

POLARIZED PHOTOACOUSTIC, ABSORPTION AND FLUORESCENCE SPECTRA  
OF CHLOROPLASTS AND THYLAKOIDS ORIENTED IN POLYVINYL ALCOHOL FILMS

D. FRACKOWIAK\*, L. LORRAIN\*, D. WROBEL\*\* AND R.M. LEBLANC\*

\*Centre de recherche en photobiophysique  
Université du Québec à Trois-Rivières  
Trois-Rivières, Québec, Canada, G9A 5H7\*\*Institute of Physics  
Poznan Technical University  
60-965 Poznan, Poland

Received October 7, 1984

---

**SUMMARY:** The polarized photoacoustic, absorption and fluorescence spectra of chloroplasts and thylakoids in unstretched and stretched polyvinyl alcohol films were measured. The intensity ratios of fluorescence bands at 674 nm, 700 nm, 730 nm and 750 nm, and the polarized fluorescence excitation spectra are strongly dependent on light polarization and film stretching. In stretched films, thylakoids exhibit predominantly 674 nm emission. The ratio of photoacoustic signal to absorption is different for light polarized parallel and perpendicular to film stretching. This difference is large in the region of chlorophyll *a* and carotenoids absorption in which the fluorescence excitation spectra are also strongly dependent on light polarization and film stretching. The observed spectral changes are explained by reorientation of pigment molecules influencing the yield of excitation transfer between different pigments. © 1985

Academic Press, Inc.

---

Light-harvesting pigments of chloroplast lamellae are functionally organized to give efficient transfer of absorbed excitation energy to the reaction centers. Various pigments and their spectroscopically different "forms" (created by the interaction of molecules with environment) are differently oriented with respect to thylakoid membrane (1). Various forms of pigments have strongly overlapping spectra but different orientations, therefore it is easier to investigate them by polarized than by natural light spectroscopy. To draw conclusions concerning the orientation of pigment molecules with respect to membrane plane, one has to know the localization of transition moments (TM) in the molecular frame. Such investigations for various chlorophylls have already been made (1, 3). The

---

**ABBREVIATIONS:** PAS-photoacoustic spectra; LD-linear dichroism; V-vertical; H-horizontal; light polarization: n, natural, ll, parallel,  $\perp$ , perpendicular; F-fluorescence;  $\alpha$ -optical absorption coefficient; LHC-light harvesting complexes; PS-photosystem.

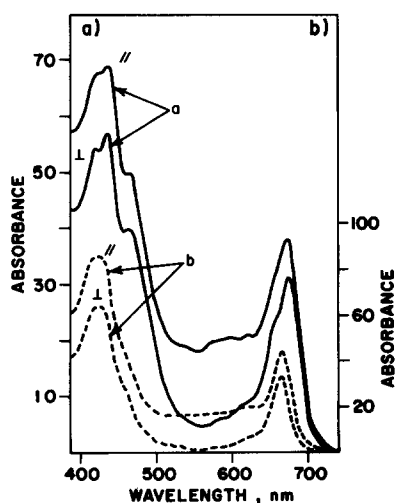
experimental approaches to study the polarized absorption property of chloroplasts are numerous and quite different, e.g., microspectrophotometry (4), orientation by air-drying, spreading (5) or gel squeezing (6). However, measurements of polarized fluorescence of aligned chloroplasts are much more scarce (1, 6, 7) whereas no polarized photoacoustic spectrum is reported yet. It is the purpose of the present study to report the most important photophysical properties of oriented chloroplasts and thylakoids using polarized light spectroscopy.

### MATERIALS AND METHODS

Chloroplasts and thylakoids are isolated from pea leaves (8) and introduced into polyvinyl alcohol (PVA) film which was stretched as described elsewhere (9). Stretching causes axial orientation of sample, but thylakoids exhibit also some orientation in the plane of PVA film. Methods of measuring polarized photoacoustic, absorption and fluorescence spectra have been described previously (2, 10).

### RESULTS AND DISCUSSION

Fig. 1 shows polarized absorption spectra of chloroplasts and thylakoids in stretched PVA films. Linear dichroism ( $LD = \frac{A_{||} - A_{\perp}}{A_{||} + 2A_{\perp}}$ ) of chloroplasts at 434 nm and 674 nm is equal to 0,067 and 0,061, respectively, whereas for thylakoids at both these wavelengths, it is equal to 0,101. It is known (11), that red band of chlorophyll a (chl a) is related to  $Q_y$  (0,0) TM which is located in porphyrin ring along a line connecting I and III pyroles, whereas Soret band is a superposition of several vibronic transitions with various (X or Y) polarization.



