Transmembrane orientation of α-helices and the organization of chlorophylls in photosynthetic pigment–protein complexes

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Received 20 August 1984

UV CD and IR spectra of the water-soluble bacteriochlorophyll-protein antenna isolated from *Prosthecochloris aestuarii* indicate that about 50% of the protein is in a β-sheet conformation while for the dominant antenna complexes isolated from bacteria (B800-850) and from green plants (LHC), the α-helix (45%) is more abundant than the β-sheet (~ 10%) conformation. Furthermore, IR dichroism studies show that the α-helical segments of a large variety of intrinsic membrane Chl-protein complexes (antenna and reaction centers) are tilted on the average at 30–35° away from the membrane normal. The observation that in these complexes the Chl planes are also tilted at about the same angle suggests that the transmembrane orientation of the α-helices determines the positioning of the Chl molecules in photosynthetic membranes.

Chlorophyll–protein complex *α-Helix* *β-Sheet* *UV circular dichroism* *Infrared dichroism* *Transmembrane orientation*

1. INTRODUCTION

In photosynthetic membranes, the Chl molecules are thought to be non-covalently bound to the protein backbone of the intrinsic membrane proteins. This conclusion has been derived mostly from biochemical isolation of these Chl–protein complexes [1,2] but also from various spectroscopic investigations such as linear dichroism [3] and resonance Raman [4]. Furthermore the determination by X-ray crystallography of the structure of an antenna Chl–protein complex from the photosynthetic bacterium *Prosthecochloris aestuarii,* which demonstrated the actual binding of the BChl molecules to amino acid residues (mostly histidyl) in a β-sheet pocket, has led to the hypothesis that such pigment–protein interactions are rather general [5]. However, the fact that this BChl–protein complex is water-soluble makes it atypical for a model of the more usual photosynthetic complexes which are always hydrophobic membrane proteins.

By comparing the UV CD and IR spectra of the water-soluble complex to those obtained with several other antenna and reaction center complexes from plants and bacteria, we demonstrate that transmembrane α-helices rather than β-sheets are likely to play a role in the binding of Chls in vivo.

Abbreviations: CD, circular dichroism; IR, infrared; Chl, chlorophyll; BChl, bacteriochlorophyll; LHC, light-harvesting complex

2. MATERIALS AND METHODS

The BChl *a*–protein, prepared according to [6], was a generous gift from Dr Olson. The B800-850 was prepared from chromatophores of *Rhodopseudomonas sphaeroides* (strain 2.4.1) by incubation for 20 min in the dark at 0°C the chromatophores (*A* at 850 nm, 50 cm^{-1}) in 20 mM Tris–HCl (pH 7.5) with 1% lauryldimethylamine oxide. The antenna complex was recovered from centrifugation (2 h, 200 000 × *g*) on a sucrose density gra-

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00145793/84/$3.00

dient (0.6–1.2 M). The LHC, prepared according to [7], was reincorporated in lipid vesicles as in [8].

UV CD in solution and IR spectra on samples air-dried on CaF_2 discs were recorded and analyzed as in [8].

3. RESULTS AND DISCUSSION

3.1. *Analysis of UV CD and IR data*

The UV CD data indicate both a large similarity between the B800-850 complex (fig.1A) and the LHC [8] and a significant difference with the water-soluble complex (fig.1B). For these 3 Chl–protein complexes, the relative amount of α-helices, β-sheets and aperiodic structures, as estimated from the curve-fitting program [8,9], are given in table 1.

Using the BChl *a*–protein model derived from the X-ray data [5], the proportion of residues involved in the regions of β-sheet conformation can be estimated at about 50%, a value similar to that obtained from the UV CD curve-fitting procedure (table 1). Furthermore the bacteriorhodopsin in the purple membrane from *Halobacterium halobium* is largely α-helical [10] and from the CD spectra we have obtained a value of 68% α-helix [8] which compares well with the value of 72% derived from a recent model which fits best the known amino acid sequence to the structural map [11]. The relative amounts of the secondary structures which have been obtained from the UV CD data of two proteins differing largely in the ratio of α-helix to β-sheet conformation being in good agreement with the values obtained by more direct structural methods [5,11], it thus seems legitimate

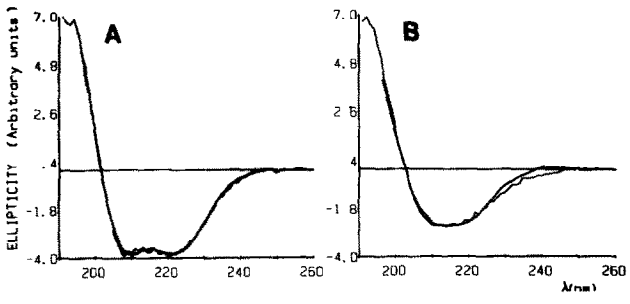

Fig.1. UV CD spectra (40 runs average) of (A) B800-850 complex in 1% lauryldimethylamine oxide. (B) Water-soluble BChl *a*–protein. Experimental (——) and calculated (▬) spectra.

