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Steady-state and time-resolved polarized light spectroscopy of the green plant light-harvesting complex II

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The major chlorophyll *a/b* light-harvesting complex from spinach thylakoid membranes was analyzed by steady-state polarized light spectroscopy at 4 K and by one-color and two-color pump-probe spectroscopy at room temperature. Steady-state absorption, linear dichroism and circular dichroism spectra indicate that the Chl $Q_{y(0-0)}$ absorption region is characterized by at least six transitions with significant differences in absorption, orientation and rotational strength. Steady-state low-temperature fluorescence spectra suggest that the fluorescence arises for a large part from several energetically similar species that form a circularly degenerate oscillator in the plane constituted by the two long axes of the particle. The possible presence of special red-absorbing pigments at low temperature is discussed. The time-resolved data suggest that the kinetics of chlorophyll $b \rightarrow a$ excitation energy transfer, as well as those of downhill excitation transfer among chlorophyll *a* spectral forms, are heterogeneous with both sub-picosecond and picosecond lifetime components.

Introduction

The major collector of solar energy in green plants and algae is the Chl *a/b* containing light-harvesting complex II (LHC-II). The complex serves as a peripheral antenna of Photosystem II and is organized as a trimer of largely homologous apoproteins with apparent molecular masses of 27 and 25 kDa [1]. The structure has been resolved at 6 Å resolution by electron crystallography [2], in which the positions and approximate orientations of the planes of the porphyrin rings of the 15 chlorophylls in every monomer were disclosed. All porphyrin rings were found to be oriented almost perpendicularly to the plane of the membrane, and arranged in two levels near the upper and lower surfaces of the thylakoid membrane. The shortest center-to-center distances ranged from 9–14 Å within one plane and from 13–14 Å between planes [2].

In view of the newly discovered structural data, it would be meaningful to develop more detailed descriptions of structure–function relationships in the Chl *a/b* antenna. New ideas about the phosphorylation mechanism have recently been presented [3]. More refined descriptions of the electronic spectroscopy and mechanisms of energy transfer would also be appropriate. For this purpose, we isolated LHC-II trimers in the presence of the non-ionic detergent *n*-dodecyl β-D-maltoside and reevaluated the spectroscopic properties by steady-state polarized light techniques at 77 K [4] and by one-color and two-color pump-probe techniques at room temperature [5]. The Chl *a/b* ratio in the preparations was found to be about 1.5, suggesting that each monomer contains 9 Chl *a* and 6 Chl *b* molecules. Some of the steady-state spectroscopic features differed from those observed with less mildly treated material, but showed a striking resemblance to those observed in intact chloroplasts, indicating that relatively native complexes have been prepared with dodecylmaltoside.

In this contribution, we will discuss some new spectroscopic features of LHC-II, obtained by steady-state polarized light techniques at 4 K and by recent one-color and two-color pump-probe techniques at room temperature [5].

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Abbreviations: CD, circular dichroism; Chl, chlorophyll; LD, linear dichroism; LHC-II, light-harvesting chlorophyll *a/b* complex from Photosystem II; *A*, absorbance; PS, Photosystem.

Steady-state spectroscopy

Trimeric LHC-II complexes were isolated in dodecyl maltoside as described in Ref. 5 and analyzed by steady-state spectroscopy as in Ref. 4, but at 4 K in a helium flow cryostat (Oxford). The 4 K absorption spectrum (Fig. 1B, dotted line) shows peaks at 675.5, 670.5 and 649 nm and shoulders near 661 and 639 nm. The most obvious difference with the 77 K spectra occurs around 670 nm, where at 4 K a clear peak is observed. The 4 K LD spectrum (Fig. 1A, full line) exhibits a very sharp peak at 676 nm in the Chl *a* $Q_{y(0-0)}$ absorption region. In the Chl *b* $Q_{y(0-0)}$ absorption region, negative peaks appear at 656 and 649 nm, and a positive peak appears at 641 nm. This indicates that the Chl *a* transitions at 676 and the Chl *b* transitions at 640 nm are at small angles with the plane constituted by the two long axes of the particle, and that the Chl *b* transitions at 656 and 649 nm are at angles clearly larger than 35° (the magic angle). The Chl *a* transitions at 670 nm do not show significant LD, suggesting an average orientation close to the magic angle.