**Fig. 1** Polarized absorption spectra of chloroplasts (a) and thylakoids (b) in stretched PVA (polarization ||,  $\perp$  shown in graphs).

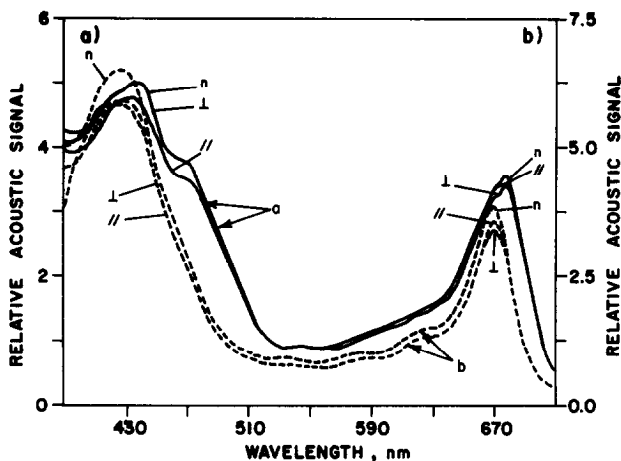


Fig. 2 Photoacoustic spectra of chloroplasts (a) and thylakoids (b) in stretched PVA. (n, natural; ||, parallel and  $\perp$ , perpendicular).

For chl a oriented along the  $Q_y$  direction, LD in red band is higher than in Soret band (2). Similar values of LD at both red and Soret bands of chloroplasts and thylakoids could be due either to the orientation of their chl a around axes located between Y and X directions or to the orientation of TM of red or Soret band out of the membrane plane. It is known that X rather than Y direction of chl a ring is oriented out of the membrane plane (1), therefore the second hypothesis seems to be plausible.

Fig. 2 shows polarized photoacoustic spectra (PAS) of stretched samples, and table I gives PAS amplitude divided by the optical absorption coefficient  $(\frac{PAS}{\alpha})$  for natural  $(\frac{PAS}{\alpha})_n$  or polarized  $(\frac{PAS}{\alpha})_{||}$ ;  $(\frac{PAS}{\alpha})_{\perp}$  light. The increase of  $(\frac{PAS}{\alpha})_n$  values with stretching is related with the increase in thermal conductivity of film. This effect was investigated and discussed previously (10), but the difference between  $(\frac{PAS}{\alpha})_{||}$  and  $(\frac{PAS}{\alpha})_{\perp}$  observed for the same film stretching are related to various yield of thermal deactivation of excitation of molecules oriented parallel and perpendicular to the stretching direction. For unstretched films,  $(\frac{PAS}{\alpha})_{||} = (\frac{PAS}{\alpha})_{\perp}$ , but as a result of stretching,  $(\frac{PAS}{\alpha})_{\perp} > (\frac{PAS}{\alpha})_{||}$  is observed. Difference between  $(\frac{PAS}{\alpha})_{\perp}$  and  $(\frac{PAS}{\alpha})_{||}$  is highest for chl b Soret band (464 nm) and in region of carotenoids absorption (500 nm). Higher value of  $\frac{PAS}{\alpha}$  for chl b than that for chl a Soret bands is explained by lower fluorescence yield of chl b. Lower  $(\frac{PAS}{\alpha})_{||}$  than  $(\frac{PAS}{\alpha})_{\perp}$  can be due to the increase of the excitation energy

