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ORIENTATION OF PHOTOSYSTEM-I PIGMENTS

INVESTIGATION BY LOW-TEMPERATURE LINEAR DICHROISM AND POLARIZED FLUORESCENCE EMISSION

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Using absorption and fluorescence experiments at low temperature with polarized light on oriented samples, the orientation of PS-I-related pigments, both in green plants and in *Chlamydomonas reinhardtii*, has been investigated on isolated pigment-protein complexes and intact thylakoids. The following observations have been made. (i) The isolation procedure of PS I_{110}, PS I_{65}, LHC I and CP0) particles from pea and *C. reinhardtii* do not alter significantly the intrinsic orientation of the pigments inside the complexes; (ii) Chl *b* is a structural component of PS I, linked to the peripheral antenna, with an orientation with respect to the thylakoid plane different from that observed in the main light-harvesting complex (iii) PS I_{65} (i.e., 'core' PS I) of pea and *C. reinhardtii* contains identical chromophores having the same orientation with respect to the geometrical longest axis (axes) of the complexes. (iv) LHC I and CP0 (i.e., PS I 'peripheral antenna') of pea and *C. reinhardtii* have identical oriented chromophores, except that a long-wavelength component with a high anisotropy is only present in green plants. This set of pigments, which absorbs at 705–725 nm, has the same orientation as the dipoles emitting F_{735} and also as the Q_Y transition of P-700. (v) All the long-wavelength fluorescence properties of the various studied membranes are explained by these data on isolated PS I complexes: wild-type *C. reinhardtii* and Chl-*b*-less barley fluoresce from the core pigments, while a CP1 deficient mutant of *C. reinhardtii* and wild-type barley fluoresce from the antenna pigments.

Introduction

In all the photosynthetic organisms with two photosystems, the fluorescence emission spectra at 77 K usually exhibited three peaks at 685, 695 and 705–740 nm [1–4]. Although the long wavelength fluorescence band had always been attributed to PS I pigments, its position depends on the organism. In pea, spinach and wild-type barley thylakoids, the PS I fluorescence emission at 77 K peaks at 735 nm while it is shifted to 722 nm in a chlorophyll-*b*-less mutant which lacks the PS I peripheral antenna [5]. In the wild-type strain of *C. reinhardtii* this peak is at 717 nm and it is shifted to 707 nm in mutant lacking in the PS I reaction center [6]. PS I 'core' and 'peripheral antenna' have recently been isolated in both higher plants and *C. reinhardtii* [6,7,8]. Whereas PS I peripheral antenna in *C. reinhardtii*, refered to as CP0, displays a 77 K emission peak at a shorter wavelength than that of the PS I core (707 nm vs. 715 nm), the peripheral antenna in pea (LHC I) has its 77 K fluorescence maximum at longer

Abbreviations: Chl, chlorophyll; LD, linear dichroism; PS I, Photosystem I; LHC, light-harvesting complex.

wavelength than the PS I core (732 nm vs. 722 nm). Whether these observations imply differences in the organization of PS I pigments or not remains to be elucidated.

Polarized light spectroscopy is an effective tool to gain structural information on absorbing or fluorescing dipole inside an anisotropic particle [9]. The recent introduction of squeezed polyacrylamide gels as an orientation technique [10] provides the opportunity to orient particles of very different sizes ($1 \cdot 10^{-6}$–$5 \cdot 10^{-8}$ m) from intact membranes to isolated pigment-protein complexes [11]. Low temperature spectroscopy (77 K) can be performed on these gels and it has been previously shown that the linear dichroism (LD) spectra of purified thylakoids in gels have the advantage of being devoid of optical artifacts [11]. These last properties allow the use of this technique to compare the orientation of a chromophore inside the membrane with those of the isolated pigment-protein complex [12–14].

We present in this paper linear dichroism and polarized fluorescence emission spectra on isolated PS I_{110}, PS I core and PS I peripheral antenna complexes derived from pea, wild type barley and its Chl-*b*-less mutant, wild type *C. reinhardtii* and its M_{19} mutant, lacking in the PS I 'core'. The aim of the present study is to identify patterns of absorption and fluorescence anisotropies in different isolated particles in an attempt: (i) to quantitate the extent of structural perturbation upon particle isolation; (ii) to identify the individual Chl proteins which are the components of the antenna and core reaction center of PS I; (iii) to investigate the origin and orientation of the long wavelength absorbing and fluorescing dipoles in the intact thylakoids.

Materials and Methods

Peas are grown in a greenhouse and harvested 8–10 days after sowing. Wild type and mutant cells of *C. reinhardtii* were grown in Tris-acetate-phosphate medium at 200 lux (cool fluorescent light). PS I_{110} and PS I_{65} particles from pea and from *C. reinhardtii* were prepared by Triton solubilization and sucrose gradient centrifugation according to Ref. 7. PS I particles from the Chl-*b*-less barley mutant were obtained by polycrylamide gel electrophoresis in the presence of deoxycholate [15]. LHC I from pea was prepared by further extraction of PS I_{110} by zwittergent treatment [8,16]. CP0 from *C. reinhardtii* was prepared by solubilization of the thylakoids in the presence of 1% SDS and polyacrylamide gel electrophoresis in the presence of SDS [6].

$LD = A_{\parallel} - A_{\perp}$ spectra were directly measured in the apparatus described in Ref. 17 using linearly polarized light with the plane of polarization modulated between the vertical and horizontal directions at a frequency of 100 kHz. With synchronous detection the difference $A_{\parallel} - A_{\perp}$ is directly obtained. Absorption is measured by recording the averaged modulated signal of the transmitted light reaching the photomultiplicator. Absorption is calculated as $\log I_0/I$ (I_0 and I are the intensity of transmitted light with or without sample). LD and A used in the above text refer to these two measurements. τ_A parameter (see below) is calculated as the LD/A ratio.

Polarized fluorescence emission spectra were recorded at 77 K on the same samples oriented in squeezed polyacrylamide gels. Excitation light at 640 nm was provided by a dye laser-Argon laser combination and a double monochromator was used to analyse fluorescence spectra. Spectra $F_{\parallel}$ and $F_{\perp}$ are recorded twice separately; the τ_F parameter is then calculated as $F_{\parallel} - F_{\perp} / \frac{1}{3}(2F_{\parallel} + F_{\perp})$. $A_{\parallel}$, $A_{\perp}$, $F_{\parallel}$, $F_{\perp}$ refer to the absorption and fluorescence of light polarized parallel and perpendicular to the long axis of the oriented objects [11]. Spectra were digitized on a Tracor Northern 1710. A variable temperature cryostat (SMC, France) was adjusted for low temperature measurements.

