The Origin of the Low-Energy Form of Photosystem I Light-Harvesting Complex Lhca4: Mixing of the Lowest Exciton with a Charge-Transfer State

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ABSTRACT The peripheral light-harvesting complex of photosystem I contains red chlorophylls (Chls) that, unlike the typical antenna Chls, absorb at lower energy than the primary electron donor P700. It has been shown that the red-most absorption band arises from two excitonically coupled Chls, although this interaction alone cannot explain the extreme red-shifted emission (25 nm, ~480 cm^{-1} for Lhca4 at 4 K) that the red Chls present. Here, we report the electric field-induced absorption changes (Stark effect) on the Q_y region of the Lhca4 complex. Two spectral forms, centered around 690 nm and 710 nm, were necessary to describe the absorption and Stark spectra. The analysis of the lowest energy transition yields a high value for the change in dipole moment, $\Delta\mu_{710nm} \approx 8\ D f^{-1}$, between the ground and excited states as compared with monomeric, $\Delta\mu = 1$ D, or dimeric, $\Delta\mu = 5$ D, Chl *a* in solution. The high value of the $\Delta\mu$ demonstrates that the origin of the red-shifted emission is the mixing of the lowest exciton state with a charge-transfer state of the dimer. This energetic configuration, an excited state with charge-transfer character, is very favorable for the trapping and dissipation of excitations and could be involved in the photoprotective mechanism(s) of the photosystem I complex.

Received for publication 1 October 2008 and in final form 20 November 2008.

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Photosystem I (PSI) is a multisubunit pigment-protein complex located in the thylakoid membrane of higher plants and algae where one of the first steps of solar energy conversion by light-driven electron transport takes place. It consists of two separable subunits: the PSI core and the peripheral light-harvesting complex I. The light-harvesting complex I, responsible for the absorption of light and the transfer of energy to the PSI core, contains in plants four subunits: Lhca1, 2, 3 and 4 (1,2). The most striking spectroscopic feature of PSI is the presence of red chlorophylls (Chls), i.e., Chls that absorb at longer wavelength than the primary electron donor P700, and that exhibit nontypical Chl behavior: a large absorption bandwidth (400–500 cm^{-1} at 4 K) due to both homogeneous and inhomogeneous broadening, and an extremely red-shifted emission (absorption at 708 nm and emission at 733 nm for Lhca4 at 4 K), i.e., 480 cm^{-1} (3), the most dramatic Stokes spectral shift observed in a photosynthetic complex so far. In higher plants, these forms are mainly associated with the outer antenna, although low energy absorption forms are also present in the core (4).

It has been shown that the red-most absorption band in the PSI complex arises from an excitonically coupled dimer of Chls (5–8), although this interaction alone cannot account for the extreme red-shifted emission and the large bandwidth. A complementary explanation for both effects is the presence of a charge-transfer (CT) state mixed with the lowest exciton state of the dimer (9). Although the involvement of a CT state in the spectroscopic properties of the red Chls has been proposed by several authors for both the red forms of the core and the antenna (3,10–13), no direct evidence has been provided to date. To reveal the origin of the red Chls is a key aspect in PSI research because of the very pronounced effect that these low energy forms have on the energy transfer and trapping in the whole complex (14).

In this study, we have focused our attention on Lhca4, the subunit with the red-most shifted emission. To investigate the CT character of its lowest exciton state, we have performed Stark spectroscopy, a technique extremely sensitive to the changes in the electronic charge distribution of pigments upon excitation. This technique monitors the spectral changes induced by an externally applied electric field in absorption or emission spectra. A detailed description of the theoretical background, experimental setup, and analytical methods of Stark spectroscopy is found elsewhere (15). In simple terms, for a randomly oriented sample, the Stark spectrum ($Abs_{F\text{on}} - Abs_{F\text{off}}$) is described by the Liptay formalism as a linear combination of the zeroth, first, and second derivatives of the absorption spectrum (16). The molecular parameters change in polarizability, $\Delta\alpha$, and change in dipole moment, $\Delta\mu$, between the excited and ground states, scale with the first and second derivatives, respectively, of the absorption spectrum. Thus, quantitative simultaneous analysis of the absorption and Stark spectra

