Linear-Dichroism Measurements on the LH2 Antenna Complex of *Rhodopseudomonas Acidophila* Strain 10050 Show that the Transition Dipole Moment of the Carotenoid Rhodopin Glucoside Is Not Collinear with the Long Molecular Axis

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We have applied linear-dichroism experiments to determine the orientation of the transition dipole moment, corresponding to the main absorption band of the carotenoid, rhodopin glucoside, in the light-harvesting complex LH2 from *Rhodopseudomonas acidophila* strain 10050. The crystal structure of this LH2 complex is known and MO calculations have been performed that indicate that the transition dipole moment is 9.1° off axis from the extended π-electron conjugated chain (Dolan, P. M.; Miller, D.; Cogdell, R. J.; Birge, R. R. Frank, H. A. *J. Phys. Chem. B* **2001**, *105*, 12134−12142). Our experimental results confirm that the transition dipole moment is not oriented along the long axis of the carotenoid molecule, and they are in excellent agreement with the MO calculations. However, they disagree with the linear-dichroism results of the same authors. Possible reasons for the discrepancy are discussed.

Introduction

Nowadays, the crystal structures of many photosynthetic pigment−protein complexes are known. For a detailed understanding of the functioning of these complexes, an accurate correlation is needed between their structure and steady-state and time-resolved spectroscopic properties. Polarized absorption techniques appear to be instrumental for establishing such a correlation. For instance, linear-dichroism and polarized-fluorescence measurements can be applied to determine the orientations of the pigment transition dipole moments with respect to the molecular frame of the pigment−protein complex, whereas circular dichroism and again polarized fluorescence measurements can provide information about the orientations of the transition dipole moments with respect to each other.[1−3] These dipole orientations together with the distances between the pigments govern the interactions between pigments and as such are crucial elements determining the shape and intensity of the absorption spectra and the rate of transfer of excitation energy. Several linear-dichroism studies have been performed on membrane-bound pigment−protein complexes prepared from photosynthetic organisms, deducing the orientations of carotenoid molecules.[4−7] Using these techniques, we have recently managed to get a fairly accurate understanding of the spectroscopic properties in the Q_y absorption region around 800 nm of the BChl *a* containing FMO antenna complex of *Pc. aestuarii*,[8] taking the transition dipole moment along the line through the N_1 and N_3 atoms (Y axis) in the BChl *a* molecules. With the use of linear-dichroism and polarized-fluorescence measurements on macroscopically oriented LH2 complexes from *Rb.*

sphaeroides, it could be determined that the Q_y transition dipole moments corresponding to both the B800 and the B850 absorption bands lie almost parallel to the (membrane) plane of the complex, the possible deviation being only a few degrees.[9] The crystal structure of the very similar LH2 complex from *Rps. acidophila* revealed that indeed all BChl *a* molecules have their Y axis nearly parallel to this plane.[10] In an extensive study on the circular-dichroism properties of many LH2 complexes from different organisms, it was among others concluded that the pigment organization in LH2 from *Rb. sphaeroides* and *Rps. acidophila* is very similar and that the bacteriochlorophylls' Q_y transition dipole moments lie nearly parallel to the membrane plane of the complex, i.e., nearly perpendicular to the quasi-rotational symmetry axis.[11,12] More complex systems such as plant antennae are also studied with these methods: with the help of linear dichroism and absorption spectroscopy it was possible to elucidate several characteristics of the CP29 higher-plant antenna protein, such as the Q_y transition dipole moment vectors of the chlorophylls,[13] as well as the Q_y transition energy and chlorophyll a/b affinity of each binding site.[14]