The observed anisotropy of absorption or fluorescence proceeds from two physical origins, i.e., the intrinsic orientation of a chromophore within the particle, and the 'mosaic spread' parameter, ϕ, describing the degree of orientation of the collection of particles. If θ_A measures the angle between absorbing dipoles and the long axis of the particle, and if we assume perfect orientation of all the long axis of the particles ($\phi = 1$) we have:

$$\frac{A_{\parallel} - A_{\perp}}{3A} = \frac{\mathrm{LD}}{3A} = \frac{3\cos^2\theta_A - 1}{2} = S_A \qquad (1)$$

When only a fraction of the particles are perfectly oriented this expression is transformed into:

$$LD/3A = S_A\phi = \tau_A \quad (2)$$

If θ_F measures the angle between a fluorescing dipole and the long axis of the same particle, we have:

$$\frac{F_{\parallel} - F_{\perp}}{3F} = S_F\phi = \tau_F \quad (3)$$

However, as other authors use $A_{\parallel}/A_{\perp}$ (dichroic ratio) and $F_{\parallel}/F_{\perp}$ (fluorescence anisotropy), the values of these ratios will be given simultaneously. Maximal for τ_A and for $A_{\parallel}/A_{\perp}$ (τ_F and $F_{\parallel}/F_{\perp}$, respectively) are obtained at identical wavelength. Since the mosaic spread parameter may vary from one preparation to another, we have selected for the data presented in the tables the highest values of τ_A and τ_F among those obtained with samples of identical spectroscopic composition. Error bars on τ_A and τ_F are not specified in the text, but given in the tables.

The mosaic spread parameter cannot be easily estimated. However, provided that τ_A and τ_f are measured on the same oriented specimen, it is possible to compare directly the orientation of the absorbing and emitting dipoles. For different particles a constant ratio measured at selected wavelengths can also indicate a correlation between the orientation of various subsets of pigments. This index has been used to compare the orientation of pigment beds in thylakoids and in isolated particles and will be further detailed in the discussion.

Results

Fig. 1 shows that the polypeptide content of PS I_{110} from pea can be reconstructed by the sum of the polypeptide patterns of PS I_{65} and LHC I, as described in Ref. 8; the same result is also obtained with PS I_{110}, PS I_{65} and CP0 of *C. reinhardtii*.

PS I_{110} particles

The 100 K LD and absorption spectra of PS I_{110} obtained from pea and *C. reinhardtii* are shown on Fig. 2a and b. Their main components are described in Table I. The spectra are nearly identical except that a long-wavelength shoulder ($C_{705-725}$) is present only in the PS I_{110} from pea. In both particles the component with the highest LD/A ratio peaks at 697 nm. This is shown in Fig. 3 for the PS I_{110} of pea. The additional $C_{705-725}$ in pea has the same dichroic ratio as at 697 nm. Fluorescence emission spectra are shown in Fig. 2c and d. PS I_{110} from pea has its maximal fluorescence at 735 nm with an anisotropy of 1.54 ($\tau_F = 0.132$). The dichroic ratio is 1.57 ($\tau_A = 0.137$) at 697 nm. PS I_{110} from *C. reinhardtii* fluorescence at 715 nm with an anisotropy of 1.2 ($\tau_S = 0.060$); the dichroic ratio is 1.43 ($\tau_A = 0.112$) at 697 nm.

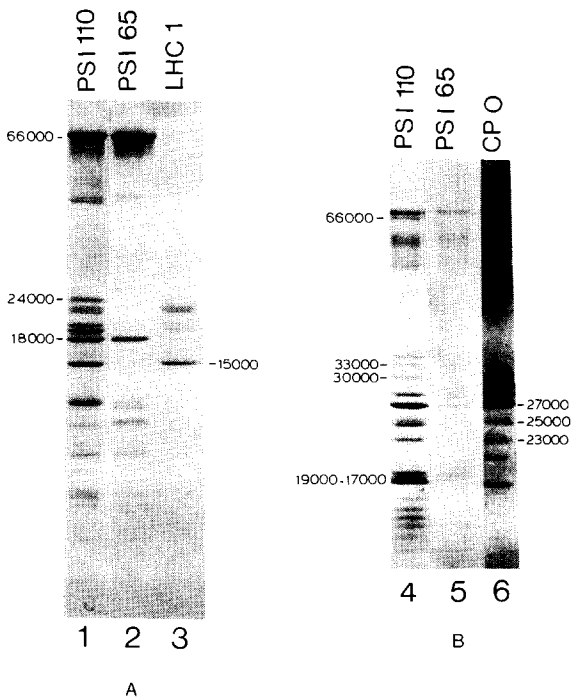

Fig. 1. Polypeptide composition of (A) PS I_{110}, PS I_{65} and LHC I from pea; (B) PS I_{110}, PS I_{65} and CP0 from *C. reinhardtii*; gel (a) was run in 12–18% acrylamide with 8 M urea; gel (b) in 7.5–15% acrylamide. Approximate molecular weights are indicated. A slight contamination of CP0 by polypeptide 18 kDa and of PS I_{65} by 27 kDA is observed in gel (b).

PS I_{65} particles

The LD and absorption spectra of PS I_{65} from either pea or *C. reinhardtii* are indistinguishable; the spectra of the complex isolated from pea are shown in Fig. 4a. Pea fluorescence emission spectra are shown in Fig. 4b. PS I_{65} from pea has its maximum fluorescence emission at 722 nm with

