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TRANSMEMBRANE ORIENTATION OF α-HELICES IN THE THYLAKOID MEMBRANE AND IN THE LIGHT-HARVESTING COMPLEX

A POLARIZED INFRARED SPECTROSCOPY STUDY

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The structure and orientation of the major protein constituent of photosynthetic membranes in green plants, the chlorophyll *a*/*b* light-harvesting complex (LHC) have been investigated by ultraviolet circular dichroism (CD) and polarized infrared spectroscopies. The isolated purified LHC has been reconstituted into phosphatidylcholine vesicles and has been compared to the pea thylakoid membrane. The native orientation of the pigments in the LHC reconstituted in vesicles was characterized by monitoring the low-temperature polarized absorption and fluorescence spectra of reconstituted membranes. Conformational analysis of thylakoid and LHC indicate that a large proportion of the thylakoid protein is in the α-helical structure (56 ± 4%), while the LHC is for 44 ± 7% α-helical. By measuring the infrared dichroism of the amide absorption bands of air-dried oriented multilayers of thylakoids and LHC reconstituted in vesicles, we have estimated the degree of orientation of the α-helical chains with respect to the membrane normal. Infrared dichroism data demonstrate that transmembrane α-helices are present in both thylakoid and LHC with the α-helix axes tilted at less than 30° in LHC and 40° in thylakoid with respect to the membrane normal. In thylakoids, an orientation of the polar C=O ester groups of the lipids parallel to the membrane plane is detected. Our results are consistent with the existence of 3–5 transmembrane α-helical segments in the LHC molecules.

Introduction

In a previous paper, we have presented direct evidence that in native photosynthetic membranes of green plants and of photosynthetic bacteria, the α-helical segments of the intrinsic proteins are on the average preferentially oriented along the normal to the membrane plane [1]. However, these membranes are heterogeneous and contain several chlorophyll-protein complexes. A more critical evaluation of the structure of the photosynthetic membrane requires an estimation of the contribution of each purified protein complex to the overall structure of the membrane. In the chloroplast of green plants, three main chlorophyll-protein complexes have been recognized: the light-harvesting complex (LHC), the Photosystem I complex and the Photosystem II complex. The LHC accounts for about 50% of the total membrane proteins [2] and apart from its role of major antenna, it contributes to the formation of grana stacks [3]. Furthermore, important interactions between Photosystem II and LHC take place and the transverse membrane arrangement of these complexes has been described [4].

In order to investigate the contribution of the

Abbreviations: LHC, light-harvesting complex; DMPC, dimyristoylphosphatidylcholine; Chl, chlorophyll; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

LHC to the overall orientation of the protein secondary structures within the thylakoid membrane, the isolated purified LHC has been reconstituted into lipid vesicles and has been compared to the native pea thylakoid membrane. This was done by analyzing the ultraviolet circular dichroism and the polarized infrared spectra of both native and reconstituted membranes. The native orientation of the pigments in the LHC reconstituted in vesicles was controlled by monitoring the polarized absorption and fluorescence spectra. Conformational analysis of the LHC indicates that 44% of the protein is α-helical. By measuring the infrared dichroism of the peptide absorption bands of air-dried oriented LHC in vesicles, we have estimated that the average tilt angle of the α-helical protein segments with respect to the bilayer normal is less than 30°. A preliminary summary of these results has already been presented [5].

Materials and Methods

Isolation of thylakoids

Chloroplasts are isolated from pea leaves using a 10 mM Tricine-NaOH buffer (pH = 7.8) containing 0.4 M sorbitol as described elsewhere [6]. Thylakoids were prepared from the chloroplasts using 0.1 M sorbitol in 5 mM EDTA (pH 7.0).

Preparation and reconstitution of LHC membranes

The LHC was purified according to Burke et al. [6]. After Mg^{2+}-induced precipitation, the LHC was redissolved in 5 mM EDTA. For reconstitution experiments, the purified LHC (with a Chl *a*/*b* ratio not greater than 1.2) was dialyzed overnight against 10 mM Tricine-NaOH buffer (pH 7.8) containing 10 mM KCl/10 mM EDTA/1.5% Triton X-100.

