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Comparison of α-helix orientation in the chromatophore, quantasome and reaction centre of *Rhodopseudomonas viridis* by circular dichroism and polarized infrared spectroscopy

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A comparison of the protein structure in chromatophores, quantasomes and reaction centres of *Rhodopseudomonas viridis* is made by investigating ultraviolet circular dichroism and polarized infrared spectra of the intact chromatophore membrane, the isolated quantasome and the reaction centre reconstituted into phosphatidyl choline vesicles. A quantasome (photoreceptor unit) is made of a reaction centre surrounded by a ring of antenna complexes. Conformational analysis of the circular dichroism data indicates that intact chromatophores and quantasomes contain more α-helical structure (57%) than reaction centres (47%). Infrared dichroism data show that α-helical segments are on the average closer to the membrane normal in quantasomes ($\phi_\alpha = 28°$) than in reaction centres ($\phi_\alpha = 38°$C). This suggests a higher content of α-helices and a better orientation of transmembrane α-helical rods in the antennae surrounding the reaction centre. Our data are discussed in view of the results previously obtained by infrared dichroism of reaction centre films of *Rps. sphaeroides* (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, Dordrecht, The Netherlands) and by X-rays of reaction centre crystals of *Rps. viridis* (Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1984) J. Mol. Biol. 180, 385–398). Our results also imply that the secondary structure and orientation of the protein is comparable in bacteriochlorophyll *a*- and bacteriochlorophyll *b*-containing reaction centres. Furthermore, our data on the orientation of the α-helices in the reaction centre of *Rps. viridis* imply that the C-2 symmetry axis observed in the model derived from X-ray crystallography is oriented in vivo along the normal to the membrane plane.

Introduction

A general transmembrane organization of the α-helices in hydrophobic photosynthetic chlorophyll-protein complexes has been recently proposed on the basis of ultraviolet circular dichroism (CD) and infrared dichroism studies [1]. This is in agreement with the sequence analysis of several antenna and reaction centre polypeptides predicting hydrophobic domains which could fit to transmembrane α-helices [2–5] and with the recent X-ray diffraction analysis of crystallized reaction centre of *Rps. viridis* [6]. Furthermore, the chromatophore membranes of this bacterium appear highly suitable for structural studies. In particular, electron microscopy of chromatophores from *Rps. viridis* has shown repeating structural units

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Abbreviation: CD, circular dichroism.

organized in a two-dimensional hexagonal lattice [7–9]. Each unit has a central core surrounded by a ring of antenna complexes [8]. Recently, these structural units, called quantasomes, have been isolated and characterized [10,11]. The respective organization of the antenna and reaction centre bacteriochlorophylls in quantasomes has been investigated [12]. The quantasomes offer the opportunity for characterizing both reaction centre and long-wavelength antenna (B 1015) type), since an active light-harvesting complex has not been isolated, and thus no direct structural analysis is available.

In this paper, using CD and polarized infrared spectroscopies, we compare the secondary structure and orientation of the proteins in chromatophores, quantasomes and reaction centres of *Rps. viridis*. Our data show that the intact chromatophore and the photoreceptor unit contain more α-helical structure (57%) than the isolated reaction centre (47%). Furthermore, these α-helices are, on the average, closer to the normal to the membrane in quantasomes than in reaction centres, suggesting a better orientation of transmembrane α-helical rods in the surrounding antennae.

Materials and Methods

Chromatophores of *Rps. viridis* were isolated according to Ref. 9. Quantasomes were prepared by treating chromatophores with lauryldimethylamine oxide and sodium deoxycholate as described in Ref. 11. The quantasomes and the chromatophores were purified by centrifugation on a sucrose density gradient. For infrared studies the quantasomes were reaggregated by dialysis (2 days) against 10 mM Tris (pH 8)/0.01% sodium azide/5 mM sodium ascorbate at 10°C, and then pelleted. Reaction centres were isolated as reported by Clayton and Clayton [13], and further purified by a high performance liquid chromatography step [14] in which lauryldimethylamine oxide detergent was exchanged for sodium cholate. They were reconstituted in soybean phosphatidylcholine vesicles according to Ref. 15.

Ultraviolet CD spectra of membranes and of solubilized proteins were measured with a Jobin-Yvon Mark V dichrograph linked to a Micral 80-31 B computer. For the infrared dichroism spectra, intact or reconstituted membranes were oriented by air-drying on CaF_2 windows. We have demonstrated that such films of chromatophores or reaction centres were active with respect to the primary donor oxidation [16]. Polarized infrared spectra were recorded on a Perkin Elmer 180 spectrometer equipped with a wire grid polarizer and linked to a Hewlett Packard 9825 A computer. The method for the infrared dichroism analysis and calculation of the average tilt angle of the α-helices has been previously described [15,17].

