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The secondary structure content of pigment-protein complexes from the thylakoids of two Chromophyte algae

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The light-harvesting complex and Photosystem I have been isolated from thylakoids of two chromophyte algae by digitonin solubilisation and sucrose-gradient centrifugation. The α-helic and β-structure content was determined by ultraviolet circular dichroism. The values obtained were approx. 40% α-helix and approx. 14% β-structure for the light-harvesting complex of both *Pavlova lutherii* and *Phaeodactylum tricornutum*. For Photosystem I the values were approx. 55% α-helix and 7% β-structure for both algae. It is concluded that for all photosynthetic antennae containing chlorophyll the dominant secondary structure is α-helix.

Introduction

There is now a large body of evidence to support the concept of α-helices as the membrane-spanning components of intrinsic membrane proteins. For photosynthetic membranes, this evidence includes studies by X-ray diffraction [1] polarized infrared spectroscopy [2–4] as well as the theoretical consideration of primary amino acid sequence [5–7]. In the case of proteins which bind chlorophyll(s), the chlorophyll(s) may be bound to these membrane spanning α-helices as demonstrated by X-ray crystallographic studies of bacterial reaction centres [1] and by the inaccessibility of pigments to the exterior application of acid to higher plant thylakoids [8].

In the absence of X-ray crystallographic data, the protein secondary structure content of soluble proteins can be estimated from ultraviolet circular dichroism spectra using data derived from soluble proteins of known secondary structure, e.g., Ref. 9. This method has also been recently applied to photosynthetic membranes from both bacteria and green plants and to their intrinsic membrane proteins isolated by detergent fractionation or reconstituted in lipid vesicles [2–4,10–12].

The most abundant intrinsic thylakoid protein of green algae and higher plants is LHC II which binds chlorophyll *a* and *b* in approximately equal amounts and comprises approx. half the protein and chlorophyll of the thylakoid [13]. For this protein, ultraviolet CD spectroscopy predicts an α-helical content of 44% [10] which is consistent with either three [5] or four [14] membrane-spanning α-helices as suggested by hydropathy plots of the primary sequence and antibody binding data. Less data are available on the chlorophyll proteins

Abbreviations: CD, circular dichroism; Chl, chlorophyll; LHC, light-harvesting complex; LHC II, light-harvesting complex of Photosystem II; PS I, Photosystem I; PS II, Photosystem II; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymehyl)ethyl]glycine.

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of Photosystems I and II but both the ultraviolet CD [1] and the primary sequence [15,16] are consistent with a higher α-helical and lower β-structure content compared to that of LHC II.

The principal chlorophyll *a*-binding proteins of PS I and PS II are common to all plants, but there are considerable differences in light-harvesting proteins. In the chromophyte algae the apoproteins of all chlorophyll *ac*-carotenoid complexes are in the range of 17–24 kDa [17–19] but no primary sequence data are available. There is, however, some evidence of immunological cross reactivity between LHC II of higher plants and chlorophyll *ac* proteins (Hiller, R.G., Larkum, A.W.D. and Wrench, P.M., unpublished data). As a contribution to the structure of chlorophyll *ac*-binding proteins we have investigated the α-helical content of the principle light-harvesting chlorophyll proteins and of PS I from two groups of chromophyte algae by means of ultraviolet CD spectroscopy.

Materials and Methods

Cultures of *Pavlova lutherii* and *Phaeodactylum tricornutum* were originally obtained from the culture collection of CSIRO Division of Fisheries, Hobart, Australia 7000. They were grown on the laboratory bench with additional continuous light provided by a 60 W tungsten light with a 20 cm filament. The growth medium was Provasoli's enriched sea water [20] bubbled with air. Cultures were harvested by centrifugation at $1000 \times g$ for 10 min after 14 days growth.