The detailed orientations of the transition dipole moments of carotenoids have received less attention in the literature, and as pointed out by Dolan et al.,[15] it has often been tacitly assumed that the direction of the strongly allowed transition moment lies along the long axis of the π-electron conjugated chain, which is defined as a vector **S** drawn from the first carbon in the conjugated system to the last. Using this assumption, for instance, Croce et al.[16] used linear-dichroism (LD) results on the LHCII antenna complex from green plants to deduce the orientation of the neoxanthin molecule in this complex. However, recent experimental and computational studies[17] have shown that this assumption is not in general correct, and Dolan et al.[15] concluded from MO calculations that the transition

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moment of the carotenoid, rhodopin glucoside, in the LH2 complex from *Rps. acidophila* lies 9.1° off the long structural axis of the molecule. The LH2 complex of *Rps. acidophila* is in fact a very suitable complex for studying this dipole orientation because its (C_9-symmetric) structure is known at 2.5 Å resolution.[10] In the same paper of Dolan et al.,[15] the authors report on linear-dichroism measurements on membranes, containing the same LH2 complex, to correlate the experimentally observed LD to the calculated orientation of the transition dipole. However, no firm conclusions could be drawn from these experiments. In fact, careful reinspection of their experimental data indicates that the measured spectrum is not in agreement with the MO calculations (see below). Therefore, we have decided to accurately reinvestigate the LD of LH2 from *Rps. acidophila*. Instead of using membranes, we used isolated LH2. We obtain different experimental results, which appear, however, to be in excellent agreement with the orientation of the carotenoid dipole moment as obtained from the MO calculations by Dolan et al.[15]

Materials and Methods

Cells of *Rps. acidophila* LH2 strain 10050 were grown, and LH2 was prepared as described before.[11] Absorption and LD measurements at room temperature were performed in uncompressed and compressed polyacrylamide gels as in Nussberger et al.[18] Polyacrylamide gels were prepared with the following concentrations of components: 20 Mm TRIS (pH = 8.0), 0.15% v/v LDAO, 15% v/v acrylamide, 0.5% v/v *N,N*-methylenebis-acrylamide (BIS), 0.03% v/v *N,N,N′,N′*-tetramethyl-para-phenylenediamine (TEMED), and 0.02% v/v APS. Before adding the gel initiator ammonium persulfate (APS), *Rps. acidophila* LH2 was added to the solution to obtain a final absorption (*A*) of 0.5 at the NIR absorption maximum, around 850 nm. The mixture was quickly poured into a home-built gel press, and after the gel was formed, it was squeezed in both the *x* and *y* directions from an initial width of 1.3 cm to a final of 1.0 cm, allowing it to expand along the *z* direction.

The way of orientation of the complexes in the sample with respect to the light direction, and the methodology followed to analyze the results was described extensively before.[1,2,19,20] Use is made of the following relation:[21]

$$\frac{\text{LD}}{3A} = -\left\langle \frac{1}{2}(3\cos^2\alpha - 1) \right\rangle \varphi \qquad (1)$$

where *A* is the isotropic absorption which is measured on an unsqueezed gel that has identical properties (optical path length and LH2 concentration) as the squeezed gel on which the LD is measured. α denotes the angle between the transition moment and the C_9-symmetry axis (perpendicular to the plane of the LH2 complex), and the brackets ⟨...⟩ denote weighted averaging over all transitions in the complex, contributing to the absorption at a particular wavelength. The factor φ depends on how well the particles are oriented in the gel and this value always lies between −0.5 and 1. For disklike complexes such as LH2, this factor is expected to be negative[1,2] and this was experimentally demonstrated to be the case for the LH2 complex of *Rb. sphaeroides*.[9] The reduced LD (LD/3*A*) spectrum is expected to be constant over an absorption band if the transition dipole moments of the corresponding transitions have the same orientation with respect to the symmetry axis; that is, the shape of the LD spectrum is in that case the same as that of the absorption spectrum in that particular wavelength region. The actual value of φ cannot directly be determined from the LD spectrum, but by taking the ratio of the reduced LD for two
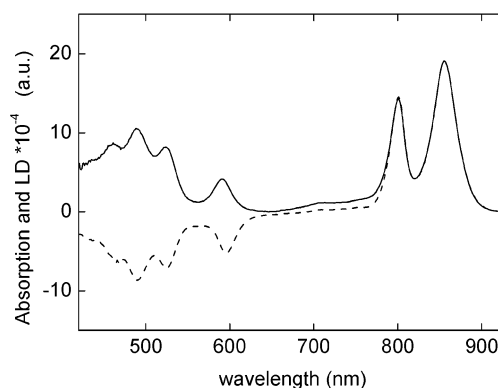

