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ORIENTATION AND LINEAR DICHROISM OF CHLOROPLASTS AND SUB-CHLOROPLAST FRAGMENTS ORIENTED IN AN ELECTRIC FIELD

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SUMMARY

Whole or broken spinach chloroplasts, bacterial chromatophores and CPI chlorophyll · protein complexes in aqueous suspensions at room temperature can be oriented in externally applied electric fields. The orientation is observed by monitoring the electric field induced linear dichroism (LD). With whole chloroplasts a detectable LD signal is observed using voltages as low as 2–3 V (50 Hz alternating voltage) across an 0.3 cm electrode gap, and nearly complete orientation is observed at fields of 30 $V \cdot cm^{-1}$. The wavelength dependence of the LD signals using either orienting electric fields ($\vec{E}$) alone, or magnetic fields ($\vec{B}$) alone, are similar but opposite in sign with $\vec{E}$ and $\vec{B}$ pointing in the same direction. The chloroplasts tend to orient in such a way that the membrane planes are parallel to $\vec{E}$. The CPI complexes and bacterial chromatophores require much higher electric fields for orientation than whole chloroplasts (for CPI complexes $E > 2000\ V \cdot cm^{-1}$); rectangular, millisecond duration, voltage pulses are utilized for the observation of electric field induced LD spectra in these cases. Oriented CPI complexes exhibit LD maxima of the same sign at 685 and at 440 nm. The oriented chromatophores exhibit an LD spectrum of either positive or negative sign, depending on the wavelength. The mechanisms of the orientation are discussed.

INTRODUCTION

A detailed knowledge of the orientation of chlorophyll and other pigments in vivo is necessary for an understanding of the structure of photosynthetic membranes. The orientation of chloroplasts or whole algae cells in magnetic fields of 10 kG or more [1] provides a convenient laboratory reference frame for the study of the anisotropic optical properties of photosynthetic membranes. Using techniques such as

Abbreviation: CP complex: chlorophyll · protein complex.

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linear dichroism [2, 3], polarized fluorescence [3–6] and anisotropy of light scattering [7], it has been demonstrated that chlorophyll in vivo tends to be highly oriented. The longer wavelength absorbing forms of chlorophyll appear to be more oriented than the shorter wavelength forms; the Q_y (red) transition moment vectors of most of these forms tend to lie close to or within the membrane plane.

Whole chloroplasts can be fractionated into smaller Photosystem-I and Photosystem-II enriched particles [8, 9], and into basically two types of chlorophyll · protein complexes [10–13], termed CP I and CP II complexes [12].

To gain an understanding of how these chlorophyll · protein complexes are embedded in the whole membrane and how they account for the overall optical anisotropy of the membranes, it is desirable to study the relative orientation of the pigment molecules in these protein complexes. Unfortunately it is not feasible to orient particles which are smaller than about one micron in size using the magnetic fields which are readily available in most laboratories. Because the size of most subchloroplast fragments of interest is considerably less than one micron, while the size of the chlorophyll · protein complexes is of the order of 100 Å, the magnitude of the induced magnetic moment of these particles is not sufficient to cause their orientation in an external magnetic field.

The interaction of electrostatic permanent or induced dipole moments with an external electric field provides another mechanism of orientation of particles. Since the electrical potential energy thus obtained is significantly larger than the magnetic potential energy, even small particles like viruses, spinach quantasomes [14], or macromolecules such as proteins and DNA can be oriented in electric fields [15].