Results

Secondary structure analysis of the proteins in chromatophores, quantasomes and reaction centres

The relative extent of α-helical, β-sheet and aperiodic structures was estimated by analyzing experimental ultraviolet CD spectra (195–260 nm region) as a linear combination of reference protein spectra [18] using a general least-squares program [15,19]. Experimental and calculated (best-fit) ultraviolet CD spectra of purified chromatophores, solubilized quantasomes and reaction centres are presented in Fig. 1. Optical effects such as light-scattering and absorption flattening in membrane sheets will not be described here as they have been extensively discussed for purple membrane [20,21] and for some photosynthetic chlorophyll-protein complexes [19,22]. However, it can be noticed that the quality of the fit between experimental and calculated CD spectra (expressed either by the difference curve – Fig. 1 – or by the root-mean-square deviation $\bar{\sigma}$ – Table I) is better for the detergent-solubilized reaction centre than for the chromatophore membrane or the quantasome. The percentages of α-helix and β-sheet for the three types of samples are compared in Table I. It appears that the proportion of α-helix is higher in chromatophores (58%) or quantasomes (56%) than in reaction centres (47%), suggesting a different α-helical content in the reaction centre and its surrounding antennae. We have previously reported similar values of protein secondary structure for the reaction centre of *Rps. sphaeroides* [15,23]. The isolated reaction centre of *Rps. viridis* contains four polypeptides, the well-known L, M and H protein subunits of apparent molecular weight of 24000, 28000, 35000, respectively, and

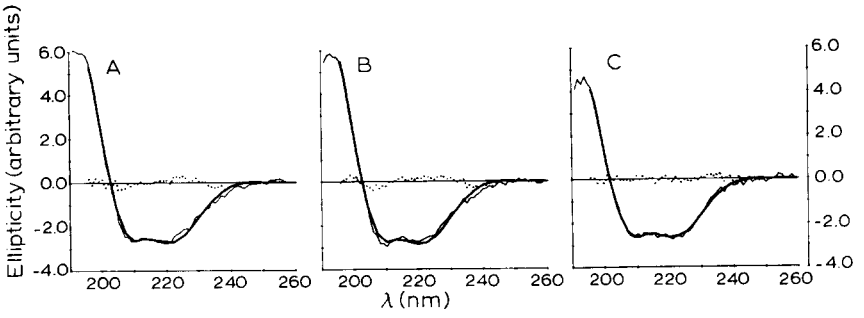

Fig. 1. Ultraviolet CD spectra (40 runs average) of (A) purified chromatophores, (B) quantasomes after dialysing 24 h against 1%) sodium deoxycholate, 20 mM Tris (pH 8), (C) reaction centres in 0.5% sodium cholate. Experimental (thin line), calculated (best-fit) (thick line) spectra and difference (· · · · · ·) between experimental and calculated spectra.

an additional membrane-bound cytochrome (M_r, 38 000). The 47% α-helical value reported here for the reaction centre of *Rps. viridis* refers to the whole protein, including cytochrome. Table I also indicates a higher content of β-structure (up to 25%) in chromatophores and quantasomes, compared to other photosynthetic membranes and hydrophobic chlorophyll-protein complexes [1]. However, some caution must be exercised in the calculation of the extent of β-structure from CD membrane spectra. Optical artifacts such as differential flattening would result in an overestimation of β-sheet [20]. Indeed, infrared absorption spectra of chromatophores, reconstituted reaction centres (data not shown) and quantasomes (Fig. 2) exhibit comparable amide I and amide II frequencies at 1658 and 1547 cm^{-1}, respectively, characteristic of α-helical and random structures, with only small contributions from the β-sheet structure at 1685 and 1635 cm^{-1}.The band at 1738 cm^{-1} is mainly due to carbonyl C=O ester from intrinsic lipids. A small C=O ester/amide I ratio is observed in quantasome, indicating a partial loss of lipids after treatment of chromatophores with detergent, as previously reported [9].

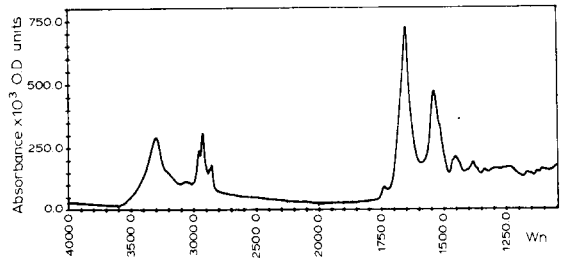

Fig. 2. Infrared absorption spectrum of an air-dried film of quantasome.

