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EXCITATION ENERGY TRANSFER IN THE LIGHT-HARVESTING CHLOROPHYLL *a*/*b* · PROTEIN

RICHARD L. VAN METTER*

Department of Physics and Astronomy, University of Rochester, Rochester, N.Y. 14627 (U.S.A.)

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SUMMARY

The "light-harvesting chlorophyll *a*/*b* · protein" described by Thornber has been prepared electrophoretically from spinach chloroplasts. The optical properties relevant to energy transfer have been measured in the red region (i.e. 600–700 nm). Measurements of the absorption spectrum, fluorescence excitation spectrum and excitation dependence of the fluorescence emission spectrum of this protein confirm that energy transfer from chlorophyll *b* to chlorophyll *a* is highly efficient, as is the case in concentrated chlorophyll solutions and in vivo. The excitation dependence of the fluorescence polarization shows a minimum polarization of 1.9 % at 650 nm which is the absorption maximum of chlorophyll *b* in the protein and rises steadily to a maximum value of 13.8 % at 695 nm, the red edge of the chlorophyll *a* absorption band. Analysis of these measurements shows that at least two unresolved components must be responsible for the chlorophyll *a* absorption maximum. Comparison of polarization measurements with those observed in vivo shows that most of the depolarization observed in vivo can take place within a single protein. Circular dichroism measurements show a doublet structure in the chlorophyll *b* absorption band which suggests an exciton splitting not resolved in absorption. Analysis of these data yields information about the relative orientation of the $S_0 \rightarrow S_1$ transition moments of the chlorophyll molecules within the protein.

INTRODUCTION

In an effort to understand better the structure and dynamics of the photosynthetic unit numerous workers have for over a decade extracted detergent solublized pigment protein complexes from photosynthetic membranes (for recent reviews see Thornber [1, 2]). In recent years two well characterized pigment protein complexes have emerged; CPI or *P*-700-chlorophyll *a* · protein which contains the heart of Photosystem I and CPII or light-harvesting chlorophyll *a*/*b* · protein which shows no photochemical activity and is believed to serve primarily as the antenna for Photo-

Abbreviation: CPII, light-harvesting chlorophyll *a*/*b* · protein; CPI, *P*-700-chlorophyll *a* · protein.

* Present address: Eastman Kodak Research Laboratories, 2000 Lake Avenue, Rochester, N.Y. 14650, U.S.A.

system II. This work will be limited to a consideration of CPII which has been isolated from chlorophyll *b* containing higher plants and algae by Thornber and coworkers [3–5] as well as other workers [6–8]. It has been reported to contain three molecules of chlorophyll *a*, three molecules of chlorophyll *b* and one molecule of carotenoid per protein molecule having a total molecular weight of 35 000 daltons [3]. The optical properties of CPII which have been reported include: (1) the absorption spectrum [3, 4, 6, 8–12], (2) the fluorescence emission spectrum (for ref. see 1 and 2) and (3) the circular dichroism spectrum [10, 13].

The existing data are not adequate because they provide little information about the detailed structure of the protein, in particular the orientation and placement of chromophores, or its possible organization and orientation within the lamellae. The structure of a bacteriochlorophyll protein from green algae has been determined by Fenna and Matthews [14, 15] using X-ray crystallography but no similar studies have been reported for CPII or CPI. The goal of this work is to extend the present understanding of CPII to include the orientation of the $S_0 \rightarrow S_1$ transition moments of the pigment molecules within the protein, and to reexamine the interaction among these pigments and between the pigments and protein. This information provides an important insight into the structure and dynamics of the photosynthetic unit by characterizing a component of the photosynthetic unit which is intermediate in size and complexity between monomeric chlorophyll solutions and intact photosynthetic membranes which have both been studied extensively.

In Materials and Methods we describe the preparation of CPII and the optical instruments used in these studies including a newly constructed fluorescence polarization instrument capable of measuring excitation, excitation polarization and emission polarization spectra. Following this the absorption, circular dichroism, fluorescence excitation, fluorescence emission, excitation fluorescence polarization and emission fluorescence polarization spectra are presented. The next section contains an analysis of the data together with an extension of the presently available theories of fluorescence depolarization to the general case of an arbitrary number of absorbing and emitting transition moments in the limit of complete incoherent energy transfer. This theory enables a detailed analysis of the fluorescence polarization data. The main results are then summarized including a brief discussion of their implications.

MATERIALS AND METHODS

Chloroplast preparation

Spinach leaves purchased at a local market were washed, deveined and then homogenized in a minimal amount of chilled buffer (50 mM Tris · HCl, pH 8.0) using a Waring Blendor. The resulting homogenate was filtered through four layers of cheese cloth, then centrifuged at $1000 \times g$ for 1 min and the supernatant retained. Centrifugation at $40\,000 \times g$ for 20 min yielded a pellet which was washed with buffer and recentrifuged at $40\,000 \times g$ for 20 min. The final pellet was resuspended in a minimal amount of 50 mM Tris · HCl, 5 mM $MgCl_2$, 20 mM sodium ascorbate, pH 8.0 and when necessary stored below 0 °C.

Isolation of light-harvesting chlorophyll a/b · protein

Spinach chloroplasts were solubilized by stirring for 30 min at 4 °C in 50 mM

Tris · HCl, 5 mM $MgCl_2$, 1 % sodium dodecyl sulfate, pH 8.0 so that sodium dodecyl sulfate/chlorophyll = 10/1 (w/w). This solution was then centrifuged at 27 000 × *g* for 10 min and the supernatant either used immediately or stored below 0 °C. Polyacrylamide gels were prepared using Electrophoresis Purity Acrylamide and *N,N'*-methylene-bis-acrylamide available from Biorad Laboratories and were made to a final concentration of 7.5 % acrylamide, 0.125 % *N,N'*-methylene-bis-acrylamide, 0.125 % amonium persulfate, 0.05 % *N,N,N',N''*-tetramethylethylenediamine, 50 mM Tris · HCl, 0.125 % sodium dodecyl sulfate, pH 8.0. Gels 5 mm in diameter and 11 cm long were preelectrophoresed for 15 min at 120 V using a running buffer of 50 mM Tris · HCl, 0.125 % sodium dodecyl sulfate pH 8.0. The supernatant was then applied to the gels such that in gels to be used for fluorescence measurements less than 1 μg of chlorophyll was applied to each gel. Electrophoresis for 90 min at 120 V with the temperature maintained at 2 °C yielded three distinct green bands in the gels the second of which is the light-harvesting chlorophyll *a/b* · protein described by Thornber [1, 2]. When desired, gels were removed from the support tubes, sectioned, the CPII containing section reinserted into shorter tubes of identical inside diameter which were filled with running buffer and sealed with dialysis membrane. The protein was then electrophoresed into free solution and used immediately. Temperatures were maintained below 2 °C during this entire procedure.