The procedure for reconstitution of LHC with phospholipids was based on that of McDonnel and Staehelin [7]. Sonicated dimyristoylphosphatidylcholine (DMPC, Sigma Chemical Co.) vesicles (10 mg/ml) were added with stirring to the solubilized LHC (Chl = 2 mg/ml) to give a Chl : lipid ratio of 0.8 (wt/wt). The protein/lipid mixture was subjected to two cycles of rapid freeze-thawing with liquid nitrogen, then allowed to stand 1 h at room temperature before Bio-Beads SM-2 (Bio-Rad, Richmond, CA) were added for another 1 h with gentle stirring. The sample was then freeze-thawed and sonicated twice for 30 s periods. The suspension was dialyzed for 24 h against 10 mM Tricine-NaOH (pH 7.8) containing 10 mM KCl. The incorporation of LHC into DMPC liposomes was checked by ficoll-density gradient analysis [8]. The orientation of the LHC in the vesicles was characterized by monitoring the low-temperature polarized absorption and fluorescence spectra of reconstituted membranes oriented by the polyacrylamide gel technique [9].

For infrared dichroism analysis, the native or reconstituted membranes were washed and pelleted at $150\,000 \times g$ for 1.5 h and finally resuspended in deionized water. Orientation of the membranes was achieved by air-drying the suspension onto CaF_2 discs [1], so that the stacking of the vesicles was mainly parallel to the disc.

Polarized infrared spectra were recorded on a Perkin-Elmer 180 double-beam spectrometer equipped with a common beam Perkin-Elmer wire-grid polarizer and linked to a Hewlett-Packard 9825 A computer. Ultraviolet circular dichroism (CD) spectra were measured with a Jobin-Yvon Mark V dichrograph linked to a Micral 80-31B computer. The method for the infrared dichroism analysis and calculation of the average tilt angle of the α-helices from the ultraviolet CD and polarized infrared spectra has been described in our previous papers [1,10]. In order to remove the major source of reflection occurring at the membrane/air interface, the infrared dichroism spectra were measured with the air-dried membrane multilayers covered with spectroscopic grade paraffin oil (nujol). As previously noticed [11], nujol improves the baselines of the infrared dichroism spectra. We have found that this procedure does not significantly change the extent of dichroism of the various lipid and amide bands nor the visible linear dichroism spectrum.

Results

Orientation of pigments

By comparing the low temperature linear dichroism (LD) in the visible spectral range of isolated LHC and native thylakoids, it has been previously concluded that the in vivo orientation of the various pigments within the LHC is pre-

served during the isolation [12].

The low temperature LD spectra of LHC reconstituted in DMPC vesicles (data not shown) is identical to those reported for isolated LHC [12]. Moreover, the low-temperature polarized fluorescence emission of LHC reconstituted in vesicles (Fig. 1) is identical to the one of isolated LHC (Tapie, P. and Breton, J., unpublished data). Fig. 1 shows that the dipoles responsible for the 681 and the 698 nm emission bands are oriented preferentially parallel to the bilayer plane as they are parallel to the plane of the aggregate in the case of isolated LHC. Both the LD and polarized fluorescence data therefore indicate that all the visible chromophores keep their native orientation during the reconstitution procedure.

Conformational analysis of the polypeptides in the thylakoid membrane and in the LHC

Ultraviolet CD spectroscopy has been used to obtain information on the protein secondary structure of purified LHC in Triton X-100 or in DMPC vesicles. The extent of α-helical structure in proteins of thylakoids and LHC was estimated by fitting experimental CD spectra to sums of published reference spectra [13] using a general least-squares program kindly supplied by Dr. K. Lintner.

