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Chlorophyll dichroism of three-dimensional crystals of the light-harvesting chlorophyll *a*/*b*-protein complex

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Thin three-dimensional crystals of the light-harvesting chlorophyll *a*/*b*-protein complex from pea chloroplast membranes were investigated by microspectrophotometry. Measurements of tilted crystals revealed pronounced linear dichroism in a direction perpendicular to the crystal plane but no observable dichroism in the crystal plane. Q_y transition moments of chlorophylls were rotationally isotropic with respect to the crystal plane and partially aligned in this plane. Q_x transition moments showed no preferential orientation and appeared to be wholly isotropic. The orientation of transition moments in the crystal was consistent with three-fold symmetry of the light-harvesting complex indicated by the crystal structure. The linear dichroism of the crystals reflected the optical properties of the complex in the photosynthetic membrane, indicating a highly symmetrical arrangement of antenna chlorophylls.

Chlorophyll-protein complex; Membrane protein crystal; Light-harvesting complex; Microspectrophotometry; Linear dichroism

1. INTRODUCTION

The light-harvesting chlorophyll *a*/*b*-protein complex (LHC II) is the most abundant membrane protein involved in plant photosynthesis, binding and organizing roughly 50% of all chlorophyll in green plants [1]. The complex is an integral membrane protein found primarily in chloroplast grana. The LHC II apoprotein consists of several polypeptides of similar molecular mass [2,3] and amino acid composition [4,5]. The major polypeptide of pea LHC II has a molecular mass of 25 kDa [2,6,7]. Each LHC II polypeptide binds a total of 15 chlorophyll (Chl) molecules [5,8] and 2–4 carotenoids [9,10]. The ratio of Chl *a*/Chl *b* of crystalline LHC II is 1.15 [11], indicating 8 Chl *a* and 7 Chl *b* per monomer.

LHC II can be induced to form two-dimensional [7,12] and three-dimensional crystals in the shape of thin hexagonal plates or octahedra [11]. The three-dimensional structure of two-dimensional LHC II crystals at 16 Å resolution has been solved by electron microscopy and image analysis [12]. The projected structure of hexagonal plates used in this study which consist of stacked two-dimensional crystals has been determined at 7 Å resolution by electron microscopy, image analysis and electron diffraction [13]. Structure analysis has shown that LHC II is a trimer and has three-fold symmetry [12,13]. Analytical ultracentrifugation revealed that LHC II is a trimer also in detergent solution [5].

We investigated the linear dichroism of thin hexagonal plates of LCH II using a microspectrophotometer that allows small protein crystals to

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be rotated and tilted. Our findings are wholly consistent with the results of the structure analysis and provide new insights into the orientation of chlorophylls in the complex.

2. MATERIALS AND METHODS

2.1. *Crystallisation*

Thin hexagonal plates of LHC II were grown from detergent solution by vapour diffusion as described [11]. Crystals suitable for microspectophotometry measured approx. 100 μm across and were 2 to 3 μm thick, as estimated by light microscopy. They grew within 4–5 days and were stable for several weeks.

2.2. *Spectroscopic methods*

Absorption spectra of crystals were measured using a single beam microspectrophotometer in which protein crystals mounted in a microcell could be rotated through 360° and tilted by ± 35°. A detailed description of the apparatus has been reported elsewhere [14]. A drop of mother liquor containing thin hexagonal plates of LHC II was placed on a microscope cover slide with a 10 μm spacer foil and sandwiched with a second cover slide. At these dimensions of the microcell, a thin crystal of roughly 100 μm diameter was parallel to the face of the cell to within ± 2°. Spectra were recorded as described [15] at wavelengths ranging from 550 to 750 nm or at the absorbance maximum of the Chl *a* Q_y band, 670 nm. The slit width was 0.5 mm, corresponding to a spectral band width of 3 nm. All measurements were made at room temperature.

