Biochimica et Biophysica Acta 808 (1985) 455–463
Elsevier

BBA 41801

Orientation of pigments in phycobilisomes of *Porphyridium* sp. Lewin. A linear dichroism study utilizing electric and gel orientation methods

A.G. Gagliano [a,c], J. Hoarau [b], J. Breton [a] and N.E. Geacintov [c]

[a] *Service Biophysique, Département de Biologie, C.E.N. de Saclay, 91191 Gif-sur-Yvette Cedex,* [b] *Laboratoire de Structure et Métabolisme des Plantes associé au C.N.R.S. (LA 40), Université Paris-Sud, Bât. 430, 91405 Orsay Cedex (France) and* [c] *Chemistry Department, New York University, New York, NY 10003 (U.S.A.)*

(Received March 22nd, 1985)

Key words: Phycobilisome; Linear dichroism; Pigment orientation; (*Porphyridium* sp. Lewin)

The linear dichroism spectra of oriented membranes prepared from the red algae *Porphyridium* sp. Lewin, of isolated phycobilisomes, and of fractions containing either phycoerythrin alone or phycoerythrin and phycocyanin, oriented by pulsed electric fields and by squeezed polyacrylamide gel orientation techniques, were studied. The intact membranes and the phycobilisomes were stabilized by glutaraldehyde fixation. The phycoerythrin- and phycocyanin-containing fractions were derived from dissociated phycobilisomes by osmotic shock and sucrose density gradient centrifugation. In intact membranes the 545 and 570 nm transitions of phycoerythrin, the 620 nm phycocyanin and 655 nm allophycocyanin transitions, as well as the Q_y transition moment of chlorophyll *a*, appear to have an overall parallel inclination with respect to the membrane plane. Utilizing both the electric field and gel-squeezing methods, similar linear dichroism spectra are obtained for isolated phycobilisomes, except in the region of 570 nm. This difference is ascribed to a partial dissociation of phycoerythrin rods from the phycobilisomes in polyacrylamide gels. A further degradation of the rods into their component discs appears to occur in the presence of glycerol. The linear dichroism spectra of the phycoerythrin, and phycoerythrin + phycocyanin fractions, suggest that the 545 nm phycoerythrin transition is perpendicular to the plane of the discs, while the approx. 570 nm phycoerythrin and the 620 nm phycocyanin transition moments tend to be oriented more parallel to the plane of the discs. In polyacrylamide gels the linear dichroism spectra are superpositions of contributions from intact and from smaller degradation fragments of phycobilisomes. Glutaraldehyde-fixed phycobilisomes in aqueous suspensions (without glycerol or polyacrylamide) appear to be intact as verified by sensitized fluorescence spectroscopy; the electric linear dichroism spectra of these suspensions thus accurately represent the linear dichroism characteristics of integral phycobilisome structures.

Introduction

Phycobilisomes are supramolecular aggregates of phycobiliproteins which serve as energy-collecting antennae in red algae and in cyanobacteria. These pigment proteins provide additional absorption bands in the 450–650 nm spectral region in which the absorption of light by the chlorophyll pigments tends to be weak. The energy absorbed by the accessory pigments is transferred by radiationless resonance energy-transfer processes according to the pathway phycoerythrin → phycocyanin → allophycocyanin → chlorophyll. Structural models, based on electron microscopy, biochemical and biophysical studies, have been proposed for the arrangement of these chro-

Abbreviations: Chl *a*, chlorophyll *a*; LD, linear dichroism.

moproteins in phycobilisomes [1,2]. In this work we have utilized pulsed electric-field techniques [3,4] and polyacrylamide gel squeezing methods [5] to study the linear dichroism of isolated phycobilisomes, some of their dissociated fragments, intact whole membranes, as well as membranes devoid of phycobilisomes. On the basis of the linear dichroism spectra, information on the relative orientations of the various pigments has been obtained.

Materials and Methods

Phycobilisome-containing membranes were isolated from *Porphyridium* sp. Lewin cells (strain No. 637, Indiana University Collection, Bloomington, IN) suspended in 0.75 M phosphate buffer (pH 6.9) by French-press treatment followed by sucrose gradient centrifugation. Isolated phycobilisomes were obtained by utilizing the method of Gantt and Lipschultz [6]. Membranes without phycobilisomes were obtained by subjecting whole cells to osmotic shock (distilled water) treatment followed by breaking the cells further in a French press. The cellular extracts thus obtained were centrifuged in a sucrose gradient and the green fraction devoid of phycoerythrin and phycocyanin pigments was isolated in the usual manner.

