the Q_x electronic band, though due to the fast ($\lesssim 100$ fs)

relaxation to Q_y , the majority of the population has already relaxed by 250 fs.

The transient infrared absorption (TIR) spectrum of LHCII are shown in Fig. 2(a). Similar TIR spectra of LHCII have been reported previously, however those experiments were performed at room temperature. [6] An interesting positive feature appears at $\sim 1680 \text{ cm}^{-1}$. This band shows a rapid decay of its high-frequency wing, followed by the slower decay due to the overall relaxation to the ground state. Observing the 2DEV spectra in Fig. 2(d-f), it becomes clear the contribution to the high frequency side of this band in the TIR is due to a separate spectral feature (at $\omega_1 = 15400 \text{ cm}^{-1}$ and $\omega_3 = 1690 \text{ cm}^{-1}$) from the long-time component. This signal originates when the excitation is initially created in the Chl *b* region of the spectrum ($\omega_1 = 15400 \text{ cm}^{-1}$), and is replaced by a band at $\omega_3 = 1680 \text{ cm}^{-1}$ as the excitation is transferred from Chl *b* to Chl *a*. There is some indication that the negative band at 1670 cm^{-1} that is associated with Chl *b* remains populated out to rather long t_2 and may even have some initial population when the Chl *a* band is excited ($\omega_1 = 14800 \text{ cm}^{-1}$). This may be an indication of a low energy state involving Chl *b* that has not previously been identified, representing a significant amount of mixing of a Chl *b* state into the Chl *a* band.

To demonstrate that the spectral dynamics observed in the 1680 cm^{-1} region are consistent with this interpretation, we show in Fig. 2(b-c) simulated 2DEV spectra for a model electronic dimer. In these simulations, we see that the electronic relaxation can be followed via the dynamics of the vibrations on both the ground and excited electronic states. At early t_2 , the spectrum shows a clear asymmetry between peaks due to initially exciting the different excitons, which becomes symmetric at long values of t_2 due to the electronic relaxation. These spectral dynamics are very similar to what we see in the spectra of LHCII.

In addition to the major bleach features, there are also several strong electronic excited state features apparent in the TIR, with two major bands at $\omega_3 = 1600 \text{ cm}^{-1}$ and 1640 cm^{-1} . The higher energy band splits into separate bands at 1635 cm^{-1} and 1655 cm^{-1} at longer times. The precise origins of all of these features is not obvious when compared to the spectra of isolated Chl *a* and *b* in Fig. 1. The two separate bands near 1640 cm^{-1} are immediately apparent in the Chl *a* band, but they are far less well resolved for the Chl *b* band. As the Chl *b* population relaxes the splitting of this band become far more apparent, but it does not fully resemble the Chl *a* band until rather long times. This may be indicative of the relaxation within the Chl *a* manifold subsequent to the fast relaxation of the Chl *b* states. More refined assignments of these bands should provide greater detail of the dynamics of electronic relaxation within LHCII.

4. Conclusion

We have presented TIR and 2DEV spectra of isolated Chl *a* and Chl *b* and the LHCII pigment-protein complex. The spectra clearly reveal the dynamics of the energy transfer from Chl *b* to Chl *a*, and open questions about the details of the single exciton manifold in this complex. With assignments of the observed spectral bands from electronic structure calculations and mutant studies, we expect that these data will reveal, with unprecedented detail, the spatio-energetic dynamics of electronic excitations in this important piece of the photosynthetic apparatus.

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