Measurements of linear dichroism (LD) in the plane of the crystals (*x,y* plane, see fig.1) were performed by rotating the polarisation vector of the incident light in steps of 15° through an arc of 180°. The intensity of the transmitted radiation was recorded and absorption spectra were generated by forming $-\log I/I_o$ where I_o was the intensity transmitted by an area adjacent to the crystal.

LD in a direction perpendicular to the crystal plane was obtained from a series of spectra (or absorption measurements at 670 nm) in steps of 5° from −35° to +35°. Four sets of data were recorded at each tilt angle: one spectrum (or one peak intensity at 670 nm) of the crystal and of an adjacent reference area each, with the polarisation vector of the incident beam first parallel and then perpendicular to the tilt axis. Tilt series were recorded with the crystal tilted around the *x* axis as in fig.1, or with the crystal rotated by 90° in the *x,y* plane. The spectrum (or the absorbance at 670 nm) was calculated for each setting and normalised to the absorption spectrum of the untilted crystal using the parallel polarised spectrum as a reference to compensate for the increase in path length due to specimen tilt [15]. The reduced linear dichroism (LD/*A*, where *A*, the absorbance of untilted crystals, was equal to 1) in the *z* direction was determined by plotting the observed differences of absorbance for each tilt angle against $\sin^2\alpha$ and extrapolating to 90° tilt angle by linear least squares regression.

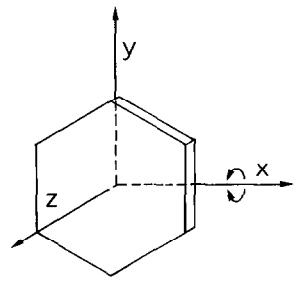

Fig.1. Schematic diagram of a hexagonal crystal of LHC II showing the orientation of crystal axes for microspectrophotometry.

3. RESULTS

Single crystals selected for microspectrophotometry had an absorbance at 670 nm of roughly 1.5. In the Soret region of the spectrum (400–480 nm), the absorbance was about 3, too high to record differences in absorbance with the required accuracy. With our apparatus, the maximum absorbance for which reliable dichroism measurements could be made was limited to 2.5 due to light scattered and diffracted at the edges of the crystal. The area of crystals that were thinner than the ones selected tended to be too small. LD measurements were therefore restricted to the chlorophyll Q absorption bands in the 550–700 nm range. The absorption spectrum of untilted hexagonal crystals (fig.2) was very similar to spectra of octahedral crystals (not shown) and

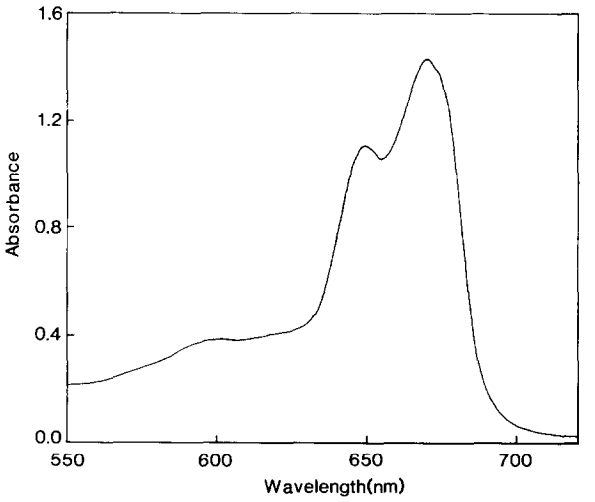

Fig.2. Absorption spectrum of an untilted hexagonal crystal of LHC II. The Chl *a* and Chl *b* Q_y bands at 670 nm and 652 nm, respectively, are well resolved. The broad band between 560 nm and 640 nm is largely due to Chl *a* and Chl *b* Q_x transitions.