In this paper, we describe the orientation in electric fields of whole chloroplasts, subchloroplast fragments, chlorophyll · protein (CP I) complexes and bacterial chromatophores suspended in aqueous solutions. For relatively large particles such as whole chloroplasts, a weak alternating field of only 30 $V \cdot cm^{-1}$ (50 Hz) is sufficient to achieve maximum orientation. Such an alternating field technique has been employed many years ago by Lauffer to orient tobacco mosaic virus particles [16], and has been discussed from a theoretical point of view by Benoit [17]. Smaller particles, such as the chlorophyll · protein complexes, chromatophores or subchloroplast fragments, are best oriented using short (1–10 ms) pulsed electric fields (approx. 3000 $V \cdot cm^{-1}$) to minimize the heating effects which are associated with high voltages applied to solutions of relatively low resistance (of the order of 1000 $\Omega \cdot cm^{-2}$). The orientation is revealed by the linear dichroism exhibited by the oriented particles. The mode of orientation of the chloroplasts in electric fields is compared to their orientation in magnetic fields. Furthermore, possible mechanisms of orientation in electric fields are discussed; evidence is presented that the action of the electric field is predominantly an effect in which the entire particle is oriented.

METHODS

Voltage sources and sample holder

The sample holder was similar to the one described elsewhere [15]. It consisted essentially of two parallel platinum strips (2 × 1 mm) which were held 3-mm apart by teflon spacers and which served as the electrodes. This assembly was immersed into a

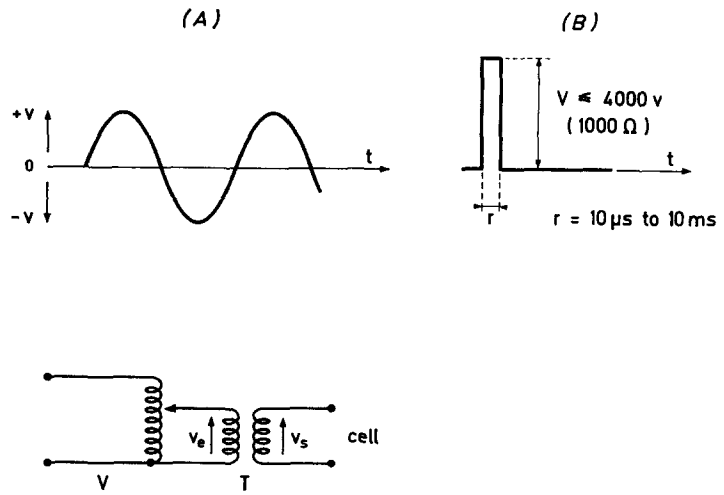

Fig. 1. (A) The time dependence of the alternating voltage (50 Hz) applied to the sample is shown (top). The method for obtaining alternating voltages from about 1–1000 V (r.m.s.) using a Variac V connected to the house mains (220 V, 50 Hz) and a step-up transformer T (the ratio $V_S/V_E = 4.5$) is shown schematically (bottom). (B) Output properties of the voltage pulser utilized in these experiments.

1 × 1 cm cuvette containing the suspension or solution. A thermocouple was inserted into a hole in one of the spacers and was used to monitor the temperature of the suspensions.

The two types of voltages applied to the electrodes are illustrated in Fig. 1. For the larger particles (e.g. chloroplasts) it was convenient to apply an alternating voltage to the electrodes. This voltage (up to 1000 V) was obtained conveniently by utilizing a combination of a step-up transformer and a step-down autotransformer; the latter was utilized mainly to obtain the desired values of relatively low voltages (below 30 V).

To orient bacterial chromatophores and CP I chlorophyll · protein complexes, it was necessary to utilize rectangular voltage pulses of the type shown in Fig. 1B, since higher values of the electric field were necessary to orient these relatively small particles. A special pulse generator which is capable of delivering a 4000 V pulse (cell resistance $\gtrsim$ 1000 Ω) for up to 10 ms was constructed.