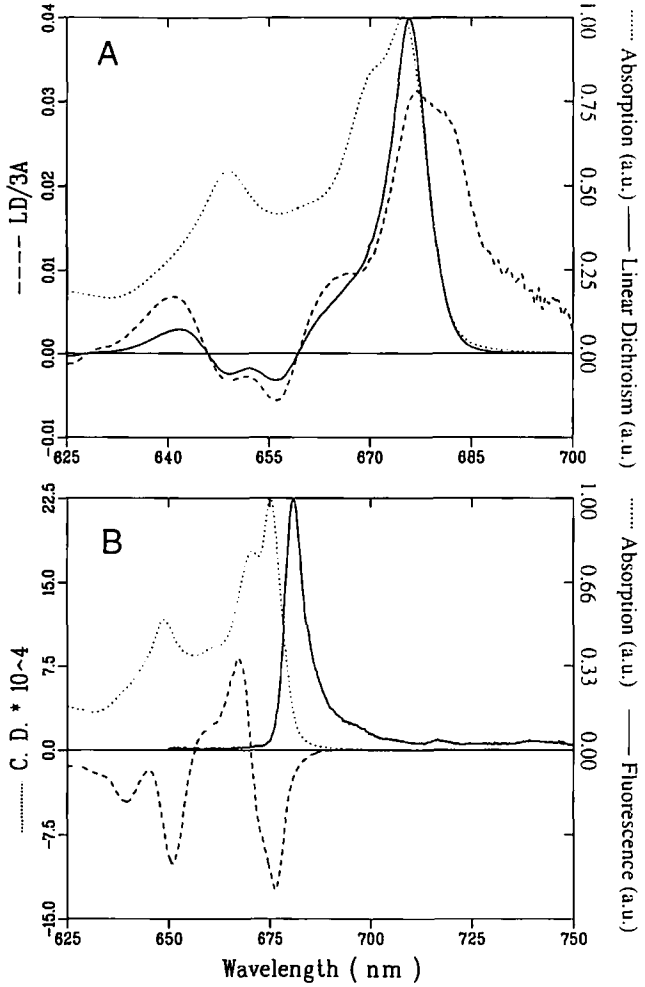

Fig. 1. (A) Full line: linear dichroism spectrum at 4 K of LHC-II, oriented in a two-dimensionally squeezed polyacrylamide gel, recorded with an optical bandwidth of 3 nm. Dotted line: absorption spectrum at 4 K of LHC-II, recorded with an optical bandwidth of 3 nm. Dashed line: reduced dichroism (LD/3*A*) spectrum of LHC-II at 4 K. (B) Full line: fluorescence spectrum of LHC-II at 4 K. Excitation wavelength: 436 nm, optical bandwidth: 1.5 nm. Dashed line: circular dichroism spectrum at 4 K of LHC-II, recorded with an optical bandwidth of 3 nm. Dotted line: absorption spectrum at 4 K of LHC-II, recorded with an optical bandwidth of 1 nm.

