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# ORIENTATION OF THE PIGMENTS IN THE THYLAKOID MEMBRANE AND IN THE ISOLATED CHLOROPHYLL-PROTEIN COMPLEXES OF HIGHER PLANTS

## III. A QUANTITATIVE COMPARISON OF THE LOW-TEMPERATURE LINEAR DICHROISM SPECTRA OF THYLAKOIDS AND ISOLATED PIGMENT-PROTEIN COMPLEXES

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**The low-temperature linear dichroism spectrum of thylakoids oriented in polyacrylamide gel can be adequately described by a linear combination of the corresponding spectra of particles of light-harvesting complex, Photosystem I and Photosystem II, isolated by Triton X-100 extraction. The main conclusions which can be derived from this observation are: (1) The in vivo orientation of the pigments within each of the three complexes is not significantly affected by the extraction and purification procedures. (2) The various photosynthetic pigments are oriented roughly to the same extent in each of the three main biochemical constituents of the thylakoid. (3) All the complexes investigated behave like ellipsoids, the largest dimensions of which are lying in the plane of the photosynthetic membrane.**

## Introduction

In the previous papers of this series [1,2], we have demonstrated that low-temperature linear dichroism (LD) spectra of pea thylakoids and of various chlorophyll-protein complexes derived from the former could be obtained free of scattering artifacts, provided a suitable technique was used. In Ref. 1, we have made the unexpected observation that the linear dichroism spectra of thylakoids and of the extract obtained by solubilizing these thylakoids with Triton X-100 were very similar. By analyzing the 100 K LD spectra of the various particles prepared by the action of this detergent [2], we have shown that characteristic features appearing in the LD spectra of the light-harvesting complex (LHC) and Photosystem I (PS I) particle could be easily recognized in the LD spectrum of the thylakoids [2]. These observations indicate that the in vivo orientation of the various pigments is probably not greatly disturbed during the isolation and purification processes. This prompted us to analyse the relative contributions of each of the three separate complexes in the 100 K LD spectrum of the intact thylakoid.

## Materials and Methods

LHC was isolated from thylakoid membranes by solubilizing with the non-ionic detergent Triton X-100 (Sigma. Chemical Co) [3]. The PS I particle ($PS\ I_{110}$) was also isolated with the same detergent treatment [4]. PS II was isolated according to the

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Abbreviations: PS, photosystem; LHC, light-harvesting complex.

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technique of Mullet and Arntzen [5] in which thylakoids were first treated with Triton X-100 and finally solubilized using digitonin and octyl-β-D-glucopyranoside (Sigma. Chemical Co). This technique also yields LHC, which has been used in this study for comparison. The PS II particle produced by this procedure is contaminated by the presence of LHC [5], but the extent of this contamination can be reduced by a subsequent detergent treatment with sodium cholate in the presence of urea [5]. The LD spectra were digitized with a Tracor Northern 1710 apparatus at 0.2 nm per channel. Fitting of the spectra was done using a general purpose least-squares programme [6].

$A_{\parallel}$ and $A_{\perp}$ refer to the absorption of light polarized, respectively, parallel and perpendicular to the longer axis of the oriented object, i.e., that axis orientating perpendicularly to the direction of squeezing as assumed in Ref. 1.

## Results and Discussion

*The 'fingerprint' of LHC orientation in vivo*

In Ref. 1 we noted that the LHC LD spectrum at room temperature was rather insensitive to the state of aggregation and/or the environment of the complex. In the present study, we have further extended this observation to low-temperature spectra and to a different biochemical preparation of the same complex, i.e., LHC isolated by digitonin and octylglucopyranoside solubilisation (data not shown). In all of these samples we have noticed that the ratio of the amplitude of the sharp negative LD signal at 648 nm to the amplitude of the 676.5 nm peak was constant (within 5%). Furthermore, this 648 nm signal, which is absent in the PS I spectra, is only present in the spectra of LHC-containing material. These observations have led us to propose the hypothesis that the 648 nm LD component belongs exclusively to the LHC and does characterize the in vivo orientation of this complex.