The 190–260 nm region of the CD spectra of thylakoids and of isolated LHC is presented in Figs. 2 and 3. A comparison of these spectra with those of soluble proteins suggests that these spectra are not severely distorted by light scattering or

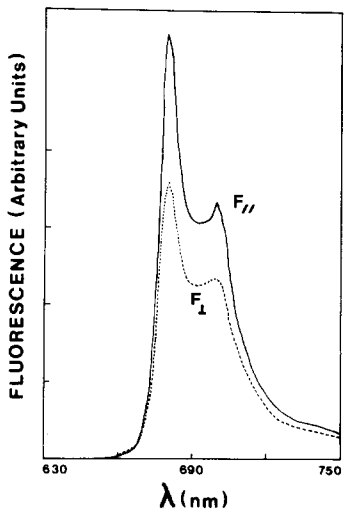

Fig. 1. The 90 K polarized fluorescence emission spectra of LHC reconstituted in DMPC vesicles. The reconstituted LHC was withdrawn from the ficoll-density gradient and oriented by the polyacrylamide gel squeezing technique. $F_{\parallel}$ (———) and $F_{\perp}$ (- - - - - -) refer to the fluorescence of light polarized parallel and perpendicular to the long axis of the oriented particles. Excitation wavelength at 645 nm.

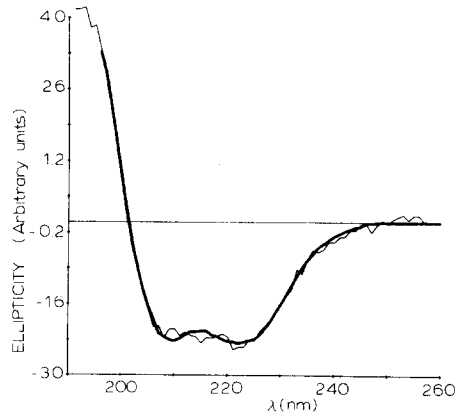

Fig. 2. Comparison of the ultraviolet CD experimental spectrum (40 runs average) of the native thylakoid membrane (———) with the calculated curve (_____) representing the best fit (between 195 and 260 nm) with Chen et al. reference spectra [13]. The experimental spectrum has been shifted by 2 nm towards shorter wavelengths.

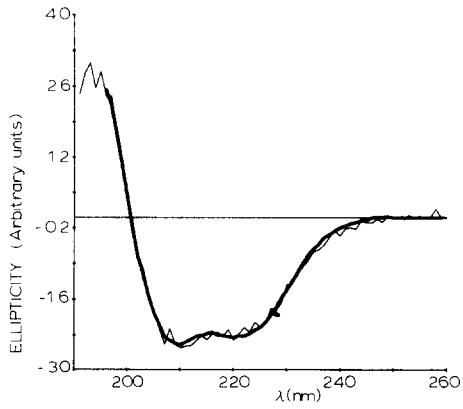

Fig. 3. Comparison of the ultraviolet CD experimental spectrum (40 runs average) of isolated LHC in Triton X-100 (———) with the calculated curve (_____) representing the best fit with Chen et al. reference spectra [13]. A blank of Triton X-100 having a matched Triton X-100 concentration has been subtracted from the LHC spectrum. The experimental spectrum has been shifted by 1 nm towards shorter wavelengths.

TABLE I

ORIENTATION OF α-HELICES IN THYLAKOID AND LHC

Average obtained from four different air-dried samples for each type of preparation.

	Thylakoid (56 ± 4% α-helix)		LHC (44 ± 7% α-helix)	
	Amide I	Amide II	Amide I	Amide II
D	1.14	0.90	1.19	0.89
D_α	1.26	0.83	1.46	0.77
ϕ_α	34°	35°	22°	25°

absorption flattening. In the following calculations, it has been assumed that the reference spectra obtained with soluble proteins by Chen et al. [13] can also be used for the conformational analysis of photosynthetic membrane proteins. However, it has been observed that a shift by 1–2 nm of the experimental spectrum to shorter wavelengths usually leads to closer fits between experimental and calculated spectra. The percentages of α-helix in thylakoids and in LHC are given in Table I. It appears that a large proportion of the thylakoid membrane is in the α-helical structure (56 ± 4%), while the main thylakoid membrane protein, i.e., the LHC, is 44 ± 7% α-helical. We consistently observed slightly more variations for different preparations of LHC than for the thylakoid ones. Furthermore, the thylakoid α-helix content reported in this study is higher than the value of 40% obtained by Menke and Hirtz [14]. This could be due to the use of a different species (*Antirrhinum majus*) and/or of a different method of preparation of the thylakoids.