The orientation was observed by monitoring the linear dichroism induced by the applied electric field. The linear dichroism was measured using a dichrograph constructed in this laboratory. Light from a xenon lamp was passed through a grating monochromator, partially attenuated at a frequency of 300 Hz using a mechanical chopper and then passed through a Glan polarizer. A photoelastic modulator (PEM-3, Morvue, Tigard, Oregon) whose driving voltage was automatically adjusted to produce a half-wave retardation at each wavelength was used to produce alternatively vertically and horizontally polarized light at a frequency of 100 kHz. The light beam thus modulated then passes through the sample cuvette and is incident onto a photomultiplier tube. A feedback loop adjusts the driving voltage of the photomultiplier tube so that the output signal at 300 Hz is kept constant; this device provides an electronic correction for the effects of spectral response of the monochromator and the

photomultiplier tube, for the spectral dependence of the lamp output and the fluctuations in its intensity, for the absorbance of the sample, etc. The 100 kHz component of the photomultiplier tube output is demodulated using a lock-in amplifier which delivers a signal proportional to the linear dichroism of the sample. This signal is fed into an X-Y recorder which is used to record the wavelength dependence of the linear dichroism.

When highly conducting solutions, or suspensions of small particles were utilized which necessitated the use of the pulsed electric fields, the monochromator was fixed at one particular wavelength, and the linear dichroism signal was fed into an oscilloscope rather than into the X-Y recorder. This signal could also be signal averaged by utilizing an Intertechnique Didac 800 multichannel analyzer in the signal averaging mode. In these cases, the wavelength dependence of the linear dichroism was recorded point by point.

Materials

The preparation of the chloroplasts is described elsewhere [18]. The chloroplasts were suspended in a 0.4 M sucrose, Tris buffer (20 mM, pH 8), 20 mM KCl solution. The subchloroplast fragments were prepared by subjecting whole chloroplasts to ultrasonic treatment and to a 90-min centrifugation at 20 000 $\times g$. The preparation is comparable to the one described by Jacobi and Lehman [19] and the chloroplast fragments in the supernatant are thus less than approx. 1000 Å in size.

The CP I complexes were kindly supplied by Dr. Acker of this department, while the bacterial chromatophores were supplied by Dr. Reiss of the Laboratoire de Photosynthèse, Gif-sur-Yvette. The CP I complexes [20] and the bacterial chromatophores [21] were prepared by procedures similar to those published elsewhere. With these samples, high electric fields were necessary in order to achieve an observable orientation.

The electrical resistances of all the solutions or suspensions were determined with a 1000 Hz impedance bridge.

RESULTS AND DISCUSSION

Orientation of whole chloroplasts and chloroplast fragments in alternating electric fields

Whole spinach chloroplasts exhibit an orientation in alternating electric fields as low as 10 V $\cdot$ cm^{-1} and maximum orientation is achieved at about 30 V $\cdot$ cm^{-1}. This corresponds to an applied voltage of only 10 V across the 3 mm electrode gap. Characteristic linear dichroism spectra for whole chloroplasts and chloroplast fragments are shown in Fig. 2 and are compared to the linear dichroism obtained with the same sample of whole chloroplasts oriented by a saturating magnetic field. The broken chloroplasts do not orient in magnetic fields because of their small size (estimated to be of the order of or less than 1000 Å). The electric field dependence of the orientation is shown in Fig. 3 (the orientation is expressed in terms of the linear dichroism ΔA, which is defined by Eqn. 1 below).

Using a single rectangular pulse corresponding to an electric field of 800 V $\cdot$ cm^{-1} (10 ms width), it was not possible to orient whole chloroplasts, even though a much smaller alternating field suffices to achieve complete orientation. However, if a series of such rectangular pulses, spaced about 1 s apart, is utilized, orientation of the