TABLE I

| Sample<br>(elongation)<br>( $\frac{\Delta L}{L} \times 100$ ) | Light<br>polarization | Ratio of photoacoustic signal to optical absorption<br>coefficient at various $\lambda$ (nm) |      |      |      |                       |       |       |       |
|---|-----------------------|--|------|------|------|-----------------------|-------|-------|-------|
|   |                       | 410  | 414  | 420  | 430  | $\lambda$ (nm)<br>464 | 500   | 670   | 675   |
| Chloroplasts<br>0%  | n                     | 5,87   | 5,90 | 6,44 | 6,50 | 7,03                  | 7,66  | 7,95  | 7,99  |
| Chloroplasts<br>300%  | n                     | 7,13   | 7,08 | 8,10 | 8,23 | 8,89                  | 8,98  | 10,07 | 10,26 |
|   |                       | 6,45   | 6,35 | 7,14 | 7,71 | 7,42                  | 7,38  | 9,60  | 9,96  |
|   | ⊥                     | 8,70   | 8,49 | 9,31 | 9,38 | 10,15                 | 11,37 | 10,90 | 11,34 |
| Thylakoids<br>0%  | n                     | 4,38   | 4,40 | 5,06 | 5,12 | 5,64                  | 5,66  | 6,03  | 6,47  |
| Thylakoids<br>300%  | n                     | 7,14   | 7,10 | 8,37 | 9,06 | 10,90                 | 9,08  | 10,21 | 13,03 |
|   |                       | 5,71   | 5,67 | 6,63 | 6,86 | 7,87                  | 5,74  | 8,36  | 11,02 |
|   | ⊥                     | 8,13   | 7,90 | 8,91 | 9,14 | 14,34                 | 18,83 | 10,26 | 14,77 |

transfer among parallel oriented molecules tunnelling excitation energy to some strongly fluorescent chromophores.

Some polarized components of fluorescence are shown in Fig. 3. Each spectrum is normalized with respect to highest peak intensity. Intensities of bands are given in Table II. The fluorescence spectrum of chloroplasts and thylakoids consists of four broad bands located at about 674 nm, 700 nm, 730 nm and 750 nm (F674, F700, F730, F750). In chloroplast spectra F674 and F700 bands are not resolved. Comparing half band-widths in Fig. 3 with those of individual form of chlorophylls (12), one can conclude that several forms of pigments contribute to each fluorescence band. The F674 depends on the emission from the following components: 1) F680 due to light harvesting complexes LHC absorbing at about 670 nm, 2) of chl b F665 (13, 14) and probably also of 3) fluorescence of chl a absorbing in 660 nm region which can be fluorescent in PVA film (15). F700 is related to the chlorophyll-protein antenna complexes (F685) and reaction center complexes (F695), both belonging to PS II (16). F730 and F750 are due to chl a from PS I absorbing at shorter and longer wavelength regions, respectively (13, 14). All spectra were obtained for an excitation at 420 nm, where X and Y polarized absorptions of chl a are overlapped, but Y polarization predominates (LD>0). The polarisers at the excitation and the emission are selecting absorbing

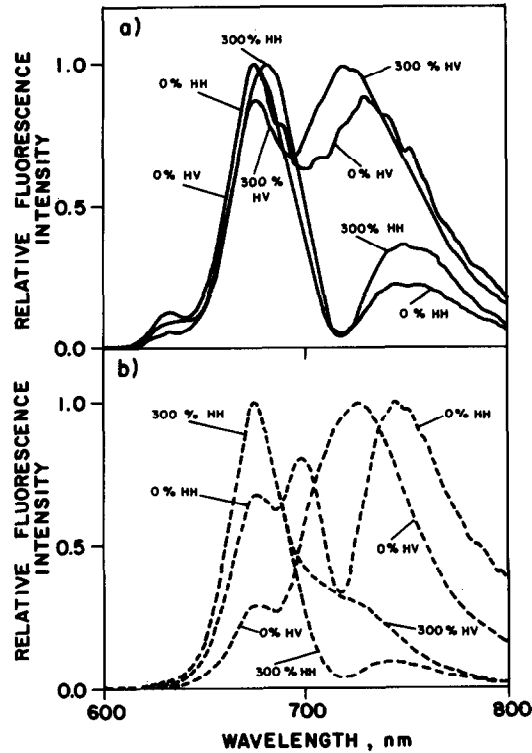


Fig. 3 Fluorescence spectra of chloroplasts (a) and thylakoids (b) in unstretched,0%,and stretched,300%,PVA. H-horizontal, V-vertical polarization of fluorescence (first index) and excitation (second index) light.