Orientation of proteins in the thylakoid membrane and in the reconstituted LHC

Absorption and polarized infrared difference spectra of air-dried thylakoids and reconstituted LHC membranes are compared in Figs. 4 and 5 from 4000 to 1000 cm^{-1}. These spectra displayed marked similarities in the frequencies and in the dichroisms of the peptide and lipid bands. In thylakoids as well as in LHC, the frequencies of the amide bands (the amide A NH stretching at 3300 cm^{-1}, the amide I C=O stretching at 1657 cm^{-1} and the amide II NH bending at 1547 cm^{-1}) indicate the presence of some α-helical structure [15]. A shoulder on the amide I band at 1515 cm^{-1} is attributable to the aromatic ring stretching modes of the tyrosine residues [16]. Several lipid bands are easily identified: the C=O stretching ester at 1738 cm^{-1}, the PO_2^- stretching transition at 1250 cm^{-1}, the various CH stretching and bending modes around 2900, 1470 and 1380 cm^{-1} [17].

Polarized infrared difference spectra ($A_{\parallel} - A_{\perp}$) indicate that in both native and reconstituted membranes, the lipid as well as the peptide groups are oriented with respect to the membrane plane. Qualitatively, a positive $A_{\parallel} - A_{\perp}$ dichroism signal is associated with the alignment of the oscillators at less than 55° from the membrane normal [1]. The dichroism signal for the three major amide bands is found to be qualitatively the same for thylakoid and reconstituted LHC and it is indicative of oriented peptide segments in both membranes. The positive signals for the amide A and amide I bands and the negative signal for the amide II band are consistent with an alignment of the axes of the α-helix segments preferentially along the normal to the membrane, since in these structures the amide A and amide I transitions tend to be aligned along the helix axis, while the amide II transition tend to have an orientation perpendicular to this axis [18].

A weak shoulder around 1675 cm^{-1} can be reproducibly observed on the infrared dichroism spectra of LHC. However, it has no pronounced counterpart in the absorption spectra and it is also less visible in the infrared dichroism spectra of thylakoids. This small signal which indicates the presence of some oriented C=O groups could be due to the presence of carbonyls from either the chlorophyll molecules or some β-conformation (β-turns and/or antiparallel-pleated sheets) in the polypeptide chain.

In both thylakoid and LHC, several of the lipid bands exhibit a dichroism. In particular, the infrared dichroism spectra of thylakoids (Fig. 4B) show that the polar C=O ester tend to lie preferentially in the membrane plane. Such an orientation of the lipids has been previously detected in native chromatophores [10]. It must be noticed that on Figs. 4B and 5B, the dichroism of the lipid acyl chains is hidden by the absorption of nujol. Furthermore, in reconstituted LHC (Fig. 5B), the ob-

