POLARIZATION OF THE 515 nm EFFECT ON CHLOROPLASTS ORIENTED BY A MAGNETIC FIELD.

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Summary : The polarization of the light-induced absorbance change known as the "515 nm effect" has been studied on spinach chloroplasts oriented by a 10 kG magnetic field H. The exciting flashes are unpolarized and the analyzing beam is polarized either // or ⊥ to H. The only influence of H is to orient the lamellae perpendicular to its direction. The kinetics of the transients are identical for both polarizations, but the magnitudes (ΔA) are different. The degree of polarization ($p = \Delta A_{//} - \Delta A_{\perp} / \Delta A_{//} + \Delta A_{\perp}$) is high : + 12.5 % at 515 nm, + 25% at 475 nm; it becomes negative in the region 482-502 nm. These results are discussed in relation to the intrinsic orientation of the pigments within the photosynthetic membranes and to the orientation of the pigments with respect to the hypothetical transient electric field.

A transient light-induced absorbance change occuring in chloroplasts suspensions, known as the "515 nm effect" or "field indicating absorption change" (1), has been interpreted as an electrochromism due to a light-induced electric field acting on the chloroplast pigments within a membrane behaving like a charged capacitor (2-4). Recent studies (5-8) have shown that these pigments are partly oriented with respect to the normal to the membrane plane.

We thought that it was possible to get more informations on the electrochromic effect, and about the orientation of the pigments within the membrane, from a study of the polarization of the 515 nm effect on chloroplast membranes oriented under physiological conditions. Our results indicate a large polarization of the 515 nm effect, with a complex wavelength dependence.

MATERIAL AND METHODS.

Biological material. Chloroplasts were prepared from spinach leaves by grinding with a Waring blendor (4°C, 10 s) in 0.4 M sucrose - 0.02 M sodium pyrophosphate pH 8.0. The homogenate was filtered through 16 layers of cheese-cloth and centrifuged at 2 000 g for 2 minutes. The pellet was rehomogenized in 0.4 M sucrose - 0.02 M Tris pH 8.0 - ficoll 6%.

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Measurement of absorbance changes. A square cuvette (8 x 8 mm) containing the chloroplast suspension (about 20 μg of chlorophyll per ml) was inserted in an horizontal magnetic field H (10 kG), as depicted in fig.1. The

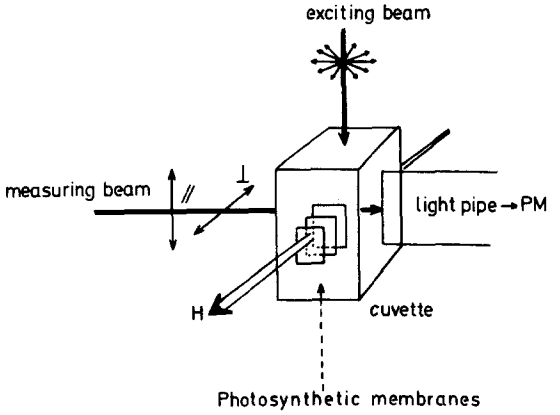

Figure 1. A schematic representation of the spatial position of the cuvette, of the exciting and measuring beams and of direction of the magnetic field H. The small rectangles represent the stacked photosynthetic lamellae.

magnetic field orients the lamellae perpendicular to its direction (6-8). The suspension was excited vertically, through a light guide, by unpolarized light from a xenon flash tube (10 μs) filtered by a Balzers (Calflex) interference filter and a Schott RG 630-3 mm filter. The excitation was saturating for the 515 nm effect. The measuring beam was polarized with a two-position polaroid polarizer HN 38, either parallel (for measurement of $\Delta A_{//}$) or perpendicular (for measurement of $\Delta A_{\perp}$) to the membrane plane. The intensity of the measuring beam ($\Delta\lambda$ = 2 nm) was 2,0 ergs.cm^{-2} s^{-1} at 515 nm: its effect on ΔA was found to be negligible. The light going through the cuvette was collected by a 20-cm light guide (10 x 20 mm, placed against the cuvette wall) and measured by a shielded photomultiplier operating at constant voltage and output. The intensity of the measuring beam was slightly varied for other wavelengths. The signal/noise ratio was improved by the use of a multichannel analyzer (further details are given in ref. 9).

