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A linear dichroism study of photosynthetic pigment organisation in two fucoxanthin-containing algae

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The orientation of the pigments in cells, thylakoids and isolated pigment-protein complexes of two chromophytic algae, containing fucoxanthin as the principle carotenoid, has been investigated by linear dichroism spectroscopy. The Q_y transition of both chlorophyll *a* (chl *a*) and chlorophyll *c* (chl *c*) is oriented at $< 35°$ to the plane of the thylakoid membrane and to the long axis of the cells. The fucoxanthin transition, centred at 540 nm, is oriented in the same manner. In the isolated light-harvesting complexes, in compressed polyacrylamide gels, the linear dichroism spectra suggest a different orientation of all pigments, i.e., $< 55°$ to the direction of applied force or $> 35°$ to the long axis of the particle. It is demonstrated that this orientation is an artefact arising from decomposition of the light-harvesting complex during its preparation in the compressed polyacrylamide gel. The pigments of isolated PS I and PS II complexes retain the orientation they show in thylakoids and whole cells.

Introduction

Light-harvesting complexes have now been isolated by diverse methods from a wide variety of chl *c*-containing algae [1–11]. These complexes are of varying apparent molecular weight but their polypeptides are relatively few and fall in the range of 16–24 kDa [12]. Although many complexes have been isolated, there have been few studies by physical techniques, such as circular or linear dichroism, which might give insight into the organisation of pigments. In the diatom *Cylindrotheca fusiformis*, the chl *a*/*c* complex was suggested [13] to have the Q_y transition of chl *a* oriented perpendicular to the long axis of the complex in contrast to its orientation in the fucoxanthin chl *a*/*c* complex, Photosystem I and intact thylakoids. In the brown alga *Dictyota dichotoma* [14], it has been suggested that the fucoxanthin chl *a*/*c* light-harvesting complex changes shape on isolation, such that the orientation of both fucoxanthin and chl *a* with respect to the direction of the applied force is opposite to that in the membrane. In both the isolated complex and the membranes, no LD signal corresponding to the Q_y of chl *c* could be seen, suggesting that it lies close to 55° to the membrane normal. In contrast, the chl a/c_2 complex in *Chroomonas* (Cryptophyta) had the oriented Q_y transitions of both chl *a* and chl c_2 parallel to the long axis of the complex and this reflected their orientation in thylakoids and cells [15]. A similar situation exists with respect to chl *a* and *b* in the light-harvesting complex (LHCII) and thylakoids of green algae and higher plant [17–21].

In this paper we report on the organisation of pigments in thylakoids and chlorophyll-protein complexes of representatives of two groups of algae containing fucoxanthin and chl *c*, for which some background data are already available [2,8,22]. Our LD results do not agree well with those reported by other workers for light-harvesting complexes containing fucoxanthin [13,14]. The differences may, however, be resolved if destabilisation of the light-harvesting complex during preparation of the compressed acrylamide gels is taken into account. This destabilisation results in a change of sign of the LD signal for both fucoxanthin and the Q_y chl *a* transition and in a shift of the latter to lower wavelengths, compared to the situation in thylakoids.

Materials and Methods

Light-harvesting and photosystem complexes were isolated by solubilisation of algal thylakoids in digitonin and subsequent separation by sucrose-density centri-

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Abbreviations: Chl, chlorophyll; LD, linear dichroism; SDS, sodium dodecyl sulphate; PS I, Photosystem I; PS II, Photosystem II; LHC, light-harvesting complex.

fugation [8,22]. Absorbance, and LD spectra were obtained as described in the preceding paper [15]. Rapidly set gels were made by doubling the ammonium persulphate concentration to 0.1% and plunging the gel tube into an ice-bath.

Results

The absorbance and LD of cells and washed thylakoids are shown in Fig. 1A and B for *Phaeodactylum* and *Pavlova*, respectively. For both algae, the LD values of cells and thylakoids are very similar, with strong positive signals for the Q_y transition of chl *a* situated at slightly longer wavelengths than that of the main absorbance. The spectra also suggest a weak positive LD for the Q_y transitions of chl *c*, at approx. 640 nm in *Phaeodactylum*. Both organisms show a region of positive LD from 500 to 570 nm, which contains at least two components; that around 540 nm we attribute to the long-wavelength component of fucoxanthin [16].