Harvested cells were washed once in 0.6 M sorbitol, 50 mM Tricine and 20 mM KCl (pH 7.4). After resuspension in the same medium, they were broken by a single passage through a French pressure cell at 82.75 MPa. Unbroken cells and debris were removed by centrifugation at $1000 \times g$ for 10 min. Thylakoids and thylakoid fragments were pelleted by centrifugation at $15\,000 \times g$ for 30 min, washed once in 50 mM Tricine and 20 mM KCl (pH 7.4) and resuspended in the same medium at a chlorophyll *a* concentration of 150 $\mu g \cdot ml^{-1}$. Digitonin was added to give a final concentration of 1% w/v and the mixture was incubated in the dark for 4 h. It was then centrifuged at $30\,000 \times g$ for 30 min and the supernatant applied to a linear gradient (10%–40%) of sucrose containing 0.1% digitonin. The gradients were centrifuged for 16 h at $240\,000 \times g$ in a Beckman SW41 rotor. 3–4 pigmented bands were resolved as shown in Fig. 1. The centre of the bands labelled A, B and C in Fig. 1 was removed and stored at $-20\,^{\circ}C$ until required.

For ultraviolet CD measurements, the complexes were freed of sucrose by passage down PD10 columns (Pharmacia) equilibrated with a solution containing 25 mM Tricine, 10 mM KCl (pH 7.4) and 0.1% digitonin. The complexes were then concentrated by centrifugation in Amicon 10 microconcentrators. Ultraviolet CD measurements were made with a Jobin-Yvon dicrograph Mark V linked to a Micral 80-31B computer, using an 0.1 mm cell. Spectra were replicated 40 times, then averaged and the data converted to values for % α-helix and % β-structure by the method of Chen et al. [9] as described in Ref. 2, 10 and 11.

Integrity of the light-harvesting Chl *ac*-fucoxanthin complex was monitored by energy transfer from chlorophyll *c* to chlorophyll *a* as shown by its fluorescence emission peaking at 680 nm on excitation with light of 460 nm.

Results

The separation of the thylakoid complexes of *Phaeodactylum tricornutum* investigated here is shown in Fig. 1. Similar results were obtained for *Pavlova lutherii* and resemble the patterns obtained for the fractionation by digitonin of Fucus thylakoids [21]. Attribution of the functional roles of the pigment bands is on the following criteria. Band A is enriched in chlorophyll *c* and fucoxanthin and has a principle polypeptide of 17 kDa which is characteristic of the main light-harvesting chlorophyll-protein prepared by two different methods [18,19]. Band B is tentatively assigned to PS II as it is enriched in a polypeptide of 47 kDa and deficient in chlorophyll *c* and fucoxanthin compared to the thylakoids. Band C is a PS I complex as shown by a chlorophyll *a*/*c* ratio of more than 10, an absorbance maximum of 677 nm, considerable enrichment in P-700 and a principle polypeptide of 70 kDa.

The α-helix and β-structure composition for the light-harvesting complex (Band A of Fig. 1) and for Photosystem I (Band C of Fig. 1) are