Figure 1. Room-temperature absorption (solid) and LD (dashed) spectra of the *Rps. acidophila* LH2 complex. The absorption spectrum is scaled to the LD spectrum for better comparison. Note that in the 800−900 nm region the absorption and LD spectra overlay almost perfectly.
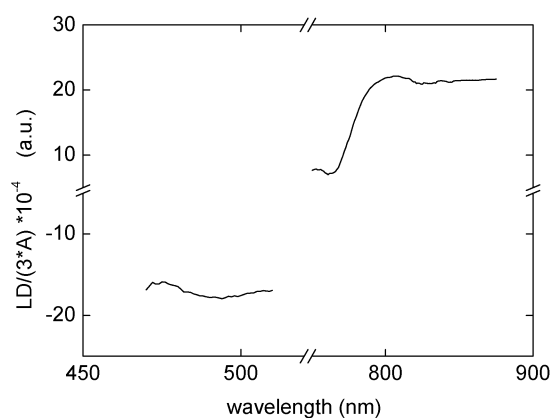

Figure 2. Reduced LD spectrum (LD/3*A*) of the Rps. acidophila LH2 complex. Only the Q_y and carotenoid regions are shown.

different absorption bands, this parameter drops out and this ratio only depends on the angles α for both bands. By comparing the reduced LD for the carotenoid absorption band to that of the BChl *a* Q_y absorption bands, it is possible to accurately determine the angle α for the carotenoid transition moment because this angle for the Q_y transitions is known to be close to 90°. Even when it is assumed that this angle is 80°, the calculated value for the carotenoid transition moment hardly differs (see below).

Results and Discussion

The LH2 complex of *Rps. acidophila* exhibits a pronounced LD signal as can be seen in Figure 1. In the Q_y region, the (normalized) absorption and LD spectra coincide almost perfectly, with peaks at 801 and 855 nm. Consequently, the reduced LD spectrum (Figure 2) is more or less constant across the entire Q_y region. The signals are positive, in agreement with the fact that α is close to 90° and the orientation factor φ is negative.

In the Q_x region, the situation is different. The LD is negative, and the peak is somewhat red-shifted with respect to the corresponding absorption peak (596 vs 592 nm). This is explained by the fact that the B850 and B800 pigments have different orientations for their *X* axes, whereas their Q_x absorption spectra slightly differ. The *X* axis for the B850 pigments is oriented more or less parallel to the symmetry axis but the one of the B800 pigments is close to parallel to the plane of the complex. Therefore, it is straightforward to conclude that the Q_x band of the B850 pigments is somewhat red-shifted with respect to that of the B800 pigments.[9,22]

Letters

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TABLE 1: Positions, Reduced LD Values, and Calculated Angles for the Qy B800 and B850 and Carotenoid Dipoles

dipole	position (nm)	LD/3A × 10^{-4} (a.u.)	LD/3A normalized[a]	α experimental	α crystal
Q_y B850	855	21.5	−0.49	85.5°	82.5° (N_B-N_D)[b]
			−0.45	80°	
Q_y B800	801	21.9	−0.5	90°	81.5°(N_B-N_D)[b]
			−0.46	81.2°	
R. glucoside	488	−17.6	0.4	39.2°	31.8° (*S*) 38° (*μ*)
			0.37	40.2°	

