THE STATE OF CHLOROPHYLL AND CAROTENOID IN VIVO II - A LINEAR DICHROISM STUDY OF PIGMENT ORIENTATION IN PHOTOSYNTHETIC BACTERIA

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SUMMARY : Similar linear dichroism (LD) spectra have been obtained for oriented samples prepared from photosynthetic bacteria containing either parallel lamellae (Rhodopseudomonas palustris) or spherical chromatophores (Rhodopseudomonas spheroides) in the spectral region from 300 to 1,000 nm. Our data indicate that the far-red Q_Y transition moment of the bacteriochlorophyll (BChl) molecules is oriented nearly parallel to the plane of the membrane, and that the Q_X transition moment (590 nm) is tilted out of the membrane plane at an angle greater than 45°. The transition moment of the carotenoid molecules is shown to point out of the membrane plane. The LD spectrum of the R-26 mutant of R. spheroides allows the assign ment of the polarization direction of the main BChl transitions in the Soret region.

New information concerning the orientation of pigments in photosynthetic membranes has been obtained recently through polarized light spectroscopy using oriented samples (1-5). Applying a sensitive technique of LD measurement to spinach chloroplasts, oriented by different methods, a high degree of orientation with respect to the plane of the membrane of both the pigments and the structural proteins has been reported in the spectral region from 180 to 800 nm (1 , 2). Geacintov et al. (3 , 4) used fluorescence polarization and LD measurements in the red region to show that intact algae and chloroplasts can be oriented by a magnetic field. Using R. palustis, Morita and Miyasaki (5) measured the LD for intact cells oriented by flow and chromatophores oriented by air-drying in the spectral range from 550 to 1,000 nm. The orientation they found for $BChl_a$ is similar to the orientation we reported for Chl_{680} in spinach chloroplasts (1).

The study reported here is aimed at comparing the LD spectra of bacteria containing spherical chromatophores and those containing lamellar chromatophores as well as resolving the LD signals in the carotenoid absorbing region and the Soret band of BChl.

MATERIAL AND METHODS

Three Rhodopseudomonas types have been studied : R. palustris, R. spheroides Y (wild-type), and R. spheroides R-26 (carotenoidless mutant). The bacteria were grown anaerobically under illumination using Hutner-modified medium. The chromatophores were obtained either by French press or by ultrasonic treatment followed by differential centrifugation [+]. In order to orient intact cells we used a 10.5 kG magnetic field. The spreading technique was used to orient both intact cells and chromatophores. Chromatophores were also oriented by air-drying on thin glass plates. Details concerning the techniques used to orient the photosynthetic material as well as the LD measurement technique and the analysis of the polarization data have been reported previously (1 , 2).

RESULTS

Intact cells : Large LD signals were detected when R. palustris cells were oriented by a magnetic field or by the spreading technique ; in contrast, no LD signals were observed with R. spheroides cells under the same conditions. The magnetic field (10.5 kG) was not great enough to saturate the orientation of R. palustris. In order to prevent phototactic motion we used a high viscosity medium (Ficoll 20 %).

Chromatophores : Large LD signals were observed using chromatophores isolated from each of the three organisms used in this study when they were oriented by spreading or air-drying. On the other hand, no LD signals were observed when the chromatophores were submitted to the magnetic field.

It is noteworthy that, whichever orientation technique is used, the LD spectra obtained are always identical in shape for each type of bacteria we used ; only the overall magnitude of the signals differ. The highest degree of orientation was achieved by orienting chromatophores by the air-drying technique. The LD spectra (solid lines) together with the corresponding absorption spectra (dashed lines) are presented in figures 1-3 . Dichroism measurements at several wavelengths are given in Table I for chromatophores oriented by the air-drying technique.

DISCUSSION

We were unable to orient isolated chromatophores using the 10.5 kG magnetic field. This fact indicates that the in vivo orientation of the BChl molecules relative

+ The intact cells and chromatophores were generously given by Mrs Clément-Métral and Reiss-Husson (Laboratoire de Photosynthèse, C.N.R.S., Gif-sur-Yvette).