TABLE II  
INTENSITIES OF POLARIZED FLUORESCENCE COMPONENTS

| Sample            | Slit<br>(nm) | Polarization | Intensity (arbitrary units) |      |      |      |
|-------------------|--------------|--------------|-----------------------------|------|------|------|
|                   |              |              | $\lambda$ (nm)              |      |      |      |
|                   |              |              | 674                         | 700  | 730  | 750  |
| Chloroplasts 0%   | 2,0          | HH           | 2,65                        | 1,10 | 0,50 | 0,70 |
|                   | 2,0          | HV           | 0,96                        | 0,62 | 0,86 | 0,68 |
| Chloroplasts 300% | 2,5          | HH           | 2,28                        | 1,31 | 0,72 | 0,98 |
|                   | 2,5          | HV           | 1,36                        | 1,15 | 1,44 | 1,04 |
|                   | 2,5          | VV           | 1,15                        | 1,18 | 1,92 | 1,36 |
|                   | 2,5          | VH           | 1,59                        | 1,19 | 0,92 | 1,18 |
| Thylakoids 0%     | 2,0          | HH           | 1,25                        | 1,49 | 1,23 | 1,81 |
|                   | 2,0          | HV           | 0,84                        | 1,65 | 2,74 | 1,85 |
| Thylakoids 300%   | 1,5          | HH           | 19,92                       | 5,76 | 1,86 | 2,00 |
|                   | 1,5          | HV           | 13,80                       | 5,76 | 3,87 | 2,23 |
|                   | 1,5          | VV           | 11,26                       | 5,05 | 4,06 | 2,48 |
|                   | 1,5          | VH           | 17,70                       | 5,42 | 1,75 | 2,12 |

(sample axes position: Horizontal (H))  
(H: horizontal, V: vertical polarization, first notation concerns excitations, second,fluorescence)

and emitting molecules of given ( $\parallel$  or  $\perp$ ) mutual orientation of transition moments (TM). From Fig. 3 and Table II, it follows that light polarization and film stretching change strongly the relative intensities of fluorescence bands. In unstretched and in stretched chloroplasts and thylakoids samples, the ratio  $F_{HH}/F_{HV}$  (notation as in Fig. 3) is higher for F674 and F700 than for F730 and F750. It means that emission TM of molecules emitting F674 and F700 are forming smaller angles with absorption TM of chl a molecules absorbing in 420 nm region than TM of molecules responsible for F730 and F750 emission. F730 is polarized  $\perp$  to the direction of stretching ( $F_{HV} \gg F_{HH}$ ), but it is excited efficiently also by V polarized light (high  $F_{VV}$ ). F750 shows low polarization in all samples. In stretched thylakoid samples, the intensity of F674 increases dramatically. This band becomes much higher than all other bands. The stretching changes the orientation of some chromophores. The reorientation influences mutual excitation energy transfer between pigments. Consequently, the ratios of the band intensities including F730/F700, which is related with light intensity adaptation *in vivo* (17, 18), are changed.

Fig. 4 shows some fluorescence excitation spectra. The relative intensities of their maxima depend on the fluorescence band, the polarization of excitation and fluorescence beams, and the film stretching. All these features can be demonstrated for chl b Soret band and for carotenoids absorption (about 500 nm) because they do not overlap with other bands. F674 is excited strongly by carotenoids absorption in stretched chloroplasts and in unstretched and stretched thylakoids. Excitation is more efficient in  $F_{VH}$  than in  $F_{HH}$  components. Thylakoids are oriented in PVA plane, chloroplasts improve this orientation with stretching. The carotenoids have to be oriented in thylakoids plane, but rather parallel to X than to Y direction of main fraction of chl a absorbing in 420 nm region, because  $F_{VH} > F_{HH}$  is observed. F730 shows very efficient excitation of chl b absorption, especially in  $F_{VH}$  component of chloroplasts. It increases with film stretching. In thylakoids contribution from chl b to F730 are lower. It is known that Y polarized transitions of chl b are directed out of thylakoid plane (1), therefore for thylakoid which are well oriented in PVA film plane, the

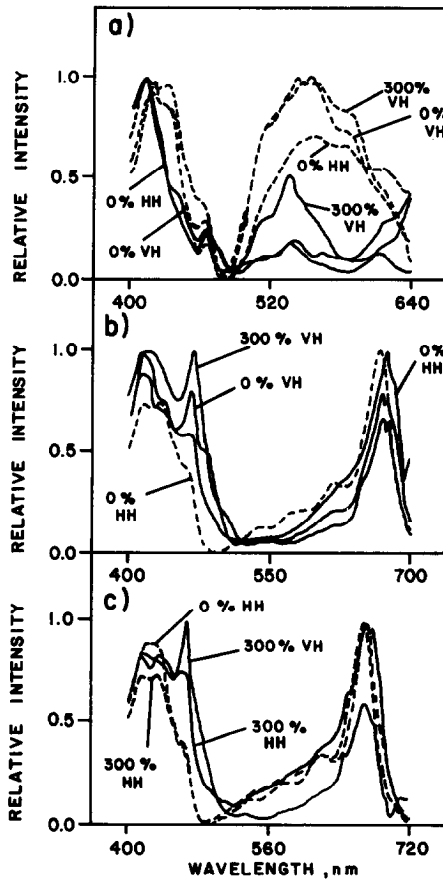


Fig. 4 Fluorescence excitation spectra of F674 nm (a) F730 nm (b) and F750 nm (c). Chloroplasts—continued, thylakoids—interrupted line; notation of polarization as in Fig. 3. Film stretching (%) is  $\frac{\Delta \lambda}{\lambda} \times 100$  shown in graphs.