[a] LD/3A is normalized so that the angle α for the Q_y B800 transition is 90° (upper line) or 80° for the Q_y of B850 transition (lower line). [b] The Q_y transition dipoles of the BChls lie slightly off the N_B−N_D direction.[5]
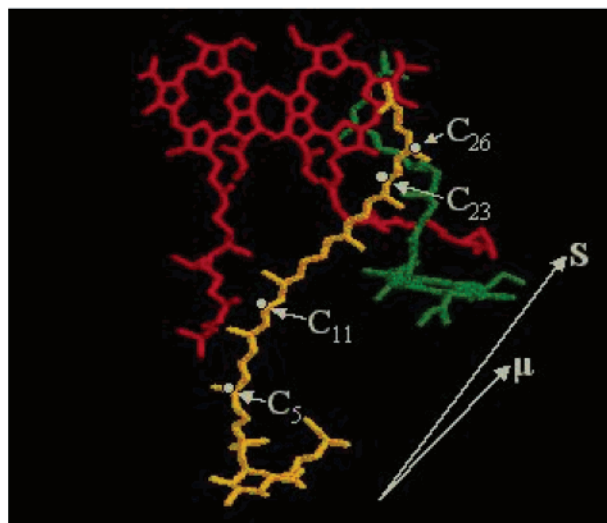

Figure 3. Structural building block of *Rps. acidophila*, consisting of three BChls: one B800 (green) and two B850 (red), and one carotenoid molecule (orange). The long structural axis vector *S* connects the carbon atoms C5 and C26, whereas the transition dipole moment *μ* stretches from the C11 to the C23 carbon atom.

The most interesting wavelength region for this study is located around 500 nm where the carotenoid absorption dominates. The most pronounced carotenoid absorption bands at 488 and 524 nm give rise to negative peaks at 489 and 524 nm in the LD spectrum. The absorption peak at 461 nm corresponds to the LD peak at 468 nm. The fact that the latter peak positions do not coincide indicates the presence of some additional underlying absorption with a transition moment orientation that is different from that of the carotenoid. This can also be seen in the reduced LD spectrum, the absolute value of which starts to decrease upon going to the blue. We ascribe this additional absorption to BChl *a*. Therefore, for calculating the angle α for the carotenoid, we use the value of the reduced LD at 488 nm where the carotenoid absorption is most pronounced. However, when we use the peak at 524 nm, the obtained value is actually almost identical.

It is now possible to estimate the orientation of the carotenoid transition dipole, making use of the fact that the transition dipole moments in the Q_y region are more or less perpendicular to the symmetry axis. Assuming that the angle α for the B800 dipole moment (which leads to a slightly higher LD than the B850 dipole) is 90° (see above) leads to an angle of 39.2° for the transition dipole of the carotenoid, using the reduced LD at 488 nm (use of the LD at 524 nm leads to a value of 38.1°). Assuming that the angle α for the B850 dipole moment is 90° leads to an angle of 38.8°. The choice of 90° is not very critical for the finally obtained angle $α_{car}$ for the carotenoid dipole moment. Even if an angle of 80° is assigned to the B850 band, which is smaller than the known values from literature, one

obtains an angle of 40.2°, i.e., only 1° higher than obtained above. Taking into account the uncertainty in the literature values for the angles in the Q_y region and the experimental uncertainties in the reduced LD in this study, we arrive at a final value for the angle of the carotenoid transition dipole of 39.5° ± 1.5°, where the value of the error margin should be considered as a conservative estimate. It is interesting to note that this angle is almost identical to the value obtained at 512 nm for LH2 from *Rb. sphaeroides*,[9] which corresponds to sphaeroidene, the carotenoid in that particular complex. The latter angle can be directly calculated from the reported value of $P_2(\cos β^μ)$ = 0.39 ± 0.03, where P_2 is the second-order Legendre polynomial and $β^μ$ is the angle between the absorption transition dipole moment and the symmetry axis of LH2. This means that α (=$β^μ$) = 39.6° ± 1.2°. Note that a similar angle of 41° ± 2° for the same complex was already found in 1981.[22]