Orientation of proteins

Infrared dichroism spectra ($A_{\parallel} - A_{\perp}$) of air-dried chromatophores, quantasomes and reconstituted reaction centres are presented in Fig. 3. These spectra exhibit characteristic positive dichroism signals for the amide A (N-H stretching at 3300 cm^{-1}) and amide I (80% C=O stretching at 1658 cm^{-1}) and negative dichroism signals for the amide II (60% N-H bending at 1547 cm^{-1}) and the C=O ester stretching (at 1738 cm^{-1}) bands, indicative of oriented peptide and lipid groups. With our conventions [17] a positive dichroism is associated with the alignment of the transition at less than 55° from the membrane normal. It must also be noticed that for comparable amide band absorbance, the linear dichroism signals of the amide bands are always smaller on reaction centres spectra than on quantasomes and chromatophores spectra (Fig. 3). Qualitatively, this already suggests

TABLE I

ESTIMATION OF THE PERCENTAGE OF α-HELIX (α) and β-STRUCTURE (β) from ultraviolet CD spectra

$\bar{\sigma}$ is the root-mean-square deviation between calculated (best-fit) and experimental spectra data.

	α (±5%)	β	$\bar{\sigma}$
Chromatophore	58	25	0.067
Quantasome	56	23	0.083
Reaction centre	47	14	0.055

TABLE II

ORIENTATION OF α-HELICES

Average obtained from five different air-dried samples for each type of preparation. D is the experimental dichroic ratio for the amide I band; D_α is the corrected dichroic ratio for the only α-helices; ϕ_α is the average tilt angle of the α-helix axes with respect to the membrane normal; ϕ_{min} and ϕ_{max} are lower and upper ϕ_α limits taking into account the effect of varying D within its experimental limits.

	D	D_α	ϕ_α	ϕ_{min}	ϕ_{max}
Chromatophore	1.16 ± 0.03	1.29	33°	29°	37°
Quantasome	1.20 ± 0.03	1.36	28°	24°	31°
Reaction centre	1.09 ± 0.02	1.20	38°	35°	41°

a lesser extent of orientation of the peptide groups in reaction centres than in quantasomes.

Quantitative determination of α-helix orientation is given in Table II. Using the percentage of α-helical structure calculated from the ultraviolet CD spectra, the experimental dichroic ratio $D = A_{\parallel}/A_{\perp}$ is corrected to yield the α-helix dichroic ratio $D_\alpha = A_{\parallel\alpha}/A_{\perp\alpha}$. From D_α the average tilt angle ϕ_α of the α-helix axes with respect to the membrane normal can be deduced. In Table II, D, D_α and ϕ_α have been calculated for the amide I band. Taking into account the variation in the experimental dichroic ratio D, limits of ϕ_α are also presented. It appears that α-helical segments are on the average closer to the membrane normal in quantasomes ($\phi_\alpha = 28°$) and chromatophores ($\phi_\alpha = 33°$) than in reaction centres ($\phi_\alpha = 38°$).

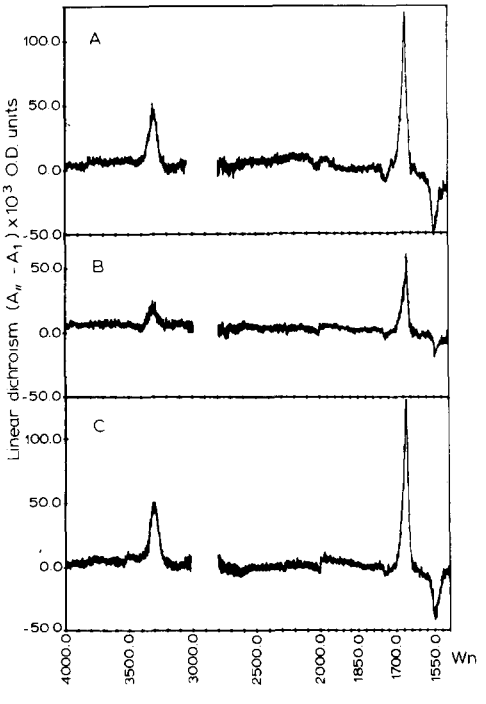

Fig. 3. Infrared dichroism spectra ($A_{\parallel} - A_{\perp}$) of (A) chromatophores, (B) reconstituted reaction centres,(C) quantasomes. The air-dried films were covered with nujol as in Ref. 23. These linear dichroism spectra correspond to identical amide I absorption for the different samples.