Different fractions derived from phycobilisomes were obtained by osmotic shock treatment of the above phycobilisome preparation, followed by dialysis against distilled water for 6 h at 4°C to a final concentration of sodium phosphate of 0.02 M. These preparations were concentrated by a factor of 10–20 by adding Ficoll to the dialysate. The solution thus concentrated was subjected to sucrose gradient centrifugation (sucrose gradient, 0.3–1.2 M; 0.001 M sodium phosphate). Centrifugation was carried out for 15 h at 4°C (Beckman rotor SW31, 40 000 rpm, 250 000 × g at the bottom of the tube).

The light fraction (S values of 9–11 S) contains essentially phycoerythrin, while the intermediate fraction (11–14 S) contains both phycoerythrin and phycocyanin; the heaviest fraction (20–24 S) contains only phycoerythrin; the S values were calculated according to McEwen [7], and the pigment composition of these fractions was determined spectrophotometrically. In this work we have studied only the intermediate and the heavy fractions. A rough estimate of the molecular weights of these two fractions can be made utilizing the equations of Martin and Ames [8] for proteins subjected to sucrose gradient centrifugation; for the 11 S fraction a molecular weight of 250 000 is estimated, while for the 20–24 S fraction values in the range of 600 000–700 000 are obtained.

In almost all experiments the various particles (intact membranes, phycobilisomes, subphycobilisome fractions) were stabilized by crosslinking with glutaraldehyde [9,10]. This was necessary in the electric linear-dichroism experiments, because in this technique a relatively low ionic strength must be maintained in the suspending medium in order to avoid possible deleterious Joule heating effects. Thus the suspensions were treated with glutaraldehyde (0.3% by volume), and the phosphate buffer was eliminated by exhaustive dialysis. The cross-linked phycobilisomes and membranes were then subjected to a second sucrose density gradient centrifugation. Fluorescence measurements on the isolated phycobilisomes (glutaraldehyde-fixed) indicate that the normal energy-transfer sequence phycoerythrin → phycocyanin → allophycocyanin is maintained; this suggests that the structural integrity of isolated phycobilisomes is not destroyed by the glutaraldehyde treatment.

The measurement of the electric linear dichroism of suspensions of photosynthetic particles utilizing short (5 ms) voltage pulses (approx. 2400 $V \cdot cm^{-1}$) has been described in detail elsewhere [3,4]. In the case of the electric-field strengths utilized in this work, the mechanism of orientation involves the induced electric polarizability of the particles, rather than their permanent dipole moments (if any). For an understanding of the results described here, it is necessary to recall only that the particles tend to orient with their greatest dimension parallel to the direction of the applied electric field. Thus, in the case of whole membranes for example, the electric-field lines due to the applied voltage tend to be in the planes of the oriented membranes [3].

Some of these particles were also oriented by the polyacrylamide gel technique [5]; upon squeezing the gels, the largest dimension of the particles tend to align in a plane which is perpendicular to

the applied force. Thus, the dichroism of particles oriented by the squeezed gel and electric field techniques can be compared.

The linear dichroism is designated by:

$$\Delta A = A_{\parallel} - A_{\perp}, \quad (1)$$

where $A_{\parallel}$ is the absorbance measured with the electric field of the probing light beam oriented parallel to the direction of the applied electric field direction, or parallel to the direction of the applied force in the gel squeezing technique. The perpendicular polarization is defined by $A_{\perp}$ in both cases.

Results and Discussion

Electric linear dichroism of phycobilisomes, whole membranes and membranes devoid of phycobilisomes

We first compare the linear dichroism spectra obtained with isolated phycobilisomes, whole membranes (containing the phycobilisomes), and membranes without phycobilisomes (Fig. 1). In the latter case, it is found that the orientation of chlorophyll *a* is similar to the orientation observed in the case of a barley mutant which is devoid of chlorophyll *b* [11]; in both cases the Q_y transition of chlorophyll *a* tends to be in the plane of the membranes (within 20–25° [12]), thus giving rise to a positive dichroism ($\Delta A > 0$) in the 670–680 nm region of the spectrum (Fig. 1A). A positive linear dichroism throughout the entire spectral range investigated (500–750 nm) is also observed for the electrically oriented phycobilisomes (Fig. 1B), and the phycobilisome-containing whole membranes (Fig. 1C).

After taking into account variations in the backgrounds of the LD spectra due to light scattering, and normalizing the LD spectra in Fig. 1A and C at 680 nm (Chl *a* band), it can be shown that the sum of these two LD spectra in Fig. 1B and C at 545 nm for phycoerythrin (phycobilisomes + [membranes without phycobilisomes]) is approximately equal to the LD spectrum of the whole, phycobilisome-containing membranes (Fig. 1C). These results indicate that the isolated phycobilisomes orient in an electric field in a similar manner whether they are isolated or attached to the membranes [13].