*The LD spectrum of PS II*

We have previously reported [2] the 100 K LD spectrum of a PS II particle which, as shown by polyacrylamide gel electrophoresis, was contaminated by a variable amount (30–60% on a chlorophyll *a* basis) of LHC. In accordance with the above hypothesis, we have subtracted from the LD of our contaminated particle (Fig. 1a) the contribution of LHC normalized on the 648 nm LD component. This contribution is shown in Fig. 1b. The resulting LD spectrum of the 'purified' PS II is shown in Fig. 1c. Several observations indicate both the validity of our approach and that the spectrum of Fig. 1c can be identified with the 100 K LD spectrum of PS II: (i) The contribution of LHC which had to be subtracted from the spectra of several PS II preparations contaminated to different extents is in qualitative agreement with the amount of the contamination as estimated by polyacrylamide gel electrophoresis. (ii) Together with the elimination of the 648 nm component from the spectrum shown in Fig. 1a, the subtrac-

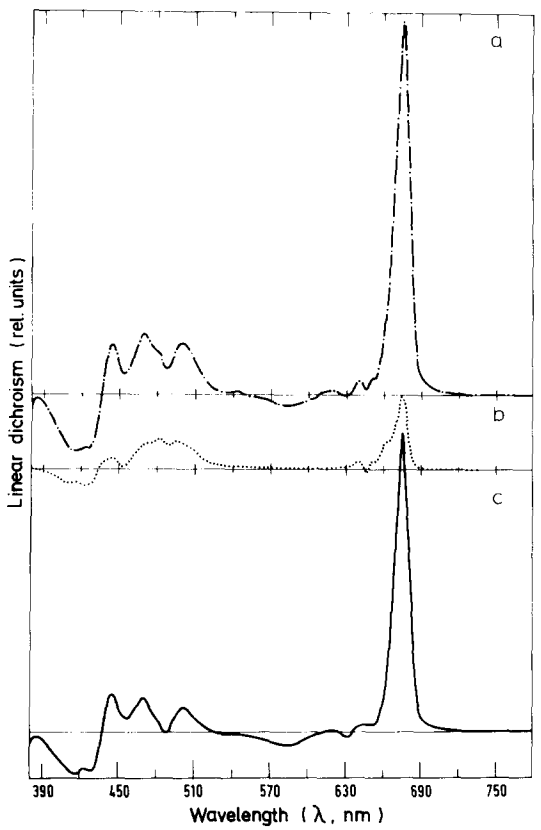


Fig. 1. LD spectra at 100 K. (a) Experimental spectrum of a PS II particle contamined by LHC (see text) (· – ·). (b) Experimental spectrum of the LHC contribution in the spectrum of a; assessment is performed in the 648 nm region, as described in the text (· · · · · ·). (c) Calculated spectrum of a 'pure' PS II particle (spectrum a – spectrum b) (———).

tion also eliminates the 482 nm shoulder which has been previously attributed to chlorophyll *b* in the LHC [2]. (iii) Finally, the LD spectrum presented in Fig. 1c is very similar to that obtained (spectrum not shown) with a biochemically pure PS II prepared from spinach by the cholate-polyacrylamide gel electrophoresis technique [7]. In this last particle the LHC contamination has been estimated to be less than 3% by polyacrylamide gel electrophoresis performed under denaturating conditions (Acker, S., personal communication). The observation that these two LD spectra appear identical in the 630–660 nm region strengthens the validity of our assumption regarding the assigment of the 648 nm component.

*Reconstitution of the thylakoid LD spectrum*

An approach similar to that used to eliminate the contribution of LHC from the PS II LD spectrum has been used to subtract the contribution of LHC in the LD spectrum of thylakoids. The resulting spectrum was then deconvoluted by least-squares fitting in the 630–735 nm region using the PS I and 'purified' PS II LD spectra. The results of this mathematical analysis are presented in Fig. 2 where the LD spectrum of the thylakoid and those of the three particles (each with its own relative weight) are depicted. A difference spectrum between the thylakoids and the sum of its three components is also presented in Fig. 2. In the red spectral region the fit is satisfactory and cannot be significantly improved upon allowing a possible shift in the LD spectrum of the thylakoid. When the computer is allowed to fit directly the thylakoid spectrum with the three individual components, a small residual 648 nm signal is present in the difference spectrum. This observation led us to use the procedure of subtracting the contribution of LHC from the thylakoid spectrum prior to the curve fitting. The amount of each of the three biochemical components in terms of the area under the LD signal of the main $Q_y$ transitions of chlorophyll *a* (665–735 nm) is presented in the caption of Fig. 2. The fit, however, is worse in the blue region with prominent negative peaks at 486 and 455 nm in the residue. Only slight decreases in the magnitude of this residue can be observed when the spectra are fitted over the whole spectral range (380–735 nm). Several hypotheses can be proposed to explain this signal of unknown origin. For example, a limited pigment disorientation could occur on one or several of the complexes during the purification process. Alternatively, some pigments embedded either in a pigment-protein complex or directly in the lipid bilayer could be lost during the separation steps. During the isolation of LHC and PS I, a faint yellow-green band can be observed at the top of the gradient after the centrifugation [3]. The absorption spectrum of this band shows a large contribution from carotenoids and a smaller one from chlorophylls. The 100 K LD spectrum of this fraction in squeezed polyacrylamide gel presents three pronounced negative peaks at about 670, 485 and 455 nm. Although we have not

