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The organisation of photosynthetic pigments in a cryptophyte alga: A linear dichroism study

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Linear dichroism spectra have been obtained for cells, thylakoid membranes, light-harvesting complex and the Photosystem I of *Chroomonas*. In the light-harvesting complex, a form of chlorophyll *c* (chl *c*) shows strong dichroism at 648 nm, the Q_y chlorophyll *a* (chl *a*) transition is split at low temperature but only that absorbing at 674 nm is strongly dichroic. We conclude that the 660 nm component of the Q_y transition of chl *a* is oriented close to 55° to the membrane normal (or the direction of the applied force) whereas that at 674 nm is at > 55°, i.e., close to the plane of the membrane and the plane of largest cross-section of the particle. The orientation of these transitions is in agreement with that observed in the intact cells. Phycoerythrin within the thylakoid lumen is not preferentially oriented. The orientation of chl *a* in Photosystem I resembles that previously reported for other photosynthetic organisms, i.e., the long-wavelength Q_y transitions are oriented parallel to the membrane plane.

Introduction

In photosynthetic bacteria [1,2], green algae [3] and higher plants [4–6], considerable insight into the organisation of photosynthetic pigments has been obtained from circular and linear dichroism spectroscopy. In the most favourable cases the orientation of individual molecules may be obtained, as in the reaction centre of *Rhodopseudomonas*. Together with X-ray crystallography [7] a correlation between the structure and the spectroscopy of the reaction centre could be inferred [2].

In less favourable cases, such as light-harvesting complexes, where many pigment species are present, LD can assist the band assignment in absorption spectra and determination of the angle(s) of chromophores with respect to the plane of the membrane. There are extensive studies of green algae and higher plants by both techniques but little information is available for the heterogenous group of the chromophyte algae and none at all for the cryptophytes. The latter group of algae possess, in addition to PS I and PS II, two light-harvesting systems, a membrane-bound intrinsic chl *a* c_2 carotenoid complex and a phycobilin located in the intrathylakoid space [8–12]. The energy gap between the fluorescence emission of phycoerythrin and the Q_y transtition of chl *a* may be bridged by chl c_2, a novel pigment, analogous to allophycocyanin [13], or close juxtaposition of a special form of phycoerythrin to an intrinsic chl protein. Either of the last two hypothetical intermediates might be preferentially oriented and thus detectable by LD.

It may be anticipated that Photosystems I and II, together with their associated inner antennae of chl *a*, will be organised in a similar manner in all plants, but the light-harvesting systems, with their multiplicity of pigments, may well show differences. In recent studies of chromophytes it was noted that in the diatom *Cylindrotheca fusiformis* the chl *a* *c*-fucoxanthin light-harvesting complex was organised with the Q_y transitions of the chl *a* and *c* parallel to the long axis of the particle and to the membrane plane, whereas the chl *a* *c* complex was organised with an opposite orientation [14]. From a study of the brown algae *Dictyota dichotoma* different conclusions were reached as to the orientation of chl *a* and *c* in the light-harvesting fucoxanthin complex [15]. In *Dictyota* the orientation of chl *a* was perpendicular to the long axis of the isolated light-harvesting complex and chl *c* showed no dichroism [15].

In this study we report on the organisation of photosynthetic pigments in a cryptophyte alga as deduced from linear dichroism.

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Abbreviations: Chl chlorophyll; LD linear dichroism; CD circular dichroism; PE phycoerythrin; PS I, Photosystem I; PS II, Photosystem II.

Materials and Methods

Chroomonas CS 24 (CSIRO Division of Fisheries, Hobart, Australia 7000) was grown in Provasoli's enriched sea water, as described previously [9]. The cells were washed in 0.5 M potassum phosphate, 0.3 M potassum citrate (pH 7.2) and broken by a single passage though a French pressure cell at 70 MPa. After removal of intact cells and debris by centrifuging at $2000 \times g$, the thylakoids were obtained by centifuging at $30000 \times g$. Thylakoid membranes were prepared by washing twice in 50 mM Tricine, 20 mM KCl (pH 7.5). Chlorophyll-protein complexes were prepared by solubilising the thylakoids in digitonin (1% final concentration, 200 μg chl *a*) and separating them by ultracentrifugation through a linear 10–40% sucrose gradient made in 50 mM Tricine KCl, 20 mM KCl and containing 0.1% digitonin [9]. Absorbance spectra were recorded by means of either a Philips 8-200 spectrophotometer or the single beam spectrophotometer described by Tapie et al. [16].