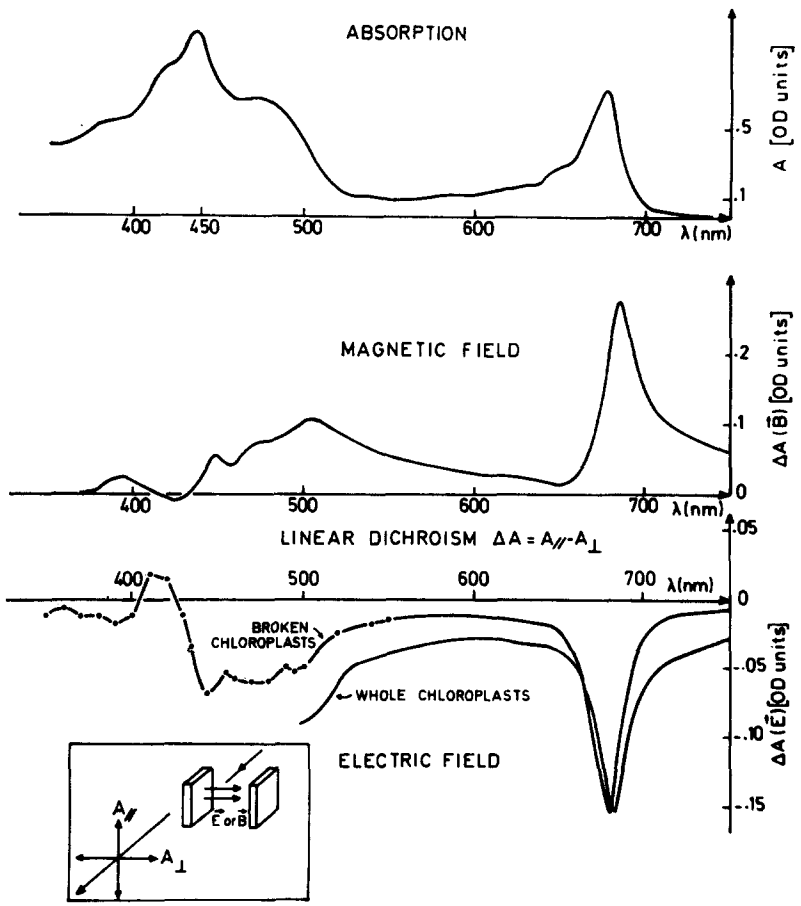

Fig. 2. Absorption spectrum (A) and linear dichroism spectra in magnetic ($\Delta A(\vec{B})$) and electric ($\Delta A(\vec{E})$) fields of whole and broken chloroplasts. The insert shows the directions of the electric ($\vec{E}$) and magnetic ($\vec{B}$) fields, and the polarization of the light with respect to the electrodes. The linear dichroism in terms of the absorbances $A_{\parallel}$ and $A_{\perp}$ is defined in Eqn. 1. Absorbances of all suspensions employed $A_{680\,nm} = 0.6-0.7$. Whole chloroplasts: alternating field, 4.7 V · cm^{-1}, 0.4 M sucrose, cell resistance, 16 700 Ω · cm^{-1}. Broken chloroplasts (in 2 M sucrose): 200 000 Ω · cm^{-1}; 2-ms rectangular pulses, 1670 V · cm^{-1}; (an alternating field of 300 V · cm^{-1}, results not shown in the figure, gives the same results as the rectangular pulses in the $\lambda > 550$ nm region). The voltages in all the electric field experiments were chosen to give the same magnitude for the ΔA signals at 680 nm.

whole chloroplasts can also be achieved. In the case of the single rectangular pulses, the field is on for only 10 ms. The rotational relaxation time of particles of the size of whole chloroplasts is of the order of several seconds [1], therefore, since the duration of the pulse is much shorter than the rotational relaxation time, the orientation achieved is negligible. Thus, even a relatively strong force characterized by the 800 V · cm^{-1} field cannot bring about a detectable (sufficiently large) orientation when a single 10 ms wide pulse is applied, since the orientational relaxation time is much longer than the time interval during which the force is applied. However, if a series of such pulses is