Absorption

All absorption measurements were made using a Perkin-Elmer Coleman 124 double beam spectrophotometer. When measurements were made on gel sections specially constructed holders were employed. Otherwise standard 1 cm quartz cuvettes were used.

Chlorophyll a to chlorophyll b ratio

The method of Arnon [16] was used to determine [chlorophyll *a*]/[chlorophyll *b*] in 80 % acetone extracts of CPII which had been electrophoresed into free solution as described above. Absorption measurements were made as described above.

Circular dichroism

Circular dichroism measurements were made using a Jasco model J-40 circular dichroism spectrophotometer on freshly prepared CPII samples in free solution. These measurements were made at room temperature.

Fluorescence and fluorescence polarization

An instrument to measure fluorescence and fluorescence polarization was constructed by the author [17]. Intensities were measured using photon counting techniques and polarizations were obtained as described by Parker [18]. The total fluorescence intensity, properly corrected for the polarization of the sample was found using an expression given by Weber [19] and was corrected for the wavelength dependence of the exciting light in relative quantum yield measurements. The linearity of the instrument was verified using neutral density filters and the wavelength calibration of the monochromators was checked with the 6328 Å line of a helium neon laser. The polarization of chlorophyll *a* in castor oil was measured and found to agree with mea-

surements reported by Gouterman and Stryer [20] verifying the calibration and alignment of the instrument. In all measurements narrow band filters were used to check for the presence of polarization artifacts due to scattered light as described by Whitmarsh and Levine [21].

RESULTS

Absorption spectrum

The absorption spectrum of light-harvesting chlorophyll *a*/*b* · protein measured at room temperature in polyacrylamide gel is presented in Fig. 1a. It shows absorption maxima in the red at 672 nm and 652 nm which can be attributed to chlorophyll *a* and chlorophyll *b* respectively. In the blue region distinct peaks appear at 438 nm and 471 nm which correspond to the Soret absorption bands of chlorophyll *a* and chlorophyll *b*, respectively, and a shoulder near 475 nm which has been tentatively attributed

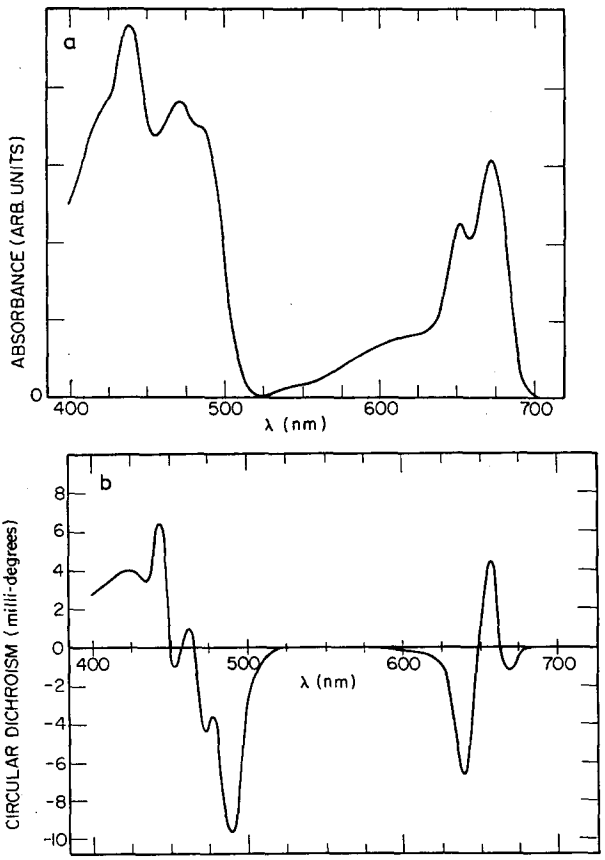

Fig. 1. (a) The absorption spectrum of CPII taken in a freshly prepared polyacrylamide gel at room temperature. (b) Circular dichroism spectrum of CPII which has been electrophoretically removed from a polyacrylamide gel. The absorbance at the 652 nm chlorophyll *b* absorption maxima was $A = 0.25$.

to the presence of carotenoid [1, 2]. This spectrum is in good agreement with those previously reported for chromatographically [3, 4, 8, 11] and electrophoretically [9, 22] prepared light-harvesting chlorophyll *a*/*b* · protein. The greatest differences between this and previously reported spectra are a larger ratio of absorption at 672 nm to that at 652 nm in this spectrum, as well as a more pronounced shoulder at 475 nm. These differences are generally small and may reflect differences in the purity or condition of the samples used or variations among the different species used in some cases. The results obtained by computer deconvolution of the absorption spectrum using the gaussian components proposed by French et al. [24, 25] and used recently to analyze the low temperature absorption spectrum of CPII [12] are shown in Fig. 2. There are only two prominent components in the chlorophyll *a* region at 670 nm and 677 nm which appear in a ratio of about 2 : 1. In the absence of corresponding circular dichroism components (see below) this suggests that two chlorophyll molecules absorb at 670 nm and one at 677 nm in the protein, a conclusion which will be further supported by analysis of the fluorescence polarization data in Theory and Analysis.

The ratio of chlorophyll *a* to chlorophyll *b* was measured using samples which had been electrophoretically removed from the polyacrylamide gels. Using the method of Arnon [16] [chlorophyll *a*]/[chlorophyll *b*] was measured to be 1.07 ± 0.06 in the samples studied. This is in general agreement with that reported by other workers [1–3, 23] and in good agreement with the assertion that each protein contains three chlorophyll *a* and three chlorophyll *b* molecules [1–3].