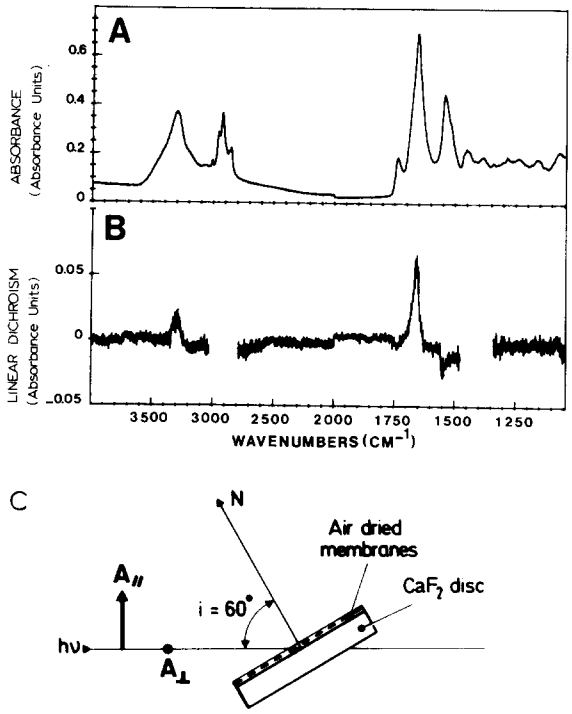

Fig. 4. (A) Infrared absorption (without nujol) and (B) dichroism (with nujol) spectra of air-dried thylakoids. (C) Schematic representation of the infrared dichroism measurement for air-dried oriented membranes on CaF_2 disc.

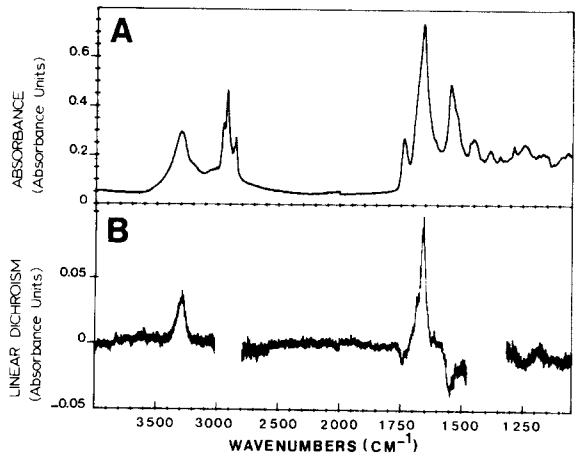

Fig. 5. (A) Infrared absorption (without nujol) and (B) dichroism (with nujol) spectra of air-dried LHC reconstituted in DMPC vesicles.

served negative dichroism at 1250 cm^{-1} is attributed to oriented DMPC phosphate groups as has been already observed in pure DMPC liposomes [19]. In thylakoids, the dichroism of the small absorption band at 1250 cm^{-1} cannot be measured. This can be correlated with the lipid composition of the photosynthetic membrane in which galactolipids account for about 80% of the total polar lipids [20].

For a quantitative determination of α-helix orientation, the experimental dichroic ratio $D = A_{\parallel}/A_{\perp}$ must be corrected to account for the extent of α-helix contribution (56% for thylakoid, 44% for LHC) to yield the α-helix dichroic ratio, $D_\alpha = A_{\parallel_\alpha}/A_{\perp_\alpha}$ [1,10]. From D_α, the tilt angle ϕ_α of the α-helix axes with respect to the membrane normal can be calculated [1,10]. These D, D_α and ϕ_α values are summarized in Table I. The dichroic ratios of the amide I and II bands lead to almost identical values for the same preparation, i.e., 34° and 35° in thylakoid, 22° and 25° in LHC. In order to include the extremes in the range of possible α-helix contents (Table I) and using the case of assumed perfect membrane ordering, upper limits for ϕ_α are placed at 40° for thylakoids and 30° for LHC. As discussed previously [1,10], the likely imperfect ordering of the multilayers will make the actual value be smaller than this.

Discussion

Since its first isolation [6], the structure of the light-harvesting complex has been investigated by a variety of biochemical and biophysical techniques. The apoprotein of LHC is predominantly composed of one or two structurally related polypeptides [21,22] in the 27 kDa range. Its amino acid composition shows a high percentage of hydrophobic residues [22]. The amino acid sequence of the major pea polypeptide has been recently determined, yielding a molecular weight of 24435 for the 228 residues (Ref. 23; see also Ref. 30). From both the sequence analysis [23] and the effect of trypsin on the intact thylakoid [22], it was deduced that the N-terminal segment is exposed to the chloroplast stroma. However, it is still unknown if the carboxyl terminal group is disposed towards the thylakoid lumen [22]. The oligomeric nature of LHC in vivo has been suggested by polyacrylamide gel electrophoresis analysis in the presence of various detergents, which gives evi-