For linear dichroism (LD) measurements, the cells, membranes or the complexes recovered from the sucrose gradient were set in polyacrylamide gels and uniaxially compressed to approx. 60% of their original length. LD spectra were measured with linearly polarised light modulated at a frequency of 100 kHz between vertical and horizontal directions. The difference $A_{\parallel}$-$A_{\perp}$ (defined as LD) was obtained by synchronous detection and the spectra digitised on a Tracor Northern 1710. The spectra have been normalised so that the absorbance or LD peak is 1 at the red maximum and so that positive LD values indicate an angle of $> 55°$ to the membrane normal or the direction of applied pressure in the case of squeezed polyacrylamide gels and hence to the normal to the plane of largest cross-section of the particle [16].

Results

In *Chroomonas* the thylakoids are arranged parallel to the long axis of the cell. Orienting the long axis of the cells perpendicular to the compression axis of the squeezed polyacrylamide gel therefore results in orientation of the thylakoids. The absorbance and LD spectra of oriented cells at room temperature are shown in Fig. 1a. The prominent features of the LD may be assigned on the basis of their wavelength to carotenoids and chlorophylls, rather than PE, which absorbs around 550–570 nm. This was confirmed by the LD of washed thylakoid membranes, which was indistinguishable from that of the cells (Fig. 1b). To assign the bands in the LD spectra of the cell, those of the chl *a* c_2 light-harvesting complex and Photosystem I were measured separately. (Fig. 2) The light-harvesting complex is clearly responsible for the positive LD in the cells at 470 nm, 644 nm, and at least part of that at 685 nm as well as for a major part of the complex negative LD from 400 to 460 nm and from 560 nm to 615 nm. The most prominent features of the Photosystem I LD are the positive bands at 442 nm and 685 nm. The former can be seen as a peak in the whole cell LD.

Below the light-harvesting complex in the sucrose-density gradient, a band which contained the polypeptides of 44 and 47 kDa characteristic of PS II, as well as those of 24 and 20 kDa, belonging to the light-harvesting complex, was observed. The LD of this component was very similar to that of the light-harvest-

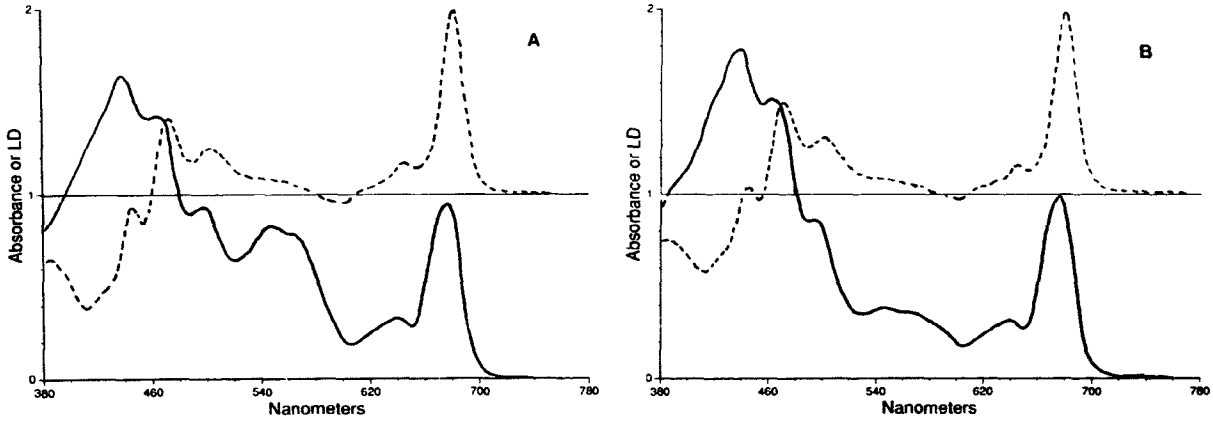

Fig. 1. Absorbance and LD spectra of (a) cells and (b) washed thylakoids of *Chroomonas* CS24 oriented in squeezed polyacrylamide gels at room temperature. (Absorbance, ———; LD, - - - - - -.)