Circular dichroism

The circular dichroism spectrum of light-harvesting chlorophyll *a*/*b* · protein prepared electrophoretically as described above is shown in Fig. 1b. Similar spectra have been observed in chromatographically prepared samples. The results in the red region of the spectra consist of a pronounced doublet structure centered at the absorption maximum of chlorophyll *b*. This is similar but not identical to the observations of other workers [10, 13], which circumstance leads to an important difference in interpretation. The doublet structure centered about 648 nm resembles that which is observed in strongly coupled dimers [26, 27] and is evidence that at least two of the chlorophyll *b* molecules in the protein are strongly coupled. In the Soret region of chlorophyll *b* there is additional complex structure, however, no significant structure is centered about either of the chlorophyll *a* absorption maxima. The data suggest that the shoulder observed near 475 nm may be partly due to exciton components of chlorophyll *b* as well as carotenoid. Neither chlorophyll *a* nor chlorophyll *b* show structure similar to these observations in dilute solutions of polar solvents [13, 26] but rather show small circular dichroism of a single sign within any given absorption band. The present data are most easily explained by assuming that the chlorophyll *b* molecules in the protein are in relatively close proximity therefore exhibiting strong exciton coupling while the chlorophyll *a* molecules are probably not strongly coupled. A comparison of the circular dichroism observed in chloroplast fragments with that observed in the chlorophyll · protein complexes extracted from them [13] has shown that significant changes occur upon extraction. It is not yet clear whether or not this indicates denaturation of CPII by the extraction process, a consideration which is of obvious importance to the work reported here.

Fluorescence excitation and emission spectrum

Fig. 2 shows the absorption and fluorescence excitation spectrum of CPII. In addition the relative quantum yield as a function of wavelength is shown. The quantum yield is seen to be independent, within experimental error, of the wavelength of excitation over a range which includes excitation into both chlorophyll *a* and chlorophyll *b*. This is a clear indication that the energy transfer between chlorophyll *b* and chlorophyll *a* is occurring at a rate sufficient to allow equilibration of the excitation energy among all emitting chromophores within the complex. This is certainly a minimal requirement for the complex to be functioning as in vivo, where chlorophyll *b* to chlorophyll *a* energy transfer has been long established [26]. The efficiency of

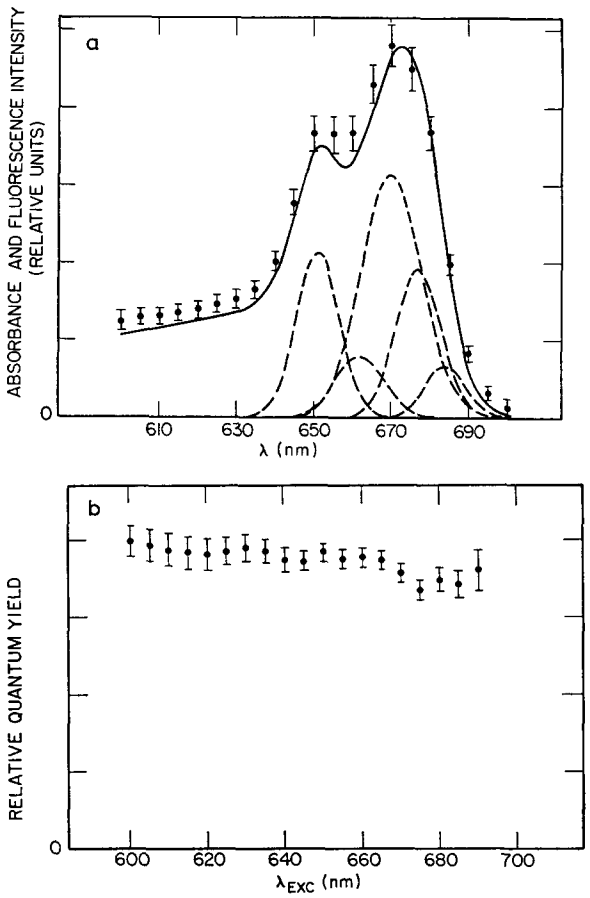

Fig. 2. (a) The absorbance of CPII (—) shown in comparison with the fluorescence excitation spectrum (●) corrected as described in Materials and Methods. The fluorescence emission was viewed at 730 nm with a spectral width of ±14 nm. Gaussian components (- - - - -) obtained by computer deconvolution of the absorption spectrum from 640 nm to 690 nm assuming the four universal forms of chlorophyll *a* [12] at 662 nm, 670 nm, 677 nm and 684 nm, a chlorophyll *b* component at 651 nm and a broad component at 630 nm (not shown). (b) The relative quantum yield of CPII as obtained from the data in part (a). The fluorescence measurements were made with the sample temperature maintained at approx. 2 °C.

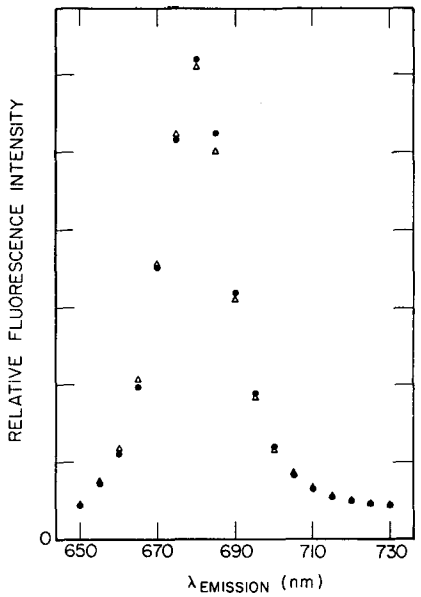

Fig. 3. The emission spectrum of CPII excited at the chlorophyll *a* absorption maxima at 436 nm (△) and the chlorophyll *b* absorption maxima at 470 nm (●). The excitation bandwidth was 6.3 nm in both cases. The typical tolerance in these data is ±2 % based on the reproducibility of individual measurements. The sample temperature was maintained at approx. 2 °C.

chlorophyll *b* to chlorophyll *a* transfer is also demonstrated by measuring the fluorescence emission spectrum when exciting primarily chlorophyll *b* or chlorophyll *a*. Uncorrected emission spectra for excitation at 470 (chlorophyll *b*) and 436 nm (chlorophyll *a*) are shown in Fig. 3. To within experimental error the emission in these two cases is identical, indicating complete equilibration of the excitation energy over the emitting molecules before emission.

