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

I. DETERMINATION OF OPTIMAL CONDITIONS FOR LINEAR DICHROISM MEASUREMENT

PHILIP HAWORTH *, CHARLES J. ARNTZEN *, PIERRE TAPIE and JACQUES BRETON **

Service de Biophysique, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette Cedex (France)

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The nature and possible causes of polarized light-scattering artefacts in linear dichroism measurements are investigated. Using criteria described in this article, the available orientation techniques have been critically assessed in order to obtain the linear dichroism spectra of thylakoids and of pigment-protein complexes isolated from pea. It is demonstrated here that the polyacrylamide gel squeezing technique of Abdourakhmanov et al. (Abdourakhmanov, I.A., Ganago, A.O., Erokhim, Yu.E., Solov'ev, A.A. and Chugunov, V.A. (1979) Biochim. Biophys. Acta 546, 183–186) does not lead to pigment degradation and that the linear dichroism spectra obtained in these conditions are essentially free of scattering artefacts. The linear dichroism spectra of light-harvesting complex isolated in different states of aggregation or incorporated into phospholipid vesicles are compared to the spectra of thylakoids. This comparison indicates: (1) that the isolation procedure of Burke et al. (Burke, J.J., Ditto, C.L. and Arntzen, C.J. (1978) Arch. Biochem. Biophys. 187, 252–263) leads to light-harvesting complex in which the in vivo orientation of pigments is preserved; (2) that the antenna chlorophyll *a* molecules of this complex have a significant degree of orientation with respect to the plane of the thylakoid.

Introduction

It is now recognized that most, if not all, of the photosynthetic pigments are bound to intrinsic proteins [1]. Several of these pigment-protein complexes have been isolated and models, consistent with the postulate of Singer and Nicholson [2], have been proposed for the arrangement of these complexes in the photosynthetic membrane. At best, however, these models project only a rough overall view of the organization of the membrane [3].

In terms of their orientation, the molecular organization of pigments in photosynthetic systems can be described at three different levels [4,5]. Firstly, by the angle between chromophores in each of the isolatable pigment-protein complexes. Secondly by the angle between the chromophore and the membrane plane. Finally, the question arises of how pigments belonging to adjacent complexes (in the membrane) are organized with respect to each other in order to achieve the cooperative effect of photosynthesis. In the case of purple bacteria, several of the questions related to the first two criteria have been answered [5]. This was made possible because of the availability of purified pigment-protein complexes containing only a small number of chromophores per particle. In the case of green plants the second type of order has mostly been investigated and there is little informa-

* Permanent address: MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 U.S.A.
** To whom correspondence should be addressed.

tion on either the orientation of the chromophores in the isolated pigment-protein complexes or on the orientation of these particles with respect to the thylakoid plane.

The technique of linear dichroism (LD), which measures the differential absorption of orthogonally plane-polarized light ($LD = A_{\parallel} - A_{\perp}$) is used to obtain information on the specific orientation of a given chromophore in an anisotropic sample [5]. Although straightforward in principle, application of this type of analysis to green plant structures has produced results somehow difficult to interpret. In early studies [6–10], a slight dichroism of chlorophyll in vivo was detected only in the red spectral range. This was interpreted as either an artefact of structural anisotropy (form dichroism) or a low degree of orientation of a long-wavelength form of chlorophyll *a*. More recently [11,12] linear dichroism spectra of oriented thylakoids and chloroplasts have been obtained over the whole visible spectrum and these spectra indicate that form dichroism was not responsible for the observed signal. After thorough investigation of various artefacts which might distort the linear dichroism spectra, Breton et al. [12] and Geacintov et al. [13] have concluded that the linear dichroism signal of oriented chloroplasts was largely due to significant orientation of the in vivo pigments. However, the contribution of an S-shaped signal, originating from an effect of polarized light scattering was noticed in these spectra [12,13]. In view of this artefactual contribution, which has the wavelength dependence of the anomalous dispersion, reservations were expressed [12–14] concerning the possibility of quantitative and unambiguous interpretation of the linear dichroism data.