After solubilising the membranes with digitonin, the components were fractionated on linear 10–40% sucrose gradients. For both organisms, a similar pattern [8,21] was obtained; at the bottom of the gradient a Photosystem I band and towards the top a main light-harvesting complex running into a photosystem II band just below it. The LD and absorbance for the Photosystem I component is shown in Fig. 2. Only the Photosystem I from *Pavlova* is shown, (Fig. 2a) since that of *Phaeodactylum* was almost identical. The positive LD Q_y transition peaking at 683 nm is especially prominent and additional positive bands are located at 444, 474 and 506 nm. That at 444 nm can be assigned to the Soret band of chl *a* whereas those at 475 and 506 nm are due to carotenoids. When the LD spectrum of light-harvesting chl *a*/*c*-fucoxanthin component of *Pavlova* was obtained (Fig. 2b) it was entirely negative,

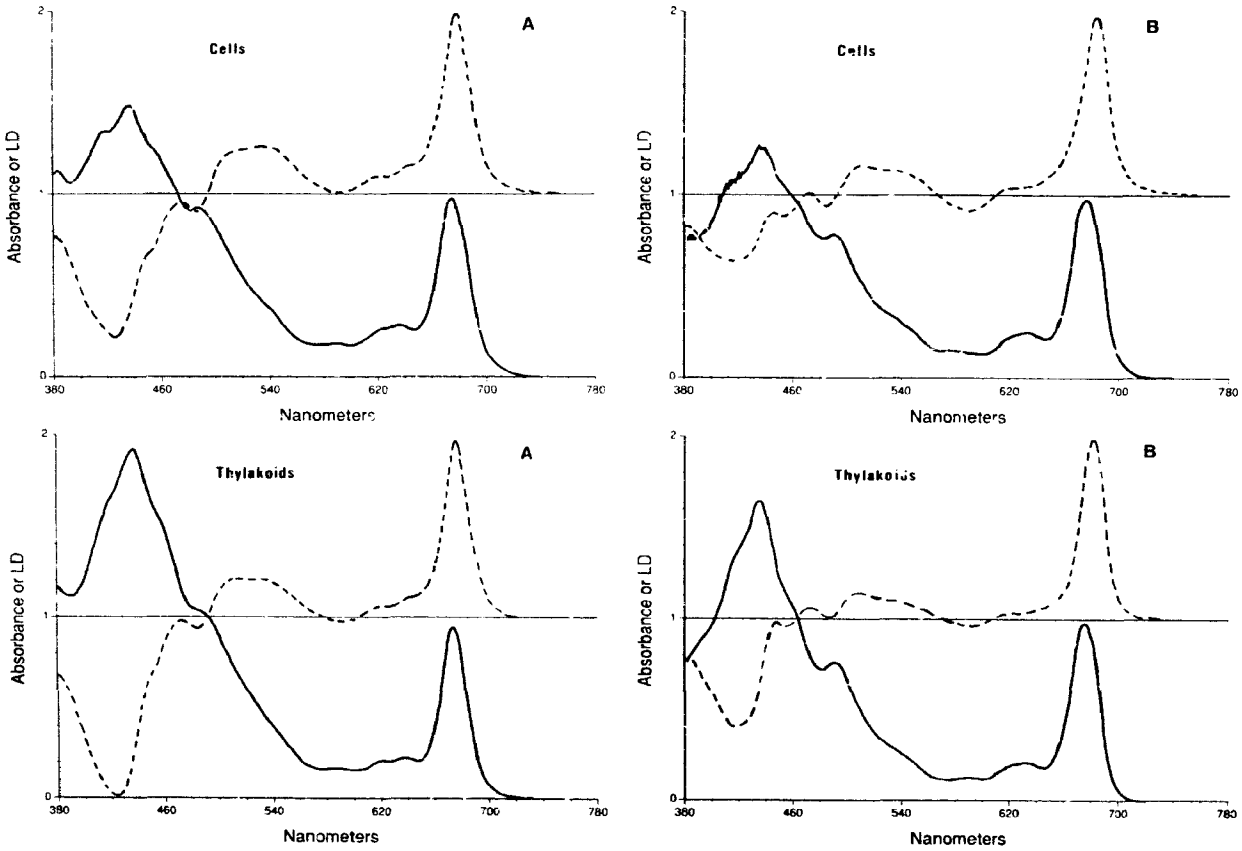

Fig. 1. Absorbance and linear dichroism spectra of cells and thylakoids at 20°C. (a) *Phaeodactylum*; (b) *Pavlova*. Absorbance (———); LD, (- - - - -).

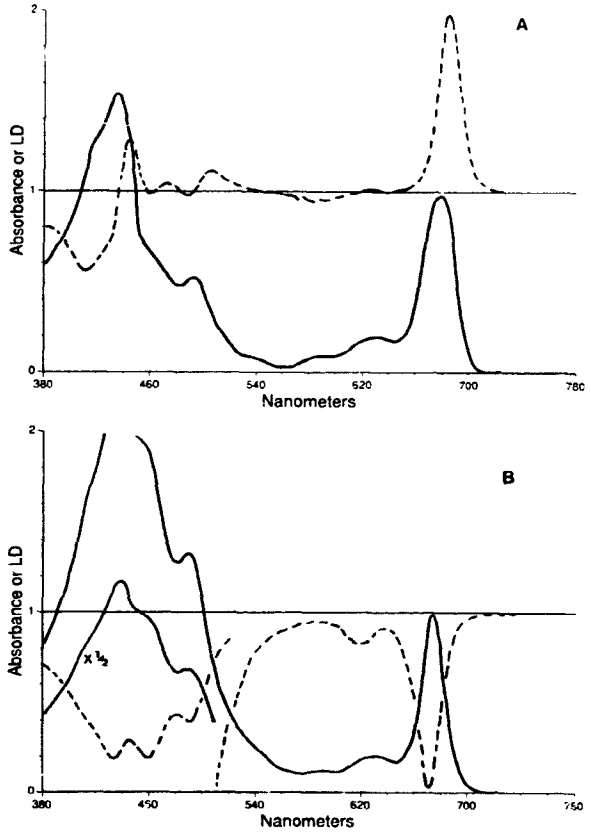

Fig. 2. Absorbance and linear dichroism of *Pavlova*. (a) Photosystem I; (b) light-harvesting complex at 20°C. Absorbance, (———); LD, (- - - - - -).

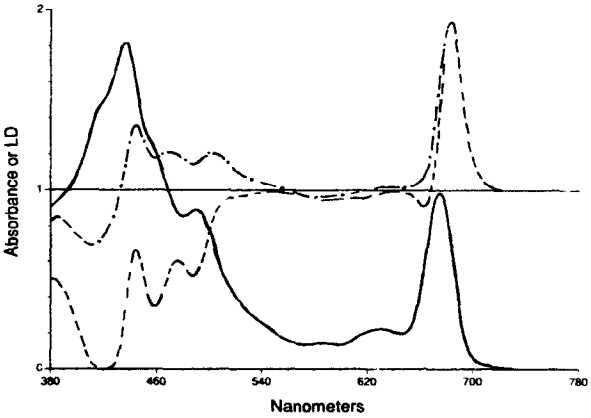

Fig. 3. Absorbance and linear dichroism of the digitonin-solubilised *Pavlova* thylakoids. Absorbance (———); LD (- - - - - -); LD spectrum after correction for the contribution of destabilised light-harvesting component (— · — · —).

and closely resembled an inverted absorbance spectrum. A similar LD spectrum was obtained for the light-harvesting complex of *Phaeodactylum* (results not shown). Since no prominent negative LD is apparent in