Fluorescence polarization spectrum

The excitation wavelength dependence of the fluorescence polarization when emission is viewed at 730 nm is shown in Fig. 4. Similar spectra were observed when the emission was viewed at 712 nm and 696 nm but in these cases the excitation wavelength was restricted to wavelengths below 680 nm and 670 nm respectively to avoid possible scattering artifacts. The essential features of these spectra are:

1. A polarization of 1.9 % at 650 nm which indicates nearly complete depolarization of the fluorescence sensitized by the $S_0 \rightarrow S_1$ transitions of chlorophyll *b*.

2. The limiting value of polarization is not reached at the absorption maxima of chlorophyll *a*, but instead the polarization rises monotonically throughout this entire absorption band only reaching a limiting value of 13.8 % near 700 nm.

The first feature can be most easily understood by assuming that the absorption dipole transition moments of the chlorophyll *b* possess a nearly spherical symmetry [29]. Since the circular dichroism spectra indicate a strong interaction among the chlorophyll *b* molecules, it seems reasonable to expect the transition moments to be those in the exciton representation [30]. The second feature is only compatible with

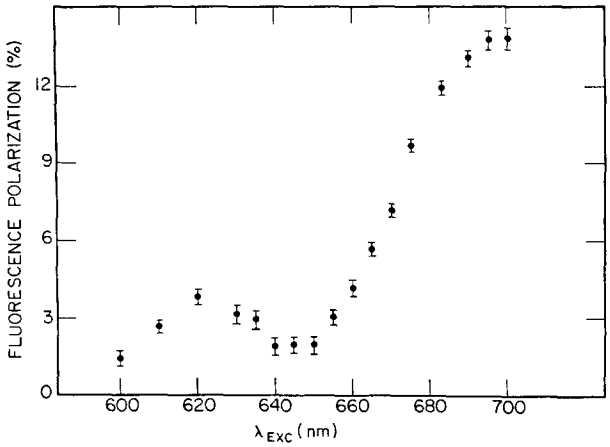

Fig. 4. The excitation wavelength dependence of the fluorescence polarization of CPII. The emission was viewed at 730 nm with a spectral width of ±14 nm. The spectral width of the exciting light was ±5 nm and the sample temperature was maintained at approx. 2 °C.

the assumption that there are at least two different spectral components in the chlorophyll *a* absorption region. This is consistent with computer deconvolutions of the absorption spectrum of CPII at room [17] and low temperatures [12] which show at least two major gaussian components at 670 nm and 677 nm as well as other minor chlorophyll *a* components. In Theory and Analysis the wavelength dependence of the fluorescence polarization will be shown to be analyzable in terms of two chlorophyll *a* molecules absorbing at 670 nm and one at 677 nm as suggested by the absorption spectra. Polarization in the region from 600 to 640 nm is due to absorption into the vibrationally excited Q_y and Q_x states of chlorophyll *a* and chlorophyll *b* and will therefore not be analyzed in detail. In these measurements the possibility of scattering artifacts has been ruled out by the addition of narrow band filters as described by Whitmarsh [21] and the depolarization due to rotation during the fluorescence lifetime can be shown to be smaller than the error bars in all measurements [17].

Small values of the fluorescence polarization measured in vivo have been interpreted in terms of a large range of excitation energy migration [21, 31]. A comparison of the fluorescence polarization measured in isolated CPII with measurements made in vivo show that most or all of the depolarization observed in vivo can take place within a single protein. The dependence of the fluorescence polarization on the state of the reaction center of Photosystem II observed in vivo is therefore not easily explained in terms of the range of exciton migration among only CPII molecules. An alternative explanation is that such observations indicate a change in the fluorescence yield of highly ordered chlorophyll *a* molecules close to the reaction center.

Fig. 5 shows the emission wavelength dependence of the fluorescence polarization observed when exciting at 470 nm. The wavelength dependence gives a clear indication that the fluorescence is being emitted in more than one gaussian spectral component of the protein, since the emission wavelength dependence of fluorescence polarization of monomeric chlorophyll *b* and chlorophyll *a* are known to be slowly monotonically descreasing functions of wavelength [17]. Consistent with our earlier assumption that the chlorophyll *b* transition moments are nearly spherically symmetric

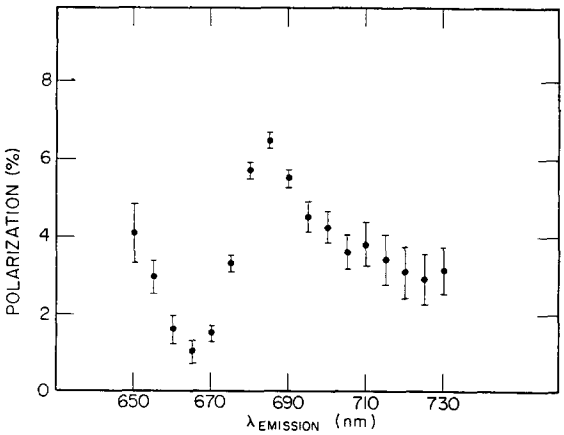

Fig. 5. The emission wavelength dependence of the fluorescence polarization. Excitation wavelength is 470 nm with a spectral width of ±6.3 nm. The emission spectral width was ±5 nm and the sample was maintained at approx. 2 °C.

is the observation that the emission polarization becomes nearly zero at an emission wavelength of 665 nm which is where chlorophyll *b* is expected to emit. Qualitatively similar spectra are observed when exciting at 436 nm (chlorophyll *a*) or 630 nm.