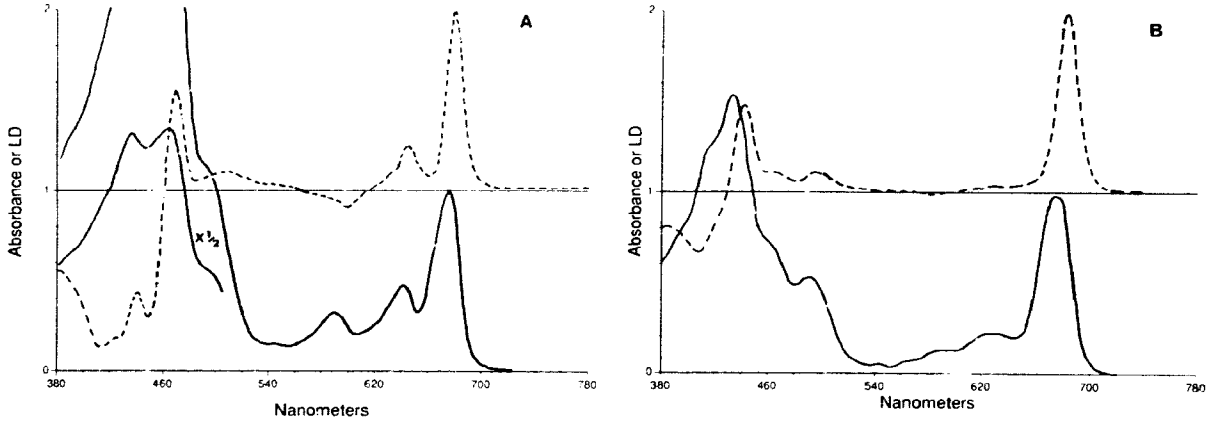

Fig. 2. (a) Absorbance and LD spectra of light-harvesting chl a-c_2 complex at room temperature. (Absorbance, ———; LD, - - - - - -.) (b) Absorbance and LD spectra of Photosystem I at room temperature. (Absorbance, ———; LD, - - - - - -.)

ing component. However, when the contribution of the light-harvesting complex was removed by three different normalisations (zero LD at 470 nm or 640 nm or that all the chl c absorbance belonged to the light-harvesting complex), there remained a positive PS II-like LD component at 678 nm with some structure at