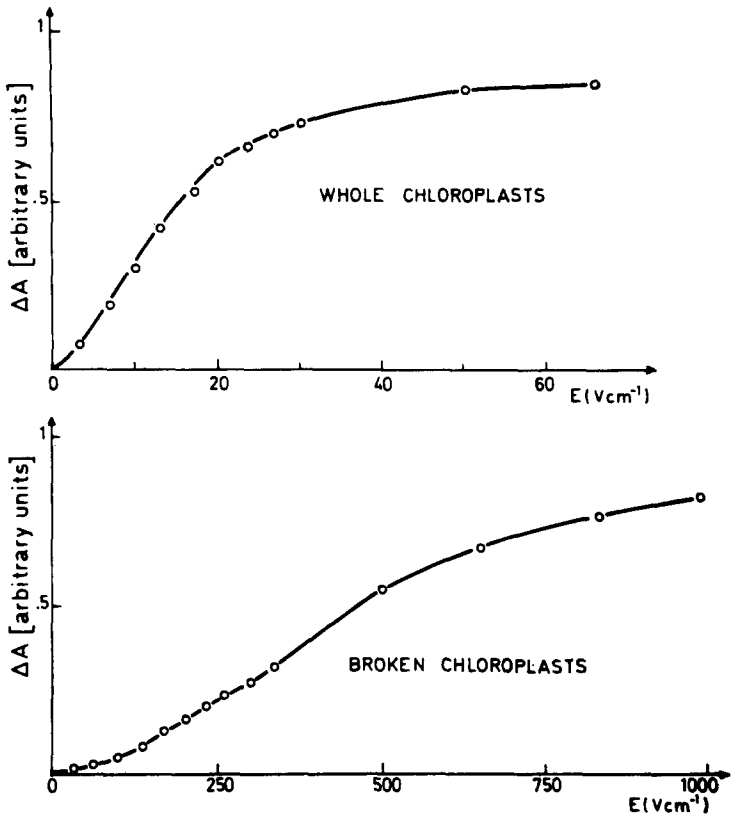

Fig. 3. Electric field dependence of the orientation of chloroplasts. The extent of orientation is proportional to the linear dichroism (Eqn. 1). The 50-Hz alternating voltage was used in these experiments. Whole chloroplasts: $A_{680\,nm} = 0.5$; 0.4 M sucrose, 16 700 $\Omega \cdot cm^{-1}$. Broken chloroplasts: $A_{680\,nm} = 1.4$; 2 M sucrose, 200 000 $\Omega \cdot cm^{-1}$.

used, and if the spacing between pulses is also much shorter than the relaxation time of the particles, the effects of the successive pulses will be cumulative and a considerable orientation can be attained when the length of the pulse train is comparable to the orientational relaxation time.

The use of alternating sinusoidal fields to orient whole chloroplasts is based on these considerations. The electric field alternates at a frequency of $2 \times 50 = 100$ Hz and the chloroplasts are too large to follow the field. However, the maximum extent of orientation is achieved after about 30–60 s. In this case, the electric field vibrates in a plane and there is no electrical force perpendicular to this plane. The chloroplasts thus orient themselves with respect to this plane in order to achieve the minimum potential energy. The chloroplasts are of course too large to follow the alternating field within this plane (which we verified experimentally).

As shown in Fig. 1A, experimentally an alternating electric field is easily obtained from the house mains using a step-up and step-down transformer. Thus, a specialized voltage pulse generator or other special equipment is not required and the alternating field technique offers an inexpensive and simple method for the study of

the properties of oriented chloroplasts under physiological conditions.

The electric field orientation technique has one disadvantage: heating effects. A compromise therefore has to be reached between the maximum field strength to be utilized, the ionic strength of the solution, and the length of time during which the electric field is to be applied. With a thermocouple immersed in the solution, the heating effects can be monitored conveniently during the course of the experiment, which can be terminated if the joule heating effects are considered to be unacceptable.

In the case of the whole chloroplasts (see Fig. 2), the resistance of the cell (measured at 1000 Hz with an alternating current impedance bridge) was 5000 Ω, since an isotonic solution of relatively high ionic strength was utilized. However, since only a modest a.c. field is needed to orient these chloroplasts, the heating effects were negligible (a maximum of 2 °C rise within a given time, required to record an entire linear dichroism spectrum, was allowed). With broken chloroplasts on the other hand, a higher field is required for the orientation since the particles are smaller. Heating effects are thus much more important and were minimized by reducing the ionic strength and increasing the viscosity (by adding sucrose) of the suspension. The resistance of the cell in this case was 60 kΩ (Fig. 2). Saturation is not reached even at a field of 1000 $V \cdot cm^{-1}$ and it was not possible to increase the field beyond this value because of heating effects.