In this first of a series of papers we have critically assessed the linear dichroism spectra of pea thylakoids oriented by a variety of techniques. In so doing, we hope to select a technique which (i) gives a linear dichroism spectrum with minimal distortion due to scattering artefacts and (ii) is suitable for low temperature linear dichroism analysis of both thylakoids and isolated pigment protein complexes. We will demonstrate in this paper that these requirements are best fulfilled using the polyacrylamide gel squeezing technique. In addition we present evidence that, under the proper conditions, linear dichroism spectra of thylakoids and of a light-harvesting complex can be obtained essentially free of the scattering artefact.

Materials and Methods

Preparation of samples for linear dichroism

Chloroplasts were isolated from pea leaves using 0.1 M Tricine buffer (pH 7.8) containing 0.4 M sorbitol as described elsewhere [15]. Thylakoids were prepared from these chloroplasts by osmotic shock using 0.1 M sorbitol in 5 mM EDTA (pH 7.0). Detergent fractionation of the thylakoids was achieved by treatment with Triton X-100 (Sigma Chemical Co.) as described by Burke et al. [15]. Light-harvesting complex was isolated and purified using the procedure described in the same paper [15]. During the course of this investigation we have used light-harvesting complex in different states of aggregation. Light-harvesting complex removed directly from the sucrose gradient has an intense red fluorescence [15]; the work of Mullet and Arntzen [16] has revealed that this is the smallest isolated particle of light-harvesting complex. This sucrose fraction we have termed 'monomeric' light-harvesting complex. Treatment of this material with 5 mM $MgCl_2$ [15] purifies the preparation by aggregating the light-harvesting complex. A further treatment of this fraction with EDTA causes a break-up of the multi-sheet structure to give large single-sheet arrangements [16] we have called 'sheet' light-harvesting complex. Unilamellar phospholipid vesicles containing 'sheet' light-harvesting complex were prepared by the cholate dilution technique of Brunner et al. [17] using soybean L-α-phosphatidylcholine (type II-S; Sigma Chemical Co.). The size of all samples used for linear dichroism studies was determined using Amicon ultrafiltration equipment with Millipore (MF) filters (Millipore Corp.) of various pore sizes.

Orientation of samples for linear dichroism

We have employed a variety of techniques for the orientations of thylakoids and isolated light-harvesting complex for linear dichroism. The techniques referred to as dry film orientation and spreading orientation were performed according to Breton et al. [12]. Orientation of samples in stretched poly(vinyl alcohol) (Type III, Sigma Chemical Co.) films was in accordance with the method of Bolt and Sauer [18]. The polyacrylamide gel orientation technique we

have used is the method developed by Abdourakhmanov et al. [19]. The gel mixture contained acrylamide (10%, w/v), *N,N'*-methylenebisacrylamide (0.3%, w/v), glycerol (50–60%, v/v), *N,N,N',N'*-tetramethylethyldiamine (0.03%, v/v) and freshly prepared ammonium persulfate (0.05%, w/v) in distilled water. Orientation of the sample was achieved by squeezing the gel to 60–70% of its original length as described by Swarthoff et al. [20].

Optical methods

Absorption spectra of samples were determined using a Cary 17 spectrophotometer. Linear dichroism was measured with the apparatus described in Ref. 21, with the sample located as close as possible (usually 1–2 cm) from the phototube. In this article, $A_{/\!/}$ 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. 20.

Results and Discussion

1. Origin of the scattering artefact in linear dichroism measurement

Absorption and scattering are the two major events which have to be considered in the interaction of light with matter (molecule, particle). Far away from an absorption band, the intensity and spatial distribution of scattered light is primarily governed by two factors. The first is related to the size of the object in comparison to the wavelength (λ) of the incident radiation [22]. For small particles the intensity varies as a function of λ^{-4} while for large particles it is independent of λ and is proportional to the size of the particle [22]. When the ratio of sample dimension to wavelength is approximately unity, the intensity of the scattered light changes abruptly (a property used to determine particle size as a function of light scatter). The second parameter is related to the difference in polarizability (or refractive index, on a macroscopic scale) between the object and the surrounding medium.

In a particle which contains pigments, the polarizability changes when the wavelength of the incident light is varied in the region of the absorption band. This polarizability variation induces both the absorption and the scattering phenomena. Furthermore, the scattering intensity depends non-linearly upon the local pigment concentration. Latimer and Rabinowitch [23] have analyzed in detail scattering (at 90°) by *Chlorella* cells. They have shown that the wavelength dependence of this effect has the shape of anomalous dispersion. Furthermore, these authors were able to account for their experimental scattering curves by a calculation based upon the Mie scattering theory. In these model calculations the cells were replaced by homogeneous spheres with an isotropic refractive index.