THEORY AND ANALYSIS

Circular dichroism

A general theory of the exciton contribution to the optical rotation of polymers has been given by Tinoco [32]. The dipole strength, rotational strength and transition frequency for each exciton state are given as

$$D_K = \boldsymbol{\mu}_K \cdot \boldsymbol{\mu}_K = \left(\sum_{j=1}^{N} C_{jK}\boldsymbol{\mu}_j\right) \cdot \left(\sum_{j=1}^{N} C_{jK}^{*}\boldsymbol{\mu}_j\right) \tag{1}$$

$$R_K = Im(\boldsymbol{\mu}_K \cdot \boldsymbol{m}_K) = Im\left(\sum_{j=1}^{N} C_{jK}\boldsymbol{\mu}_j\right) \cdot \left\{\sum_{j=1}^{N} C_{jK}^{*}\left[\left(\frac{\pi\nu_0 i}{c}\right)(\boldsymbol{R}_j \times \boldsymbol{\mu}_j) + \boldsymbol{m}_j\right]\right\} \tag{2}$$

$$\nu_K = \nu_0 + \sum_{i=1}^{N}\sum_{j=1}^{N} C_{iK}C_{jK}^{*}V_{ij}/h \tag{3}$$

in terms of the monomer electric dipole transition moment $\boldsymbol{\mu}_j$, magnetic dipole transition moment $\boldsymbol{m}_j$ and the coefficients of the states which diagonalize the interaction Hamiltonian V_{ij} taken to be

$$V_{ij} = \frac{1}{|\boldsymbol{R}_{ij}|^3}\left\{\boldsymbol{\mu}_i \cdot \boldsymbol{\mu}_j - 3\frac{(\boldsymbol{\mu}_i \cdot \boldsymbol{R}_{ij})(\boldsymbol{\mu}_j \cdot \boldsymbol{R}_{ij})}{|\boldsymbol{R}_{ij}|^2}\right\} \tag{4}$$

where $\boldsymbol{R}_{ij}$ is the displacement vector between two monomers. Since the $S_0 \rightarrow S_1$ transition moments of the chlorophylls have been shown to be predominantly electric dipole in nature [29], Eqn. 2 simplifies to

$$R_K = \frac{\pi \nu_0}{c} \left(\sum_{j=1}^{N} C_{jK} \mu_j \right) \cdot \left(\sum_{j=1}^{N} C_{jK}^* [R_j \times \mu_j] \right) \tag{5}$$

This formalism has been used recently to interpret absorption and circular dichroism data of bacteriorhodopsin trimers in the purple membrane of Halobacterium halobium [33, 34]. In this case electron microscopy had established the C_3 symmetry of the membrane and the theory was used to completely determine the orientation of the chromophores with respect to the center of symmetry. In applying this theory to the interaction among chlorophyll *b* molecules in CPII such a detailed analysis is not possible, for a number of reasons: (1) there is no a priori reason to assume C_3 symmetry; (2) the absorption spectrum of the chlorophyll *b* trimer cannot be satisfactorily deconvoluted into exciton components because of the extensive overlapping of the vibronic states of chlorophyll *a*; and (3) it is impossible to obtain in situ monomer data for chlorophyll *b* as was done for bacteriorhodopsin in the purple membrane. It is possible however to obtain a limited amount of useful information from a semi-quantitative analysis of the data.

The dipole transition moments μ_K of the exciton states of a trimer are not in general orthogonal to one another. A special class of trimer geometries which insures the orthogonality of $[\mu_K]$ are those possessing C_3 symmetry. The observation that the polarization is near zero for excitation into, as well as emission from, chlorophyll *b* requires that the transition moments be orthogonal and that the dipole strength of the exciton D_K all be nearly equal. In addition the C_3 symmetry assures that a doublet will appear in the circular dichroism spectrum while in general a triplet could be expected. It is therefore reasonable to assume C_3 symmetry because of its qualitative consistency with the data as well as its simplicity. This allows the geometry of the trimer to be completely specified by two angles θ and ϕ defined such that θ is the angle between each dipole transition moment and the symmetry axis and ϕ is the angle between the projection of each transition moment in the plane of the trimer and the vector from the center of symmetry to each monomer as shown in Fig. 6a. Assuming that the dipole strengths of the exciton states are identically equal puts no constraint on ϕ but requires $\tan^2\theta = 2$, giving $\theta = 54.7°$ and $\theta = 125.3°$. The sign of the interaction V_{ij} however depends on θ and ϕ as

$$V_{ij} = \frac{|\mu_i|^2}{|R_{ij}|^3} \{(1 - \tfrac{3}{2}\sin^2\theta) - \tfrac{3}{4}\sin^2\theta(1 - 4\cos^2\phi)\} \tag{6}$$

which for the values of θ specified above predicts $V > 0$ when $\phi < 60°$ and $V < 0$ when $\phi > 60°$. There are then four cases to be considered which are shown in Fig. 6b. Cases (a) and (d) can be eliminated immediately because the circular dichroism bands are predicted in the wrong order. The difference between case (b) and (c) is more subtle. They differ physically because while the plus and minus states carry equal rotational strength, which predicts that the circular dichroism spectrum due to the exciton splitting should pass through zero at the midpoint in energy between the states, they do not carry equal oscillator strength. The minus states collectively carry twice the oscillator strength of the plus state. We therefore expect the absorption maxima to lie closer to the minus states. The absorption spectrum shows the chlorophyll *b* absorption maximum at 652 nm, while the exciton contribution to the circular dichroism bands is