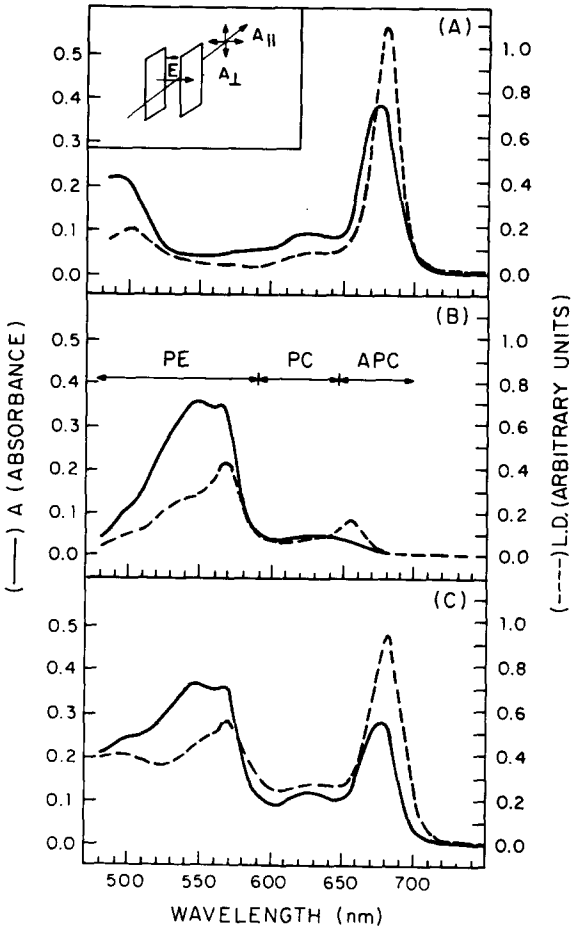

Fig. 1. Absorption and linear dichroism spectra (LD) in aqueous suspensions (electric field orientation) of (A) membranes without phycobilisomes (B) glutaraldehyde-fixed phycobilisomes, and (C) whole membranes (with phycobilisomes). PE, phycoerythrin; PC, phycocyanin; APC, allophycocyanin.

Linear dichroism of phycoerythrin and phycocyanin fractions

The pigments phycoerythrin and phycocyanin are chromoproteins arranged in disc-like shapes which are stacked to form cylindrical rod-like structures in the phycobilisomes. These cylindrical structures containing both phycoerythrin and phycocyanin are arranged radially about a core containing APC [14,15].

The light fraction (11–14 *S*) obtained by sucrose density gradient centrifugation of osmotically shocked phycobilisomes contains phycoerythrin and phycocyanin pigments in equal proportions according to the absorption spectra (Fig. 3), and

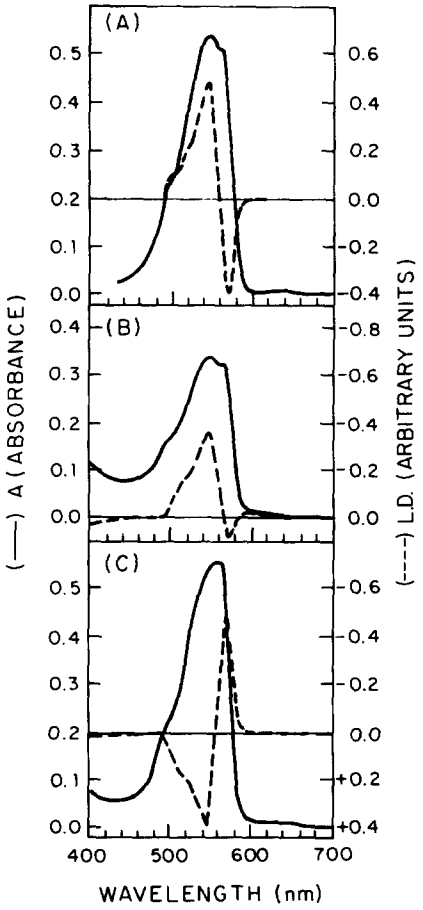

Fig. 2. Absorption and linear dichroism spectra of the $S =$ 20–24 (PE) fraction. (A) Electric field orientation in aqueous suspension, not glutaraldehyde-fixed; (B) squeezed polyacrylamide gel, no glycerol added; (C) squeezed polyacrylamide gel containing 50% glycerol by volume. (B) and (C) glutaraldehyde-fixed samples. Note that the polyacrylamide gel LD spectra are inverted in order to facilitate comparisons with the electrically oriented samples.

as determined from the relative extinction coefficients of these two pigments.