Editor: Janos K. Lanyi.

doi: 10.1016/j.bpj.2008.11.043

gives values for $\Delta\alpha$ and $\Delta\mu$ that are expressed in terms of the local field correction f, a parameter that accounts for the internal electric field felt by the chromophore because of the protein environment (theoretical calculations estimate $1.1 > f > 1.3$ (15)). A measure of the degree of charge separation between the ground and excited states is given by $\Delta\mu$, the key parameter to determine when the presence of CT states is investigated. $\Delta\alpha$ is related to the deformability of the electronic cloud of a molecule and provides information about the electronic properties of a chromophore interacting with the protein matrix.

The Liptay formalism, however, presents limitations when applied to photosynthetic systems: it is formulated for noninteracting molecules with isolated absorption bands with constant electrooptic parameters across each band and does not account for the field-induced mixing of (excitonic) states that can lead to new transitions. Another source of uncertainty when applying the Liptay formalism is the description of the absorption bands with Gaussian shapes that do not describe the vibrational side bands. Several examples of the failure of the method can be found in the literature (17,18). This problem can be overcome by combining the disordered exciton model with the modified Redfield Theory, in which more realistic band positions and band shapes are obtained from the simultaneous fit of absorption, fluorescence, linear dichroism, circular dichroism, and triplet-minus-singlet spectra (19). Although there are conceptual limitations underlying the Liptay formalism, it is useful, as a first approximation, for obtaining an estimation of the $\Delta\mu$ (the analytical limitations are more important for $\Delta\alpha$ (15)). By comparing the $\Delta\mu$ of interest with the $\Delta\mu$ for monomeric (1 D) and dimeric (5 D) Chl a in solution (the large increase upon dimerization was ascribed to the CT character of the dimer excited state) (20), it is concluded that for transitions arising from a dimer, when $\Delta\mu \geq 5\ \mathrm{D}f^{-1}$ the excited state is mixed with a CT state.

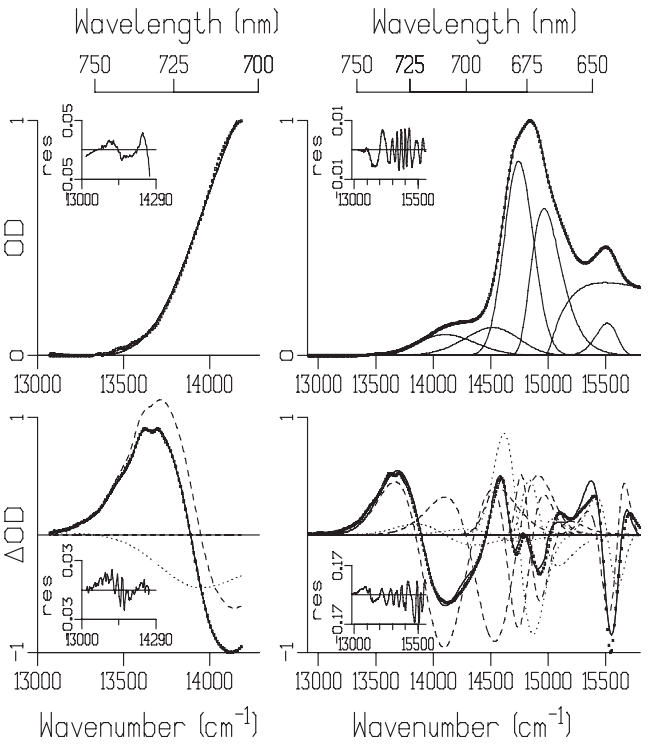

FIGURE 1 Normalized absorption (*upper panel*) and Stark (*lower panel*) spectra of Lhca4 at 77K. (*Dots*) data points, (*solid lines*) fit results, (*dashed lines*) second derivatives, (*dotted lines*) first derivatives. Insets show the residuals of the fit. Absorption spectrum fitted with a spline line shape (*left panels*) and with skewed Gaussians (*right panels*). The sample was in a glycerol buffer glass (57% glycerol v/v) containing 10 mM Hepes pH 7.5, 0.06% β-DM, sucrose ~0.4 M. The Stark spectra was measured at magic angle $\aleph = 54.7^{\circ}$ ($\aleph$ is the angle between the externally applied electric field and the polarization of the measuring light) and at a field strength of $F = 2.375 \times 10^5$ V cm^{-1}. The absorption maximum is OD 0.66 at 674 nm, the Stark minimum is ΔOD -1.8×10^{-4} at 643.5 nm.