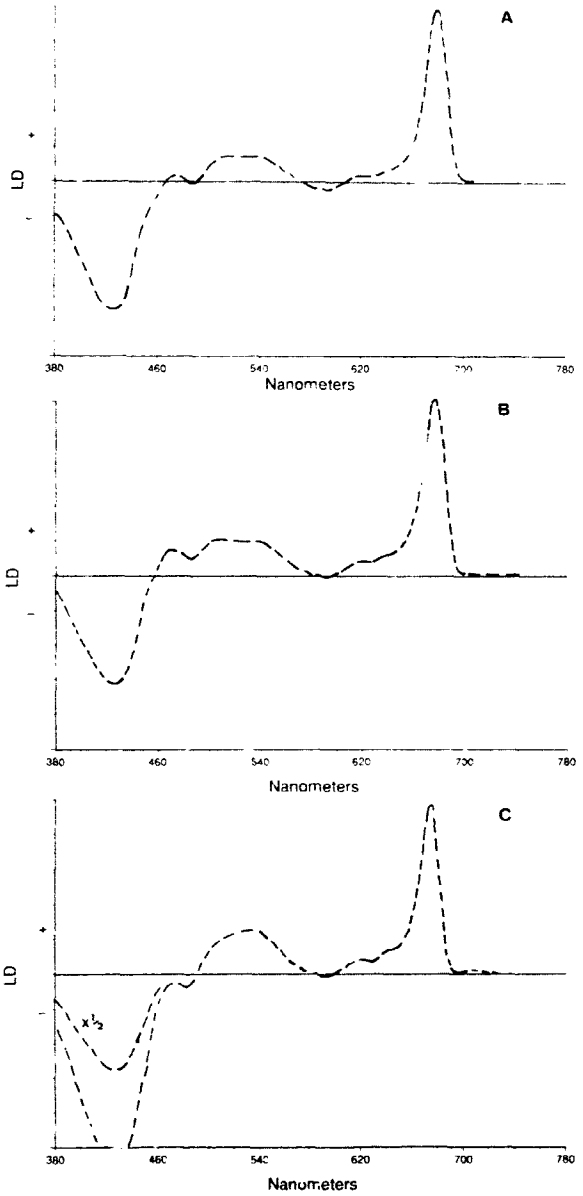

Fig. 4. Computed linear dichroism of the light-harvesting complex in thylakoids. (a) *Pavlova*; LD of thylakoids – (LD of PS I+PS II calculated from digitonin-solubilised thylakoids); (b) *Pavlova*; LD of thylakoids – (LD of isolated PS I and PS II complexes); (c) *Phaeodactylum*; LD of thylakoids – (LD of isolated PS I and PS II complexes).

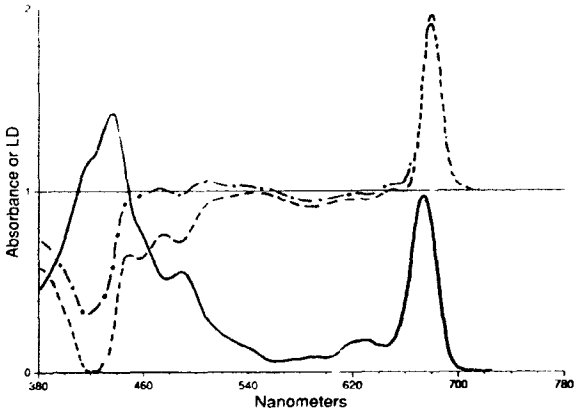

Fig. 5. Absorbance and linear dichroism of *Pavlova* PS II complex. Absorbance, (———); LD, (- - - - -); LD corrected for contamination by LHC (- · - · -).