The heavy fraction (20–24 *S*) contains mostly phycoerythrin as is evident from the absorption spectra (Fig. 2). These absorption spectra exhibit structure at 545 and 570 nm, and a shoulder near 500 nm both in the aqueous suspensions and in the polyacrylamide gels without glycerol (Fig. 2A and B, respectively). However, the 570 nm peak disappears when glycerol (50%) is added to the gels (Fig. 2c).

In Fig. 2A and B the absorption spectra of the 20–24 *S* fraction are similar to the absorption spectra of the B-phycoerythrin (B-phycoerythrin) fraction from phycobilisomes derived from *Porphyridium cruentum* [9,16–18]. In the latter case, however, the long-wavelength maximum occurs at 563–565 nm, and not at 570 nm. Besides B-phycoerythrin another phycoerythrin chromoprotein called b-phycoerythrin has been identified [16,18]. The polypeptide composition of these phycoerythrin pigment forms is rather complex [19]; native B-phycoerythrin has a molecular weight of 240 000–275 000 dalton [16,20] and is composed of three dissimilar protein subunits α, β and γ according to the stoichiometric ratio $(\alpha\beta)_6\gamma$; b-phycoerythrin is polydisperse and consists of α and β subunits only [16]. The $(\alpha\beta)_6$ unit is believed to be a double-disc hexamer stabilized by the γ-subunit. In these subunits the longest wavelength transition (563–565 nm) exhibits an exciton-like circular dichroism spectrum which suggests that at least some of the phycoerythrobilin chromophores interact strongly with one another [16]. Consistent with this hypothesis are the observations that the degradation of the phycoerythrin hexamers leads to a loss of the long-wavelength absorption band and to a decrease of the exciton-like circular dichroism signal [9,17]. Analogous observations have been reported for C-phycoerythrin derived from cyanobacterial phycobilisomes upon dissociation of the B-phycoerythrin aggregates into smaller subunits [21,22]. Thus, the disappearance of the structure at 570 nm in 50% glycerol (Fig. 2C) is probably due to a dissociation of the phycoerythrin aggregates into smaller subunits.

The 20–24 S fraction. The approximate molecular weight of 600 000–700 000 estimated for the particles in this fraction suggests that this preparation may contain dodecamer aggregates of phycoerythrin. The absorption and linear dichroism spectra of these preparations are shown in Fig. 2. In Fig. 2A, in the electric-field case, the shoulder at approx. 570 nm is apparent, which indicates that the larger phycoerythrin aggregates are present, as discussed above. The linear dichroism spectra are characterized by a negative structure at 570 nm and a positive maximum at 545 nm. Identical spectra are obtained either with or without glutaraldehyde fixation of these prepara-

tions in aqueous suspensions. In order to interpret these results we recall that the rod-like stacked disc structures which are expected to be present in these preparations, orient with their long axis parallel to the electric field. Accordingly, since for the 545 nm transition $\Delta A > 0$, the transition moments are oriented parallel to the axis of the rods, or perpendicular to the planes of the disc. The approx. 570 nm transition moment on the other hand appears to be oriented within the planes of the discs.

In the gel without glycerol (Fig. 2B) the absorption spectra are the same as in water, but the linear dichroism signal, while qualitatively similar, exhibits a smaller negative peak at 570 nm. This spectrum was obtained with a glutaraldehyde-fixed sample; without glutaraldehyde fixation, the LD spectrum is inverted in sign: the LD signal is positive at 570 nm, and is negative at approx. 545 nm (data not shown). In the gel in the presence of 50% glycerol the signs of the LD bands (Fig. 2C) are also inverted relative to those observed in Fig. 2B and the 570 nm structure characteristic of aggregated phycoerythrin subunits is no longer apparent in the absorption spectrum. It is also important to note that the data in Fig. 2C are the same with and without glutaraldehyde crosslinking (data not shown). The effects of glycerol on the LD and on the absorption spectra can be understood if it is assumed that the rods dissociate into smaller disc-like components in the presence of glycerol. The smaller negative LD peak at 570 nm in Fig. 2B suggests that such a partial dissociation with glutaraldehyde-fixed preparations may also take place in the presence of acrylamide alone. In the absence of crosslinking, such a degradation is complete, since the LD spectra in the gels without glycerol are similar to those obtained in the presence of glycerol.