Comparison of orientation of chloroplasts in magnetic and electric fields

It is now established that chloroplasts tend to orient with their lamellar planes perpendicular to the magnetic field [1, 2, 22, 23]. This mode of orientation is depicted schematically in the upper part of Fig. 4. We define the linear dichroism with reference to the insert in Fig. 2. If we arbitrarily designate the absorbance of the solution with the polarization vector oriented parallel to the planes of the electrodes as $A_{\parallel}$, and the perpendicular component of the absorbance as $A_{\perp}$, then the linear dichroism in the presence of either the electric field ($\Delta A(\vec{E})$), or the magnetic field ($\Delta A(\vec{B})$), is designated by

$$\Delta A = A_{\parallel} - A_{\perp} \tag{1}$$

By adopting this definition of $A_{\parallel}$ and $A_{\perp}$, the sign of ΔA is consistent with the one adopted in refs. 2 and 3 in which the linear dichroism of magnetically oriented chloroplasts is described. We note further from the insert in Fig. 2, that the directions of the electric ($\vec{E}$) and magnetic fields ($\vec{B}$) were taken parallel to each other in these experiments.

Using the same sample of whole chloroplasts (a typical absorption spectrum is shown in the upper part of Fig. 2) the linear dichroism ΔA was determined using either an electric field alone, or a magnetic field alone to orient the chloroplasts.

In the magnetic field case ΔA is positive in the red region of the absorption spectrum (Q_y band of chlorophyll). This indicates, as pointed out elsewhere [1–3], that the Q_y transition moments of chlorophyll *a* tend to be oriented close to or within the planes of the lamellae.

In the presence of the electric field, however, ΔA is negative. $\Delta A(\vec{B})$ and $\Delta A(\vec{E})$ appear to be mirror images of each other.

The absolute value of ΔA in the electric field is always smaller than the linear

dichroism in the magnetic field. For four different samples of chloroplasts oriented either in saturating electric or magnetic fields, the following ratio of the linear dichroism was obtained:

$$\left|\frac{\Delta A(\vec{B})}{\Delta A(\vec{E})}\right| = 1.77-1.87 \tag{2}$$

Even though the degree of orientation is complete in both cases, $|\Delta A|$ is significantly smaller in the case of the electric field. This fact, in addition to the observation that the signs of $\Delta A(\vec{E})$ and $\Delta A(\vec{B})$ are opposite, leads us to propose that the lamellar planes of the chloroplasts tend to line up parallel to the direction of the electric field lines. This orientation is depicted in Fig. 4.

With the Q_y transition moment vectors of the chlorophyll molecules oriented, on the average, randomly within the membrane planes [2–4], a lamellar plane oriented as shown on the extreme left, (i) in Fig. 4B, does not contribute to ΔA. This is due to the fact that the light beam is oriented perpendicular to this plane and therefore $A_{\parallel} = A_{\perp}$ for (i). The other possible orientations, for example (ii) and (iii) in Fig. 4B are equally likely to occur as orientation (i), or any other orientation as long as $\vec{E}$ lies in the planes of the lamellae. For these orientations $A_{\perp}(\vec{E}) > A_{\parallel}(\vec{E})$ and thus $\Delta A(\vec{E})$ in the 680 nm region has a negative sign.

In the case of the magnetic field, the membranes are all lined up in the same direction with respect to the light beam and $A_{\parallel}(\vec{B}) > A_{\perp}(\vec{B})$ for all the lamellar planes. It should be noted that the expected theoretical factor in Eqn. 2 is 2.0; this factor arises because of the averaging of all possible orientations of the planes (see Fig. 4B) for the electric field orientation case, whereas such an averaging is not appropriate in the case of the magnetic field (Fig. 4A). Thus the experimentally observed ratios of 1.77–1.87 are not far from the theoretically expected values.