An additional level of complexity is encountered when dealing with oriented objects for which both the anisotropy of polarizability and the shape of the object have to be further taken into account. The wavelength dependence of the polarization of light scattered (at 90°) from an oriented suspension of *Chlorella* has been analyzed by Swenberg and Geacintov [24]. Using a simple model in which the cell is equated to an homogeneous thin disc, they were able to explain the experimental spectra only when some orientation of the chlorophylls was taken into account. Such an orientation of the pigments induces an anisotropy of the dielectric tensor characterizing the polarizability properties of the object. In turn, the components of this dielectric tensor can be estimated from proper measurements of the optical properties of the oriented object. However a precise determination is possible only for pigment-containing systems of known geometry such as thin planar layers in which the dimension is large compared to the wavelength. In such cases, coherence effects take place [24], leading to polarized selective reflection [12]. Such effects have been described for monolayers of pigments [25] and quantitatively accounted for in a detailed study of chlorophyll *a* in lipid bilayers [26].

The influence of polarized scattering on linear dichroism spectra arises from the property of a linear dichrograph to detect in a limited solid angle the light transmitted through the sample. If the amount of differential polarized scattering is high, in comparison to differential polarized absorption, a distortion of the linear dichroism will be observed. This distortion, which we call scattering artefact, has been observed in the linear dichroism of photosynthetic membranes from green plants [12–14,27].

From our discussion of polarized light scattering it

can be inferred that the magnitude of the scattering artefact is dependent upon a variety of factors: the ratio of particle size to the wavelength; the shape of the oriented particle; the refractive index difference between sample and surrounding medium; and, finally, the geometry of the measuring instrument. The influence of these various factors on the quality of linear dichroism spectra has apparently not been considered sufficiently in some recent publications. For example, Biggins and Svejkovsky [28] have reported the appearance of a pronounced shoulder at 690 nm in the linear dichroism spectra of magneto-oriented chloroplasts upon addition of Mg^{2+}. A more recent study [29], however, suggests this is not the case. Since Mg^{2+} is known to induce thylakoid stacking, the lack of reproducibility might be due to changes in either the angle of light collection or the geometry of the pigmented layers. We have noticed (unpublished data) that the shape of the linear dichroism spectrum of magnetically oriented intact chloroplasts is also a function of the distance between sample and photodetector. These observations bear some resemblance to an analogous effect reported for the circular dichroism of chloroplasts [30].

Although scattering occurs over a wide spectral range, we can make a quantitative assessment of the above-mentioned artefact only in the 730–780 nm range. In this region there is no pigment absorption and therefore no linear dichroism. Consequently any observed signal in this region, i.e., a deflection from the baseline, is assumed to be due to the scattering artefact.

2. Comparison of available orientation techniques

Several techniques have been used to orient various photosynthetic materials macroscopically [5]. The specific aim of this study limits our choice to only those techniques which can orient both thylakoids and isolated chlorophyll-protein complexes. Furthermore, for a given technique to be of potential interest here, several criteria have to be fulfilled: no degradation of biological material; minimal scattering distortion and feasibility of low-temperature spectroscopy. If more than one of the orientation techniques fulfill these requirements, that technique with the largest LD/*A* ratio will be selected [5]. On the basis of these criteria we have eliminated magnetic field orientation, which cannot be applied to thylakoids and isolated complexes. Electric field orientation was eliminated because it cannot be used at low temperature and gives a small value of LD/*A*. The dry-film technique was abandoned because of pigment degradation caused by the increasing detergent concentration when dealing with isolated complexes. The stretching of transparent films made of either gelatin or poly(vinyl alcohol) and containing pigmented particles does induce orientation of those particles. This approach has been recently applied to reaction centers [31] and antenna complexes [18] isolated from photosynthetic bacteria. Biggins and Svejkovsky [14] have also used poly(vinyl alcohol) films to orientate both thylakoids and isolated pigment-protein complexes from higher plants. In our hands, however, this technique produces linear dichroism spectra with a rather large variability. This variation is, at least in part, related to the drying time of the poly(vinyl alcohol) films. Prolonged drying (2–3 days) at 4°C caused a blue shift in the absorption spectrum; faster drying (1–2 h) in a dry airstream at room temperature reduced this effect but did not abolish it. This pigment degradation led us to reject this technique also. The linear dichroism spectrum of thylakoids oriented by the film-stretching