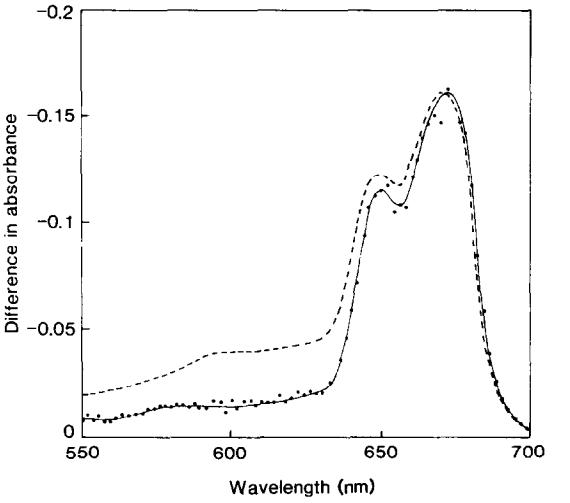

Fig.3. Difference spectrum between the untilted hexagonal LHC II crystal and the crystal tilted by ± 30° (——). Differences in absorbance between the tilted crystal and the normalized spectrum of the untilted crystal are indicated for each wavelength (●). The spectrum of an untilted crystal, scaled to the maximal difference at 670 nm (– – –) is shown superimposed on the difference spectrum.

of the complex in detergent solution [8]. The characteristic Q_y bands of Chl *a* at 670 nm and of Chl *b* at 652 nm were more highly resolved than in the solution spectrum. A broad absorption band between 560 nm and 640 nm represented the Q_x transitions of Chl *a* and Chl *b* [16,17]. The Soret bands of Chl *a* at 436 nm and Chl *b* at 474 nm [8] as well as the carotenoid shoulder at 487 nm [10] were visible, although poorly resolved, due to the high absorbance of the crystals at these wavelengths.

Rotating the polarisation vector of the incident light had no effect on the observed absorption spectrum for untilted crystals, indicating an absence of LD in the *x,y* plane. However, difference spectra, obtained by subtracting perpendicularly polarized spectra of tilted crystals from the normalized spectrum of an untilted crystal, revealed significant LD in a direction perpendicular to the *x,y* plane. This was evident from a decrease in intensity of the Chl *a* and Chl *b* Q_y bands in the spectra of tilted crystals. The decrease of both bands was proportional to $\sin^2\alpha$, where α is the absolute tilt angle, but did not depend on the choice of tilt axis in the *x,y* plane. A typical difference spectrum for a tilt angle of ± 30° is shown in fig.3. Comparison with the spectrum of the untilted crystal (fig.2) indicates that the Q_y bands of Chl *a* and Chl *b* were diminished roughly in the same proportion. Data from four tilt series were combined and plotted against $\sin^2\alpha$. The reduced

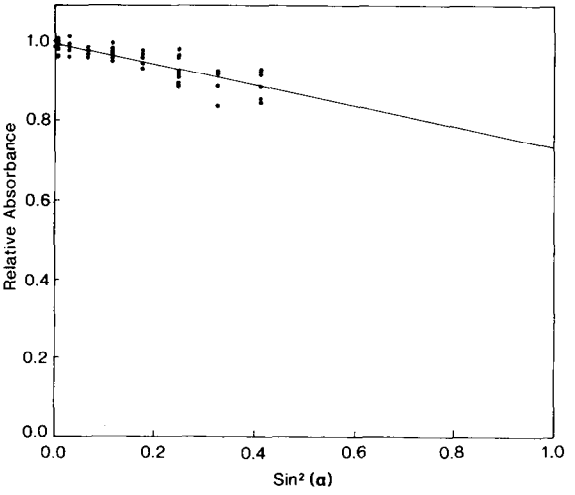

Fig.4. Linear dichroism of hexagonal plates of LHC II as a function of tilt angle α. Tilt series were recorded to ± 35°. Extrapolation to 90° gave a reduced dichroism of 0.26 ± 0.05.

dichroism in the z direction was found to be 0.26 ± 0.05 by extrapolation to 90° tilt angle.

In contrast, tilted and untilted crystals absorbed equally well in the Q_x region of the spectrum (560–630 nm). The absence of differences in this region suggested that the Q_x transition dipoles had no appreciable dichroism in the z direction.