The S = 11–14 fraction. In the gels containing glycerol, the approx. 570 nm phycoerythrin and 620 nm phycocyanin transition moments exhibit a negative LD, while the 545 nm transition is characterized by a positive LD (Fig. 3). This is qualitatively similar to the behavior exhibited by the 570 and 545 nm transitions in the heavy (20–24 *S*) fraction under similar conditions (Fig. 2C). On the basis of these results, it is reasonable to assume that the 620 nm transition moments tend to be

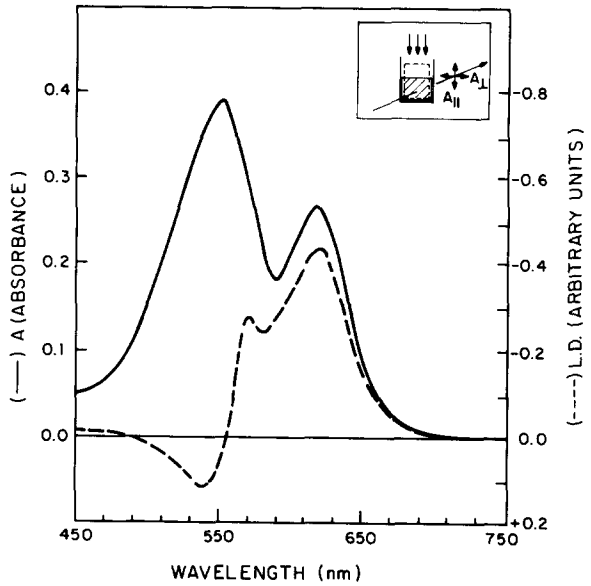

Fig. 3. Absorption and linear dichroism spectra of the $S = 11–14$ (phycoerythrin + phycocyanin) fraction in a squeezed polyacrylamide gel containing 50% glycerol by volume. Glutaraldehyde-fixed sample.

oriented like the 570 nm transitions, namely within the planes of the discs, and that this light (11–14 *S*) fraction consists mainly of dissociated discs rather than of rod-like aggregates of discs.

In electrically oriented samples both the 570 and 620 nm bands are characterized by positive LD signals [13] thus confirming the results shown for gels in Fig. 3.

Phycobilisomes in polyacrylamide gels

Squeezed polyacrylamide gels containing 50% glycerol are suitable for measuring linear dichroism spectra of photosynthetic particles at low temperatures; a better spectral resolution of the different absorption and linear dichroism bands is obtained at the lower temperatures than at room temperature [11]. Since pulsed electric field orientation methods are not suitable for low-temperature LD studies, we have investigated the feasibility of utilizing the squeezed polyacrylamide gel method for orienting phycobilisomes and studying their linear dichroism spectra. However, we have noted dramatic changes in the fluorescence emission properties of isolated cross-linked phycobilisomes upon treatment with acrylamide and/or glycerol. Notably, upon excitation at 540 nm the

ratio of fluorescence intensities F_{580}/F_{620} which is equal to 0.17 in the original material, increases to 4–5 after inclusion in the gel. This clearly indicates a substantial degradation of the phycobilisome, since the normal energy transfer sequence phycoerythrin → allophycocyanin is interrupted.

The isolated phycobilisome LD spectra are also different in the electric field and the polyacrylamide gel (with and without glycerol) cases. First of all, while the absorption spectra are similar in the aqueous solution (Fig. 1B) and in the gels (Fig. 4A, no glycerol), the linear dichroism signal appears to be reduced in the region of 570 nm. This is evident from the relative dichroism signals at 545 and 655 nm. In both the aqueous solutions and the gels, the ratio $\Delta A_{545}/\Delta A_{655} = 1.45$, while the $\Delta A_{570}/\Delta A_{655}$ ratio is 2.5 and 0.9 in aqueous solution (Fig. 1B) and in the gels without glycerol (Fig. 4A), respectively. This suggests again a partial dissociation of larger aggregates of phycoerythrin from the phycobilisome, since only the 570 nm shoulder in the linear dichroism spectrum is reduced, whereas this feature in the absorption spectrum remains intact (Fig. 4A). In the presence of glycerol in the gels (Fig. 4B), the structure at approx. 570 nm is lost both in the absorption and in the linear dichroism spectra, suggesting an even greater extent of dissociation of the phycobilisomes in the presence of glycerol.