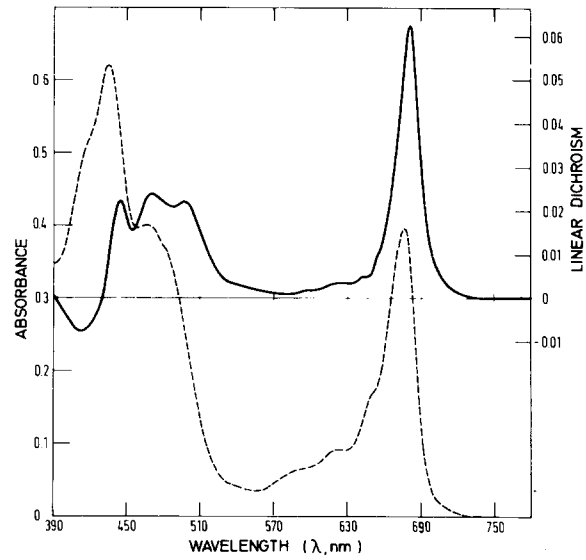

Fig. 1. Absorption (------) and linear dichroism (———) spectra of pea thylakoids oriented in a squeezed polyacrylamide gel.

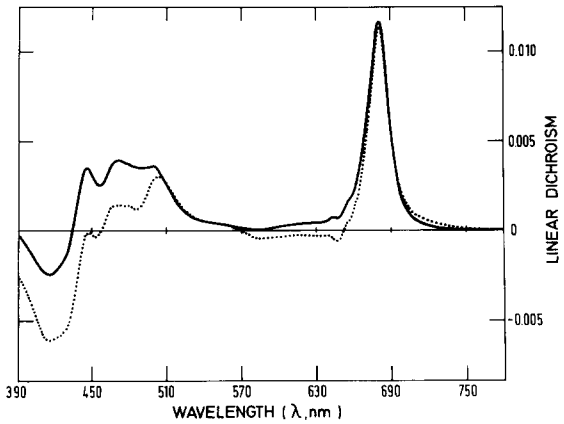

Fig. 2. Linear dichroism spectra of pea thylakoids oriented by the spreading technique (see text). The absorption at the red maximum was 0.31 A unit. The specimen was suspended either in air (· · · · · ·) or in 60% glycerol (———).

technique are, however, very similar to those reported for thylakoids oriented by the spreading technique [11,12]. This is demonstrated by comparing the low-temperature (77 K) spectra of thylakoids oriented by spreading (Fig. 3 of Ref. 12) and in poly(vinyl alcohol) films (Fig. 1 of Ref. 14).

The polyacrylamide gel squeezing technique developed by Abdourakhmanov et al. [19] will efficiently orient both intact membranes and isolated complexes derived from photosynthetic bacteria [19,20]. We have used the same technique to orient thylakoids and a variety of isolated complexes. The resulting samples were found to be optically homogenous with a high degree of orientation, suitable for low-temperature spectroscopy and showed no shift in the absorption spectrum (even for detergent solubilized complexes). The results of these studies will be reported in forthcoming publications. The room-temperature linear dichroism spectrum of pea thylakoids oriented by the gel squeezing technique, is shown in Fig. 1. For comparison, the linear dichroism spectrum of the same material oriented by the spreading technique is presented in Fig. 2 (dotted line). Note the identity of the linear dichroism spectrum of Fig. 2 (- - - - - -) to the previously published spectra [11,12]. Although there are many similarities between these spectra, the differences are significant. Thus it is important to determine whether these differences are due to a disruption of the pigment bed by the gel or to scattering differences between the samples. We note that the long-wavelength absorption maximum of the thylakoids included in the gel appears at 677–678 nm, as expected for chlorophyll *a* in vivo. In the 730–780 nm region the linear dichroism spectrum in Fig. 1 is more flat than the dotted spectrum in Fig. 2. This difference indicates a lower contribution of the scattering artefact in the case of the gel-oriented sample. In an attempt to reduce the scattering artefact for the spreading technique, the sample (glass plate bearing the oriented thylakoids) was suspended in a 60% glycerol/buffer mixture. The resulting linear dichroism spectrum is presented in Fig. 2 (solid line). This spectrum shows a reduced contribution of the scattering artefact around 750 nm and now more closely resembles the spectrum presented in Fig. 1. We note, too, that the linear dichroism spectrum of Fig. 1 appears almost identical to those reported for spinach thylakoids oriented by electric field [32]. Finally, it must be noted that upon increasing the distance between the sample and the phototube (from approx. 1 cm to approx. 20 cm), we observed no significant change in the shape of the linear dichroism spectrum for thylakoids in gels. Similar results were also observed for a variety of isolated particles.