It should be noted that the ratio of the reduced LD in the carotenoid region and that in the Q_y region as reported by Dolan et al.[15] is considerably smaller than in our case, and applying the above analysis to their data leads to an angle close to 50°. There may be various explanations for this discrepancy. First of all, their study was performed on membrane fragments, whereas we used isolated complexes. The membrane fragments also contain some LH1 complexes, and their carotenoids contribute to the absorption and possibly to the LD spectrum. In the present study, these LH1 complexes are absent. It is also possible that an additional LH2 carotenoid molecule is present in the membranes, which is lost upon isolation of LH2. However, the LH2 complexes in the present study contain only one carotenoid molecule (per B800 pigment), which is also the case for LH2 for which the crystal structure was determined. An alternative explanation for the apparent discrepancy might seem to be the different way in which the complexes were oriented in both studies (unidirectional vs bidirectional compression). However, this is only expected to lead to a different way of orientation and thus to a different value of the orientation factor $φ$; therefore, the shape of the reduced LD spectrum should remain the same, only the height, which is not relevant for the presented way of analyzing the data, is expected to differ. Finally, we would like to note that the LD spectra were measured in different ways. We used the standard method of lock-in detection where the polarization of the light was varied with a frequency of 100 kHz in order to be able to accurately determine the difference in absorption in a direct way. On the other hand, Dolan et al.[15] recorded absorption spectra with different orientations of a polarizer. The LD spectrum was determined by subtracting two spectra recorded with perpendicular orientations. Although we cannot be sure that this method leads to some deformation of the LD spectrum, we note that in general the latter method can lead to large baseline problems. We take the fact that our reduced LD spectrum in the entire Q_y region and in most of the carotenoid region is more or less constant as good evidence for the fact that baseline problems

are virtually absent in our experiments and this is supported by our previous quantitative studies as mentioned above.

We now want to compare the obtained value for α_{car} with the results of the MO calculations that were presented by Dolan et al.[15] These authors concluded that the angle between the long axis S and the transition dipole moment μ is 9.1°. The angles of these vectors with the symmetry axis of the LH2 complex were reported to be 50° and 46°, respectively. However, these values cannot be reconciled with Figure 7 in their article, in which the orientations of S (through the 5th and 26th carbon atoms of the carotenoid) and μ (through the 11th and 23rd carbon) are given with respect to the rhodopin glucoside molecule. The corresponding angles with respect to the symmetry axis are 31.8° and 38.0°. It is not entirely evident how the angles with respect to the symmetry axis that were reported by Dolan et al.[15] were calculated, but the discrepancy must be traced back to the transformation procedure, where Euler matrix multiplication was used to obtain the reported values. However, it should be noted that this procedure is in fact redundant, and it suffices to directly determine the angle between the vectors and the symmetry axis. Indeed, with our measurements we cannot estimate directly the angle of the transition dipole moment with respect to the carotenoid molecule, but the calculation of its angle with respect to the symmetry axis is adequate.

In conclusion, we have been able to accurately determine the angle α_{car} between the transition dipole moment of the strong absorption band around 500 nm of rhodopin glucoside and the (C_9-) symmetry axis of the LH2 complex. This value is 39.5° ± 1.5° and is significantly different from the angle for the long axis of the carotenoid, S, which is 31.8°. This agrees nicely with the results of the MO calculations of Dolan et al.,[15] who concluded that the transition dipole moment μ is parallel to a line through the 11th and 23rd carbon atoms in the rhodopin glucoside molecule in LH2, which makes an angle of 38° with respect to the symmetry axis, whereas the angle between S and μ is 9.1°. Moreover, the angle is very similar to the value of 39.5° that was obtained for the carotenoid in LH2 from *Rb. sphaeroides*.[9]

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