dence for the formation of dimers and/or trimers [24,25]. Furthermore, immunological studies point out to a transbilayer organization of the complex [4]. Estimation of the chlorophyll: protein ratio indicates that each polypeptide binds 11–13 Chl $a + b$ [6,22,26], although lower values have also been reported [21]. The Chl a/b ratio is typically 1.1–1.2, but little is known about the detailed organization of the pigments. A model based upon CD investigation in the visible has been proposed for the arrangement of the chlorophylls in a Chl a/b antenna fraction: a trimer of strongly interacting Chl *b* surrounded by less tightly coupled Chl *a* [27]. Furthermore, low temperature LD experiments [9,12,28] demonstrate that the specific orientation of the various LHC pigments in the native membrane is undisturbed upon isolation and purification of the complex. It has also been shown that the chlorophylls and carotenoids in LHC were well shielded from proton attack even after some external segments had been clipped with proteases [3,29] thus indicating that these pigments are probably deeply buried in a hydrophobic region of the complex.

Regular arrays of LHC have been prepared from detergent solubilized or reconstituted membranes [3,7]. Freeze-fracture micrographs of these membrane sheets revealed hexagonal planar arrays with particles of 70–90 Å diameter [3,7]. More recently, 2-dimensional crystals of LHC have been investigated by electron microscopy and image analysis [26,20–32] as well as by X-rays [26]. Two kinds of three-dimensional maps of negatively stained LHC at different resolution (16 and 30 Å) were obtained in detergent [32] and in reconstituted membranes respectively [30]. Both studies show that LHC is an asymmetric transmembrane protein with large differences in surface area exposed on both sides of the complex. The LHC molecules are organized in trimers and have an elongated shape from 59 [32] to 65 Å [30] perpendicular to the bilayer and protruding on both sides by a total of 10–20 Å. Each lattice face shows two sets of trimers (head-to-tail configuration) detected by protruding portions of the LHC molecules. Looking at the 3-dimensional map obtained by Kühlbrandt at 16 Å resolution [32], each trimer is characterized by three lobes corresponding to each LHC molecule. Each lobe appears to be composed of three or four protruding structures which extend through the lattice interior and terminate on the opposite surface in a broad protrusion (80% of the stain excluding volume is localized in the half of the molecule which contains these three or four protrusions). In Kühlbrant's model [32] these protrusions on both sides of the LHC sheet are connected by several cylindrical structures, 15–20 Å in diameter, which are somewhat reminiscent of the rods observed in the 3-dimensional map of unstained purple membrane and which have been attributed to transmembrane α-helical segments [33].

Although these various data give us some information on the primary and tertiary (or quaternary) structure of LHC as well as on its molecular arrangement in membranes, very little experimental data have been actually obtained on the secondary structure of LHC. Only one study, by X-rays diffraction [26], has indicated that some protein chains are oriented perpendicular to the plane of the bilayer. However, the resolution was too low to discriminate between α-helix or β-sheet. From our CD data, we can estimate that the contribution of β-structure is smaller than 10%, while that of α-helix is 44%. However, our CD analysis does not include a β-turn term and possible uncertainties in the estimates of β-form cannot be excluded. The infrared absorption spectrum of LHC (Fig. 5A) mainly shows the presence of α-helical and random structures in agreement with our CD data. The infrared dichroism spectrum (Fig. 5B) indicates a large amount of oriented α-helices (at 1657 cm^{-1}) with very little contribution from oriented β-structure (at 1675 cm^{-1}). These α-helices are oriented at less that 30° with respect to the membrane normal. Thus, our present investigation demonstrates that the oriented protein segments detected by Li and Hollingshead [26] are mainly α-helical.