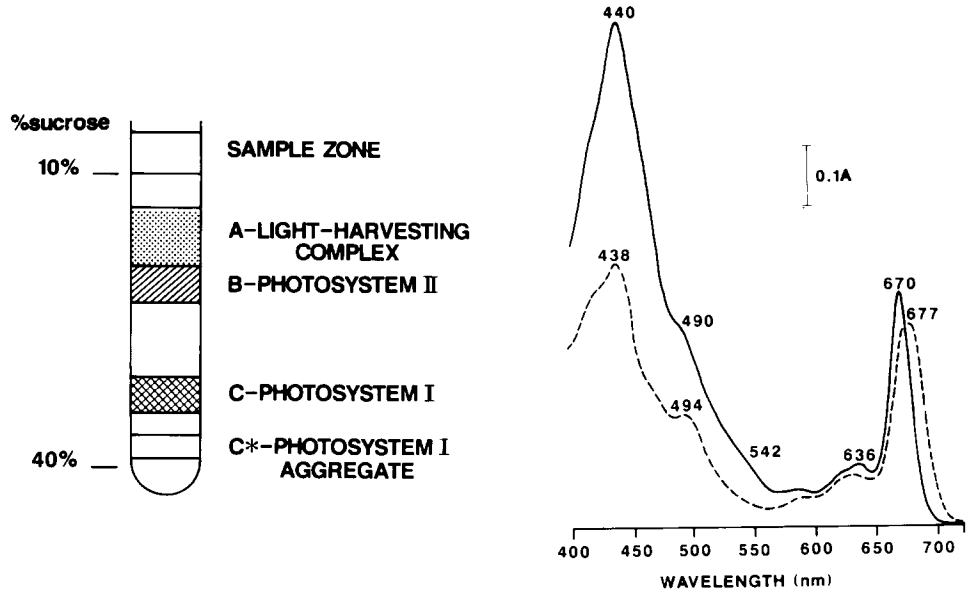

Fig. 1. Fractionation of *Phaeodactylum* thylakoid components by means of digitonin and sucrose gradient centrifugation. Band A: light-harvesting complex; Band B: Photosystem II; Band C: Photosystem I; Band C *: aggregated Photosystem I. Absorbance spectrum of band A (———); absorbance spectrum of band C (— — —).

presented in Fig. 2 and Table I. Both *Pavlova* and *Phaeodactylum* light-harvesting complexes have approx. 40% α-helix and 14% β-structure. By contrast Photosystem I from both organisms contained a greater percentage of α-helix (55%) and less β-structure (6%). Band B attributed to Photosystem II was contaminated with the tail of Band A on many gradients and occasionally by a small

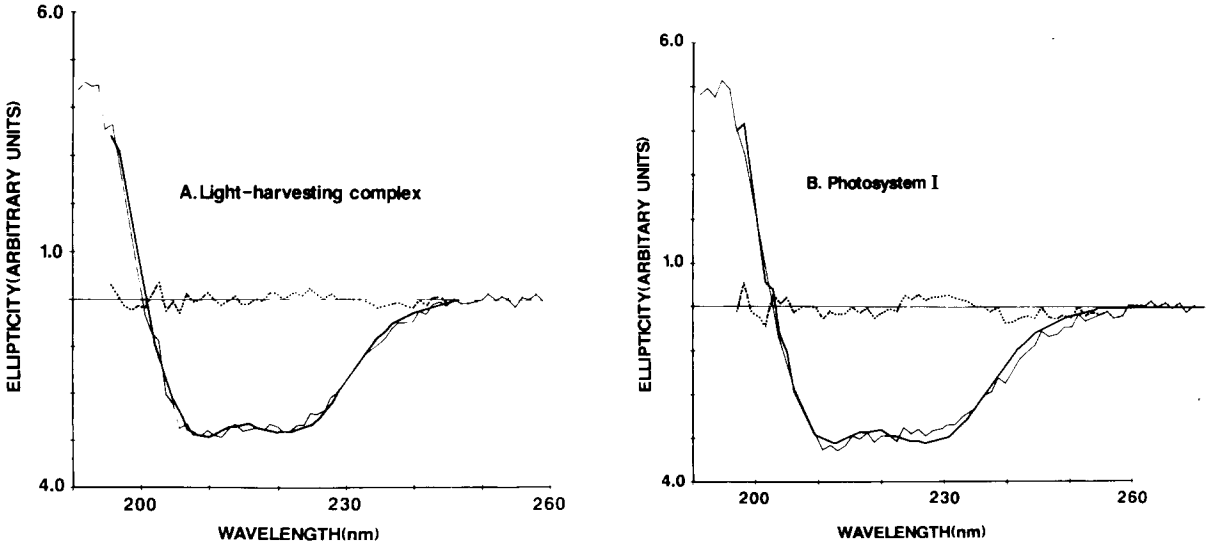

Fig. 2. Ultraviolet circular dichroism spectra (40 runs average) of *Phaeodactylum* thylakoid components: light-harvesting complex (A) and Photosystem I (B). Experimental (———), calculated spectra representing the best fit with Chen et al. reference curves [9] (———), and difference between experimental and calculated spectra (· · · · · ·). The experimental spectrum has been shifted by 1 nm towards shorter wavelengths. A blank having a matched digitonin concentration has been subtracted from the experimental spectrum.