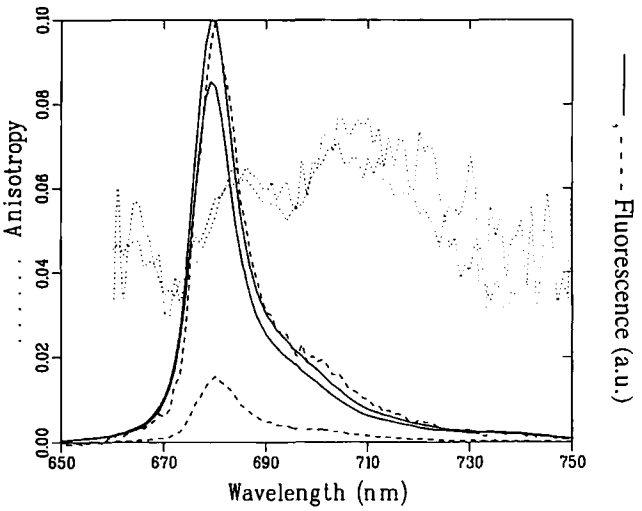

Fig. 2. Fluorescence emission spectra at 77 K of LHC-II, oriented as for linear dichroism measurements, recorded with an optical bandwidth of 3 nm. The excitation wavelength was at 610 nm and the intensities I_{vv}, I_{vh}, I_{hv} and I_{hh} were measured (the subscripts indicate the vertical or horizontal positions of the polarizers between excitation light and sample, and between sample and detector, respectively, see also Ref. 6). Full lines (upper and lower): $F_v \equiv I_{vv} + 2I_{hv}$ and $F_h \equiv I_{vh} + 2I_{hh}$ (respectively). Dashed lines (upper and lower): $F_v - F_h$ normalized to F_v and not normalized (respectively). Dotted lines: anisotropy $(F_v - F_h)/(F_v + 2F_h)$ determined in two independent experiments. The absorbance at 676 nm was 0.04.

The 4 K CD spectrum (Fig. 1B, dashed line) shows negative peaks at 676, 651 and 639 nm, positive peaks at 667 and 645 nm and shoulders near 660 and 673 nm. The latter shoulder was not resolved at 77 K [4]. These spectra suggest that in LHC-II a complicated set of excitonic interactions occurs between several Chl *a* and Chl *b* molecules, which is consistent with the relatively short nearest-neighbor separations of 9–14 Å between all Chl molecules in every monomer [2].

The 4 K emission spectrum consists of a narrow (fwhm 5.5 nm) transition peaking at 681 nm and a number of vibrational transitions at higher wavelengths (Fig. 1B, full line). The peak-wavelength is red-shifted by 1–2 nm compared to the spectra recorded at 77 K or at higher temperatures [4], suggesting that at 77 K more than one emitting species is present. This also becomes clear when comparing (Fig. 2) emission spectra detected with vertically or horizontally polarized light from macroscopically oriented particles [6]. The

difference spectrum $F_v - F_h$ (in fact the linear dichroism of the emitting species) is positive, as is expected for transitions oriented towards the plane constituted by the two long axes of the particle. This confirms the conclusion in Ref. 4 that the steady-state fluorescence arises for a large part from the Chl species absorbing at 676 nm, which are also oriented towards the plane of the particle (Fig. 1A, full line). The shape of the $F_v - F_h$ spectrum (Fig. 2, dashed lines), however, slightly differs from that of the F_v or F_h spectra, and the anisotropy (Fig. 2, dotted lines) is clearly lower on the blue side than on the red side of the main emission band. This confirms the composite nature of the 77 K fluorescence band.

The polarized excitation spectra reported in Ref. 4 have suggested that all Chl species transfer their energy very efficiently to the species responsible for the steady-state emission. In the Chl *b* absorption region, the polarized excitation spectrum showed fine structure that clearly resembled the LD spectrum. This was explained by assuming that the emitting species form a circularly degenerate oscillator in a plane, which is reflected similarly by the fluorescence polarization and the LD spectra. This suggests that the plane of the circularly degenerate oscillator is (almost) the same as the plane constituted by the two long axes of the particle. The observed low value of the polarization at 676 nm (approx. 0.1, Ref. 4) is in agreement with the idea of a circularly degenerate oscillator, provided that the energy transfer between the several Chl_{676} molecules is efficient. The gradual rise of the polarization upon raising the excitation wavelength [4] can in this context be explained by inhomogeneous broadening of the 676 nm transition and less efficient back-transfer once a relatively red-absorbing species is excited.

The orientations of the various bands in an inhomogeneously broadened spectrum should in principle be equal. Thus, if the red-most part of a spectrum is inhomogeneously broadened, the LD/*A* ratio in this part of the spectrum should be constant. Fig. 1A (dashed line) shows, however, that this ratio is only about constant between 676 and 683 nm, and that it drops considerably above 683 nm. This means that the average orientation of the transitions above 683 nm is less parallel to the plane constituted by the two long axes of the particle than of the 676 nm transitions. Consequently, most of the absorbance above 683 nm must be caused by transitions different from those peaking at 676 nm. It is, however, not likely that the features above 683 nm are caused by a special red-shifted pigment. The oscillator strength above 683 nm (at 4 K) is less than 1% of the combined Chl *a* bands (which we rather arbitrarily estimated as the total oscillator strength above 663 nm), i.e., considerably less than one red-shifted Chl *a* pigment in a trimer. Because the LD/*A* ratio above 683 nm resembles the ratio at 665–670 nm, it might be caused by broadening of the 670 nm transitions. We can only speculate about the mechanism for such a broadening. One of the possibilities to consider is lifetime-broadening, which would cause Lorentzian-shaped bands with sharp peaks and relatively pronounced tails. Preliminary calculations have suggested that a broadening of 670 nm transitions as observed in the 4 K absorption spectrum occurs with lifetimes in the range of some hundreds of femtoseconds. The lifetimes of the excited states of some of the Chl molecules absorbing at lower wavelengths might indeed be extremely short (see below).