Table 1

Estimation of α-helical (α), β-sheet (β) and aperiodic (γ) structures from UV CD data

	α	β	γ
LHC	44	8	48
B800-850	46	12	42
Water-soluble complex	30	50	20

to assume that the low (~10%) β-sheet and the ~45% α-helical contents of LHC and B800-850 (table 1) are also realistic estimates.

The IR absorption spectrum of B800-850 (fig.2A, see also [12]) is very similar to that of LHC [8] and exhibits an amide II peak at 1546 cm^{-1}, an amide I peak at 1656 cm^{-1} and only very little absorption at 1630 and 1685 cm^{-1}, characterisic of the β-sheet conformations. In contrast, the IR absorption spectrum of the water-soluble complex (fig.2B) shows a large contribution of the 1631 and 1688 cm^{-1} bands indicative of antiparallel β-pleated sheets and/or β-turns [13,14]. The frequency of the amide II band maximum at 1526 cm^{-1} and its shoulder at 1533 cm^{-1} also reflect β-conforma-

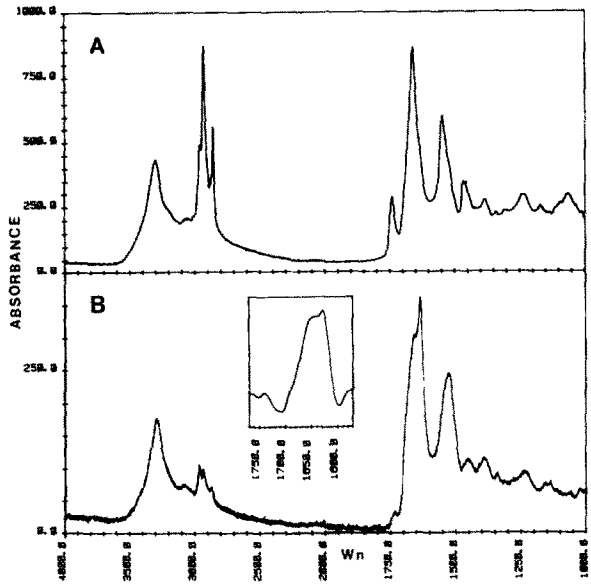

Fig.2. IR absorption spectra of air-dried samples of (A) B800-850 complex. (B) BChl *a*–protein. Inset: IR absorption spectrum of BChl *a*–protein in ^2H_2O after dialyzing for 4 h 150 μl of sample against two changes (15 ml each) of ^2H_2O.

tions. As a similar spectrum in the amide I band can be obtained when the protein is solubilized in D_2O (see inset fig.2B) this demonstrates that no major conformational change is induced by the air-drying process. The IR absorption spectrum of purple membrane [15] exhibits a main peak at 1666 cm^{-1} with only little contributions at 1630 and 1685 cm^{-1} which agrees with the small amount of β-sheet conformation found from the UV CD data. Taken together with the small contribution from 1630 and 1685 cm^{-1} bands relative to the 1666 cm^{-1} peak in the IR dichroism spectrum of purple membrane ([15] and in preparation), these observations do not support the recent proposal [16] of a large contribution of oriented β-sheets in this system. The IR absorption data on purple membrane, the water-soluble complex and the B800-850 or LHC complexes are thus in good qualitative agreement with the results from the UV CD data.

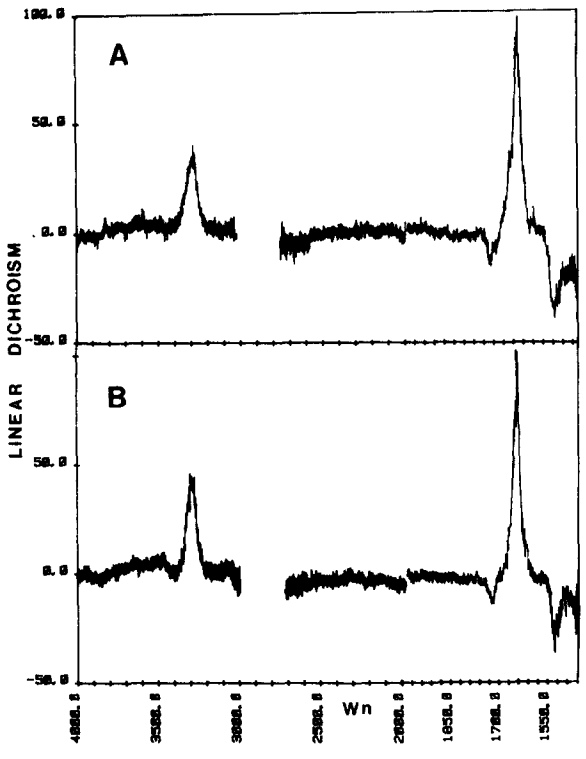

Fig.3. IR dichroism spectra ($A_{\parallel} - A_{\perp}$) of air-dried oriented samples of (A) LHC reconstituted in lipid vesicles. (B) B800-850 complex. Samples were covered with nujol [20].