In a typical experiment the suspension was allowed to stand for 5 min in the cuvette (thermostated at 3°C), with the magnetic field and the exciting flashes on (0.2 Hz); 20 signals were recorded for $\Delta A_{//}$, then 40 signals for $\Delta A_{\perp}$ and 20 signals for $\Delta A_{//}$ (or the reverse series). Measu-

rements at different wavelengths were alternated with measurements at 515 nm in order to check for long time changes of the sample. Any series of measurements indicating an evolution with time was discarded.

The orientation was checked by transferring the cuvette, the cuvette holder and the electromagnet to a spectropolarimeter that records the linear dichroism spectrum (5).

RESULTS.

Polarization of absorbance changes. Wavelength dependence. Typical curves of absorbance changes are presented in fig.2 for both polarizations (// and ⊥) of the measuring beam. At each wavelength (475 and 515 nm) the bottom trace represents the amplified difference between the signals obtained for the two polarizations. The decay is slower than the decay reported by Junge and Witt (2), a difference that can be primarly attributed to the lower temperature in our measurements. The rise-time is instrument-limited (about 100 ms) in fig. 2 ; in experiments performed with a shortertime response (5 ms) the kinetics of decay for the three curves remain identical. In a large series of experiments, the average degree of polari-

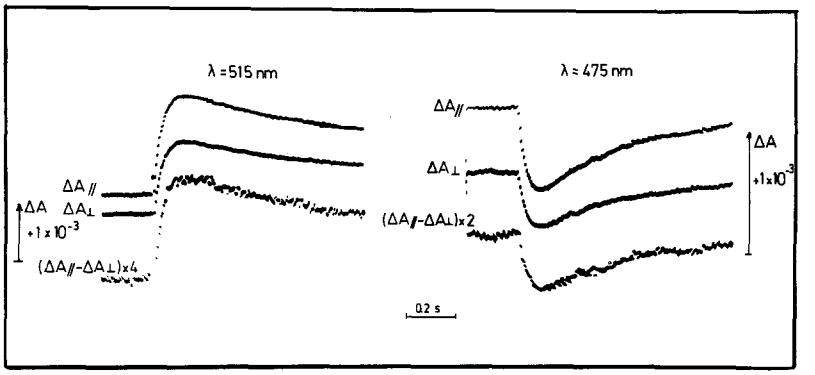

Figure 2. Oscilloscope display of the absorbance changes ΔA at 515 nm and 475 nm for chloroplasts oriented in a 10 kG magnetic field. The subscripts // and ⊥ refer to the electric vector of the measuring beam (see fig.1). The rise-time is instrument limited. Chlorophyll concentration : 2.2 x 10^{-5}M. Optical path : 0.8 cm. Temperature : 3°C. 40 transients were averaged.
The two bottom traces represent the differences between the absorbance changes measured for the two polarizations, amplified 4 x (at 515 nm) and 2 x (at 475 nm).

zation (defined as $p = (\frac{\Delta A_{//} - \Delta A_{\perp}}{\Delta A_{//} + \Delta A_{\perp}})$ is 12.5 ± 2.5 % at 515 nm. The deviations from the average are due mainly to differences from day to day preparations. Similar variations were observed in the linear dichroism of the pigments, measured with the spectropolarimeter.

