ORIENTATION OF CHLOROPHYLL IN VIVO.

STUDIES WITH MAGNETIC FIELD ORIENTED CHLORELLA

J. F. Becker, N. E. Geacintov, F. Van Nostrand* and R. Van Metter
Radiation and Solid State Laboratory and Chemistry Department
New York University, New York, New York 10003.

Received January 20,1973

SUMMARY

The orientation of whole cells of Chlorella pyrenoidosa in aqueous suspensions is due to the anisotropy in the diamagnetic susceptibility of oriented chlorophyll molecules. The lipid molecules tend to align with their long axes parallel to the magnetic field and the "red" Q_y transition moment of chlorophyll perpendicular to the field, thus in the plane of the lamellae. The plane of the porphyrin rings tends to be parallel to the magnetic field because of the large diamagnetic susceptibility perpendicular to the ring. It is concluded that the porphyrin ring planes tilt away from the lamellar plane by an angle greater than 45°. The short wavelength spectroscopic forms of chlorophyll are unoriented.

INTRODUCTION

It has been shown recently that whole cells of Chlorella, Scenedesmus and spinach chloroplasts in aqueous suspension can be oriented in magnetic fields of ten kilogauss or more (1). The suspensions of these magneto-oriented particles exhibit anisotropies in the fluorescence, absorption, and scattering of incident light. The study of these phenomena yields information about the orientation of pigments in vivo (1). In this communication it is shown that oriented forms of chlorophyll are most probably responsible for the observed magnetic phenomena, rather than the lipid molecules. Studies of the wavelength dependence of the fluorescence polarization indicate that the bulk of the chlorophyll a is highly oriented, whereas the short wavelength spectroscopic forms of chlorophyll (2, 3) are either unoriented or possess a low degree of orientation.

METHODS AND RESULTS

In order to demonstrate the orientation of the lipids, Chlorella pyrenoidosa cells (Indiana University, Culture Collection No. 395) were stained with rhodamine B. This dye is known to be preferentially solubilized by the lipids (4-6). The cells were

*Current address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

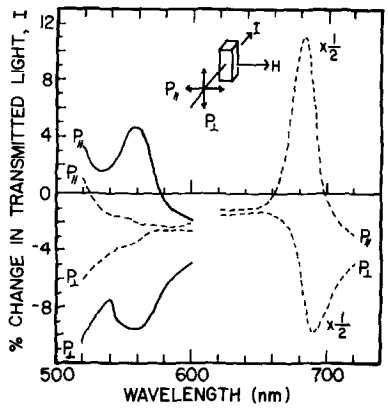

Fig. 1.

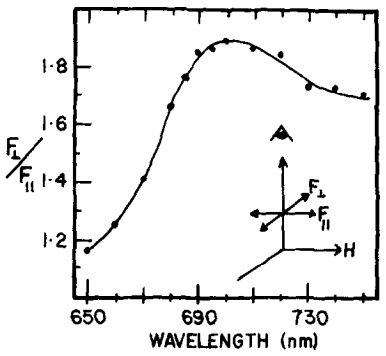

Fig. 2.

Fig. 1: Changes in transmittance of Chlorella pyrenoidosa cells stained with Rhodamine B (———), and cells from which rhodamine has been removed by washing (------). Magnetic field H = 13KG, 25°C, cell density $10^8 cm^{-3}$. $P_{||}$ and $P_{\perp}$ orientations of polarizers with respect to H. Dichroism for $\lambda > 600nm$ is unaffected by rhodamine.

Fig. 2: Fluorescence polarization ratio as a function of wavelength of a Chlorella suspension, extrapolated to zero particle density. Fluorescence viewed in a direction perpendicular to magnetic field H. $F_{\perp}$ and $F_{||}$ are the intensities with the analyzer oriented $\perp$ and $||$ to H respectively.

---

placed in fresh growth medium solution (1), which was saturated with rhodamine B, for 20 hours in the dark. The suspensions were then centrifuged, washed four times with fresh medium and finally resuspended in rhodamine-free medium. The dichroism induced by a magnetic field was determined within one half hour. The rhodamine B diffused out of the cells within 10-20 hours if the Chlorella cells were allowed to remain in rhodamine-free medium. The changes in the transmittance of stained Chlorella suspensions in a magnetic field are shown in fig. 1; (the apparatus for this type of measurement has been described elsewhere (1)). Depending on the orientation of the polarizer, maxima or minima were observed in the region of the rhodamine absorption maximum (560nm). The dichroism at wavelengths greater than 600 nm is due to chlorophyll and does not depend on the presence or absence of rhodamine.