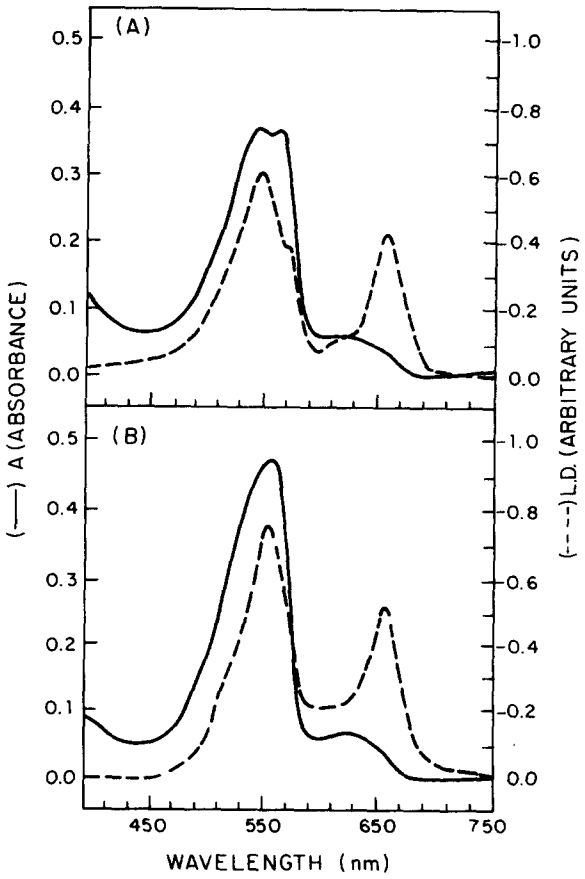

Fig. 4. Absorption and linear dichroism spectra of glutaraldehyde-fixed phycobilisomes in squeezed polyacrylamide gel, (A) without glycerol, and (B) with glycerol added.

Comparison between electric field and gel orientation techniques

Untreated phycobilisomes are stable in 0.75 M phosphate buffer, but readily dissociate at lower salt concentration. Upon crosslinking with glutaraldehyde it becomes possible to stabilize the structures so that they can be resuspended in distilled water. Accordingly, this procedure has enabled us to measure the electric dichroism of phycobilisomes either in the isolated state or still bound to the membrane. We have also investigated the possibility of studying the properties of oriented phycobilisomes in polyacrylamide gels with the goal of measuring low temperature LD spectra. Previous investigations on a variety of hydrophobic chlorophyll protein complexes have demonstrated an absence of structural degradation when these complexes were dispersed in gels [5,11,23]. In the present study however, the high concentrations of extraneous molecules (acrylamide, glycerol) required to prepare these gels leads to extensive disruption of the supramolecular organization of the discs constituting the rods in the phycobilisomes (even after glutaraldehyde fixation). This can be related to the observation that untreated phycobilisomes readily dissociate when the molarity of the phosphate buffer is decreased below 0.75 M. We have noticed that 10–20% glycerol added to crosslinked phycobilisomes or the $S = 20$–24 PE fraction leads to drastic changes in the electric dichroism spectra (data not shown). These effects are sufficient to explain the differences observed between the linear dichroism spectra of phycobilisomes shown in Fig. 1B and 4. Furthermore, in the electric-field experiments, the mechanisms of interaction of the particle with the applied field favors the orientation

of the large particles [4], while in the polyacrylamide gel all the particles tend to orient according to their anisotropy of shape. Although these differences can be used to select an orientation technique best adapted for a given type of particle, we note also that they will further complicate the comparison between the results of the two techniques in the case of a suspension where the size distribution of the particles is heterogenous.

Although the results on the inclusion of phycobilisomes in gels presented here indicate a significant degree of dissociation, we have also observed that the extent of dissociation of glutaraldehyde-treated phycobilisomes was diminished when higher concentrations of the cross-linking agent, as well as repeated treatments, were used. This indicates that it might be possible to develop conditions which are suitable for recording lineare dichroism spectra of intact phycobilisomes in gels.

Relative orientations of the chromophores

With the exception of a few linear-dichroism spectra of rather poor quality (high light-scattering level) obtained with whole algae [24,25], there is, to our knowledge, no other report on the orientation of chromophores in phycobilisomes. This is rather surprising in view of (i) the large body of spectroscopic data on the isolated chromophores [26,27], and (ii) the existence of specific models for the supramolecular organization of the building block of phycoerythrin, phycocyanin and allophycocyanin in the phycobilisome [28,29]. In this respect linear dichroism techniques seem ideally suited to check these models especially in view of our finding that in isolated phycoerythrin aggregates the 570 and 545 nm transitions exhibit dichroism of opposite signs.

The orientation of the different transition moments can, in principle, be estimated from the reduced linear dichroism $\Delta A/A$ which is related to the tilt angle θ between the transition moment and the symmetry axis of the oriented object. In thylakoid membranes this axis corresponds to the normal to the membrane plane; in the hemispherically shaped phycobilisomes this symmetry axis is coincident with the normal to the membrane plane in the intact phycobilisome-membrane structures. The reduced linear dichroism is defined as follows:

$$\Delta A/A = \tfrac{3}{2}(1-3\cos^2\theta)f \qquad (2)$$

where f is an orientational factor which describes the distribution of orientations of the symmetry axes of the oriented particles. This function f has opposite signs in the cases of orientation by the gel and the electric-field methods, thus accounting for the inversion of the LD spectra obtained by these two different methods of orientation.