All these observations indicate that the squeezing of polyacrylamide gels represents a useful orientation technique for obtaining linear dichroism spectra of isolated thylakoids with a minimum contribution of scattering artefact.

3. Effect of particle size on its linear dichroism spectrum

In this section we will further demonstrate that the linear dichroism spectra obtained using the gel squeezing technique are not distorted by scattering artefacts and give an accurate picture of pigment orientation in plant systems. In order to test these statements we have compared the linear dichroism spectra of a pigmented sample in which the particle size is varied significantly. From data presented elsewhere [22,24] we know that scattering intensity will vary as a function of particle size. Using the gel squeezing technique we have observed that the linear dichroism of sonicated thylakoids does not vary from that depicted in Fig. 1. Furthermore, we have com-

pared the linear dichroism spectra of thylakoids and of a crude Triton extract from which the various pigment-protein complexes can be isolated [15,16]. The thylakoids we prepare are approx. 5000 Å in diameter, as determined by ultrafiltration, while after treatment with 0.75% Triton X-100 and centrifugation this size is reduced by an order of magnitude. The two linear dichroism spectra were identical in shape, although the magnitude of LD/A was smaller for the Triton extract (data not shown). The implications of this observation on the interpretation of linear dichroism of thylakoids and detergent solubilized pigment-protein complexes will be described in a future paper. At this point in time it is sufficient to note that a large reduction in particle size, which must considerably change the scattering properties, does not alter the shape of the linear dichroism spectrum when determined in polyacrylamide gel. This observation was further supported when we compared the linear dichroism spectrum of various aggregation states of light-harvesting complex. The preparations named 'sheet' and 'monomeric' light-harvesting complex are defined in Materials and Methods; their linear dichroism spectra in polyacrylamide gel are shown in Fig. 3a and b. The size of 'monomeric' light-harvesting complex determined by ultrafiltration is approx. 200 Å diameter, although freeze-fracture electron microscopy data of Mullet and Arntzen [16] suggest a value nearer 80 Å. The 'sheet' complex, on the other hand, will not pass a filter of pore size 4500 Å but permeates freely a 10 000 Å filter.