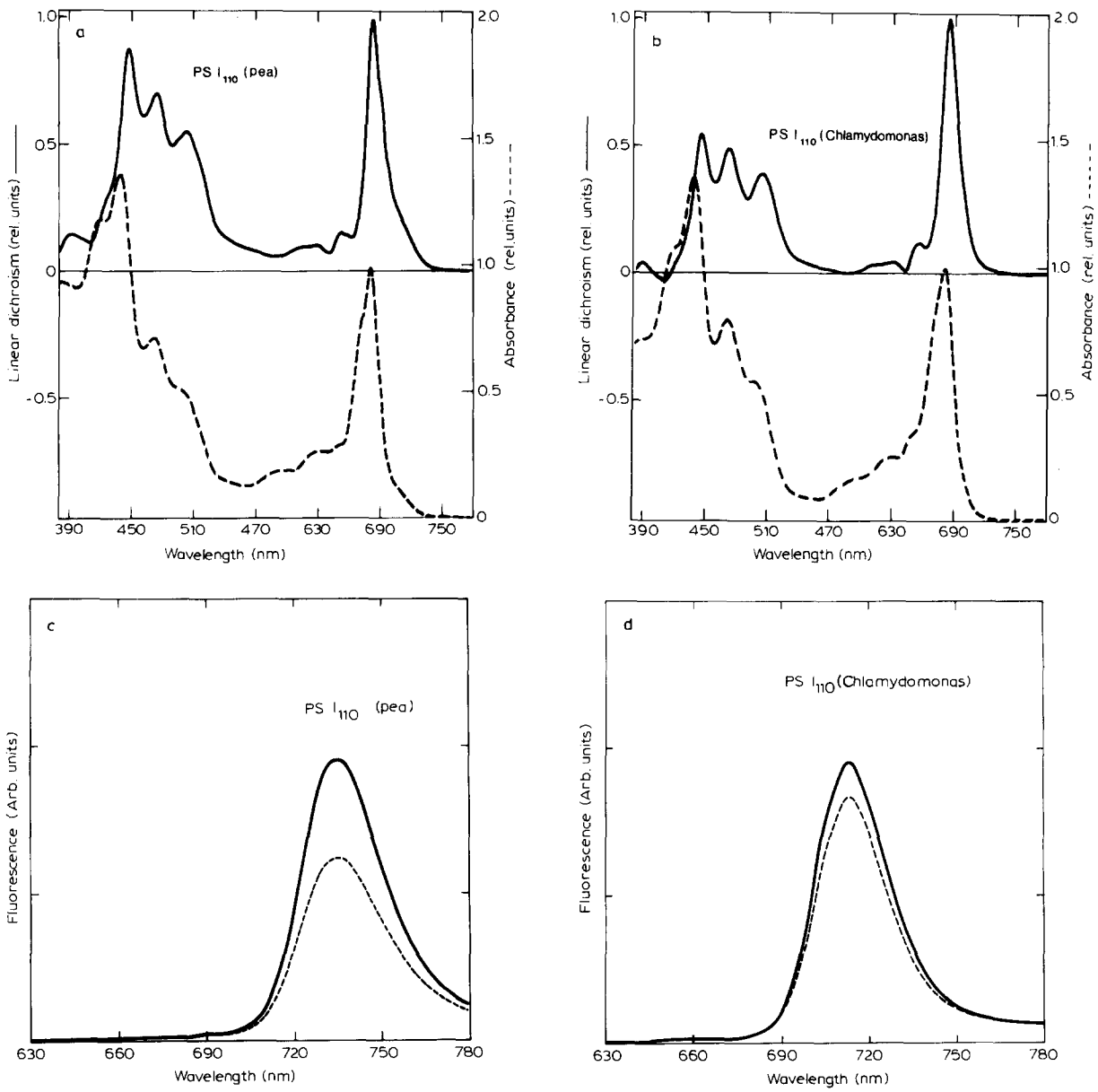

Fig. 2. L.D. (———) and absorbance (------) spectra at 100 K of PS I_{110} from pea (a) and *C. reinhardtii* (b). Polarized fluorescence emission spectra at 77 K of PS I_{110} from pea (c) and *C. reinhardtii* (d): $F_{\parallel}$ (———) and $F_{\perp}$ (------). Excitation wavelength is 640 nm.

an anisotropy of 1.05 ($\tau_F = 0.016$). The dichroic ratio is 1.21 ($\tau_A = 0.062$) at 697 nm. PS I_{65} from *C. reinhardtii* has its maximum fluorescence emission at 715 nm with an anisotropy of 1.15 ($\tau_F = 0.045$). The maximum dichroic ratio is observed at 697 nm (Fig. 3), and is 1.30 ($\tau_A = 0.083$). The dashed lines in Fig. 4a and b will be discussed in the subsection Denaturation of chlorophyll-protein complexes.

PS I peripheral antenna complexes

LD and absorption spectra of LHC I (pea) and CP0 (*C. reinhardtii*) are shown on Fig. 5a and b. The only difference which can be observed between the data of the two particles is the highly dichroic component $C_{705-725}$ present only in pea. Fluorescence emission spectra are shown in Fig. 5c and d. LHC I has its maximum fluorescence emis-

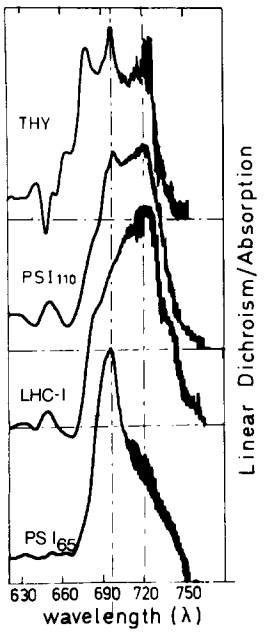

Fig. 3. LD/absorption spectra of pea thylakoid, (THY), PS I_{110}, LHC I and PS I_{65} in the red spectral region.

TABLE I

WAVELENGTHS OF THE LD AND ABSORPTION FEATURES OBSERVED IN THE VARIOUS CHLOROPHYLL-PROTEIN COMPLEXES

Absorption and LD peaks are set in italics, shoulders are set in roman.

Particles	Wavelengths for major LD components	Wavelengths for major absorption components
PS I 110 pea	*653*, 673, *681*, 695–702, 705–725	*651*, 671, *680* 694–702, 705–725
PS I 65 pea	665, 675, *686*, 695–702	668, *677*, 687 695–705
LHC I pea	*649*, *681*, 705–725	650, *671*, 678, 705–725
PS I 110 *C. reinhardtii*	*654*, 674, *682*, 695–705	*650*, 671.5, *679*, 695–705
PS I 65 *C. reinhardtii*	663, 673, *685*, 695–705	668, *676*, 686 695–705
CP0	*651*, *681*	650, *672*, 678
PS I (b^-)	664, 670, *683.5*, 695–705	668, *677*, 688 *695*–705

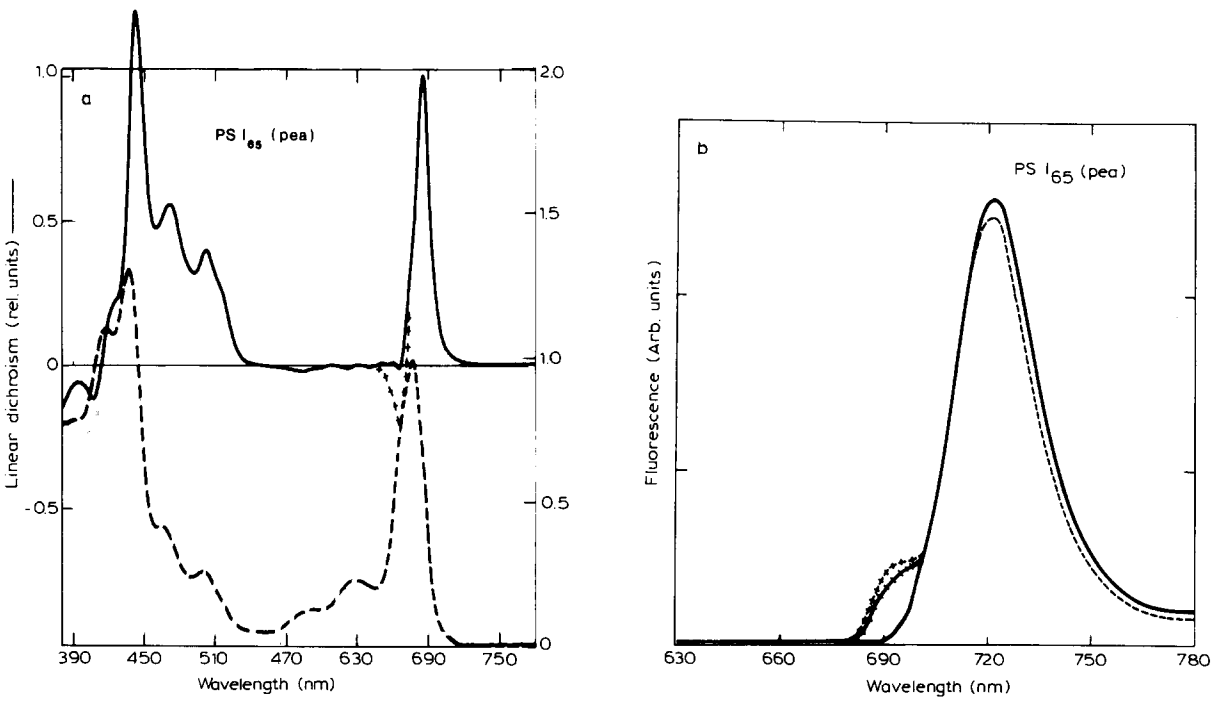

Fig. 4. PS I_{65} from pea: (a) LD (———) and absorbance (------) spectra at 100 K; (b) 77 K polarized fluorescence emission spectra at 77 K: $F_{\parallel}$ (———) and $F_{\perp}$ (------). In Fig. 4a, lines marked with crosses (+ + +) indicate the appearance, in some preparations, of a negative LD signal at 665 nm. In Fig. 4B, dashed lines marked with crosses (+ + +) indicate the appearance, in the same sample, of a fluorescence at 690 nm; $F_{\perp}$ (+ + + +) is larger than $F_{\parallel}$ (||||), which indicates a fluorescence anisotropy lower than 1.