The absorbance changes have been studied in the spectral range 450-540 nm. The kinetic parameters are the same for all the studied wavelengths (but small variations were observed from one preparation to another) and for both polarizations of the measuring beam. The degree of polarization varies widely in the studied spectral range. It is about 25% at 475 nm (fig.2) and it is negative in the range 480-500 nm. The values of $\Delta A_{//}$ and $\Delta A_{\perp}$ are plotted versus wavelength in fig.3 : the values are

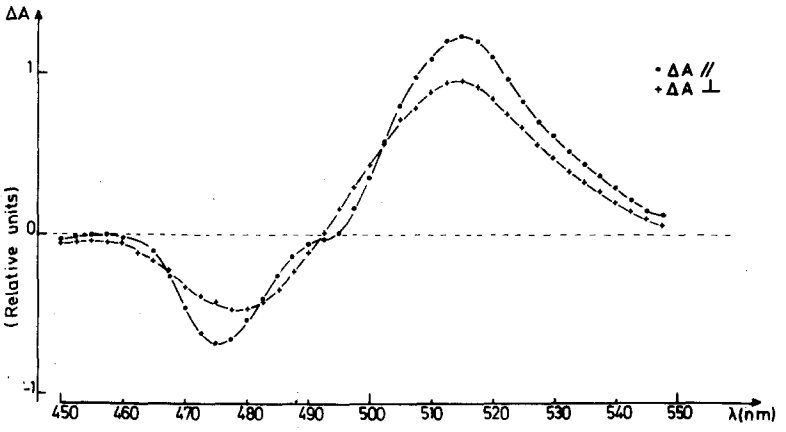

Figure 3. Wavelength dependence of absorbance changes for the two polarizations of the measuring beam. The conditions are as for fig.2. In order to correct for small day to day variations, the magnitudes of ΔA were normalized to a constant value at 515 nm, for both polarizations.

normalized for a degree of polarization of 12.5% at 515 nm. Crossing points of the two curves are reproducibly observed around 480, 490 and 500 nm.

Control experiments. Measurements of the degree of polarization of the absorbance changes are exposed to the same potential artifacts as the measurement of linear dichroism (7). A few control experiments were deviced in order to check the nature of the observed absorbance changes and the effect of the magnetic field.

A possible contribution of light scattering has been checked by

varying the solid angle of collection of the measuring light by increasing the distance between the cuvette and the light pipe from 1 mm to 25 cm. We found no change in the magnitude of ΔA (in the absence of H) or in the degree of polarization (with H).

In the absence of magnetic field no polarization effect was detected.

With a non-polarized measuring beam we found no influence of H on the kinetics of decay of the absorbance changes although the magnitude decreased slightly in the presence of H (about 5%). This effect is attributed to a decrease in the optical density of the suspension due to the orientation of the membranes parallel to the direction of the measuring beam (6).

All the experiments described above were performed with chloroplasts prepared as described under Material and Methods. With chloroplasts that had been broken (either by isolation in a low-salt buffer or by osmotic shock treatment) we found no influence of H on the absorbance changes (with and without polarization of the measuring beam). Similarly no linear dichroism could be detected with these preparations by the spectropolarimeter technique.

The decrease of the decay-time of the transients by gramicidin was checked on chloroplasts isolated in a low-salt buffer (0,175 M NaCl - 0,02 M tricine pH 8,0). In order to achieve an orientation in the magnetic field, the chloroplasts were resuspended in 0,8 M sucrose - 1 mM $MgCl_2$ - 0,01 M KCl. The orientation, measured by the linear dichroism of the pigments or by the degree of polarization of the 515 nm absorbance change, is about 80% of the control "unbroken" chloroplasts. We found that treatment with 10^{-7}M gramicidin causes an acceleration of the decay of absorbance changes by an order of magnitude, in agreement with Junge and Witt (2). The degree of polarization of the field effect, accelerated by gramicidin, is not affected by that treatment. A very slowly decaying phase (see ref.3), not accelerated by gramicidin, seems to present a degree of polarization different from the degree of polarization of the field effect. Due to its very small amplitude, this slow phase has not been systematically studied.