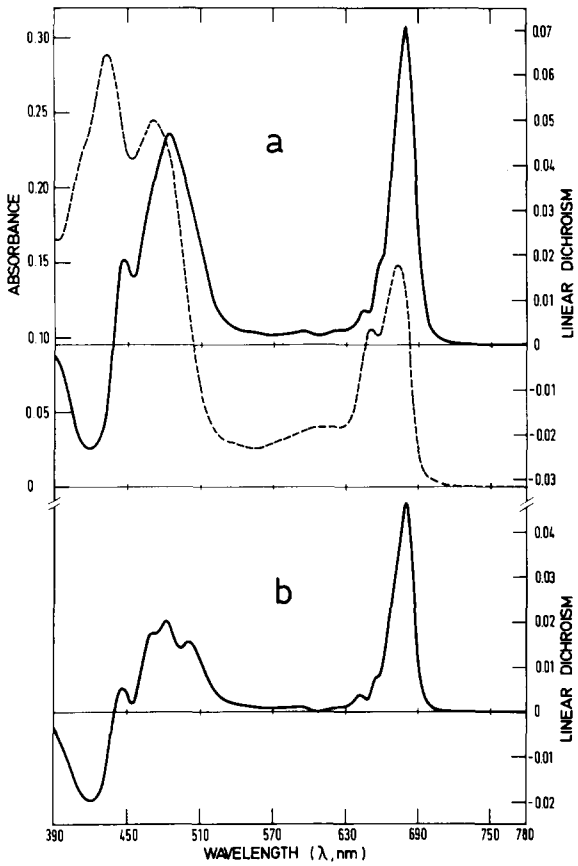

Fig. 3. Linear dichroism spectra of light-harvesting complex from pea oriented in a squeezed polyacrylamide gel. a. (———) linear dichroism and (- - - - -) absorption spectra of sheet light-harvesting complex (absorption maximum at 675 nm). b. linear dichroism spectrum of 'monomeric' light-harvesting complex as recovered from the sucrose density gradient. The difference between the two linear dichroism spectra is due to the presence of some free pigments in spectrum b (see text for explanations).

We have analyzed the linear dichroism spectra of five different preparations of 'monomeric' light-harvesting complexes. The spectrum shown in Fig. 3b is the one which presents the greatest difference compared to the linear dichroism spectrum of Fig. 3a, and all the others range between these two linear dichroism spectra. We have computed the difference linear dichroism spectrum between several of these spectra and have observed (data not shown) that they resemble the linear dichroism of a fraction of free pigments collected at the top of the sucrose gradient, just above the zone where the 'monomeric' light-harvesting complex is collected. We thus conclude that the difference in the linear dichroism spectra (Fig. 3a and b) of these two physical states of the light-harvesting complexes is due primarily to the presence of a small and variable amount of free pigments in the 'monomeric' fraction of the light-harvesting complex. The linear dichroism spectrum of light-harvesting complex inserted into unilamellar phospholipid vesicles, with diameter 5000–10 000 Å, is indistinguishable from that depicted in Fig. 3a.

A very high degree of orientation of the pigments in the 'sheet' light-harvesting complex was noticed in these experiments, especially in the case of this complex reconstituted in vesicles where the dichroic ratio ($A_{\parallel}/A_{\perp}$) at the red maximum was close to 2. In all these spectra the 720–780 nm region is flat and

remains unaltered if the distance between sample and phototube is increased from 1 to 20 cm.

Finally, a comparison of the data presented in Figs. 1 and 3 shows a marked similarity in the linear dichroism of the intact membrane and the isolated light-harvesting complex, especially on the short-wavelength side of the red band, which indicates a significant contribution of the light-harvesting complex to the linear dichroism spectrum of the thylakoid. This is further substantiated by linear dichroism spectroscopy of thylakoids, light-harvesting complexes, Photosystem I and Photosystem II particles which allow determination of the relative contribution of these three types of complex to the linear dichroism spectrum of the thylakoid (Tapie, P., Haworth, P. and Breton, J., unpublished data).

Conclusions

We have used the polyacrylamide gel squeezing technique, developed by Abdourakhmanov et al. [19], to orient both thylakoids and light-harvesting complex isolated in various states of aggregation. Efficient orientation of all samples can be achieved quickly without sign of pigment degradation. We have demonstrated that the linear dichroism spectra obtained by this procedure were free of distortion from the polarized light-scattering artefact.

The preliminary data presented here demonstrate that the various pigments bound to the light-harvesting complex are oriented with respect to an intrinsic axis within the particle. They also indicate that the isolation procedure of Burke et al. [15] does not change the in vivo orientation of the pigments with respect to this axis. Furthermore, the similarity between the linear dichroism spectra of 'monomeric' and 'sheet' light-harvesting complexes on the one hand, and of thylakoids and Triton-solubilized thylakoids on the other hand, suggests that modification of the hydrophobic and/or ionic interactions between the complexes does not alter significantly the orientation of the pigments with respect to the intrinsic axis. It also appears that the Q_Y transition moments of the chlorophyll *a* associated with 'monomeric' light-harvesting complex are highly oriented parallel to the largest dimension(s) of the particle. In membranous materials (thylakoids, 'sheet' light-harvesting complex, reconstituted vesicles) this is the direction within the plane of the membrane.

The characterization of an orientation technique which produces linear dichroism spectra accurately reflecting the true pigment orientation is of interest. With this technique we have been able to analyze at room and low temperatures the linear dichroism of isolated light-harvesting. Photosystem I and Photosystem II complexes (Haworth, P., Tapie, P., Arntzen, C. and Breton, J., unpublished data). The good quality of these spectra was a prerequisite to any precise determination of the contribution of each of these complexes to the overall linear dichroism spectrum in pursuit of our eventual goal, which is to comprehend the organization of the pigments in the photosynthetic membrane.