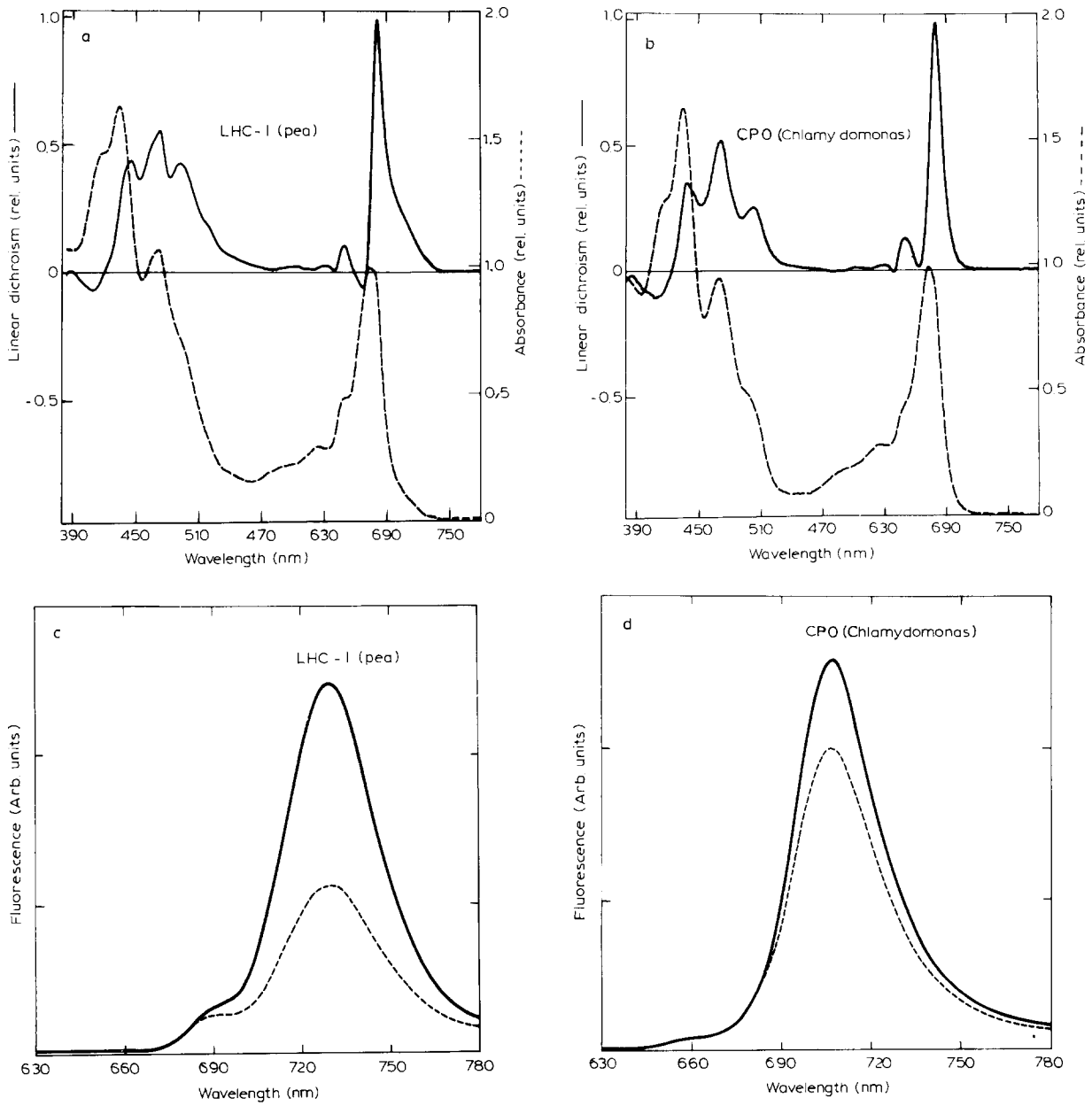

Fig. 5. LD (———) and absorbance (------) spectra at 100 K of LHC I (a) and CP0 (b). Polarized fluorescence emission spectra at 77 K of LHC I (c) and CP0 (d): $F_{\parallel}$ (———) and $F_{\perp}$ (------).

sion at 732 nm with an anisotropy of 2.22 (τ_F = 0.22). The maximum dichroic ratio is observed in the 715–725 nm region, and is 2.22 (τ_A = 0.22). CP0 has its maximum fluorescence emission at 707 nm with an anisotropy of 1.29 (τ_F = 0.081). The maximum dichroic ratio is observed at 691 nm, and is 1.38 (τ_A = 0.100).

Denaturation of chlorophyll-protein complexes

A sharp negative LD peak can be observed at 665 nm in some batches of PS I_{65} derived from either pea or *C. reinhardtii* and in LHC I from pea. This signal is correlated with the appearance of a fluorescence emission band at 690 nm which presents an anisotropy lower than 1 (Fig. 4b). In fig.

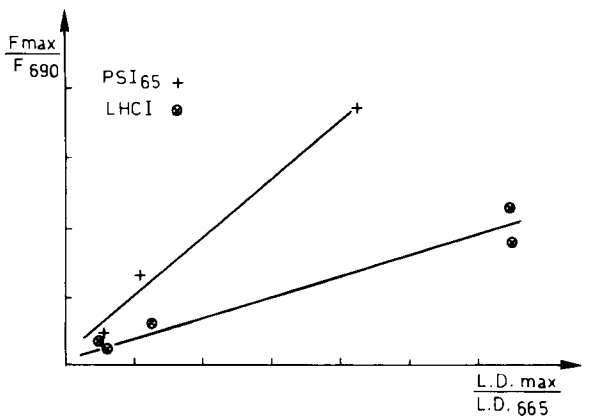

Fig. 6. Relative variation of LD_{665} and F_{690} in PS I_{65} and LHC I.

4a and b the lines marked with crosses indicate the shape of the PS I_{65} spectra when the LD_{665} and the F_{690} are present. Various samples of either PS I_{65} or LHC I isolated with constant procedures yield different extents of LD_{665} and F_{690}; the ratio LD_{max}/LD_{665} is found to be always proportional to the ratio F_{max}/F_{690} (Fig. 6). In addition the dipoles responsible for LD_{665} and F_{690} are both directed at more than 35° away from the long axis of the complexes. These observations suggest that the same pigment bed is responsible for both LD_{665} and F_{690}. A similar LD_{665} signal has been observed for a CP1 particle by Gagliano et al. [18]. They have attributed this signal to a partial denaturation of the complex. A similar conclusion was reached by Mullet et al. [8] concerning the F_{690} present in the LHC I complex. As heat treatment (50 °C for a few seconds) of the particles induces an increase in both LD_{665} and F_{690} (data not shown) we conclude that they are produced by dissociated pigments.