In both the IR absorption spectra of the B800-850 and the water-soluble complex a band is present at 1735 cm^{-1} (fig.2A,B). While in B800-850 this band can be assigned for a large part to the carbonyl esters of the phospholipids tightly associated with this complex [17,18], in the water-soluble complex it is more probable that the carbonyl esters of the BChls are involved. The proportion of 4 of these carbonyls per 100 peptide carbonyls which can be evaluated from the structure [5] is in rough agreement with the spectrum (fig.2B). However, in the absence of more detailed biochemical analysis, we cannot exclude the presence of some lipids in the water-soluble complex.

For all of the antenna samples discussed here the spectra in the visible region show little change in the absorption properties of the pigments upon dehydration. Furthermore in the case of B800-850 and of LHC the linear dichroism spectra in the visible region (not shown) demonstrate that the native orientation of the pigments [19,20] has been preserved. The strong similarity between the IR dichroism spectra of LHC (fig.3A) and of B800-850 (fig.3B) complexes together with their identical α-helical content (table 1) indicates a similar average orientation of the α-helices at 30–35° from the membrane normal [8].

3.2. *Transmembrane orientation of the α-helices and the organization of chlorophylls in vivo*

In addition to the data on the main antenna complexes discussed here, a transmembrane orientation of the α-helices has been described both in intact membranes [15] and in several other isolated Chl–protein complexes such as the bacterial reaction center from *Rps. sphaeroides* (strain R.26) [21], its LM subunit [22] and the Photosystem I particles [23]. Thus the transmembrane orientation of α-helical segments appears to be a rather general property of antenna and reaction center Chl–protein complexes which are integral membrane proteins. Furthermore the small (typically ~10%) amount of β-sheet structure which is observed in these complexes cannot provide enough space for the binding of all the pigments [8,24]. The water-soluble complex, with its large amount of β-sheet structure to which the BChl molecules are bound, is a rather atypical system and does not provide a good model for the general organization of the

pigments in vivo. In view of resonance Raman data [4] and of sequence analysis [12,25], however, it seems likely that the bonding pattern of the pigments to the amino acid residues in the intrinsic membrane pigment–protein complexes is of the same nature as the one encountered in the water-soluble complex [5].

A large number of the polypeptides of the pigment-protein complexes have been shown to span the photosynthetic membrane [26–28]. It is thus highly probable that the α-helical segments preferentially oriented towards the normal to the membrane plane which have been demonstrated by our IR dichroism studies do represent these transmembrane peptides. Although it cannot be excluded that some of the photosynthetic pigments are preferentially bound to the random coil part of the protein, it appears more likely that the α-helices are involved in this binding. Firstly we note that the LM fraction of the bacterial reaction center, which contains all of the pigments, appears slightly enriched in α-helices as compared to the intact reaction center ([22] and unpublished). Furthermore the high degree of orientation of the Chls in vivo [3] strongly suggests a binding of the pigments to anisotropic regions of the protein. In addition these pigments are well shielded from the aqueous environment and are not affected by protease treatments which clip external segments of the protein [27,29,30] thus suggesting that they are buried in a hydrophobic core such as the one provided by α-helices. Hydrophobic stretches, which probably correspond to α-helical segments, have been observed in the primary structure of several antenna [25,31,32] and reaction center [33] polypeptides.

These observations not only lend support to the notion that α-helices tilted on the average at a large angle (~60°) away from the membrane plane play a very important role in the organization of photosynthetic pigments in vivo, but also suggest that the orientation of the chlorophylls may actually be determined by the preferential transmembrane orientation of the α-helices. The large majority of the antenna Chls from plants and bacteria orient with their plane strongly tilted out of the membrane plane [3], with the exception of the B800 molecule in the B800-850 complex [19,34]. In bacterial reaction centers, the special pair is oriented rather perpendicular to the plane of the membrane [3] while a similar orientation is observed for the plane of the pheophytin molecule intermediary acceptor in bacteria and Photosystem II [3,35].

Based essentially upon our experimental data on the orientation of both the pigments and the α-helix segments of the intrinsic membrane Chl–protein complexes, we thus propose that the orientation of the bulky Chl molecules rather perpendicular to the membrane plane is in part determined by the preferential transmembrane orientation (on the average 30–35° away from the membrane normal) of the α-helical segments which account for about half of the secondary structure of these complexes.

ACKNOWLEDGEMENTS

The authors greatly acknowledge Dr J.M. Olson for his generous gift of the water-soluble complex which was essential for this work, Dr B.W. Matthews for discussions and A.M. Bardin for the measurement of the CD data. Part of this work was funded by an AFME grant (no. 3.320.1402).

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