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References

1 Thornber, J.P., Markwell, J.P. and Reinman, S. (1979) Photochem. Photobiol. 29, 1205–1216.
2 Singer, S.J. and Nicholson, G.L. (1972) Science 175, 720–631.
3 Sauer, K. (1975) in Bioenergetics of Photosynthesis (Govindjee ed.), pp. 115–181, Academic Press, New York
4 Breton, J., Vermeglio, A., Garrigos, M. and Paillotin, G. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Kassandra-Halkidiki, Greece, 1980), in the press
5 Breton, J. and Vermeglio, A. (1981) in Integrated Approach to Plant and Bacterial Photosynthesis (Govindjee, ed.), Academic Press, New York, in the press
6 Menke, W. (1943) Biol. Zentral. 63, 326–349
7 Goedheer, J.C. (1955) Biochim. Biophys. Acta 16, 471–476
8 Olson, R.A., Jennings, W.H. and Butler, W.L. (1964) Biochim. Biophys. Acta 88, 318–330
9 Sauer, K. and Calvin, M. (1962) J. Mol. Biol. 4, 451–466
10 Thomas, J.B., Van Lierop, J.H. and Ten Ham, M. (1967) Biochim. Biophys. Acta 143, 204–220
11 Breton, J. and Roux, E. (1971) Biochem. Biophys. Res. Commun. 45, 537–563
12 Breton, J., Michel-Villaz, M. and Paillotin, G. (1973) Biochim. Biophys. Acta 314, 42–56
13 Geacintov, N.E., Van Nostrand, F., Becker, J.F. and Tinkel, J.B. (1972) Biochim. Biophys. Acta 267, 65–79

14 Biggins, J. and Svejkovsky, J. (1980) Biochim. Biophys. Acta 592, 565–576
15 Burke, J.J., Ditto, C.L. and Arntzen, C.J. (1978) Arch. Biochem. Biophys. 187, 252–263
16 Mullet, J.E. and Arntzen, C.J. (1980) Biochim. Biophys. Acta 589, 100–117
17 Brunner, J., Skrabal, P. and Hauser, H. (1976) Biochim. Biophys. Acta 455, 322–331
18 Bolt, J. and Sauer, K. (1979) Biochim. Biophys. Acta 546, 54–63
19 Abdourakhmanov, I.A., Ganago, A.O., Erokhin, Yu.E., Solov'ev, A.A. and Chugunov, V.A. (1979) Biochim. Biophys. Acta 546, 183–186
20 Swarthoff, T., De Grooth, B.G., Meiburg, R.F., Rijgersberg, C.P. and Amesz, J. (1980) Biochim. Biophys. Acta 593, 51–59
21 Breton, J. (1977) Thèse Doctorat d'Etat, Université Paris-Sud
22 Van de Hulst, H.C. (1957) in Light Scattering by Small Particles, John Wiley, New York
23 Latimer, P. and Rabinowitch, E. (1959) Arch. Biochem. Biophys. 84, 428–441
24 Swenberg, C.E. and Geacintov, N.E. (1979) in Excited States in Biology (Birks, J.B., ed.), pp. 288–300, John Wiley, New York
25 Breton, J., Michel-Villaz, M., Paillotin, G. and Vandevyver, M. (1972) Thin Solid Films 13, 351–357
26 Krawczyck, S. (1981) Biochim. Biophys. Acta 640, 628–639
27 Geacintov, N.E., Van Nostrand, F. and Becker, J.F. (1974) Biochim. Biophys. Acta 347, 443–463
28 Biggins, J. and Svejkovsky, J. (1978) FEBS Lett. 89, 201–204
29 Biggins, J. (1981) Biochim. Biophys. Acta 635, 259–266
30 Philipson, K.B. and Sauer, K. (1972) Biochemistry 11, 1880–1885
31 Rafferty, C.N. and Clayton, R.K. (1978) Biochim. Biophys. Acta 502, 51–60
32 Gagliano, A.G., Geacintov, N.E. and Breton, J. (1977) Biochim. Biophys. Acta 461, 460–474