TABLE I

THE PERCENTAGE OF α-HELIX AND β-STRUCTURE IN THE LIGHT-HARVESTING COMPLEX AND PHOTOSYSTEM I DETERMINED BY ULTRAVIOLET CIRCULAR DICHROISM

Figures are presented as the mean ± S.D. ($n = 5$), $\bar{\sigma}$ is the root-mean-square deviation between calculated (best fit) and experimental spectra.

	α-helix (%)	β-structure (%)	$\bar{\sigma}$
Phaeodactylum			
Light-harvesting (band A) complex	41 ± 2	14 ± 2	0.059
Photosystem I (band C)	55 ± 2	6 ± 2	0.084
Pavlova			
Light-harvesting (band A) complex	40 ± 2	14 ± 2	0.071
Photosystem I (band C)	55 ± 4	7 ± 3	0.068

amount of PS I and only the best two preparations as judged by the Chl *a*-to-Chl *c* ratio and the position of the red absorbance maximum, were analysed, one from each organism. The mean values obtained were 59% α-helix and 7% β-structure suggesting some similarity with PS I.

Discussion

Estimates of the α-helical content of higher plant thylakoids range from 44% for LHC II to 53–56% for PS I and thylakoids, respectively (Refs. 10 and 11). From Table I, it appears that comparable α-helical values from chromophyte algae are obtained for LHC (40%) and PS I (55%) complexes. In our experiments the main light-harvesting complex comprises 70% and PS I plus PS II complexes 30% of the total chlorophyll *a* and from this and the data above we estimate the α-helix content of chromophyte algal thylakoids to be 46%.

As no sequence data are available for any chlorophyll *c*-binding proteins, it is not possible to match our observations against models derived by the methods of Kyte and Doolittle [22] or Chou and Fasman [23]. For LHC II, for which primary sequences are available, models with two [24], three [5] and four [14] membrane-spanning sequences have been proposed. The last case [14] provides the closest match with the α-helical content of 44 ± 7% determined by ultraviolet CD [10]. If a sequence of 24 amino acids is required to provide a transmembrane α-helix and an average molecular weight per residue is 110, a 40% α-helix content converts to three transmembrane α-helical segments for *Pavlova* LHC (21 kDa polypeptide). For *Phaeodactylum* LHC (17 kDa polypeptide [18]) it corresponds to 2–3 transmembrane helices. Both these calculations exclude any contribution from extra membranous α-helical regions and suggest that two transmembrane helices are likely in the LHC of both organisms.

It may be noticed that ultraviolet CD spectra of PS I are more difficult to adjust than that of LHC as reflected by a higher $\bar{\sigma}$-value (Table I) and a high amplitude of the difference curve between experimental and calculated spectra (Fig. 2B). This is probably due to distortions induced by optical effects due to aggregated PS I particles as previously observed for PS I from higher plants [11]. Preparations of *Pavlova* Photosystem I when analysed by SDS polyacrylamide gel electrophoresis invariably contain a minor peptide of 21 kDa. This reacts with antibody to the 21 kDa polypeptide of the light-harvesting complex and may indicate contamination with LHC. However, this contamination cannot be large otherwise the Chl *a*-to-*c* ratio of our band C would be less than the observed value (> 10) (Hiller, R.G., Larkum, A.W.D. and Wrench, P.M., unpublished data).

Using ultraviolet CD spectroscopy, a quantitative analysis of the protein secondary structure in several antenna from different species (bacteria and higher plants) was previously performed [3,4,10]. From these data and those here reported for chromophyte algae, it can be generally concluded that for all the investigated membrane antennae, including the B800–850 and B875 of purple bacteria as well as the LHC from higher plants and algae, the dominant secondary structure is α-helix.

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