These considerations explain both the opposite signs of $\Delta A(\vec{E})$ and $\Delta A(\vec{B})$, as

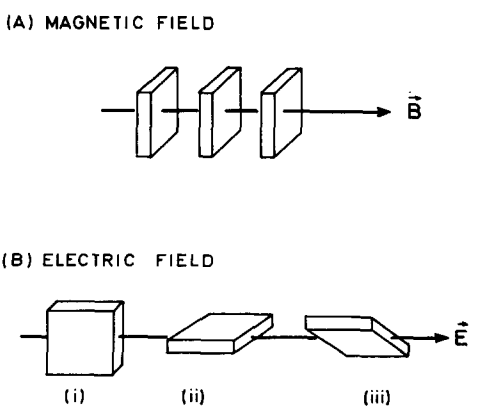

Fig. 4. Mode of orientation of chloroplasts in magnetic and electric fields. The lamellar planes of the membranes are represented by rectangular slabs.

well as the reduced dichroism in the electric field case, and are consistent with the mode of orientation proposed in Fig. 4.

Orientation of broken chloroplasts and form dichroism

Linear dichroism measurements are subject to artefacts such as form dichroism [2, 14, 24], flattening effect [3] and anisotropic selective light scattering effects [2, 3, 7, 25], all of which are capable of distorting the spectra. Form dichroism can arise even if the chlorophyll molecules are randomly oriented. All of these artefacts can be minimized by utilizing particles which are considerably smaller than the wavelength of the light. Considerations of this type have led Sauer and Calvin [14] to study the electric field-induced dichroism of small spinach chloroplast lamellar subunits about 100×500 Å in size. The results which they obtained indicated that only the longer wavelength forms of chlorophyll in these subunits exhibited orientation, whereas our results obtained with whole chloroplasts indicated a strong dichroism within the longer wavelength edge of the Q_y absorption band (Fig. 2). This is the same linear dichroism which is observed with magnetically oriented chloroplasts and is fully discussed in references 1–4.

The linear dichroism spectra of oriented whole chloroplasts and small subchloroplast lamellar fragments are compared in Fig. 2. There is a slight shift in the linear dichroism peak from 682 nm for whole chloroplasts to 680 nm for the chloroplast fragments. Otherwise the qualitative aspects of the linear dichroism are independent of the particle size and we conclude once more [2, 3] that form dichroism and other artefacts play no substantial role in these measurements. This confirms our earlier conclusions that chlorophyll in vivo possesses a high degree of orientation which accounts for the strong anisotropic optical properties of oriented chloroplasts.

Orientation of subchloroplast fragments and chromatophores with pulsed electric fields

Using alternating fields of 800 $V \cdot cm^{-1}$, a small linear dichroism signal using dialyzed solutions of the CP I chlorophyll · protein complex could be discerned with a maximum at 685 nm. However, because of the high electric fields required, the heating effects are substantial and the alternating field technique is thus not very useful for the study of CP I particles. With bacterial chromatophores no discernable orientation could be observed using alternating fields as high as 800 $V \cdot cm^{-1}$. Of course, because of the small size of the particles, neither the CP I complexes nor the chromatophores can be oriented in magnetic fields of 10–15 kG.

Small particles of this type are best oriented using short rectangular pulses of the type shown in Fig. 1B. Some examples of the orientation monitored by observing the ΔA signals induced by pulsed electric fields are shown in Fig. 5.