The absorption and Stark spectra of reconstituted Lhca4 prepared as in Croce et al. (21) are shown in Fig. 1. Six skewed Gaussian bands are necessary to obtain a satisfactory simultaneous fit of the absorption and Stark spectra. When all the fitting parameters (position, full width at half maximum (FWHM), and skewness of the absorption bands) are free, the low energy form is fitted with a single broad band (centered at 700 nm, FWHM = 970 cm^{-1}) whose derivatives do not fully describe the red tail of the Stark spectrum (see Supporting Material). According to Croce et al. (3), a second emission form at 705 nm absorbing around 690 nm is present in Lhca4. The inclusion of this second red absorption band substantially improves the quality of the fit. Several fits with similar quality are obtained when the position and the width of the lowest energy forms are fixed to 689–690 nm, FWHM = 550–800 cm^{-1}; and 708–711 nm, FWHM = 550–700 cm^{-1} (the rest of fitting parameters are free, see Supporting Material). The Gaussian deconvolution of the absorption spectra also satisfies two criteria obtained from fluorescence measurements (3): 1), the maximum of the red-most band is around 709 nm; and 2), the red-most band shows absorption in the range of 685–697 nm.

The change in dipole moment between the excited and ground states for the red-most band is deducted from seven different fits in the 630–800 nm region (for a selection, see Supporting Material) and ranges from $\Delta\mu = 6.6$ to 8.2 Df^{-1}. We also fitted the red side of the band with a spline line shape from 705 to 775 nm (Fig. 1), where the overlap with the 690 nm band is small, obtaining $\Delta\mu = 8.2\ \mathrm{D}f^{-1}$, which shows that the fit is consistent. The high $\Delta\mu$ indicates that the lowest exciton state has acquired a significant CT character or, in other words, the lowest exciton state is mixed with a CT state of the dimer. This mixing creates a vibronically broadened lowest excitonic state that couples strongly with phonons (protein lattice vibrations), resulting in a redistribution of oscillator

strength, an homogeneously broad absorption band, and an extreme red-shifted emission.

The second red absorption band, centered around 690 nm, has a value for the change in dipole moment that ranges from $\Delta\mu = 3.8$ to 6.8 Df^{-1}. This is most likely due to the spectral overlap and will not be further discussed here because it is out of the scope of this letter.

The change in dipole moment for the bulk Chls ranges from $\Delta\mu = 0.7$ to 1.1 Df^{-1}, which suggests that these are monomeric Chls. The broad band at the blue side of the spectra around 650 nm is necessary for the description of the absorption spectrum (it could account for the sum of the vibrational side bands of red and bulk Chls), although it does not contribute to the Stark spectrum ($\Delta\mu = 0.00$ to 0.04 Df^{-1}). The Chls *b* absorbing at 644.5 nm have a change in dipole moment from $\Delta\mu = 1.7$ to 1.8 Df^{-1}, which indicates that the Chls *b* behave differently from the bulk and red Chls.

We note that the mixing exciton-CT state and its strong coupling to phonons make the dimer an energetically very flexible system, highly dependent on protein vibrations and conformation. Thus, the protein could, by conformational changes (22), modulate the orientation and distance of the Chls forming the excitonically coupled dimer and the mixing with the CT state to switch between a system that transfers excitation energy to P700 and a system that dissipates excess excitation energy (photoprotective state).

In conclusion, we have demonstrated that the large bandwidth and the extreme red-shifted emission of the lowest energy form in the Lhca4 complex originates from the mixing of the lowest exciton state with a CT state of the excitonically coupled dimer.

SUPPORTING MATERIAL

Three figures and three tables are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(09)00207-0.

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