The ratio of intensities of fluorescence polarized perpendicular and parallel to the magnetic field as a function of the wavelength of fluorescence is shown in fig. 2. This polarization ratio ($F_{\perp}/F_{||}$) depends on the cell density because of reabsorption

and selective scattering effects; the values shown in fig. 2 have been extrapolated to "zero particle density" (7) and thus represent the fluorescence polarization ratios per single Chlorella cell.

DISCUSSION

From the works of Menke (4) and Goedheer (5, 6), it is known that the rhodamine B is oriented within the lipids in such a way that the absorption is greater with the E-vector vibrating parallel to the lamellar plane, than with the E-vector perpendicular to this plane. In the magnetic field, the absorption of light by the stained cell suspensions is stronger with the E-vector oriented perpendicular to the field. We therefore conclude that the planes of the lamellae tend to be oriented perpendicular to the magnetic field. This conclusion is in accord with a comparison of our own observations with those of Olson et al (8): Viewing single chloroplast lamellae on edge through a microscope, they find that the fluorescence is most intense when viewed through an analyzer oriented parallel to the lamellar plane. In our magneto-oriented cell suspensions the fluorescence is most intense in a plane perpendicular to the field, thus the lamellar planes must be perpendicular to the field, and the "red" Q_y transition moment lies either close to or in the plane of the lamellae.

We conclude further that the lipid molecules tend to be lined up with their long axes parallel to the field. Lonsdale (9) has shown that the absolute value of the diamagnetic susceptibility $|\chi_{axial}|$ is greater along the long axis of stearic acid than perpendicular to this axis $|\chi_{radial}|$. The lipid molecules should display similar anisotropies in χ. Physical considerations dictate that a cylindrical, diamagnetically anisotropic body with $|\chi_{axial}| > |\chi_{radial}|$ should line up with the direction of $|\chi_{axial}|$ perpendicular to the magnetic field (10). If the lipid molecules were responsible for the observed magneto-orientation effects, the lipid molecules would tend to orient with their long axes perpendicular to the field, and the planes of the lamellae therefore parallel to the field, which is contrary to our observations. The chlorophyll molecules therefore, represent the most likely oriented cell components which have a sufficiently high anisotropy in χ and a sufficient degree of orientation to cause the orientation of the entire cell (1). For a planar aromatic π electron system, $|\chi|$

normal to the plane is much greater than within the plane (11). Thus the planes of the porphyrin rings will tend to be parallel to the magnetic field and perpendicular to the lamellar plane. However, in a given lamellar system, the planes of the porphyrin rings may not all be parallel to each other. Therefore, it is possible to conclude only that on the average, the porphyrin planes must be inclined at an angle less than 45° to the magnetic field, and that their planes are tangent to the surface of a cone whose axis coincides with the direction of the magnetic field. Since the lamellar planes are perpendicular to the field, this orientation of chlorophyll would make the average angle between the porphyrin and lamellar planes greater than 45°. This conclusion is different from the one given by us previously (1). However, it is in accord with recent linear dichroism studies of spinach chloroplasts by an ORD technique (12, 13).

The direction of orientation of a magnetically antisotropic sample depends on (1):

$$\sum_i \Delta\chi_i V_i \approx \sum_i \Delta\chi_i N_i$$

where $\Delta\chi_i$ is the anisotropy in the diamagnetic susceptibility, V_i the effective volume and N_i the corresponding number of molecules of the i-th species. If N_ℓ and N_c, $\Delta\chi_\ell$ and $\Delta\chi_c$ are the relative numbers of molecules and anisotropies of lipids and chlorophyll molecules respectively, our experimental results indicate that $|\Delta\chi_c| N_c > |\Delta\chi_\ell| N_\ell$. The chemical composition of chloroplast lamellae extracted from spinach has been determined by Park et al (14, 15). Taking into account the unidentified lipids, a quantasome has approximately 400 lipids, 230 chlorophylls and 48 carotenoids (15). $\Delta\chi_c$ can be approximated from the diamagnetic anisotropy of phtalocyanine (11) and is -530×10^{-6}, or about twenty times greater than $\Delta\chi_\ell = -25\times10^{-6}$. The latter is assumed to be similar to $\Delta\chi$ of stearic acid (10). The ratio $\Delta\chi_c N_c / \Delta\chi_\ell N_\ell \approx 6$, and we expect the chlorophyll to dominate the diamagnetic anisotropy of the chloroplast.