the whole cell spectra such a result could indicate, either that the isolated chl *a*/*c*-fucoxanthin complexes orient with their transmembrane axes perpendicular to the direction of applied pressure, or that the pigments' orientation is altered during isolation, for example, by entering alignable detergent micelles. To further investigate the orientation of the pigments, absorbance and LD spectra were taken immediately following removal of insoluble material remaining after digitonin solubilisation of the membranes. This material was identical to that loaded onto the sucrose gradients from which the partially purified LHC complex, retaining efficient transfer from both fucoxanthin and chl *c* to chl *a*, was obtained [8]. As shown in Fig. 3 for *Pavlova*, the LD spectrum has negative peaks at 663 nm, 488 nm and 459 nm. When the LD of this component, which comprises up to 70% of the chl *a*, is removed by subtraction of the LD of the isolated LHC complex, a spectrum is obtained which is almost identical to that of the Photosystem I complex (cf. Fig. 2a). This corrected LD spectrum should also contain that of PS II in addition to that of PS I. To gain information on the orientation of the pigments of the LHC in the thylakoid membrane before solubilisation with digitonin, we corrected the LD of the thylakoid membranes by deducting that of the digitonin-soluble fraction, which is dominated by PS I + PS II (Fig. 3), from the LD of the thylakoids (Fig. 1). The resulting spectrum is shown in Fig. 4a for *Pavlova*. The Q_y band of chl *a* shows a positive LD, as does the 540 nm absorbance of fucoxanthin.

Preparations of PS II from the sucrose gradient are invariably contaminated with small amounts of LHC and the LD spectrum (Fig. 5), which has a positive peak in the chl *a* Q_y region with negative LD in the remainder of the spectrum due to LHC, reflects this. A corrected PS II LD spectrum was obtained by the following method. The absorbance of LHC included in that of the PS II complex was calculated from the chl *c* content and the LD of an LHC preparation of equal absorbance was subtracted from the original PS II LD. The resulting LD spectrum for both *Pavlova* (Fig. 5) and *Phaeodactylum* (results not shown) closely resembles that of higher plant PS II [18].

An alternative LD spectrum for that of the LHC in the membrane, to that shown in Fig. 4a, was obtained by subtracting that of the LD of isolated PS I (to give zero LD at 700 nm) and then the corrected PS II (to give zero LD at 695 nm) from that of the LD of the

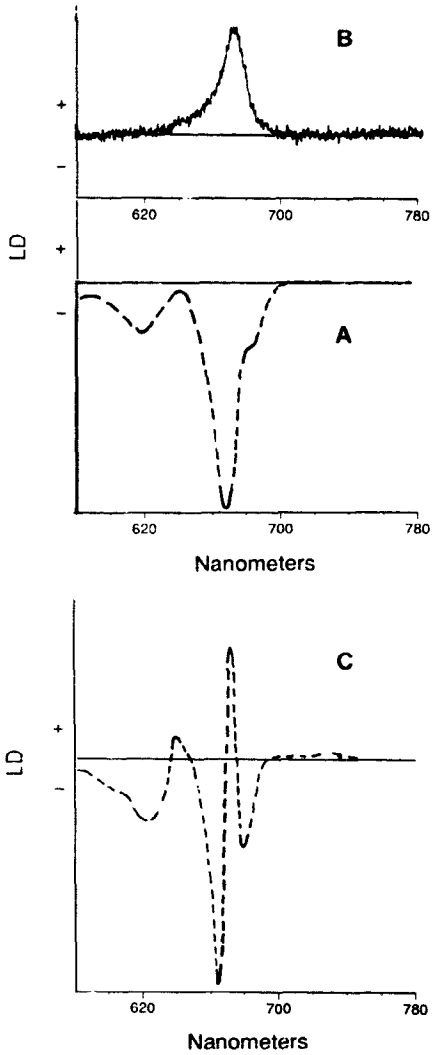

Fig. 6. Linear dichroism of *Phaeodactylum* LHC. (a) Initial LD at 20°C in a gel set rapidly on ice. (b) Initial LD at T_0–LD after 1 h incubation at 20°C. (c) LD at 100 K of rapidly-set gel.

thylakoids. The results for *Pavlova* obtained by this second method are very similar to those obtained previously (cf. Figs. 4A and B). The LD spectrum of *Phaeodactylum* LHC in the thylakoid membrane, also obtained by the second method, is shown in Fig. 4C. From the three spectra it can be concluded that the Q_y transitions of both chl *a* and chl *c* in the LHC are oriented < 35° to the plane of the membrane, as is the fucoxanthin transition over the range 505–560 nm. The strongly negative LD from 380–480 nm is made up of contributions from the Soret bands of chl *a* and chl *c* as well, perhaps, as from further contributions from carotenoid.