Linear combination of PS I_{65} and LHC I features

The PS I_{110} absorption and LD spectra have been reconstituted by least squares curve fitting using linear combination of PS I_{65} and LHC I or PS I_{65} and CP0 spectra for pea and *C. reinhardtii*, respectively. The least squares procedure was identical to the one used in Ref. 13. The spectra were arbitrarily normalized to the same value. Results of the fitting are:

0.55 ± 0.10 (PS I_{65}) + 0.45 ± 0.010 (antenna) in absorption

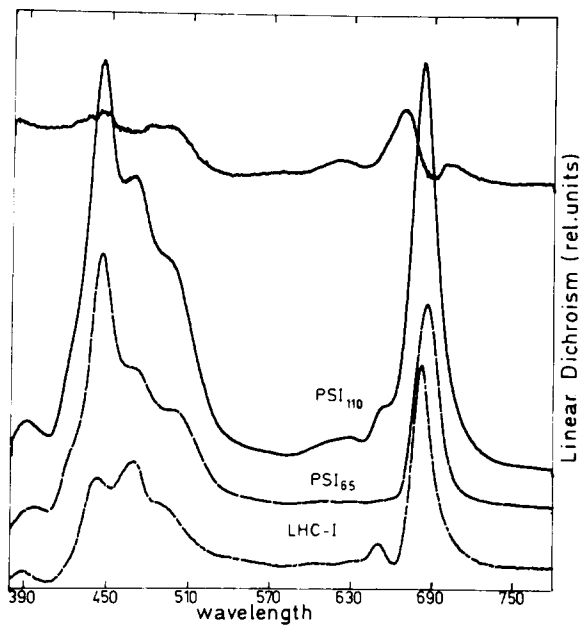

Fig. 7. Linear combination of room temperature LD spectra of PS I_{65} and LHC I reconstitute LD spectra of PS I_{110}; contributions are 1 : 1. The difference between experimental and calculated spectra is shown in the upper part of the figure.

0.45 ± 0.10 (PS I_{65}) + 0.55 ± 0.1 (antenna) in LD

The amplitude of the difference between the experimental and the calculated spectra of PS I_{110} is similar for absorption and LD (Fig. 7. : LD)

Polarized fluorescence spectra of intact thylakoids

The polarized fluorescence emission spectra of thylakoids from pea (identical to that in the wild-type strain of barley), from a Chl-*b*-less mutant strain of barley, from the wild-type and M19 mutant strains of *C. reinhardtii* are shown respectively on Fig. 8 a, b, c and d. The corresponding values of τ_A and τ_F in the various particles at matching wavelength are given in Tables II, III, IV and V.

The PS I_{110} from pea, barley, the LHC I from pea and the intact thylakoid in this strain display a maximum fluorescence emission at 735 nm. The corresponding maxima in the τ_A (λ) spectra are at 697 and 720 nm in PS I_{110} and thylakoid, 720 nm in LHC I. The corresponding τ_A and τ_F values are depicted in Table II.

The PS I_{65} from pea, the PS I particle extracted

from the Chl-*b*-less barley mutant and the intact thylakoids in this strain display a maximum fluorescence emission at 720–722 nm. The corresponding maximum in the τ_A (λ) spectra in these particles is at 697 nm. In Table III we show that the τ_A [697]/τ_F [722] ratios are identical in the three particles.

PS I_{110}, PS I_{65} and intact thylakoids of wild type *C. reinhardtii* display the same maximal fluorescence emission at 715 nm. In both cases, the maxima in the τ_A (λ) spectra are at 697 nm. The corresponding τ_A and τ_F maximal values are depicted in Table IV.

C. reinhardtii M_{19} mutant thylakoid and CP0 complexes display their maximal fluorescence at 707 nm. In these particles the τ_A maximal value is situated at 691 nm. The corresponding τ_A and τ_F values are depicted in Table V.

Discussion

Through a comparison of both the 77 K fluorescence emission spectra and the respective polypeptide profiles of isolated chlorophyll-protein complexes and intact membranes from different strains of *C. reinhardtii*, Wollman and Bennoun have shown that the CP0 complex, of low mobility in SDS polyacrylamide gel electrophoresis, corresponds to a PS I peripheral antenna [6]. According to these authors the long wavelength fluorescence emission proceeds from CP1 in the wild type strain (F_{715}) and from CP0 in the absence of CP1 as is the case in the M19 mutant strain (F_{707}). According to Anderson [19], two distinct complexes containing the PS I reaction center can be isolated in higher plants by SDS polyacrylamide gel electrophoresis: $CP1_a$ which displays a 77 K maximum of fluorescence at 735 nm and CP1 with a fluorescence maximum at 722 nm. Chl *b* is present in $CP1_a$ but not in CP1 [20] and four additionnal polypeptides are present in the $CP1_a$ (24, 22, 14 and 10 kDa polypeptides) as compared to I. Mullet et al. [8] prepared PS I_{110} particles using sucrose gradient centrifugation of detergent solubilized thylakoids from pea. This complex has been further dissociated in PS I_{65} and LHC I [8]. The polypeptides detached for PS I_{110} particles during the preparation of PS I_{65} particles are similar to those which are present in $CP1_a$ but not in CP1. They are found in another gradient fraction and form a chlorophyll-protein complex, the LHC I [8]. Using the n_{34} mutant of barley, lacking in PS I_{65} polypeptides and deficient in PS I activity, Mullet et al. have isolated, instead of the current PS I_{110} particle, another complex which has its maximal fluorescence emission at 732 nm and contains the LHC I polypeptides [8]. The F_{735} was then attributed to the PS I peripheral antenna pigments while the F_{722} is assigned to pigments of the PS I core which contains the reaction center.

Applying the CP0 preparation procedure to pea thylakoids, we failed in isolating a PS I peripheral antenna. Anderson observed that LHC I polypeptides could not be separated from $CP1_a$ by electrophoresis [20]. PS I core and peripheral antenna might then be more strongly associated in higher plants than in *C. reinhardtii*. An alternative hypothesis is that LHC I is more labile than CP0.

To characterize the various chlorophyll beds of the PS I subunits, we have compared the orientation of a given chromophore in different particles. This has led to the identification of an absorption (respectively, fluorescence) band to a same transition of identical orientation in two distinct particles. In addition we can then assess whether extraction procedures have altered the native state of the pigments in an isolated complex.