Energy transfer

In a second approach, we recorded time-resolved absorption difference profiles using one-color and two-color pump-probe techniques. The work was performed at room temperature with equipment as described in Ref. 7 and will be described in detail elsewhere [5]. Here, we will restrict ourselves to summarize and discuss the most important conclusions.

The kinetics of Chl *b* → Chl *a* excitation transfer, as well as those of downhill excitation transfer among Chl *a* spectral forms, were found to proceed for a large part in the sub-picosecond time domain. This is illustrated by the results of the two-color pump-probe experiments shown in Fig. 3, where even at the shortest time (2.5 ps) a very significant fraction of the excitation

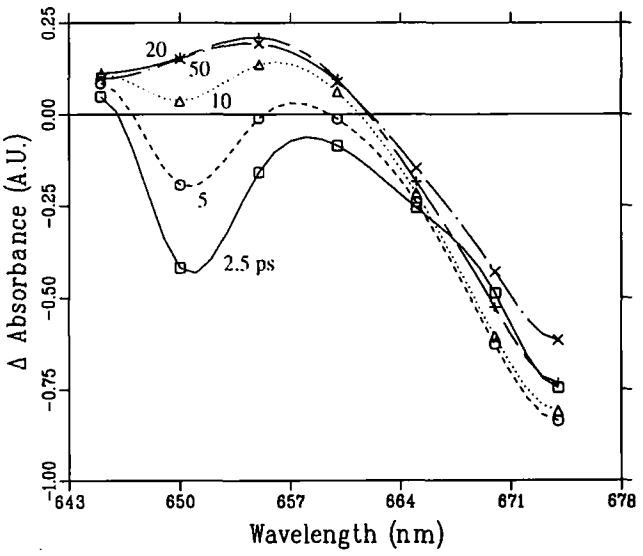

Fig. 3. Two-color difference absorption spectra of purified LHC-II complexes from spinach for fixed time delays, reconstructed from isotropic time-dependent profiles at seven wavelengths from 645 to 675 nm. The excitation wavelength was at 650 nm, which predominantly excited Chl *b*. The datapoints are the absorption at 2.5 ps (□), 5 ps (○), 10 ps (△), 20 ps (+) or 50 ps (×) after the pump pulses minus the absorption before the pump pulses. Adapted from Ref. 5.

density already resides on Chl *a* (as reflected by the intense bleaching around 675 nm) upon excitation of Chl *b*. This result confirms earlier conclusions of Eads et al. [8] on the basis of their fluorescence upconversion study. On the other hand, the results presented in Fig. 3 show that the ground state Chl *b* recovery at 650 nm also exhibits a picosecond component, which qualitatively confirms earlier one-color pump-probe experiments by Gillbro et al. [9]. Thus, our results confirm each of the two earlier seemingly contradictory conclusions and suggest that the Chl *b* → Chl *a* excitation transfer contains both sub-picosecond and picosecond lifetimes. Both groups of lifetimes were also observed in the one-color and two-color anisotropy decays [5]. The appearance of heterogeneous kinetics is in fact not unexpected, in view of the dispersion in the Chl–Chl separations and orientations [2]; the faster kinetics might, for instance, be dominated by equilibration of the excitation between the chlorophylls within one of the layers near the upper and lower surfaces of the thylakoid membrane, whereas the slower kinetics might be dominated by equilibration between layers.

In addition to the subpicosecond and 2–6 ps lifetime components, components with lifetimes of 14–36 ps and several hundreds of picoseconds were observed. The former group of lifetimes may arise from exciton equilibration between pigments of different monomers [5]; the latter remained unassigned. The observed long-time anisotropies in the one-color and two-color experiments [5] were qualitatively consistent with the steady-state polarized excitation and linear dichroism spectra [4].

Acknowledgments

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