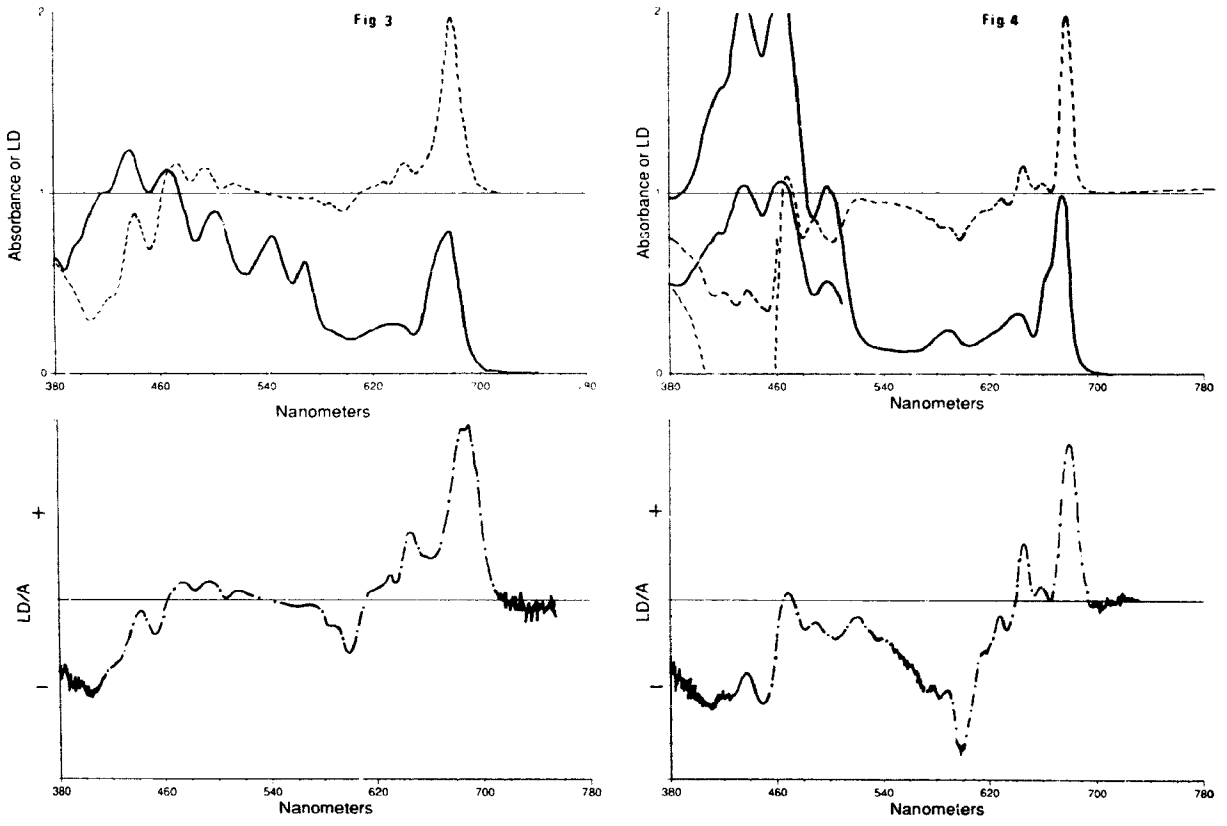

Fig. 3. Absorbance, LD and LD/A spectra of *Chroomonas* cells at 10 K. (Absorbance, ———; LD, - - - - - -; LD/A, ·–·–·.)

Fig. 4. Absorbance, LD and LD/A spectra of light-harvesting chl a-c_2 complex at 10 K. (Absorbance, ———; LD, - - - - - -; LD/A, ·–·–·.)

shorter wavelengths. There was no evidence of any negative LD in the Q_y region of chl *a* (results not shown).

To increase the spectral resolution, samples of cells and of the light-harvesting complex were cooled to low temperatures. In Fig. 3 the absorbance of cells at 10 K is shown. A notable feature of the absorbance spectrum is the clear splitting of the absorbance due to PE into two peaks, that at 545 nm due to the phycoerythrobilin chromophores of the β-subunits and that at 570 nm to cryptoviolin (PXB) of the α-subunits [12]. There are no corresponding features in the LD spectrum. In the red region, the absorbances due to chl *a* in different environments are beginning to be resolved. A positive LD at 645 nm due to chl c_2 is especially notable as is the negative region extending from 560–610 nm. Both the 645 nm positive and 560 nm–610 nm negative LD are components of the light-harvesting complex, as shown by the LD spectrum of the isolated chl-*a* c_2 complex. This observation unambiguously demonstrates that the plane of largest cross-section of this particle keeps the same orientation in the gel that it would exhibit if it were still in the native membrane. (Fig. 4). The absorbance spectrum (Fig. 4a) shows evidence of two main forms of chl *a*. The shoulder at 662 nm has little if any associated LD whereas the main absorbance peak at 674 nm is strongly dichroic. The small positive LD at 659 nm and the negative LP at 573, 583 and 592 nm have no obvious counterparts in the absorbance spectrum. All of them, however, become much more prominent in the LD/A spectrum which also shows that the long-wavelength Q_y transition of chl *a* is much more dichroic than any other component in the red region of the spectrum.

In the intact cell, interaction of PE in the intrathylakoid space with itself or some thylakoid component may lead to a PE species absorbing at longer wavelengths than 565 nm. As the light-harvesting complex contains most of the chl c_2 and at least 60% of the chl *a*, its LD was subtracted from LD of the cells on the assumption that all LD at 640 nm or 470 nm was due to the chl *a* c_2 complex. The subtraction resulted in loss of structure over the region 590 nm to 640 nm, leaving prominent Photosystem I positive peaks at 448 nm and 683 nm. A small positive LD at 570 nm is attributable to the PXB chromophore of the α-subunits of PE, which is responsible for the shoulder at 565 nm in the whole cell absorbance spectrum (results not shown).