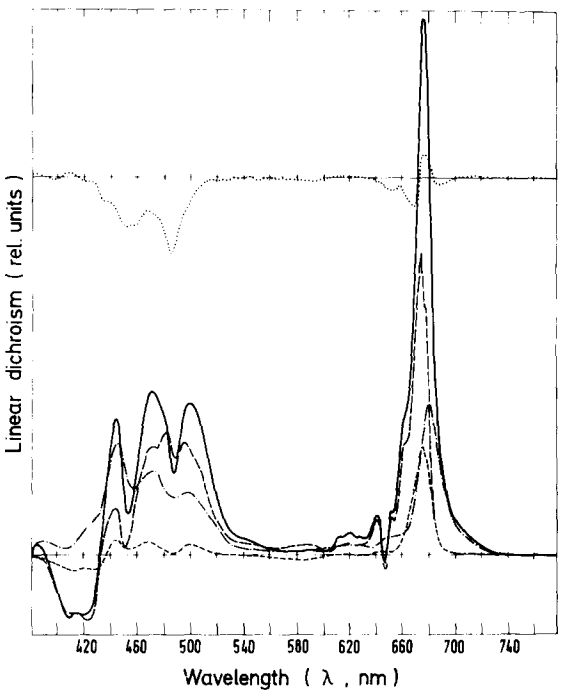


Fig. 2. Recomposition of the LD spectrum (100 K) of the thylakoid by linear combination of LHC, PS I and PS II spectra. The fit is performed in the 630–735 nm region. The difference (· · · · · ·) is shown in the whole range (380–780 nm), with normalisation determined by least-squares fitting. Thylakoids (———), LHC (– –), PS I (– · –), PS II (– – –). The relative contribution of the area of the three spectroscopic components in the total area of the thylakoid LD spectrum measured in the 665–735 nm spectral region is 46% for LHC, 36% for PS I and 18% for PS II.

characterized biochemically this fraction, we noticed a significant improvement of the fit when this component was added in the adjustments. More experiments are needed in order to clarify this point which seems related to the problem of the 'free pigments' appearing during the isolation of the various particles.

*LD spectra in the 535–635 nm spectral range*

The amplified LD signals of the thylakoids and each of its three components in this region are shown in Fig. 3 using the same scaling factors as for the spectra presented in Fig. 2. At the level of amplification required to obtain the spectra presented in Fig. 3, slight baseline differences are present and preclude a quantitative fitting of the spectra by our technique. However, most of the spectroscopic features present in the LD spectrum of the thylakoid can be equated, at least semi-quantitatively, to corresponding features of one or the other of the components. This observation indicates that the LD spectrum of thylakoids can be viewed as the sum of the LD spectra of the three separate complexes. Although the assigment of the various spectroscopic features which can be detected in these spectra to known constituents of

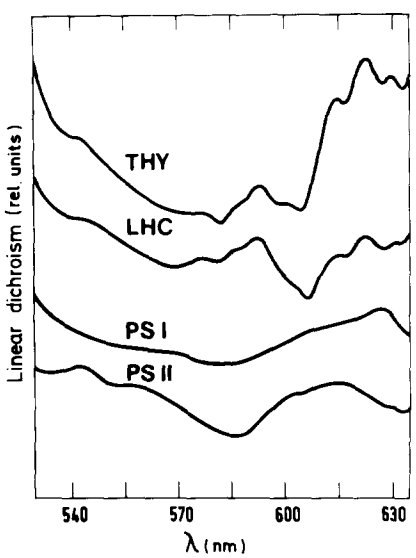


Fig. 3. Amplified LD spectra (100 K) in the 535–635 nm region. Each individual spectrum is depicted with the amplitude shown in Fig. 2. THY, thylakoid.

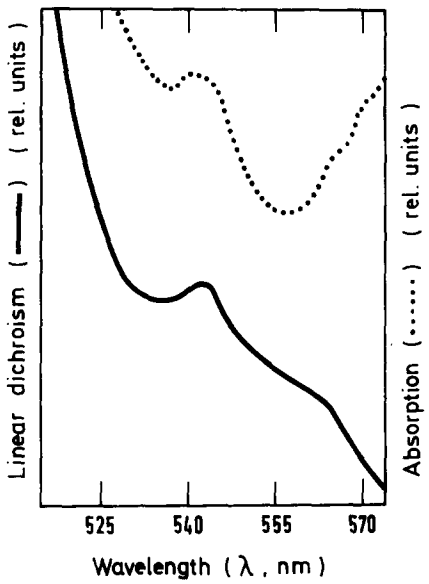


Fig. 4. Amplified absorption (· · · · · ·) and LD (———) spectra (100 K) of the PS II particle in the 520–570 nm region.