In Li's model [30], a significant portion of the LHC molecule protrudes asymmetrically by 20 Å out of the plane of the bilayer and is therefore exposed to the aqueous phase. It has been demonstrated that the N-terminal lies on the stroma side [22,23] and it can be calculated [30,34] that the first 60 residues are predominantly hydrophilic. Accordingly, this protruding region could correspond to part of the approximately 46% random

structure deduced from our CD data. A comparison can be made between the α-helix content and orientation found in bacteriorhodopsin (M_r 26 000) and LHC ($M_r \approx 24\,500$). Using the same curve-fitting procedure for both proteins, our CD data indicate an α-helical content of 70 ± 10% in purple membrane (Nabedryk, E. and Breton, J., unpublished data). Furthermore, the infrared dichroism on bacteriorhodopsin [1] gives a value (11°) for the average tilt of the seven α-helices which agree with the electron microscopy work [33]. Accordingly, our determination of 44 ± 7% α-helical content and of the 30° average tilt angle leads us to propose the existence of three to five transmembrane α-helical segments in the LHC molecule. Recent theoretical calculations from the amino-acid sequence of some intrinsic proteins [34–40] other than bacteriorhodopsin and rhodopsin, propose transmembrane α-helices as the basic structural element. Our data on several Chl-protein complexes [1,10,11] either in the native membrane (thylakoids, chromatophores) or in the isolated state (LHC, bacterial reaction center and its LM subunit) substantiate these theoretical results and suggest that transmembrane orientation of α-helices is a rather general property of both antenna and reaction center complexes. The LHC model described above is compatible with the prediction based upon hydropathy indices of three regions that could cross the thylakoid membrane as α-helices (Ref. 34 and Li, J., personal communication). However, in this prediction study, the effect of the large number of pigments (11 or 13 Chl $a + b$ and 3 Car per polypeptide), which correspond to approx. half the molecular weight of the polypeptide (24 500), was not taken into account. This situation is different from that found in bacteriorhodopsin (where only one retinal is present per polypeptide) and the large number of pigments contained within the LHC could induce perturbations in the formation and the packing of α-helices.

It is most unlikely that the Chl in LHC could be organized in a way similar to the one observed for the seven bacteriochlorophylls in the pigment-protein complex (M_r 50 000) from *Prosthecochloris aestuarii* [41]. In this soluble protein, X-rays crystallography has demonstrated that most of the protein, and especially the region containing the binding sites for the pigments, is arranged in an extended antiparallel β-sheet structure. The small amount of β-structure detected in LHC by our CD analysis exclude the possibility that several Chl could be organized in the same way as in the water-soluble protein. A similar conclusion has also been reached by Li [30]. The large hydrophilic portion of the LHC molecule cannot be a candidate for the Chl-binding sites because of the inaccessibility of the pigments to proton attack [29]. Furthermore, proteases which also clip the protein in this region do not modify this inaccessibility, thus indicating that the pigments are deeply buried in the hydrophobic interior of the complex. The precise location of the pigments as well as their orientation with respect to the stain excluding hydrophobic interior described on the LHC three-dimensional map [30,32] and to the α-helical segments must still await more precise analysis. Furthermore, the infrared dichroism data will be useful in order to draw more realistic models of intrinsic membrane proteins.

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References

1 Nabedryk, E. and Breton, J. (1981) Biochim. Biophys. Acta 635, 515–524
2 Thornber, J.P. (1975) Annu. Rev. Plant. Physiol. 26, 127–158
3 Mullet, J.E. and Arntzen, C.J. (1980) Biochim. Biophys. Acta 589, 100–117
4 Andersson, B., Anderson, J.M. and Ryrie, I.J. (1982) Eur. J. Biochem. 123, 465–472
5 Nabedryk, E., Andrianambinintsoa, S., Bardin, A.M. and Breton, J. (1983) Abstr. II-10, Workshop on Molecular