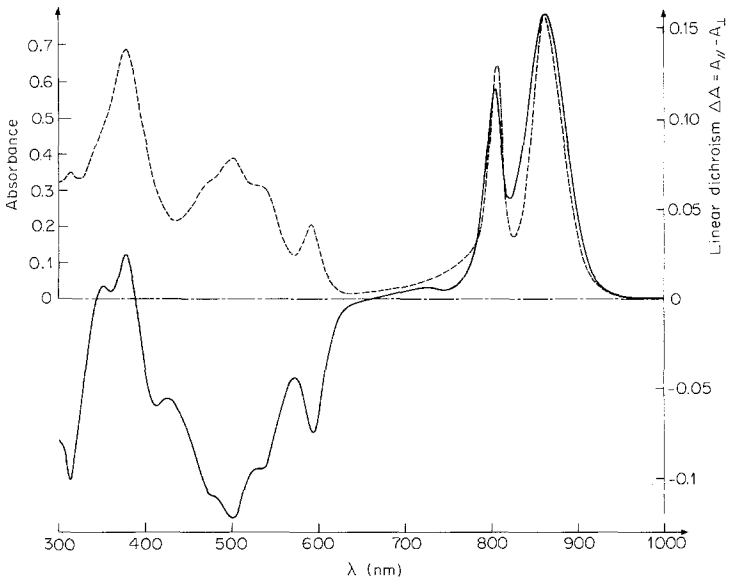

Figure 1 Absorption (- - -) and linear dichroism (——) spectra of intact cells of Rhodopseudomonas palustris. Orientation by the magnetic field.

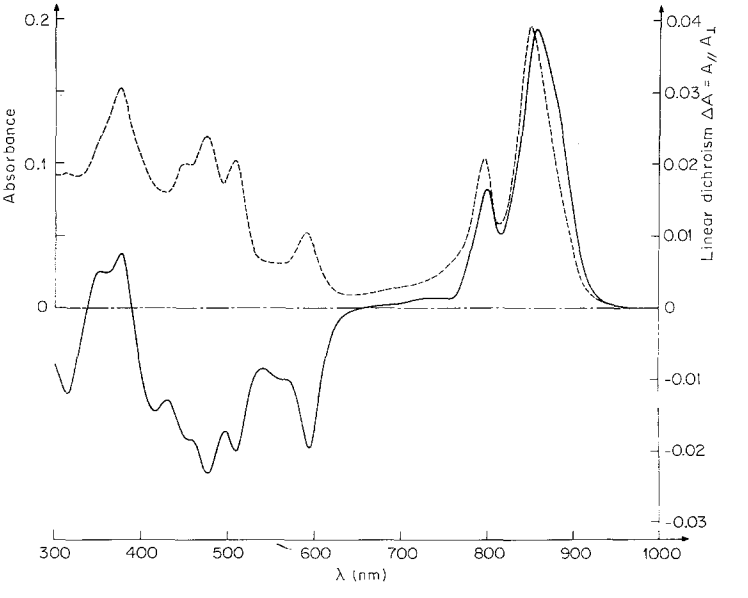

Figure 2 Absorption (- - -) and linear dichroism (——) spectra of chromatophores of Rhodopseudomonas spheroides Y. Orientation by air-drying.

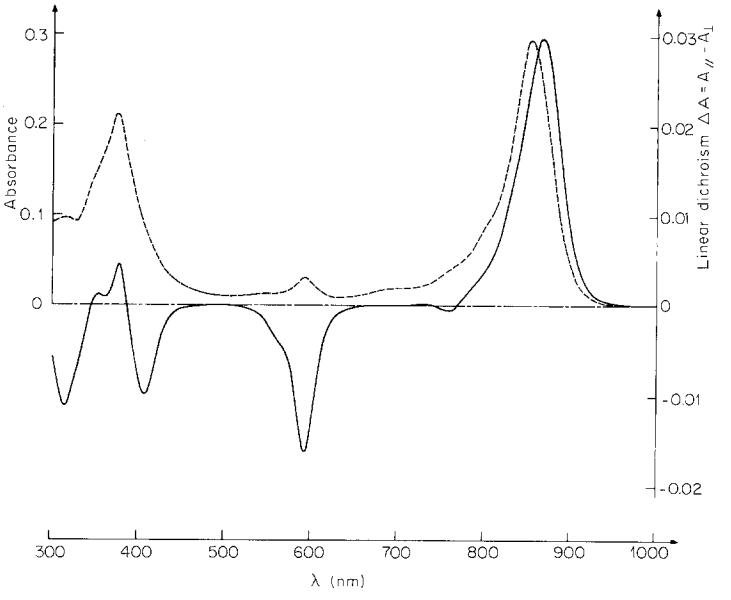

Figure 3 Absorption (- - -) and linear dichroism (——) spectra of chromatophores of Rhodopseudomonas spheroides R-26. Orientation by spreading.

to the plane of the membrane is not noticeably effected by the magnetic field. The absence of LD signals using intact cells of R. spheroides in the magnetic field is most likely a consequence of the isotropic distribution of the nearly spherical chromatophores within these cells (6). On the other hand, intact bacteria exhibiting a cylindrical symmetry of their lamellae, such as R. palustris, can be oriented by this technique. This magneto-induced orientation must be related to an anisotropy in the diamagnetic susceptibility of the membranes (4) and to the cooperative effects produced by the regular arrangement of these membranes in intact cells. Using the magnetic field we have also been able to orient the bacteria Rhodopseudomonas viridis which contains stacked parallel membranes (6). In this case we have found (unpublished) the $BChl_b$ to be oriented similar to the $BChl_a$ in R. palustris described in this report.