As we were unable to obtain, for either of the algae, LD spectra for the isolated light-harvesting complexes that showed an orientation consistent with the spectra deduced from thylakoid membranes, we altered the preparation of the squeezed polyacrylamide gels containing LHC. Gels set rapidly at 0°C, with twice the usual concentration of ammonium persulphate, gave negative spectra with a shoulder at 675–680 nm, suggesting an underlying positive component of the LHC (Fig. 6A). This shoulder disappeared after 2 h at room temperature. The difference spectrum at 20°C of the initial LD minus that after 1 h is shown in Fig. 6B. The results clearly demonstrate that a positive LD component with a peak at 675–676 nm is disappearing. The difference in initial absorbance minus that at 1 h (results not shown) indicated a loss of absorbance at 540 nm (due to fucoxanthin) together with a small bandshift in the Q_y region of chl *a*. When the gels containing the LHC are transferred rapidly to 100 K, the LD spectrum shows a mixture of positive and negative peaks in the Q_y chl *a* region (Fig. 6C), together with a positive peak due to fucoxanthin centered at 540 nm (not shown).

Discussion

From a comparable study [14] of the diatom *Cylindrotheca fusiformis*, it was concluded from the LD values of cells and thylakoids that the long-wavelength (540 nm) transition of fucoxanthin and the Q_y transition of chl *a* were oriented parallel to the membrane plane, in agreement with our results. This orientation in the same plane, of fucoxanthin and the Q_y transition of chl *a*, was also seen in the isolated light-harvesting complex, where all the LD bands were found to be positive, in contrast to our results and those of Mimuro et al. for the brown alga *Dictyota* [14]. To orient in a squeezed polyacrylamide gel, it appears necessary for a complex to form a sheet of many individual monomers. This is readily achieved in all Photosystem I and II complexes, the LHCP of higher plants [17–21] and in the light-harvesting chl a/c_2 complex of cryptophytes [15]. The complexes from *Cylindrotheca* were prepared using Triton X-100 to solubilise the thylakoids [13] rather than digitonin and this may have lead to appropriate stable association of light-harvesting complex monomers, although addition of Triton X-100 to digitonin-solubilised LHC's containing fucoxanthin usually leads to destabilisation [8,14]. Alternatively, the isolated light-harvesting complex may have been attached to some readily oriented Photosystem II complex. The polypeptide profiles were equivocal on this point. Hsu and Lee [13] also isolated a chl *a/c* complex and this was oriented in the gel with all transitions at less than 55% to the direction of applied force, just as we found for the main light-harvesting complex of both algae. Hsu and Lee noted that if the chl *a/c*-fucoxanthin complex in the gel remained at room temperature, then the positive LD, peaking at 668 nm, was replaced by a negative peak of 660 nm. The negative LD of the chl *a/c* complex was not thought to have resulted from a similar breakdown phenomenon since the negative Q_y LD peak was at 666 nm. Since LHC's containing fucoxanthin are readily dissociated by Triton X-100 [8,14] and, as shown above, the LHC complex can exist as a mixture of native and degraded forms, it seems likely that Hsu and Lee trapped their chl *a/c* complex at a stage corresponding approximately to that of our Fig. 3B (cf. Fig. 2C of ref. 13). In *Dictyota* [14] the LD signals from the LHC were essentially the same as we initially obtained, that is, negative throughout the entire visible spectrum. However, the accompanying absorbance spectrum (Fig. 5A of Ref. 14) clearly indicates that the 540 nm absorbance of stretched fucoxanthin has been lost due to heat decomposition of the LHC. It is also possible that the LD spectrum of the *Dictyota* thylakoids is distorted by a similar process, since the signals are somewhat weak and resemble that of our Photosystem I preparations.