contributions from chl b are lower than for chloroplasts. F750 has high contribution of chl b in  $F_{VH}$  component. F700 (not shown) is efficiently excited by both chl b and carotenoids absorptions. The stretching does not change the shape of excitation spectrum of F700 band. It is known that F681 and F698 which are overlapped in this emission region are polarized in thylakoid membrane plane (19).

Based on the photophysical properties that we have reported, it is possible to draw the following conclusions:

1) the changes in polarized PAS spectra caused by film stretching are accompanied by strong changes in the fluorescence and fluorescence excitation spectra;

2) mutual orientation of pigment, which can be changed by film stretching or selected by light polarization, has strong influence on excitation energy transfer among photosynthetic pigments and on their thermal deactivation of excitation.

#### ACKNOWLEDGEMENTS

This work was supported by the Natural Science and Engineering Research Council of Canada and by the Polish project RIII 13. 74.3 (for D.W.). One of us (D.F.) wishes to thank NSERC of Canada for an International Scientific Exchange Award to work in Photobiophysics Research Centre at Trois-Rivières.

#### REFERENCES

- 1) Breton, J. and Vermeglio, A. (1982) Photosynthesis: Energy conversion by Plants and Bacteria Vol. 1 (Govindjee Ed.) pp. 153-194 Academic Press, New York.
- 2) Frackowiak, D., Hotchandani, S. and Leblanc, R.M. (1983) Photobiochem. Photobiophys. 6, 339-350.
- 3) Bauman, D. and Wrobel, D. (1980) Biophys. Chem. 12, 83-91.
- 4) Goedheer, J.C. (1955) Biochim. Biophys. Acta 16, 471-476.
- 5) Breton, J. and Roux, E. (1971) Biochem. Biophys. Res. Commun. 45, 557-563.
- 6) Gonago, A.O., Gerab, G.Y. and Faludi-Daniel, A. (1983) Biochim. Biophys. Acta 783, 287-293.
- 7) Gacintov, N.E., Van Nostrand, F., Becker, J.F. and Tinkel, J.B. (1972) Biochim. Biophys. Acta 267, 65-79.
- 8) Burke, J.J., Ditto, C.L. and Arntznen, C.J. (1978) Arch. Biochem. Biophys. 187, 252-262.
- 9) Fiksinski, K. and Frackowiak, D. (1980) Spectroscopy Lett. 13, 873-899.
- 10) Frackowiak, D., Hotchandani, S., Szych, B. and Leblanc, R.M. Acta Phys. Polon. (submitted).
- 11) Petke, J.D., Maggiora, G.M., Shipman, L. and Christoffersen, R.E. (1979) Photochem. Photobiol. 30, 203-223.
- 12) Litvin, F.F. and Sineschekov, V.A. (1975) Bioenergetics of Photosynthesis (Govindjee Ed.) pp. 619-656. Academic Press, New York.
- 13) Papageorgiou, G. (1975) Bioenergetics of Photosynthesis (Govindjee Ed.) pp. 320-366. Academic Press, New York.
- 14) Govindjee and Govindjee, R. (1975) Bioenergetics of Photosynthesis (Govindjee Ed.) pp. 2-43. Academic Press, New York.
- 15) Frackowiak, D., Hotchandani, S., Fiksinski, K. and Leblanc, R.M. (1983) Photosynthetica 17, 456-459.
- 16) Nakatani, H.Y., Ke, B., Dolan, E. and Arntznen, C.J. (1984) Biochim. Biophys. Acta 765, 347-352.
- 17) Saito, K., Williams, W.P. and Bennet, J. (1984) Biochim. Biophys. Acta 724, 94-103.
- 18) Satoh, K. and Fork, D. (1983) Biochim. Biophys. Acta 722, 190-196.
- 19) Nabadryk, E., Andrianambinintsoa, S. and Breton, J. (1984) Biochim. Biophys. Acta 765, 380-387.