Interpretation of dichroic ratios and fluorescence anisotropies

The mosaic spread parameter contributes to the absolute values of LD or polarized fluorescence emission (see Materials and Methods). The dichroism of a chromophore then depends on our ability to orientate the particles to which it is bound to. It is, however, possible to compare directly the orientation of absorption and emission dipoles by performing simultaneous measurements of LD, absorption and polarized fluorescence on the same sample. Let (A) and (F) be the absorption and fluorescence of two given chromophores (a) and (f) in two particles i and j. Then;

$$\begin{cases} \tau_A(\mathrm{a,i}) = \mathrm{LD}/3A(\mathrm{a,i}) = \tau_S(\mathrm{a}) \times \phi_\mathrm{i} \\ \tau_A(\mathrm{a,j}) = \mathrm{LD}/3A(\mathrm{a,j}) = \tau_S(\mathrm{a}) \times \phi_\mathrm{j} \end{cases} \quad \begin{cases} F(\mathrm{f,i}) = S_F(\mathrm{f}) \times \phi_\mathrm{i} \\ F(\mathrm{f,j}) = S_F(\mathrm{f}) \times \phi_\mathrm{j} \end{cases}$$

and $\tau_A(\mathrm{a})/\tau_F(\mathrm{f}) = S_A(\mathrm{a})/S_F(\mathrm{f})$ is independent of the particles i and j. Accordingly, we will use the

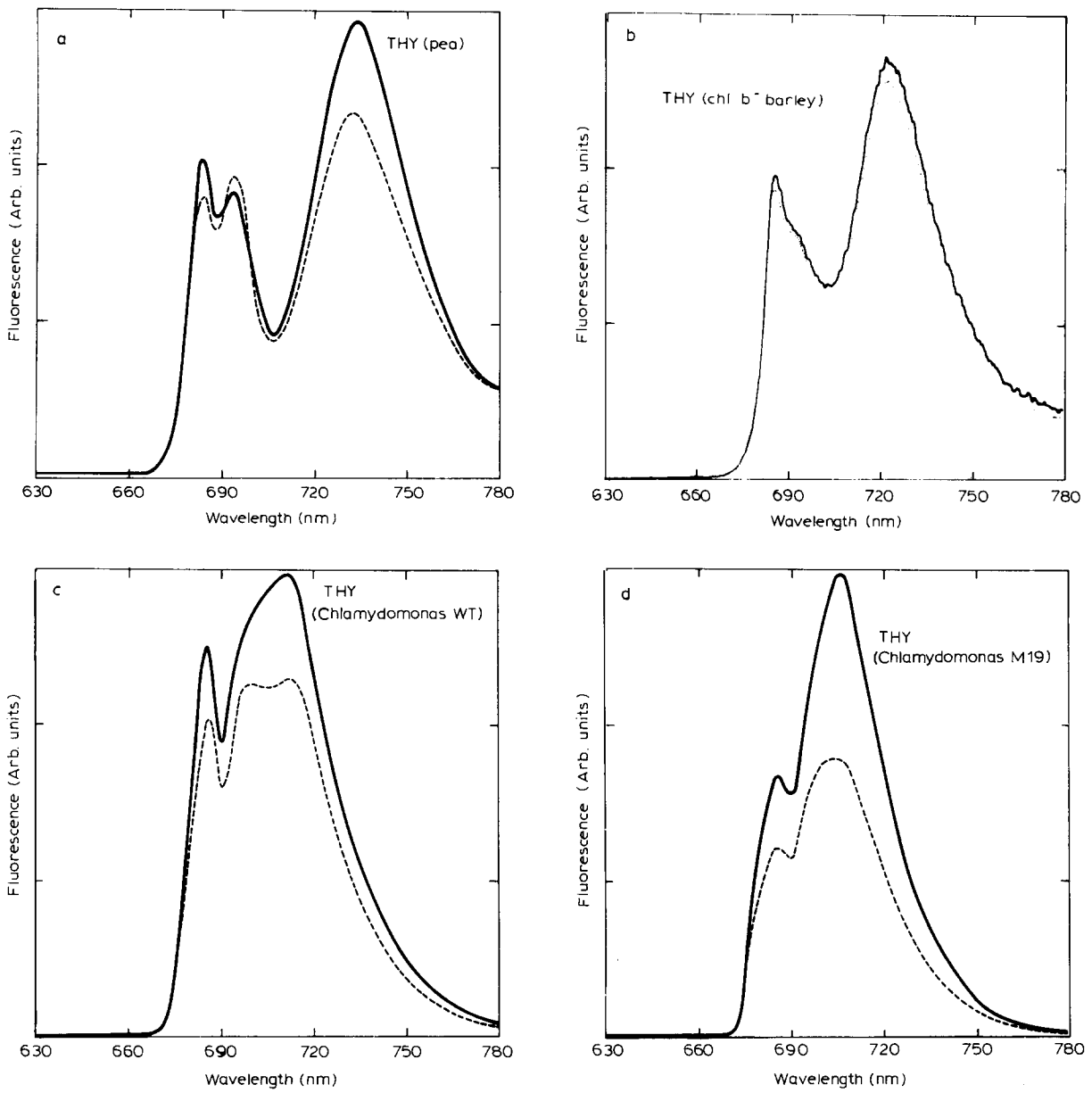

Fig. 8. Polarized fluorescence emission spectra at 77 K of thylakoids (THY) of pea (a), Chl-*b*-less barley (b), wild type (c) and M_{19} (d) mutant of *C. reinhardtii*: $F_{\parallel}$ (———) and $F_{\perp}$ (------).

observation of a constant value of this ratio as a valid indication that the orientations of both absorbing and fluorescing chromophores relative to the main axis of the particles were the same in the two samples *.

This computation is significant when only one chromophore contributes to the wavelength under study. The constant value of $F_{\parallel}/F_{\perp}$ over the

* A constant value of τ_A/τ_F in various particles does not necessarily imply that the two chromophores a and f have an orientation with respect to the long axis of the particles which is identical for all the particles, because a change in the orientation of both chromophores could fortuitously lead to the same ratio. However, such a situation seems highly improbable when analyzing pigment beds which are already characterized by an identical wavelength in absorption or emission in different particles.

TABLE II

ANISOTROPY OF F_{735} IN PEA OR WILD-TYPE BARLEY

$\sigma_A = (A_{\parallel} - A_{\perp})/3A$ and $\sigma_F = (F_{\parallel} - F_{\perp})/3F$ values observed on thylakoids and chlorophyll-protein complexes presenting a 735 nm maximal fluorescence. Error values are indicated. THY, thylakoid.

	τ_A (697)	τ_A (720)	τ_F (735)	$\frac{\tau_A(697)}{\tau_F(735)}$
THY pea	0.092 ± 0.005	0.085 ± 0.006	0.097 ± 0.005	0.95 ± 0.12
PS I_{110} pea	0.137 ± 0.007	0.140 ± 0.007	0.132 ± 0.007	1.04 ± 0.1
PS I_{110} barley	0.107 ± 0.005	0.110 ± 0.006	0.110 ± 0.006	0.97 ± 0.12
LHC I	0.170 ± 0.009	0.220 ± 0.015	0.220 ± 0.010	0.77 ± 0.08

TABLE III

ANISOTROPY OF F_{722} IN THE CHLOROPHYLL b^- MUTANT OF BARLEY

$\sigma_A = (A_{\parallel} - A_{\perp})/3A$ and $\sigma_F = (F_{\parallel} - F_{\perp})/3F$ values observed on thylakoids and chlorophyll-protein complexes presenting a 722 nm maximal fluorescence. Error values are indicated. THY, thylakoid.