Discussion

The spectral and biochemical characteristics of the pigment-protein complexes of *Chroomonas* CS24 have been described previously [9,10]. In common with other cryptophytes [11] containing PE, no phycocyanin or allophycocyanin was detected, an absorbance peak sometimes present at 640 nm in crude PE preparations being attributable [10] to the chromophore of dissociated α-subunits of the PE. In the present study we found no evidence for an absorbing species present in whole cells and arising from interaction between PE and the thylakoids which might have been destroyed in isolating the components of the light-harvesting system.

In a series of papers on the structure of higher plant thylakoids and pigment protein complexes derived from them, it was concluded that the thylakoid LD spectra could be largely reproduced by addition of the LD spectra of isolated complexes which retained in vitro, the in vivo pigment orientation. [3,15–17]. In the present series of experiments we have made observations on a cryptophyte alga *Chroomonas* which possesses two light-harvesting systems, a water-soluble phycobilin located in the intrathylakoid space and a major intrinsic chlorophyll-protein complex. The former contributes much to the absorbance but very little to the LD spectrum. Small positive values in the 550–570 nm region might be attributed to the chromophore, PXB of the α-subunit of phycoerythin [10,19]. In contrast to the lack of LD from PE, that from chl *a*, chl c_2 and a carotenoid from the intrinsic light-harvesting component are quite apparent in the LD spectra of whole cells, as well as of washed thylakoids. It can be concluded that for *Chroomonas*, as for higher plants, the pigments' orientation in vivo is maintained in the isolated complexes.

A particular problem in the interpretation of LD spectra arises when peaks, particularly at cryogenic temperatures, have no correspondence in the absorbance spectra. Two examples of this in our experiments merit discussion. In the 10 K light-harvesting complex LD spectrum there is a small peak at 659 nm; this could be represented by an orientation of the 662 nm absorbance component at an angle just less than 55° to the membrane normal or a component absorbing at a slightly longer wavelength and oriented at an angle slightly greater than 55° to the membrane normal. In the region 580 to 610 nm a negative LD signal is observed. Much of this signal arises from the light-harvesting chl *a* c_2 complex in which the negative LD at 597 nm is as large as that at 645 nm due to the Q_y transition of chl c_2. Negative LD signals in the region 580–610 nm have been reported for LD spectra of thylakoids of a barley mutant [17] which lacks chl *b*, *Chlamydomonas* Photosystem II preparations [2,20], and, although less obvious, from thylakoids and complexes of a diatom [15]. In the latter case this negative signal was assigned tentatively to the Q_x transition of chl *a*. In the *Chroomonas* chl *a* c_2 complex at 10 K the region is composed of at least three components, centred at 573, 583 and 597 nm, and the possibility exists of a contribution from chl *c*. These signals are much

less prominent and less structured in other algae containing chl *c* [15,16].

From the data presented here, we conclude that chl *a* in cryptophytes is organised in a somewhat similar manner to that in the LHCII of chlorophytes. Preliminary sequence data indicate that some parts of the *Chroomonas* LHC are strongly conserved when compared to that of higher plant LHCII [21]. It is likely that, in the LHCII of higher plants, highly conserved regions of the protein will be involved in the juxtaposition of the LHC complex to Photosystem II or in the alignment of the emitting chl *a* molecules so these can effectively transfer their energy to the chl *a* molecules of the core complex. It had been hoped that these studies might be conclusive for or against the existence of an absorbing species which acts as an intermediate in the energy transfer from PE to chl *a*. If such a component existed it might not be detected by LD since even in the cyanobacteria, the relatively dichroic allophycocyanin was not always apparent in the LD of membranes with attached phycobilisomes [22]. It may also be noted that, by analogy with energy transfer from carotenoids to chl *a*, as in the peridinin-chl *a* protein [23], provided the distance between PE at the edge of thylakoid lumen and chlorophylls of the intrinsic thylakoid complexes is sufficiently small, no problem exists for even 100% efficiency of energy transfer.

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