Applying the spreading technique to intact cells we found that only those organisms exhibiting an anisotropy in the distribution of their lamellae with respect to the major axis of the cell gave rise to an LD signal.

Using R. palustris, a comparison of the sign of the LD signal for intact cells oriented by the magnetic field with the sign of the LD signal for chromatophores oriented by air-drying, leads us to conclude that in the magnetic field the membranes tend to lie in a manner such that the projection of their surface perpendicular to the

TABLE I

NUMERICAL VALUE OF DICHROISM MEASUREMENTS USING AIR-DRIED SAMPLES OF R. PALUSTRIS AND R. SPHEROIDES (Y AND R-26)

λ nm	Organism	$\frac{\Delta A}{A}$	$S = \frac{1-3\cos^2\phi}{2}$	ϕ
860	R. palustris	0.23	0.36	72°
	R. spheroides Y	0.20	0.30	69°
	R. spheroides R-26	0.10	0.13	60°
800	R. palustris	0.21	0.32	70°
	R. spheroides Y	0.16	0.22	65°
590	R. palustris	- 0.42	- 0.33	42°
	R. spheroides Y	- 0.37	- 030	43°
	R. spheroides R-26	- 0.51	- 0.37	40°
500	R. palustris	- 0.36	- 0.29	43°
475	R. spheroides Y	- 0.19	- 0.18	48°

$\Delta A/A$ depends upon the experimental arrangement (2). In this case A was measured with the sample perpendicular to the light beam and $\Delta A = A_{//} - A_{\perp}$ was recorded with the sample tilted at an angle of 30° with respect to the measuring beam. S is a parameter (calculated from $\Delta A/A$) that is used to compare different oriented samples. Assuming a uniform distribution of the angle ϕ between the transition moments and the normal at the lamellar plane, this angle can be calculated using the equation $S = (1 - 3\cos^2\phi) / 2$. (Details concerning these calculations are given in ref. 2).

magnetic field is a maximum. Similar results have been described using spinach chloroplasts (2,3), algae (3) and several different photosynthetic bacteria (Geacintov, personnal communication).

In an earlier paper (2) we discussed the effect of various possible artifacts on LD measurement ; textural dichroism, if present, would give rise to an LD signal of the same shape as the absorption spectrum and both polarized light reflection and polarized light scattering would distort the LD spectrum by introducing dispersive

terms that would cause the LD extrema to shift. The observation that most of the absorption and LD extrema occur at the same wavelength and that the LD spectra clearly show positive and negative bands indicate that the above mentioned artifacts can be neglected and provide strong support to the interpretation of these spectra (Fig. 1-3) in terms of pigment orientation.

Our data, using R. palustris, are in good agreement with the report of Morita and Miyasaki (5) concerning the sign of the LD for the 860, 800 and 590 nm bands, though a sharper resolution of the spectra is achieved in the present study (Fig. 1). Orientation of the pigments is not restricted to bacteria exhibiting a regular arrangement of the membranes since similar spectra are obtained using R. spheroides Y chromatophores (Fig. 2).

The Y-transitions around 860 and 800 nm give positive LD signals that are interpreted by assuming that the Y-direction of the tetrapyrrolic ring makes a small angle with respect to the plane of the membrane. There are experimental as well as theoretical reasons why the S value (Table I) calculated is smaller than the actual value (2). Hence, for R. palustris, it can be calculated (Table I) that the angle $(\frac{\pi}{2} - \phi)$ between the Y-transition direction at 860 nm and the plane of the membrane cannot be greater than 18°. The 860 nm LD peaks do not always match exactly the corresponding absorption peaks, demonstrating the composite nature of these bands. The X-transition at 590 nm gives rise to a large negative LD signal indicating that the X-direction is pointing out of the membrane plane at an angle $(\frac{\pi}{2} - \phi)$ not smaller than 47° to 50°.

Since there is no absorption by carotenoid molecules in the R-26 mutant of R. spheroides (7), the complex set of bands in the region from 300 to 500 nm (Fig. 3) is primarily due to the Soret band of the $BChl_a$ molecules. Assuming that the far-red region corresponds to a Q_Y transition and that the orange transition corresponds to a Q_X transition, it can be concluded from our data that the first transition (400 nm) of the Soret band is polarized in the X-direction, the transition at the maximum (375 nm) is plarized in the Y-direction and the transition around 310 nm is polarized in the X-direction. Very similar spectra have been obtained using Rhodospirillum rubrum G-9 (unpublished).

For R. spheroides Y and R. palustris, a three-banded LD signal is observed in the carotenoid absorbing region (Fig. 1-2). The magnitude of this negative signal leads us to conclude that carotenoid molecules are pointing out of the lamellar plane at an angle near 45°. This result is different from what has been observed for spinach chloro-

plasts (1) and chlorella (4) where more carotenoid molecules are found parallel to the membrane plane than perpendicular to it. This could be explained by differences in the structure and/or in the chemical environnement of the carotenoid molecules involved.

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