	τ_A(697)	τ_F(722)	$\frac{\sigma_A(697)}{\sigma_F(722)}$
THY b^- barley	0.080 ± 0.004	0.021 ± 0.001	3.8 ± 0.4
PS I b^- barley	0.036 ± 0.002	0.010 ± 0.0005	3.6 ± 0.4
PS I_{65} pea	0.062 ± 0.003	0.016 ± 0.001	3.8 ± 0.4

TABLE IV

ANISOTROPY OF F_{715} IN *C. REINHARDTII* WILD TYPE

$\sigma_A = (A_{\parallel} - A_{\perp})/3A$ and $\sigma_F = (F_{\parallel} - F_{\perp})/3F$ values observed on thylakoids and chlorophyll-protein complexes presenting a 715 nm maximal fluorescence. Error values are indicated. THY, thylakoid; WT, wild type

	τ_A(695)	τ_F(715)	$\frac{\sigma_A(695)}{\sigma_F(715)}$
THY (*C. reinhardtii* WT)	0.146 ± 0.007	0.094 ± 0.005	1.6 ± 0.16
PS I_{110} (*C. reinhardtii* WT)	0.112 ± 0.006	0.060 ± 0.003	1.8 ± 0.18
PS I_{65} (*C. reinhardtii* WT)	0.083 ± 0.004	0.045 ± 0.002	1.8 ± 0.18

TABLE V

ANISOTROPY OF F_{707} IN *C. REINHARDTII* (M_{19})

$\sigma_A = (A_{\parallel} - A_{\perp})/3A$ and $\sigma_F = (F_{\parallel} - F_{\perp})/3F$ values observed on thylakoids and chlorophyll-protein complexes presenting a 707 nm maximal fluorescence. Error values are indicated. THY, thylakoid

	τ_A(691)	τ_F(707)	$\frac{\sigma_A(691)}{\sigma_F(707)}$
THY (*C. reinhardtii* M_{19})	0.167 ± 0.008	0.153 ± 0.008	1.10 ± 0.11
CP0 (*C. reinhardtii* M_{19})	0.100 ± 0.005	0.08 ± 0.004	1.25 ± 0.13

whole width of their respective fluorescence emission peak suggests that such is the case in our study. On the contrary, several chromophores contribute to the LD spectra in the particles we have studied. In any of those which contain the PS I reaction center, the maximum in the LD spectrum peaks at 697 ± 1 nm with an additionnal peak at about 720 nm in the particles which show 735 nm fluorescence. We have then based our LD/A evaluation on the chromophores C_{697} and C_{720}. According to recent photoselection studies, the absorption maximum at 77 K of the special pair, P-700, is at 695.5 nm [21]. Furthermore a value of 2.7 has been reported for the anisotropy of the main absorption band of P-700 at room temperature [22]. These observations are fairly consistent with our observation of the presence of a 697 nm maximum in the τ_A (λ) spectrum of the particles containing the PS I reaction center while those lacking in the reaction center do not have this highly dichroic component.

Chlorophyll-protein complexes

PS I_{65}. The LD and absorption spectra of PS I_{65} in pea and in *C. reinhardtii* are identical. In the blue part of the spectrum one observes the presence of highly dichroic carotenoids. There is no chlorophyll *b* related absorption signal either in the blue or red part of the spectra, which is consistent with a Chl *a*/Chl *b* ratio higher than 12. The PS I particles isolated from the Chl *b*-less barley mutant display the same LD and absorption spectra as those of the two PS I_{65} particles described above [23]. This identity suggests that the pigments in the PS I core are spectrally identical and have the same orientation with respect to the geometry of the isolated complexes in the three organisms from which they are extrated.

PS I antennae. The LD and absorption spectra of the LHC I from pea and CP0 from *C. reinhardtii* are very similar. Two main differences from the spectra of the PS I_{65} particles can be observed. First, in the blue part of the spectrum the carotenoids are less dichroic in the antennae particles and, secondly, in the red part of the spectrum we note the presence of highly oriented chlorophyll at 650 nm. The present analysis of LD spectra confirms the previous finding that chlorophyll *b* is part of the PS I antenna [6,16]. Its dichroism at 650 nm indicates a single Q_Y transition oriented at less than 35° from the long axis of the complex. Using chlorophyll protein complexes oriented in stretched polyvinyl alcohol films, Biggins et al. have previously described a similar orientation for Chl *b* in PS I particles isolated from pea [24]. This orientation of the Chl *b* in the PS I peripheral antenna can be distinguished from the orientation of the Chl *b* in the main light-harvesting complex where two transitions absorbing at about 650 nm have dichroisms of opposite signs while another around 660 nm lies parallel to the main axis of the particles [12]. The only noticeable difference between the spectra of CP0 and LHC I is the presence in the latter of a highly dichroic component in the 705–725 nm region. Thus, with the exception of the $C_{705-725}$, the pigments associated with the PS I peripheral antenna in pea and in *C. reinhardtii* are spectrally identical and have identical orientations with respect to the long axis of both complexes.

PS I_{110}. The LD and absorption spectra in the PS I_{110} particles from both *C. reinhardtii* and pea are similar in most of the regions of the spectrum, except in the 705–725 nm region where a long wavelength absorbing component is observed in PS I_{110} from pea but not in *C. reinhardtii.*

LD spectra of PS I_{110} can be reconstituted by a linear conbination of those of PS I_{65} and LHC I (in pea) as indicated Fig. 7. The coefficient obtained in the least-square fitting in absorption can be correlated with the respective number of chlorophylls bound to the 'PS I core' and the 'peripheral antenna' as described by previous authors [7,27]. The difference observed between LD and absorption fitting coefficients suggests that the PS I core complex has, in vivo, a lower degree of orientation than the PS I antenna complex. The difference between the actual PS I_{110} LD spectrum and that obtained through linear combination of PS I_{65} and LHC-I spectra is shown in Fig. 7. The residual peak observed at 668 nm corresponds to the negative component present in LHC I which is due to a slight denaturation of this complex. A similar combination can be done with the spectra of *C. reinhardtii* complexes. These LD experiments show that isolation procedures which separate the PS I_{65} and peripheral antennae complexes do not alter the orientation of the pigments within the

complexes. The fluorescence data discussed below are in agreement with this conclusion. It has been shown previously [13] that the main Q_Y transitions of Chl *a* in the PS I_{110} particles have an orientation close to the thylakoid plane. We can conclude from the present study that this is also the case for the Q_Y transition of the main Chl *a* of the PS I core and PS I antenna; The Chl *b* located in the peripheral antenna is oriented rather parallel to the thylakoid plane (less than 35° with respect to the plane).

Comparison between thylakoids and isolated chlorophyll protein complexes

The comparative spectral analysis of oriented particles conducted in this study gives information on the origin of the various fluorescence bands at 77 K observed in intact thylakoids. We discuss below the characteristics of F_{735}, F_{722}, F_{715} and F_{707}.

Pea or wild-type barley. The $\tau_A(\lambda)$ spectra of pea thylakoids and of the corresponding PS I_{110}, PS I_{65}, and LHC-I particles are shown on Fig. 3. There are two maxima of equal amplitude at 697 and 720 nm in the thylakoids and PS I_{110} $\tau_A(\lambda)$ spectra. One maximum only is visible in the spectra of PS I_{65} and LHC-I, at 697 nm and 715–725 nm, respectively. The equal values of τ_A (697) and τ_A (720) in PS I_{110} and thylakoid (Table II) indicate that the chromophores absorbing at these wavelengths have the same orientation with respect to the membrane plane. An angle of less than 24° between the Q_Y long wavelength transition of P-700 and the membrane plane has been determined in previous studies [22]. Similarly, Kramer and Amesz [25] have recently calculated that the angle of F_{735} with respect to the membrane plane is less that 23.5°. In our most dichroic sample, the LHC I, the angle value for F_{735} is less than 25°. Taking into account that F_{735} is present only in particles containing $C_{705-725}$, the tilt angle values previously published for F_{735} and the Q_Y transition of P-700 are consistent with the equal dichroic ratios that we observe at 697 and 705–725 nm. That $C_{705-725}$ and F_{735} correspond to the same bed of pigments is further confirmed by the equal values of τ_A (720) and τ_F(735) in LHC I, PS I_{110}, and intact thylakoids from pea (Table II). Furthermore, the constant value of τ_A/τ_F ratio is a strong indication that C_{697} and F_{735} have an identical orientation relative to the long axis of the particle in thylakoids and isolated PS I_{110}.