Discussion

Our present CD and infrared dichroism data on reaction centres of *Rps. viridis* demonstrate that the α-helical segments account for 47% of the protein secondary structure, with the helix-axes tilted, on the average, at 38° with respect to the normal to the membrane. Assuming an identical orientation of the reaction centre polypeptides in the isolated reaction centre and in the quantasome, our infrared dichroism results imply a slightly better orientation of the α-helical segments in the ring of antennae than in the reaction centre. However, at least two effects can contribute to increase the experimental ϕ_α value observed for reaction centres. Firstly, electron microscopy of chromatophore membranes [7] and X-ray studies of reaction centre crystals [6] have shown that the reaction centre complex has a 14 nm elongated shape with a central core (the L and M subunits anchored in the 4–6 nm bilayer thickness) and two globular units (cytochrome and H subunit) protruding at each extremity. When preparing films of reconstituted reaction centres for infrared dichroism analysis, the whole protein which largely protrudes outside the bilayer, may tilt during the drying

process, and thus can lead to a lower orientation of the reaction centre long axis in the bilayer. However, this effect does not seem to occur to a large extent as variations of protein-to-lipid ratio (1/50, 1/100) during the reconstitution of reaction centres do not alter the observed amide I dichroic ratio. Secondly, the cytochrome protein part of the reaction centre accounts for about one third molecular weight of the total protein complex. Its X-ray structure is not yet reported. Assuming that α-helical segments in this cytochrome are mainly randomly organized (and not preferentially perpendicular to the bilayer) as in soluble cytochrome *c*, the exact ϕ_α value for the only α-helices of the LMH complex should be lower than 38°.

The electron density map of reaction centre crystals of *Rps. viridis* [6] clearly indicates that both the L and M subunits contain five α-helical regions, approx. 4 nm long, which could be related to the five hydrophobic domains deduced from hydropathy plots of the L and M amino acid sequences of reaction centre of *Rps. sphaeroides* [2,3] and of *Rps. capsulata* [4]. On the other hand, only one transmembrane α-helical segment is predicted from the amino acid sequence [4] of the H subunit (which possesses a large cytoplasmic domain) and is detected by X-rays [6]. This is in agreement with the ultraviolet CD spectrum of the isolated H subunit of reaction centre from *Rps. sphaeroides* which leads to a 31% α-helix content for H compared to 51% and 55% for LMH and LM, respectively (Refs. 15 and 23, see also Nabedryk, E., Breton, J., Debus, R., Okamura, M. and Feher, G., unpublished results). Furthermore, the X-ray data indicate that the ten α-helices observed in the LM subunit are tilted on the average at a small angle with respect to the C-2 symmetry axis with none of them tilted at more than 30° from this axis. As discussed above, due to the presence of the cytochrome in the reaction centre complex of *Rps. viridis*, it is probable that the experimental tilt angle (38°) is overestimated and thus the exact tilt angle for the only α-helices of the LMH complex is lower than 38° and closer to the angle observed either by X-ray of reaction centre crystals of *Rps. viridis* or by infrared dichroism of reaction centre and LM films of *Rps. sphaeroides* [15,23].

We have previously reported [17] that the tilt angle calculated from our CD and infrared data represents an average value for all of the α-helices contained in the system under investigation. The possibility that some α-helical segments are either rather parallel to the membrane, (i.e., joining transmembrane α-helices), or random (at the N- and C-terminals of the various subunits) should not be excluded. Such segments have been presumed from X-ray data [24]. Assuming a random orientation of these α-helices, we can apply a correction to our infrared dichroism data in order to calculate the average orientation of the only transmembrane α-helices. From (i) the 55% α-helical content deduced from ultraviolet CD spectra of the LM subunit from *Rps. sphaeroides* (ii) the assumption of an average of 25 residues per transmembrane α-helix and (iii) the ten transmembrane α-helices predicted by hydropathy plots [2–4] and identified by X-rays [6], it can be calculated that among the 324 aminoacids belonging to α-helical structures in the LM subunit of *Rps. sphaeroides*, 75 may be located in non-transmembrane α-helices, leading to a 42% content of purely transmembrane α-helices and an average tilt angle for these α-helices of 20–25° wih respect to the membrane normal. The X-ray data show that the transmembrane α-helices in the LM subunit of *Rps. viridis* are tilted at less than 30° with respect to the C-2 symmetry axis. Thus, our result that the only transmembrane α-helices in LM are tilted on the average at 20–25° from the normal to the membrane demonstrates that the C-2 symmetry axis is indeed aligned in vivo along the normal to the membrane plane, as also discussed in Refs. 6 and 25.

In addition to the good qualitative agreement between spectroscopic and X-ray diffraction studies, our data on the reaction centre of *Rps. sphaeroides* and *Rps. viridis* imply that the secondary structure and orientation of the protein is comparable in bacteriochlorophyll *a*- and bacteriochlorophyll *b*-containing reaction centres. The identical geometrical arrangement of the chromophores has already been discussed [25]. Furthermore, the surrounding antennae of the reaction centre of *Rps. viridis*, i.e., the B1015 polypeptides should contain transmembrane α-helical segments oriented slightly closer to the membrane normal than in the reaction centre core.

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