In the presence of an applied electric field the membrane planes are oriented along the electrical field lines thus rendering $A_{\parallel} > A_{\perp}$ for any transition which is tiled close to the plane [3]. In the case of the gels, the planes of the membranes tend to be oriented perpendicular to the direction of the applied force, and thus the linear dichroism is negative for any transition moments which tend to be oriented within the membrane planes (in order to facilitate comparisons between the electrically oriented samples and the oriented gel samples, we have inverted the gel linear dichroism spectra in the figures).

Utilizing typical electric linear dichroism data such as shown in Fig. 1, relative values of $\Delta A/A$ for the different pigment bands were estimated for isolated phycobilisomes and for whole membranes and the results are summarized in Table I. The positive values of $\Delta A/A$ indicate that the transition moments of all of the different pigments are oriented at an angle θ greater than 55°, i.e., they are all oriented within approx. 35° of the planes of the membranes, or of the planes of the largest cross section of the hemispherically shaped phycobilisomes. the reduced dichroism increases with increasing wavelength for the transitions of phycoerythrin (545 and 570 nm), of phycocyanin (620 nm) and of allophycocyanin (644 nm) in isolated phycobilisomes. Similar behavior is also noted in the case of the whole membranes (although the contributions of the phycocyanin and allophycocyanin bands are too weak to be quantified), with the Chl *a* 680 nm band exhibiting the strongest reduced linear dichroism. These observations parallel a similar trend in green algae and in spinach chloroplasts in which the reduced dichroism, and thus the tilt angle of the Q_y transition moment of chlorophyll *a* with respect to the plane

TABLE I

REDUCED ELECTRIC LINEAR DICHROISM ($\Delta A/A$) IN ARBITRARY UNITS FOR THE DIFFERENT PIGMENT FORMS IN WHOLE MEMBRANES CONTAINING PHYCOBILISOMES (MEMB + PBS), IN MEMBRANES WITHOUT PHYCOBILISOMES (MEMB), AND IN ISOLATED PHYCOBILISOMES (PBS)

All of the data were taken from Fig. 1 (electrically oriented samples). The $\Delta A/A$ values were normalized to one another at 545 and at 680 nm for ease of comparison.

Pigment	MEMB + PBS	MEMB	PBS
phycoerythrin (545 nm)	0.36	–	0.36
phycoerythrin (570 nm)	0.48	–	0.59
phycocyanin (620 nm)	0.66	–	0.82
Allophycocyanin (655 nm)	0.71	0.37	3.0
Chl *a*(680 nm)	1.0	1.0	–

of the membrane decreases as the wavelength increases [30–33].

The values of $\Delta A/A$ in Table I were arbitrarily normalized to one another at 545 nm (phycoerythrin band) to facilitate comparisons of the dichroism in the case of the isolated phycobilisomes and the membranes to which the phycobilisomes are still attached. In order to facilitate comparisons between the latter and the membranes devoid of phycobilisomes, the $\Delta A/A$ values were set equal to one another at 680 nm, the Chl *a* maximum.

It is noteworthy that the dichroism at 655 nm due to the allophycocyanin band in phycobilisomes is much higher than at the other wavelengths. The dichroism at 655 nm in the membrane + phycobilisomes case is nearly twice as large (0.71) as in the case of the membranes devoid of phycobilisomes (0.37). These relative $\Delta A/A$ values are compatible with an orientation of the allophycocyanin transition moments which are closer to the membrane planes than the Chl *a* 680 nm transition moment vectors.

Finally, according to our results on the isolated phycoerythrin fractions (Fig. 2), we can conclude from the sign of the reduced linear dichroism that the 545 and 570 nm transitions are oriented at less than, and more than 55° respectively from the axis of the rods. In intact phycobilisomes both of these transitions appear to be oriented at less than 35° away from the membrane plane. This observation thus implies that the distribution of the phycoerythrin chromophores around the axis of the rods cannot be random, but that these orientations are fixed with respect to the plane of the membrane, since a definite positive dichroism is observable when the phycobilisomes are fixed to the membranes.

Acknowledgements

This work was supported by the Centre National de la Recherche Scientifique (Action Thematique Programme No. 3374) to J.B., and by the National Science Foundation (Grant PCM-83-08190) to N.E.G.. We are grateful to Professor B.A. Zilinskas for helpful discussions and suggestions.