Chl-b-less mutant of barley. We have shown in Table III that τ_A (697) is not equal to τ_F(722) and that the τ_A (697)/τ_F (722) ratios are identical in the three particles: thylakoid and PS I from Chl *b*-less mutant of barley, and PS I_{65} of pea. Two conclusions can be deduced from these observations. (i) F_{722} has a different orientation than C_{697} relative to the plane of the thylakoid; this indicates that F_{722} and C_{697} do not originate from the same pigment bed. The absorbing dipoles corresponding to F_{722} are probably situated at longer wavelength than 697 nm, where τ_A (λ) has its maximum. (ii) The constant value of τ_A/τ_F ratio indicates that in Chl-*b*-less thylakoids and isolated complexes, the two dipoles have a constant orientation relatively to the long axis of the particle. This strengthens the assumption that F_{722} in the thylakoid of the Chl-*b*-less mutant of barley has its origin in the core PS I.

Wild-type C. reinhardtii. In contrast to the case of the PS I_{110} and PS I_{65} particles isolated from pea, those from *C. reinhardtii* have the same maximal fluorescence emission at 715 nm. The τ_A (λ) spectra for these two particles and for the intact thylakoids from the wild-type strain of *C. reinhardtii*, have the same maximum at 697 nm. We show on Table IV that the ratio τ_A/τ_F is the same in the PS I_{110} and PS I_{65} particles and is very close to that in the intact membranes. This situation appears to be similar to the case of the Chl-*b*-less mutant of barley with the dipoles responsible for F_{715} being closer to the long axis of the particle than the ones involved in F_{722}.

M_{19} mutant of C. reinhardtii. The 707 nm fluorescence observed at 77 K in the intact thylakoids of the M_{19} mutant of *C. reinhardtii* has previously been attributed to the fluorescence of the peripheral PS I antenna [6]. In these particles the maximum of τ_A (λ) spectrum is at 691 nm, compared to 697 nm in particles showing PS I activity. The similar values of the τ_A/τ_F ratios reveal that C_{691} and F_{707} have a fairly constant orientation in the thylakoid and the isolated CP0 complex: this observation confirms the previous attribution of F_{707} to CP0 pigments. According to our experiments the τ_A/τ_F value is close but not equal to 1; we do

not know presently whether the 707 nm fluorescence corresponds to the pigment absorbing at 691 nm or not.

Conclusion

A large difference in the emission wavelengths of the PS I pigments at low temperature can be noticed between green plants and *C. reinhardtii*: in the latter, the 'peripheral antenna' pigment fluorescens at shorter wavelength than the 'core' pigments, while in the former the opposite situation is observed.

Our results show that PS I 'core' chlorophyll complexes of the two organisms are composed of the same pigments with identical intrinsic orientation inside the complexes. Most of the observed spectral differences are explained by an additional pigment present only in the peripheral antenna of green plants, absorbing at 705–725 nm, fluorescing at 735 nm, and oriented with its Q_Y transition as close to the thylakoid plane (less than 24°) as the trap of PS I. Satoh and Butler [26] have measured the intensity of F_{735} and the rate of photoxydation of P-700 as function of temperature. They showed that F_{735} competes with PS I photochemistry at low temperatures. However, the function of these pigments under physiological conditions is still an open question [16].

References

1 Butler, W.L. (1961) Arch. Biochem. Biophys. 92, 413–422
2 Goedheer, J.C. (1964) Biochim. Biophys. Acta 88, 304–317
3 Boardman, N.K. (1970) Annu. Rev. Plant Physiol. 21, 115–140
4 Goedheer, J.C. (1972) Annu. Rev. Plant Physiol. 23, 87–112
5 Mullet, J.E., Burke, J.J., Arntzen, C.J. (1980) Plant Physiol. 65, 823–827
6 Wollman, F.-A. and Bennoun, P. (1982) Biochim. Biophys. Acta 680, 352–360
7 Mullet, J.E., Burke J.J. and Arntzen C.J. (1980) Plant Physiol. 65, 814–822
8 Mullet, J.E., Leto, K. and Arntzen, C.J. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. 5, pp. 557–568, Balaban International Science Services, Philadelphia, PA
9 Breton, J. and Vermeglio, A. (1982) Photosynthesis (Vol. I) Energy Conversion by Plants and Bacteria (Govindjee, ed.), pp. 153–194, Academic Press, New York
10 Abdourakhmanov, I.A., Ganago, A.O., Erokhin, Yu.E., Solov'ev, A.A. and Chugunov, V.A. (1979) Biochim. Biophys. Acta 546, 183–186
11 Haworth, P., Arntzen, C.J., Tapie, P. and Breton, J. (1982) Biochim. Biophys. Acta 679, 428–435
12 Haworth, P., Tapie, P., Arntzen, C.J. and Breton, J. (1982) Biochim. Biophys. Acta 682, 152–159
13 Tapie, P., Haworth, P., Hervo, G. and Breton, J. (1982) Biochim. Biophys. Acta 682, 339–344
14 Haworth, P., Tapie, P., Arntzen, C.J. and Breton, J. (1982) Biochim. Biophys. Acta 682, 504–506
15 Picaud, A., Acker, S. and Duranton, J. (1982) Photosynth. Res. 3, 203–214
16 Haworth, P., Watson, J.G. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 724, 151–158
17 Breton, J. (1977) Thèse Doctorat d'Etat, Université Paris Sud
18 Gagliano, A.G., Geacintov, N.E., Breton, J., Acker, S., and Remy, R., (1979) Photochem. Photobiol. 29, 415–418
19 Anderson, J.M. (1980) Biochim. Biophys. Acta 591, 113–126
20 Anderson, J.M., Brown, J.S., Lam, E. and Malkin, R. (1983) Photochem. Photobiol. 38 205–210
21 Schaffernicht, H. and Junge, W. (1982) Photochem. Photobiol. 36, 111–115
22 Breton, J. (1977) Biochim. Biophys. Acta 459, 66–75
23 Tapie, P., Acker, S., Arntzen, C.J., Choquet, Y., Delepelaire, P., Diner, B., Wollman, F.-A. and Breton. J. (1984) Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 693–696, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
24 Biggins, J. and Svejkovsky, J. (1980) Biochim. Biophys. Acta 592, 565–576
25 Kramer, H.J. and Amesz, J. (1982) Biochim. Biophys. Acta 682, 201–207
26 Satoh, K., Butler, W.L. (1978) Biochim. Biophys. Acta, 502, 105–110
27 De Vitry, C., Wollmann, F.A. and Delepelaire, P. (1983) C.R. Acad. Sci. (Paris) 297, 277–280