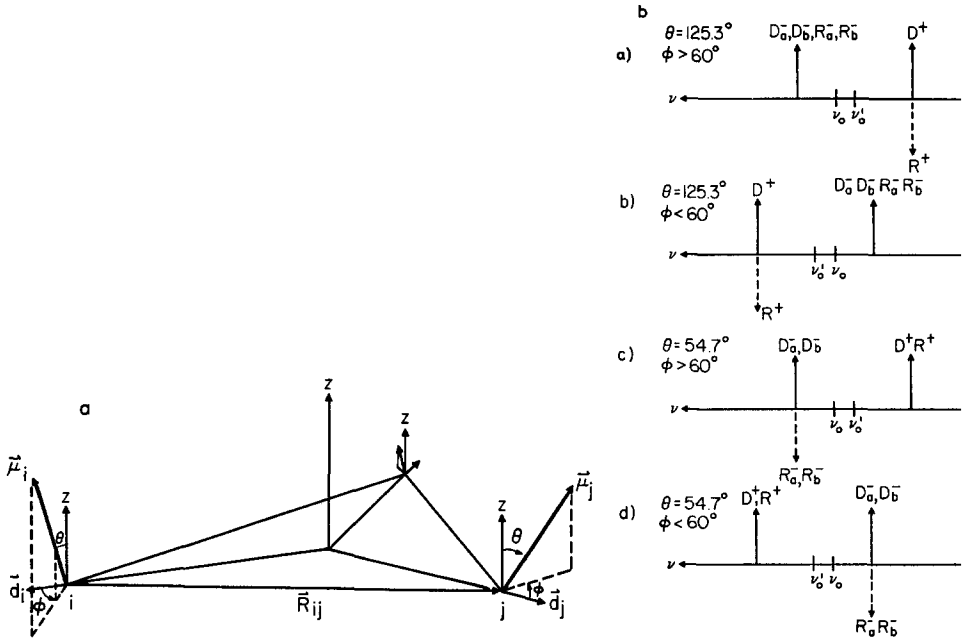

Fig. 6. (a) The geometry of the chlorophyll *b* trimer, assumed to have C_3 symmetry. The configuration of the trimer is completely specified by $|\vec{R}_{ij}|$ the intermonomer distance, θ the azmuthal angle of the transition dipole moment vector $\vec{\mu}_i$ and ϕ the angle between the vector connecting the symmetry axis to the monomer d_i and the projection of μ_i in the plane of the trimer. (b) A schematic representation of the spectral position of the trimer transitions indicating the dipole strengths D^+, D_a^-, D_b^-, and rotational strengths R^+, R_a^-, R_b^- and sign. v_0 is the assumed monomer energy and v'_0 is the position where the exciton contribution to the circular dichroism should cross zero assuming symmetric absorption bands. Four cases are shown corresponding to values of θ and ϕ which are consistent with the polarization data.

centered at approx. 648 nm, consistent only with case (b) of Fig. 6b. This conclusion is supported by a deconvolution of the absorption and circular dichroism spectra (Fig. 7) in terms of common Gaussian components described in Table I. In accordance with case (b) of Fig. 6b the chlorophyll *b* components at 644 and 652 nm have equal widths, nearly equal and opposite rotational strengths and dipole strengths in the ratio 1 : 2. The chlorophyll *a* components have been modeled as in the polarization calculation below.

In this simple semiquantitative analysis based on a chlorophyll *b* trimer with C_3 symmetry we have been able to conclude that the angle between the symmetry axis and the transition dipole moments is near 125.3° and that the angle between the projection of each transition dipole moment in the plane of the trimer and the vector from the center of symmetry to each monomer must be $0° < \phi < 60°$.

Fluorescence polarization

A general formalism to predict the fluorescence polarization properties of an aggregate containing an arbitrary number of chromophores in the limit of complete, incoherent transfer will be described. The results of previous formalisms [35] can be obtained as special cases. The specific assumptions which have been made are: (1) that

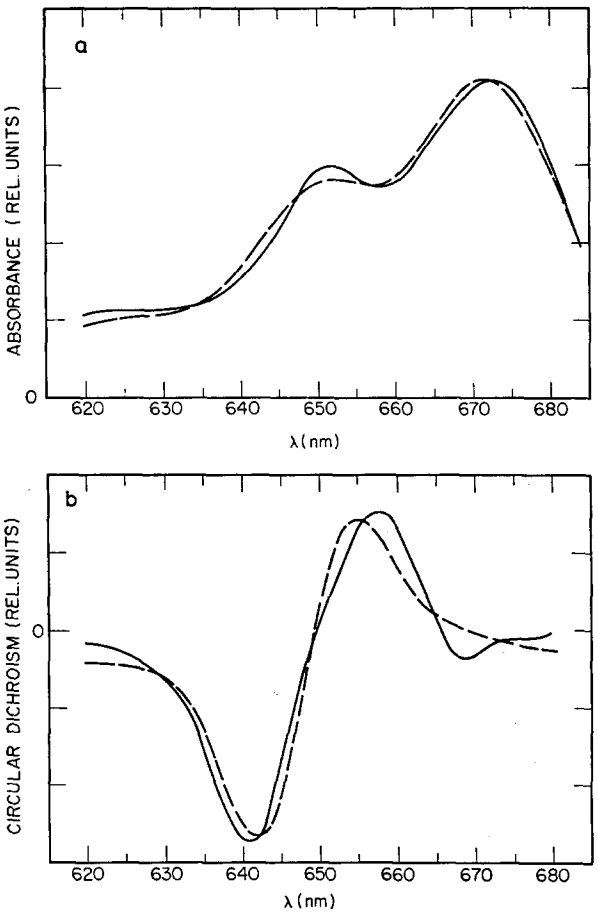

Fig. 7. A best fit of the absorption (a) and circular dichroism (b) spectra in terms of common Gaussian components. The experimental spectra are shown as solid lines and the spectra resulting from the deconvolution as dashed lines. The parameter values are given in Table I.

TABLE I

GAUSSIAN COMPONENTS OF A DECONVOLUTION OF THE ABSORPTION AND CIRCULAR DICHROISM SPECTRA OF CPII

The relative dipole strengths $^{\circ}\varepsilon_i$, spectral positions $h\nu_i$, Gaussian spectral widths $h\delta_i$ and relative rotational strengths $^{\circ}C_i$ obtained for a best fit of the data are shown.