4. DISCUSSION

In a crystal, the components of the transition dipole moments of all molecules of one pigment type add up to three mutually perpendicular vectors which define an ellipsoid (Welte, W., personal communication). The relative length of these vectors and their orientation relative to the morphological crystal axes (fig.1) can be determined by LD measurement. In the case of hexagonal plates of LHC II, a simple relationship exists between these axes and the axes of the transition dipole ellipsoid of the Chl *a* and Chl *b* Q_y transitions. The absence of dichroism on the *x,y* plane indicates that two axes of the ellipsoid are equal and lie in this plane. LD in a direction perpendicular to the *x,y* plane indicates that the third axis is shorter. The net transition dipole moments of the Chl Q_y bands can be thought of as defining an oblate rotational ellipsoid, the axes of which coincide with the crystal axes. The Q_y transition dipole moments of Chl *a* and Chl *b* molecules in the light-harvesting complex are thus orientated preferentially in the *x,y* plane of the crystal. The Q_x transition dipole moments, on the other hand, show no evidence of such orientation and hence seem to be wholly isotropic. The LD of LHC II carotenoids could not be measured. The orientation of carotenoids in the crystals is therefore unknown.

The structure analysis by electron diffraction, electron microscopy and image processing indicated that the hexagonal plates consist of layers of two-dimensional crystals. The projected structure of hexagonal plates at 7 Å resolution [13] and the three-dimensional structure of negatively stained two-dimensional crystals of LHC II at 16 Å resolution [11] has shown that the complex is a trimer, composed of three monomers related by three-fold rotation. The symmetry of the two-dimensional crystals was that of the two-sided plane group p321, corresponding to the three-dimensional space group P321, with a three-fold axis perpendicular to the *x,y* plane of the crystal. A three-fold axis in this direction is consistent with rotationally isotropic Chl transition moments in the *x,y* plane.

Since the hexagonal plates are stacks of two-dimensional crystals, and since the *x,y* plane of the hexagonal plates coincides with the plane of two-dimensional crystals, our observations can be related directly to individual molecules of LHC II. Membrane proteins in two-dimensional crystals orient themselves such that their hydrophilic segments protrude on either side from the continuous, two-dimensional lipid or detergent phase. The orientation of LHC II in the two-dimensional crystals and in the hexagonal plates therefore is the same as in the native membrane. Since LHC II is a trimer not only in the crystalline state but also in detergent solution [5] and since efficient intramolecular energy transfer between Chl molecules depends on the complex having three-fold symmetry [18], it seems very likely that it exists as a trimer in vivo and that, therefore, the optical properties measured in this study are those of the complex in the chloroplast thylakoid membrane. We conclude that the Q_y transition dipole moments of Chl *a* and Chl *b* (and therefore the *y* axes of Chl porphyrins) in the complex in vivo are partially oriented in the plane of the thylakoid membrane and are most efficient in absorbing light at right angles to this plane. The Q_x transition dipoles show no such orientation and absorb light isotropically. As one LHC II trimer contains 45 Chl molecules, one would expect Q_y as well as Q_x transition dipole moments to be more or less randomly oriented. The observation of 25% dichroism relative to the untilted crystal in a direction perpendicular to the membrane plane represents a significant degree of order.

A trimer having three-fold symmetry is the simplest, most economical macromolecular assembly to function as a rotationally isotropic antenna of light energy. It is interesting to note in this context that the crystal structures of two other, non-membrane photosynthetic antenna complexes, the bacteriochlorophyll *a* protein-complex of *Prostecochloris aestuarii* [19] and the *c*-phycocyanin of *Mastigocladus laminosus* [20], have three-fold symmetry, probably for the same reason. Three-fold symmetry has also been ob-

served in the photosystem I reaction centre, a membrane Chl-protein complex from the cyanobacterium *Synechococcus* [21]. In the case of LHC II, the partial alignment of Q_y transition moments in the membrane plane may represent an added advantage, presumably because, on average, more light reaches the LHC II in a direction perpendicular to the membrane plane due to the proximity of other Chl protein complexes in the same membrane.

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