References

1 Mörschel, E. (1981) in Photosynthesis, Structure and Molecular Organization of the Photosynthetic Membrane (Akoyunoglou, G., ed.), Vol. III, pp. 155–165, Balaban International Science Services, Philadelphia, PA
2 Gantt, E., Lipshultz, C.A. and Zilinskas, B. (1976) Biochim. Biophys. Acta 430, 375–388
3 Gagliano, A.G., Geacintov, N.E. and Breton, J. (1977) Biochim. Biophys. Acta 461, 460–474
4 Gagliano, A.G. (1982) Thèse, Université de Technologie de Compiegne, France
5 Abdurakhmanov, I.A., Ganago, A.O., Erokhin, Yu, E., Solov'ev, A.A. and Chugunov, V.A. (1979) Biochim. Biophys. Acta 546, 183–186
6 Gantt, E. and Lipshultz, C.A. (1972) J. Cell Biol. 54, 313–324
7 McEwen, R.C. (1967) Analyt. Biochem. 20, 114–149
8 Martin, R.G. and Ames, B.N. (1961) J. Biol. Chem. 236, 1372–1379
9 Gantt, E. (1969) Plant Physiol. 44, 1629–1638
10 Sofrova, D. and Papageorgiou, G.C. (1981) in Photosynthesis, Structure and Molecular Organization of the Photosynthetic Membrane (Akoyonoglou, G., ed.), Vol. III, 167–174; Balaban International Science Services, Philadelphia, PA
11 Haworth, P., Arntzen, C.J., Tapie, P. and Breton, J. (1982) Biochim. Biophys. Acta 679, 428–435
12 Breton, J., Vermeglio, A. (1982) in Photosynthesis. Energy Conversion by Plants and Bacteria (Govindjee, ed.), Vol. I, 153–194, Academic Press, New York
13 Hoareau, J., Gagliano, A.G., Geacintov, N.E. and Breton, J. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 705–708, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
14 Glazer, A.N., Williams, R.C., Yamanaka, G. and Schachman, H.K. (1979) Proc. Natl. Acad. Sci. USA 76, 6162–6166
15 Bryant, D.A., Guglielmi, G., Tandeau de Marsac, N., Castets, A.M. and Cohen-Bazire, G. (1979). Arch. Microbiol. 123, 113–127

16 Glazer, A.N. and Hixson, C.S. (1977) J. Biol. Chem. 252, 32–42
17 Fujimori, E. and Pecci, J. (1967) Arch. Biochem. Biophys. 118, 448–455
18 Gantt, E. and Lipschultz, C.A. (1974) Biochemistry (USA) 13, 2960–2966
19 Redlinger, T. and Gantt, E. (1981) Plant Physiol. 68, 1375–1379
20 Abad-Zapatero, C., Fox, J.L. and Hackert, M.L. (1977) Biochem. Biophys. Res. Commun. 78, 266–272
21 Rigby, M., Rosinski, J., Siegelman, H.W. and Sutherland, J.C. (1980) Proc. Natl. Acad. Sci. USA 77, 1961–1965
22 Zilinskas, B.A., Grabowski, J. and Campbell, S. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. II, pp. 687–690, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
23 Tapie, P., Acker, S., Arntzen, C.J., Choquet, Y., Delepelaire, P., Diner, B., Wollman, F.A., Breton, J. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 693–696, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
24 Gulayev, B.A., Tetenkin, V.L. and Bekasova, D.D. (1981) Izv. Akad. Nauk SSSR, Ser. Biol. 2, 188–201
25 De Grooth, B.G. (1980), Thesis, Leiden, The Netherlands
26 Grabowski, J. and Gantt, E. (1978) Photochem. Photobiol. 28, 39–45
27 MacColl, R. (1982) Photochem. Photobiol. 35, 899–904.
28 Scheer, H. (1982) in Light Reaction Path of Photosynthesis (Fong, F.K., ed.), pp. 7–45, Springer-Verlag, Berlin
29 Glazer, A.N. (1984) Biochim. Biophys. Acta 768, 29–51
30 Breton, J. and Roux, E. (1971) Biochem. Biophys. Res. Commun. 45, 557–563
31 Geacintov, N.E., Van Nostrand, F. and Becker, J.F. (1974) Biochim. Biophys. Acta 347, 443–463
32 Breton, J. and Geacintov, N.E. (1979) in Chlorophyll Organization and Energy Transfer in Photosynthesis. Ciba Foundation Symposium No. 61, pp. 217–236, Excerpta Medica, Elsevier/North-Holland, Amsterdam
33 Breton, J. (1985) in Photosynthetic Membranes. Encyclopedia of Plant Physiol, New Series (Staehelin, A. and Arntzen, C., eds.), Springer-Verlag, Berlin, in the press