Where the LD signals generated from complexes isolated by detergents are totally opposite to those seen in membranes or cell preparations and are essentially negative mirrors of the absorbance spectra, breakdown should be suspected, with pigments being trapped in oriented detergent micelles. It may be noted that if, during the setting of the polyacrylamide gels, the temperature rises above a critical level, even otherwise stable complexes, such as the cryptophyte chlorophyll a/c_2 light-harvesting complex, will lose their characteristic LD spectra. The LHC of *Phaeodactylum* and *Pavlova*, isolated here by solubilising the membranes in digitonin, compares closely with that obtained from *Dictyota* by means of decylsucrose [14]. Both preparations show excellent energy transfer from carotenoids and from chl *c* to chl *a*, as judged by fluorescence emission and excitation. In addition, the circular dichroism spectra are almost identical, there being a strong negative signal at 480 nm due to fucoxanthin which has a zero point at 455 nm (results not

shown). Antibodies to the LHC of *Pavlova* cross-react not only with LHC of *Phaeodactylum*, but also with the LHC of the brown alga *Colpomenia* [12]. It seems likely that all light-harvesting complexes containing fucoxanthin, chl *c* and chl *a* are similar in structure and that the reported differences in pigment orientation detected by LD arise from their different stability under the stress of polymerisation of the polyacrylamide gel.

References

1 Ingram, K. and Hiller, R.G. (1983) Biochim. Biophys. Acta 722, 310–319.
2 Friedman, A.L. and Alberte, R.S. (1984) Plant Physiol. 76, 483–489.
3 Fawley, M.W. and Grossman, A.R. (1986) Plant Physiol. 81, 149–155.
4 Owens, T.G. and Wold, E.R. (1986) Plant Physiol. 80, 732–738.
5 Caron, L. and Brown, J.S. (1987) Plant Cell Physiol. 28, 775–786.
6 Brown, J.S. (1988) J. Phycol. 24, 96–102.
7 Fawley, M.W., Morton, S.J., Stewart, K.D. and Mattox, K.R. (1987) J. Phycol. 23, 377–381.
8 Hiller, R.G., Larkum, A.W.D. and Wrench, P.M. (1988) Biochim. Biophys. Acta 932, 223–231.
9 Lichtlé, C. and Duval, J.C. (1984) in Advances in Photosynthesis (Sybesma, C., ed.), Vol. II, pp. 57–60, Martinus Nijhoff/Junk, Dordrecht.
10 Caron, L., Remy, R. and Berkaloff, C. (1988) FEBS Lett. 229, 11–15.
11 Katoh, T., Timuro, M. and Takaichi, S. (1989) Biochim. Biophys. Acta 976, 233–240.
12 Larkum, A.W.D. and Hiller, R.G. (1988) in Photosynthetic Light-Harvesting Systems (Scheer, H. and Schneider, W., eds.), pp. 153–165, W. de Gruyter, Berlin.
13 Hsu, B.D. and Lee, J.Y. (1987) Biochim. Biophys. Acta 893, 572–577.
14 Mimuro, M., Katoh, T. and Kawai, H. (1990) Biochim. Biophys. Acta 1015, 450–456.
15 Hiller, R.G., Scaramuzzi, C.D. and Breton, J. (1992) Biochim. Biophys. Acta 1102, 360–364.
16 Haxo, F.T. and Blinks, L.R. (1950) J. Gen. Physiol. 33, 389–422.
17 Haworth, P., Arntzen, C.P., Tapie, P. and Breton, J. (1982) Biochim. Biophys. Acta 679, 428–435.
18 Haworth, P., Tapie, P., Arntzen, C.P. and Breton, J. (1982) Biochim. Biophys. Acta 682, 152–159.
19 Tapie, P., Haworth, P., Hervo, G. and Breton, J. (1982) Biochim. Biophys. Acta 682, 339–344.
20 Haworth, P., Tapie, P., Arntzen, C.J. and Breton, J. (1982) Biochim. Biophys. Acta 682, 504–506.
21 Tapie, P., Choquet, Y., Breton, J., Delepelaire, P. and Wollman, F.A. (1984) Biochim. Biophys. Acta 767, 57–69.
22 Hiller, R.G., Bardin, A.M. and Nabedryk, E. (1987) Biochim. Biophys. Acta 894, 365–369.