Component	$^{\circ}\varepsilon_i$	$h\bar{\nu}_i$ (eV)	$h\delta_i$ (eV)	$^{\circ}C_i$
Chl *a*-677	0.2674	1.83267	0.0249	−14.16
Chl *a*-670	0.5349	1.85045	0.0249	13.68
Chl *b*-652	0.2811	1.90153	0.0170	72.08
Chl *b*-644	0.1405	1.92516	0.0170	−88.34
[630]	0.2128	1.96614	0.0583	−13.64

transfer between chromophores is incoherent and (2) that the distribution of excitation energy equilibrates among the emitting chromophores during times short compared with the fluorescence lifetime. With these assumptions the essential behavior of the fluorescence intensity of a single aggregate can be expressed as

$$I_{\hat{e}_1\hat{e}_2}(\nu_{\text{exc}}, \nu_{\text{em}}) \propto [\sum_i \varepsilon_i(\nu_{\text{exc}})(\hat{e}_1 \cdot \mathsf{R} \cdot \hat{\mu}_i)^2] \times [\sum_j f_j(\nu_{\text{em}})(\hat{e}_2 \cdot \mathsf{R} \cdot \hat{\mu}_j)^2] \tag{7}$$

where $\hat{e}_1$ and $\hat{e}_2$ are unit vectors representing the polarization of the exciting and emitted light respectively, $\varepsilon_i(\nu_{\text{exc}})$ is the excitation spectrum of the i^{th} chromophore, $\hat{\mu}_i$ is the direction of the absorption dipole transition moment in the coordinate frame of the aggregate, $f_j(\nu_{\text{em}})$ is the emission spectrum of the j^{th} chromophore in the aggregate, $\hat{\mu}_j$ is the direction of the emission dipole transition moment and R is the rotation matrix connecting aggregate coordinates and laboratory coordinates. The fluorescence polarization of a random solution is, by definition,

$$p(\nu_{\text{exc}}, \nu_{\text{em}}) = \frac{\langle I_{\hat{z}\hat{z}}(\nu_{\text{exc}}, \nu_{\text{em}})\rangle - \langle I_{\hat{z}\hat{x}}(\nu_{\text{exc}}, \nu_{\text{em}})\rangle}{\langle I_{\hat{z}\hat{z}}(\nu_{\text{exc}}, \nu_{\text{em}})\rangle + \langle I_{\hat{z}\hat{x}}(\nu_{\text{exc}}, \nu_{\text{em}})\rangle} \tag{8}$$

where <- - -> represents an average over all Euler angles. This average can be taken formally yielding

$$\begin{aligned}\langle I_{\hat{z}\hat{z}}(\nu_{\text{exc}}, \nu_{\text{em}})\rangle = \sum_j \sum_i \varepsilon_i(\nu_{\text{exc}}) f_j(\nu_{\text{em}}) \{&\tfrac{1}{5}(\mu_{ix}^2\mu_{jx}^2 + \mu_{iy}^2\mu_{jy}^2 + \mu_{iz}^2\mu_{jz}^2) \\ &+ \tfrac{4}{15}(\mu_{ix}\mu_{iy}\mu_{jx}\mu_{jy} + \mu_{ix}\mu_{iz}\mu_{jx}\mu_{jz} + \mu_{iy}\mu_{iz}\mu_{jy}\mu_{jz}) \\ &+ \tfrac{1}{15}(\mu_{ix}^2\mu_{jy}^2 + \mu_{iy}^2\mu_{jx}^2 + \mu_{iy}^2\mu_{jz}^2 + \mu_{iz}^2\mu_{jy}^2 + \mu_{ix}^2\mu_{jz}^2 + \mu_{iz}^2\mu_{jx}^2)\}\end{aligned} \tag{9}$$

$$\begin{aligned}\langle I_{\hat{z}\hat{x}}(\nu_{\text{exc}}, \nu_{\text{em}})\rangle = \sum_j \sum_i \varepsilon_i(\nu_{\text{exc}}) f_j(\nu_{\text{em}}) \{&\tfrac{1}{15}(\mu_{ix}^2\mu_{jx}^2 + \mu_{iy}^2\mu_{jy}^2 + \mu_{iz}^2\mu_{jz}^2) \\ &- \tfrac{2}{15}(\mu_{ix}\mu_{iy}\mu_{jx}\mu_{jy} + \mu_{ix}\mu_{iz}\mu_{jx}\mu_{jz} + \mu_{iy}\mu_{iz}\mu_{jy}\mu_{jz}) \\ &+ \tfrac{2}{15}(\mu_{ix}^2\mu_{jy}^2 + \mu_{iy}^2\mu_{jx}^2 + \mu_{iy}^2\mu_{jz}^2 + \mu_{iz}^2\mu_{jy}^2 + \mu_{ix}^2\mu_{jz}^2 + \mu_{iz}^2\mu_{jx}^2)\}\end{aligned} \tag{10}$$

where

$$\hat{\mu}_i = \mu_{ix}\hat{x} + \mu_{iy}\hat{y} + \mu_{iz}\hat{z} \tag{11}$$

in the aggregate coordinate reference frame. The polarization can then be evaluated for specific models using Eqn 8.

In order to use the above formalism a specific model was chosen for the light-harvesting chlorophyll *a*/*b*-protein. Since the chlorophyll *b* is strongly coupled and exhibits a polarization very close to zero at 650 nm we have chosen to assume that it may be represented by three equally absorbing transition moments which are mutually orthogonal and hence act as a spherically degenerate oscillator in the terminology of Gurinovich. The chlorophyll *a* absorption or excitation spectrum in the protein has been shown in Results to possess two major components using the major chlorophyll components described by Brown et al. [12]. These are at 670 and 677 nm and occur in a ratio of about 2 : 1. Since it is known that there are three chlorophyll *a* molecules per complex it was assumed that two chlorophyll *a* transition moments were at 670 nm and the position of the remaining chlorophyll *a* transition moment was allowed to

vary from 670 to 680. All transitions were modeled as gaussian dipole oscillators assuming the three chlorophyll *a* molecules were identical except for the position of the absorption maximum. Three angles θ_2, θ_3, ϕ_3 are adequate to completely define the relative orientation of three transition moments and together with λ_1 the wavelength of the absorption maximum of the low energy chlorophyll *a* molecule completely define the model since chlorophyll *b* is assumed as a spherically degenerate oscillator. We assume that emission is only from chlorophyll *a*, that it is Boltzmann distributed among the chlorophyll *a* molecules and that the absorption and emission transition moments are parallel. The chlorophyll *a* transition moments are described in the coordinates of the protein as

$$\begin{aligned}\hat{\mu}_1 &= \hat{z}\\ \hat{\mu}_2 &= \sin\theta_2\hat{x}+\cos\theta_2\,\hat{z}\\ \hat{\mu}_3 &= \sin\theta_3\cos\phi_3\hat{x}+\sin\theta_3\sin\phi_3\hat{y}+\cos\theta_3\,\hat{z}\end{aligned} \tag{12}$$