Dichroism and light scattering anisotropies indicate that the carotenoids are oriented with their long axis within the planes of the lamellae (7). Since the diamagnetic anisotropy of a carotenoid molecule is similar to that of stearic acid, the carotenoids would tend to line up with their long axes perpendicular to the field. Thus, the $|\chi|$ due to the carotenoids is maximum within the lamellar plane. The porphyrin rings also give rise to a larger $|\chi|$ within this plane than perpendicular to it, and

therefore the carotenoids add to the total magnetic susceptibility anisotropy of the lamellar system generated by the chlorophylls.

Breton et al (12, 13) have recently found that some structural proteins in intact thylakoid membranes are also oriented. Since the α-helical regions are parallel to the membrane plane and the planes of the tryptophan residues are perpendicular (13), their combined effect would also tend to orient the lamellae perpendicular to the magnetic field. Since relative amounts of oriented proteins is not known, quantitative estimates of their effect are not possible.

The fluorescence polarization ratio $F_{\perp}/F_{\parallel}$ for the particular Chlorella culture shown in fig. 2 is the highest we have ever observed. This ratio has a peak value of 1.89 in the 690-705 nm region. With other cultures and strains we have observed peak values as low as ~1.20, with the average being in the range of ~1.30 - 1.60. There is a substantial decrease in the polarization ratio at wavelengths below the 685 nm fluorescence maximum. Similar results have been obtained with spinach chloroplasts and Scenedesmus obliquus. The data in fig. 2 indicates that the different spectroscopic forms of chlorophyll in vivo have different degrees of orientation. The bulk chlorophyll forms Chl a 677 and Chl a 683 (2), as well as the longer wavelength forms appear to be highly oriented, while the short wavelength forms are either unoriented or possess a very low degree of orientation. These results are also in agreement with the linear dichroism studies of Breton et al (12, 13) on spinach chloroplasts. It may well be that the unoriented short wavelength spectroscopic forms are not closely associated with the highly oriented bulk pigments, and thus their absorption maxima are not as far red shifted as that of the oriented bulk pigments, although the association between the two forms may be close enough to ensure efficient energy transfer.

Olson, Jennings and Butler have measured the fluorescence polarization ratio (perpendicular and parallel to lamellar plane) of single chloroplasts of Euglena and found a maximum in the 710 - 730 nm region (8). The fluorescence maximum in Euglena, however, occurs at 705 nm at room temperature (16), whereas the maximum in Chlorella is at 685 nm. Quantitative comparisons between our results for Chlorella and Olson et al's for Euglena are therefore difficult to make.

ACKNOWLEDGEMENTS

This work was supported in part by the Atomic Energy Commission and National Science Foundation, and in part by equipment grants to one of us (N.E.G.) by the Research Corporation and by a National Institute of Health Grant to New York University. We acknowledge useful discussions with C.E. Swenberg, R.S. Knox, M. Pope and J. Breton.

REFERENCES

1. N.E. Geacintov, F. Van Nostrand, J.F. Becker and J.B. Tinkel, Biochim. Biophys. Acta. 267, 65 (1972)
2. C.S. French, Proc. Nat. Acad. Sci. U.S.A. 68, 2893 (1971)
3. C.N. Cederstrand, E. Rabinowitch and Govindjee, Biochim. Biophys. Acta. 126, 1 (1966)
4. W. Menke, Biol. Zentralblatt 63, 326 (1943)
5. J.C. Goedheer, Biochim. Biophys. Acta. 16, 471 (1955)
6. J.C. Goedheer, Thesis, Utrecht (1957)
7. F. Van Nostrand, Thesis, New York University (1972)
8. R.A. Olson, W.H. Jennings and W.L. Butler, Biochim. Biophys. Acta. 88 331 (1964)
9. K. Lonsdale, Proc. Roy. Soc. London A171, 541 (1939)
10. F.T. Hong, D. Mauzerall and A. Mauro, Proc. Nat. Acad. Sci. U.S.A. 68 1283 (1971)
11. K. Lonsdale, Proc. Roy. Soc. London A159, 149 (1937)
12. J. Breton and E. Roux, Biochim.. Biophys. Res. Comm. 45, 557 (1971)
13. J. Breton, M. Michel-Villaz and G. Paillotin, Biochim. Biophys. Acta. in press
14. H.K. Lichtenthaler and R.B. Park, Nature, 198, 1070 (1963)
15. R.B. Park and J. Biggins, Science, 144, 1009 (1964)
16. J.C. Goedheer, Biochim. Biophys. Acta. 88, 304 (1964)