the thylakoids is beyond the scope of this article, special mention should be made of the 543 nm signal of the PS II particles. The absorption and LD of the PS II particle in the spectral range 520–570 nm are shown in Fig. 4 and a positive dichroism can be observed for the transition absorbing at 543 nm. This signal, which is also present in the biochemically pure particle but is not seen in LHC and PS I, can be assigned to a transition from pheophytin *a* as (i) this molecule absorbs in vitro at 535 nm and (ii) the presence of this species has been demonstrated in PS II particles [8,9]. The orientation of this molecule, which plays the role of the primary acceptor for the trap of PS II, has been recently investigated by Ganago et al. [10] by measuring the light-induced polarized absorption changes of PS II particles poised at very low redox potential. These authors have demonstrated that the $Q_y$ transition (and accordingly the plane of the macrocycle) of this molecule was oriented rather perpendicular to the plane of the particle. Several observations from resonance Raman spectroscopy [11] and from the LD of pigments oriented in stretched plastic films [12] have been taken to indicate that the 543 nm band could be assigned to an out-of-plane transition. The

positive dichroism at 543 nm reported in this study * would then also imply that the pheophytin plane is rather perpendicular to the plane of the particle, a finding which is in agreement with the earlier conclusions reached by Ganago et al. [10].

## Conclusions

In a comparative study of the low-temperature absorption spectra of thylakoids versus LHC and PS I, it has recently been shown that in order to obtain accurate fitting it was necessary to include a third component in the absorption spectrum of thylakoids [13]. In the present work, we have used a more direct approach by taking into account the three biochemically isolatable particles. Furthermore, by using LD spectra we take advantage of (i) the larger difference that exists between the three complexes in LD spectra as compared to absorption spectra and (ii) the greater information content of LD spectra where not only the absorption characteristics but also the specific orientation of the pigments are involved. The most unexpected result of this study is the fact that a mere linear combination of the LD spectra of the three particles can lead to fits as good as that presented in Fig. 2. A completely different result would have been obtained provided the orientation of the pigments with respect to the symmetry axis of each of the isolated complex is not correlated to the orientation of the same pigment sets with respect to the thylakoid plane.

One of the major conclusion which can be derived from the results presented here is that the in vivo orientation of the pigments is not significantly affected by the extraction and purification processes. This is true at least for the detergent (or combination of detergents) used in this study. Such an observation is of interest not only because it provides good evidence that these preparations can be used for further structural studies but also as it indicates a valuable technique to assess the structural 'intactness' of other types of preparations. Furthermore, the good quality of the fit presented here, as well as the observation that the LD spectra of thylakoids and Triton-dissolved thylakoids are very similar [1], indicates that the pigment-protein interactions prevailing in each of these complexes are not disturbed by modification in the protein-protein and/or protein-lipid interactions which occur when the native membrane is dissolved with detergents.

Another significant conclusion is also obtained regarding the fact that the various photosynthetic pigments are oriented roughly to the same extent in each of the three main biochemical constituents of the thylakoid. Although a more quantitative description must await a comparison of the extent of orientation of the different particles, this assertion is derived from the observation that the proportion of oriented chlorophyll *a* attributed to each of the three complexes is in agreement with the relative contributions of these three constituents in the thylakoid [13,14].

Finally, it is worth noting that in the case of the thylakoid membranes our spectrum describes the orientation of the various pigments with respect to the membrane plane, while in the isolated complexes we analyse the orientation of the same set of pigments with respect to the largest dimension(s) of the complex itself. There was no a priori reason why in the membrane the direction of these largest dimension(s) should be positioned in the same way for all the three different complexes. From the good overall quality of the fits presented here we must conclude (i) that all the complexes investigated here behave like ellipsoids (oblate or prolate) and (ii) that the largest dimension(s) of these ellipsoids is (are) lying in the plane of the thylakoid membrane. This observation is of interest in two ways. Firstly, it provides a reference plane when the orientation of some component, which can be more easily detected in a particle than in the intact system, has to be determined with respect to the plane of the membrane. This is clearly demonstrated in the present study where the orientation of the primary acceptor pheophytin plane perpendicular to the largest dimension(s) of the PS II particle can be directly related to a similar orientation with respect to the thylakoid plane. Secondly, it seems to indicate that there is no transmembrane orientation of a long axis in any of the three isolatable complexes. However, before such a

* And which can be directly correlated with the positive dichroism observed for this transition in the absorbance changes spectrum [10].

conclusion can be drawn it is necessary to analyse in detail the possible changes in the external shape of the protein part of the native complex which could be induced by either the bound detergent and/or auto-aggregation of these complexes.

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