Structure and Function of Light-harvesting Pigment-protein Complexes and Photosynthetic Reaction Centers, Zürich, July 25–28
6 Burke, J.J., Ditto, C.L. and Arntzen, C.J. (1978) Arch. Biochem. Biophys. 187, 252–263
7 McDonnell, A. and Staehelin, L. (1980) J. Cell. Biol. 84, 40–56
8 Ryrie, I.J., Anderson, J.M. and Goodchild, D.J. (1980) Eur. J. Biochem. 107, 345–354
9 Haworth, P., Arntzen, C.J., Tapie, P. and Breton, J. (1982) Biochim. Biophys. Acta 679, 428–435
10 Nabedryk, E., Tiede, D.M., Dutton, P.L. and Breton, J. (1982) Biochim. Biophys. Acta 682, 273–280
11 Nabedryk, E. Tiede, D.M., Dutton, P.L. and Breton, J. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. II, pp. 177–180, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
12 Tapie, P., Haworth, P., Hervo, G. and Breton, J. (1982) Biochim. Biophys. Acta 682, 339–344
13 Chen, Y.H., Yang, J.T. and Chau, K.H. (1974) Biochemistry 13, 3350–3359
14 Menke, W. and Hirtz, R.D. (1973) Z. Naturforschung 28c, 128–130
15 Susi, H. (1969) in Structure and Stability of Biological Macromolecules (Timasheff, S.N. and Fasman, G.D., eds.), pp. 575–663, Dekker, New York
16 Bendit, E.G. (1967) Biopolymers 5, 525–533
17 Wallach, D.F.H., Verma, S.P. and Fookson, J. (1979) Biochim. Biophys. Acta 559, 153–208
18 Miyazawa, T. (1960) J. Am. Chem. Soc. 32, 1647–1652
19 Nabedryk, E., Gingold, M.P. and Breton, J. (1982) Biophys. J. 38, 243–249
20 Quinn, P.J. and Williams, W.P. (1983) Biochim. Biophys. Acta 737, 223–266
21 Bennett, J. (1983) J. Biochem. 212, 1–13
22 Mullet, J.E. (1983) J. Biol. Chem. 258, 9941–9948
23 Coruzzi, G., Broglie, R., Cashmore, A. and Chua, N.H. (1983) J. Biol. Chem. 258, 1399–1402
24 Markwell, J.P., Reinman, S. and Thornber, J.P. (1978) Arch. Biochem. Biophys. 190, 136–141
25 Anderson, J.M. (1980) FEBS Lett. 117, 327–331
26 Li, J. and Hollingshead, C. (1982) Biophys. J. 37, 363–370
27 Van Metter, R.L. (1977) Biochim. Biophys. Acta 462, 642–658
28 Haworth, P., Tapie, P., Arntzen, C.J. and Breton, J. (1982) Biochim. Biophys. Acta 682, 504–506
29 Siefermann-Harms, D. and Ninnemann, H. (1983) Photobiochem. Photobiophys. 6, 85–91
30 Li, J. (1984) Proc. Natl. Acad. Sci. U.S.A., in the press
31 Kühlbrandt, W., Thaler, T.H. and Wehrli, E. (1983) J. Cell. Biol. 96, 1414–1424
32 Kühlbrandt, W. (1984) Nature 307, 478–480
33 Henderson, R. and Unwin, P.N.T. (1975) Nature 257, 28–32
34 Tobin, E.M., Wimpee, C.F., Silverthorne, J., Stiekema, W.J., Neumann, G.A. and Thornber, J.P. (1984) In Biosynthesis of the Photosynthetic Apparatus. Molecular Biology, Development and Regulation; UCLA Symposium on Molecular and cellular Biology, New Series, Vol. 14 (Hallick, R., Staehelin, L.A. and Thornber, G.J.P., eds.), in the press
35 Capaldi, R.A. (1982) Trends Biochem. Sci. 7, 292–295
36 Senior, A.E. (1983) Biochim. Biophys. Acta 726, 81–95
37 Kosower, E.M. (1983) Biochem. Biophys. Res. Commun. 111, 1022–1026
38 Rao, J.K.M., Hargrave, P.A. and Argos, P. (1983) FEBS Lett. 156, 165–169
39 Theiler, R. and Zuber, H. (1984) Eur. J. Biochem., in the press
40 Widger, W.R., Cramer, W.A., Herrmann, R.G. and Trebst, A. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 674–678
41 Matthews, B.W., Fenna, R.E., Bolognesi, M.C., Schmid, M.F. and Olson, J.M. (1979) J. Mol. Biol. 131, 259–285