The excitation polarization data in the region from 650 to 700 nm can be interpreted quite well (Fig. 8) in terms of this model which clearly shows the necessity of having two components in the chlorophyll *a* absorption region. Failure to fit the data in the region near 650 is due to the presence of other chlorophyll *a* bands in that region which have not been included in the model. Of particular importance is the model's ability to interpret the wavelength dependence of polarization to the red of the chlorophyll *a* absorption maxima which is qualitatively different than that in monomeric chlorophyll [15, 18]. The results of a best fit to θ_2, θ_3, ϕ_3 and λ_1 show good agreement with the gaussian fit of the absorption spectrum by finding $\lambda_1 = 676.5$ with $\theta_2 = 83°$, $\theta_3 = 67°$ and $\phi_3 = 54°$. A good fit is also obtained with $\lambda_1 = 676.5$ nm, $\theta_2 = 83°$ and $\theta_3 = 67°$ and $\phi_3 = 134°$ as well as at angles related to these by reflections through the xz, yz and xy planes. Any of these descriptions, however, contain the essential asymmetry of the $S_0 \rightarrow S_1$ transition moments in the protein and should be used as the

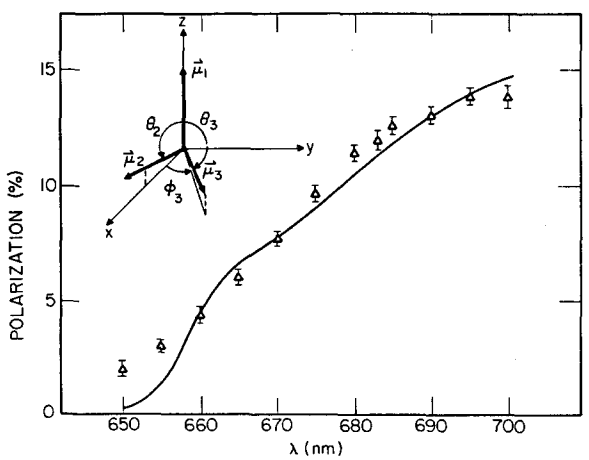

Fig. 8. The excitation wavelength dependence of the fluorescence polarization spectrum (△) as described in Fig. 4 and a best fit of the data (—) between 650 nm and 700 nm as described in the text. The parameter values obtained are $\lambda_1 = 676.5$, $\theta_2 = 83°$, $\theta_3 = 67°$ and $\phi_3 = 54°$.

starting point for calculations of the optical properties of photosynthetic membranes. The tendency to planar symmetry suggested by and observed in intact photosynthetic membranes [31, 33–35] is seen in Fig. 8 to be manifest at the level of CPII.

CONCLUSIONS

The principal results reported here are as follows:

(1) Excitation energy in CPII has been shown to equilibrate between chlorophyll *b* and chlorophyll *a* during the fluorescence lifetime by the excitation wavelength independence of the fluorescence emission spectrum including excitation primarily into chlorophyll *a* and chlorophyll *b*. Also, the quantum yield of fluorescence has been shown to be independent of excitation wavelength.

(2) Polarization measurements show that the absorption due to chlorophyll *b* leads to nearly complete depolarization while the polarization of the chlorophyll *a* absorption band can only be explained in terms of at least two chlorophyll *a* components differing in absorption wavelength. The emission wavelength dependence of polarization is also consistent with this analysis.

(3) Comparison of fluorescence polarization measured in vivo with that observed in CPII shows that the depolarization observed in vivo can occur within CPII.

(4) Circular dichroism measurements indicate strong coupling between the chlorophyll *b* molecules in the complex while this is not indicated for the chlorophyll *a* molecules. Model calculations show that a chlorophyll *b* trimer with C_3 symmetry with angles $\theta = 125.3°$ and $0° < \phi < 60°$ is consistent with the data.

(5) A detailed model is introduced to explain the fluorescence polarization data but which because of its generality can be used whenever complete, incoherent transfer is to be assumed. Within the limitations of this model the relative orientation of each of the chlorophyll *a* $S_0 \rightarrow S_1$ transition moments within the protein can be determined, and the essential asymmetry of optical properties of the protein can be described.

The results reported here provide important insight into the structure of the light-harvesting chlorophyll *a*/*b* · protein. Because polarization is very sensitive to changes in conformation it provides a useful tool to monitor changes in conformation of extracted proteins [17] in addition to providing insight into their intrinsic structure. Additional information about the structure and exciton dynamics of CPII should be obtained by using oriented samples, and making time resolved and low temperature measurements.

The knowledge of the structure of light-harvesting chlorophyll *a*/*b* · protein provided here will allow more detailed model calculations of energy transfer dynamics within the photosynthetic unit. For example, it would be useful to compare theoretical polarization and dichroism spectra of model membranes containing LHCP with corresponding spectra in vivo (see for example [31, 36–39]). The orientation of transition moments found in CPII isolated from spinach chloroplasts can be compared with that isolated from other higher plants and algae to test the hypothesis that CPII is an ubiquitous protein common to a wide range of chlorophyll *b* containing species. The techniques reported here should be useful for studying other pigment proteins. Similar studies have already been reported on the peridinin-chlorophyll *a* · protein extracted from marine dinoflagellates [40]. In the future it is hoped that the crystallization of CPII will be reported and an X-ray determination of the position and orientation of

the chromophores within the protein will be made. This will allow the data reported here to test the details of exciton dynamics which could then be predicted by extension of the unified theory of fluorescence depolarization developed for dimers by Rahman [41].

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