DISCUSSION.

The magneto-induced orientation of photosynthetic material can be explained by an anisotropy in the diamagnetic susceptibility of molecules oriented in the membrane (8). The overall magnetic anisotropy of the chloroplast will also depend upon the size, the shape and the stacking of the membranes. For chloroplasts suspended in hypotonic medium, the membrane

are swollen and are no longer parallel so that the resulting anisotropy is too small to cause an orientation in the 10 KG magnetic field, as shown by the absence of LD signals. The lack of polarization of the 515 nm absorbance change in this case precludes any direct effect of the magnetic field on the individual pigment molecules and indicates that the only influence of the magnetic field on the 515 nm effect occurs through the orientation of the membranes. The wavelength dependence, the kinetics, and the action of gramicidin allow us to identify the transients reported in this study with the 515 nm effect, as described by Witt and coworkers (1-3). If this effect results from different phenomena, one might expect to discriminate between them on the basis of their degree of polarization. The identity of the kinetics of decay $\Delta A_{//}$ and $\Delta A_{\perp}$ is a further support to the hypothesis that the 515 nm effect is an unique phenomenon.

The strong polarization effects reported in this study, though higher than those observed for the linear dichroism of the pigments in the covered spectral range (5), are of the same order of magnitude. So far, the best documented hypothesis accounting for the 515 nm effect is to consider a cross membrane delocalized electrical field that induces a Stark effect on the photosynthetic pigments (1,2). Taking this hypothesis in account, the degree of polarization that we measured would reflect the polarization of the Stark effect and it would depend upon the orientation of the pigments in the membrane with respect to the light-induced electric field. A few observations are relevant to this point :

1. Kleuser and Bücher (10) studied the electrochromism of chlorophylls a and b in monolayers. They found that the Stark effect was mainly polarized parallel to the electric field.

2. Schmidt et al (11) have been able to nearly reproduce the in vivo spectrum of the 515 nm effect by applying an electric field to multilayers of photosynthetic pigments. However the pigments composition of the layers was markedly different from that in chloroplasts. This could be due to a difference in the orientation of the pigments in the two systems.

3. We observed inversions in the sign of the degree of polarization in the 482-502 nm region (fig.4) which might reflect the difference in orientation found for the Soret band of chlorophyll b (polarized out of the membrane plane) and for carotenoids (an excess of carotenoids lying parallel to the membrane plane) (7).

Further studies are clearly needed in order to interpret in details our data and to derive more informations on the orientation of the pigments

in the photosynthetic membrane : theoretical and experimental work on the effect of an electrical field on the absorption of photosynthetic pigments having a known orientation relatively to the field. In this respect the use of oriented monolayers might be very fruitful.

Note on photoselection experiments. Recently Junge and Eckhof described photoselection experiments on randomly oriented chloroplasts (12,13). They detected an orientation of P 700 (Chl a_1), but they detected no anisotropy for the electrochromic effect at 520 nm. At first sight this seems to be in disagreement with our results. An explanation for that discrepancy may be found in the different mode of orientation : geometrical orientation of the membrane (in our experiments) or photoselection (Junge and Eckhof). The main difference however is the lower maximum theoretical value of p obtained from the photoselection technique. Using the hypothesis of a delocalized field and of the red transition moments of chlorophylls lying parallel to the membrane plane, the maximum value of p would be 1 for membrane perfectly oriented in the magnetic field but only 1/7 for the photoselection technique (14). For this last technique an incomplete orientation of the antenna pigments (or an orientation slightly out-of-plane) will further decrease p. The magnitude of $\frac{\Delta A}{A}$ in the long-wavelength region of the spectrum indicates that this decrease could be as large as 4 (7). With p = 12.5 % in our experiments, wa cannot expect p> 1% with the photoselection technique, at the limit of sensitivity of the measurements.

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