The time dependences of both the voltage pulse and the ΔA signal for broken chloroplasts are shown in Fig. 5A. It is noteworthy that the rise time of the effect (approx. 5 ms) is longer than the response time of the apparatus (approx. 1 ms) and is also slower than the fall time of the induced ΔA signal when the field is removed. This difference in the rise time and the fall time of the signal is related to the mechanism of orientation, which is discussed below. There is also a residual signal which persists for times longer than 5–10 ms after the field has been removed (the signal returns to the electric field-off value about 500 ms after the removal of the field). This component may be due to the polydispersity of the sample, but its origin is not well

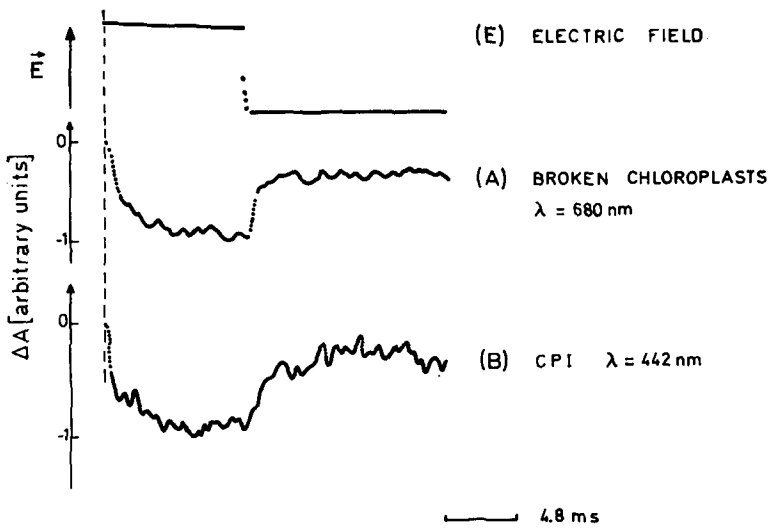

Fig. 5. Time dependence of the rectangular voltage pulses (E), and the field induced linear dichroism (ΔA) signals of broken chloroplasts (A) and the CP I chlorophyll · protein complexes (B). Broken chloroplasts: $A_{680\,nm} = 0.4$; 2 M sucrose, 1 670 V · cm^{-1}; 150 000 Ω · cm^{-1}. CP I complexes (dialyzed): $A_{675\,nm} = 0.21$; 2 M sucrose, 8000 V · cm^{-1}, 16 700 Ω · cm^{-1}.

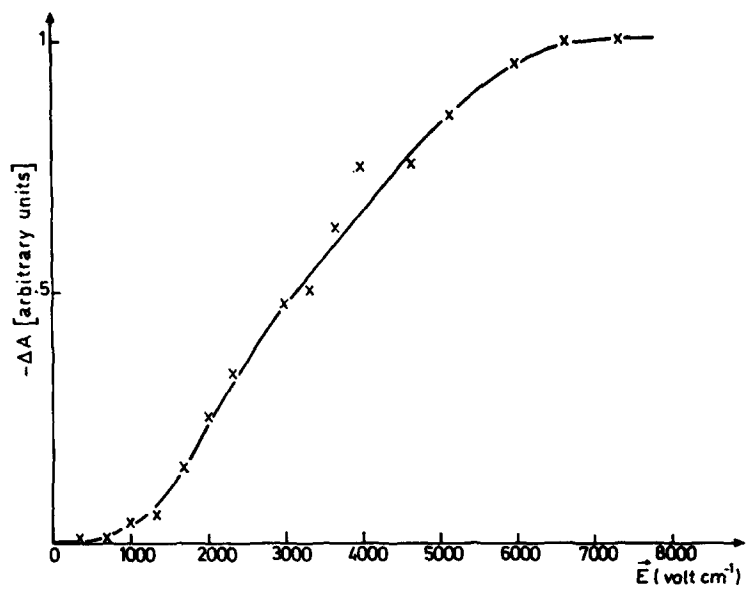

Fig. 6. Electric field dependence of the linear dichroism (ΔA) signal of the CP I chlorophyll · protein complexes. Wavelength: 442 nm. The voltage pulser was used in these experiments. $A_{442\,nm} = 0.34$; 2 M sucrose; 2-ms pulse width, 1670 Ω · cm^{-1}.

understood at this time.

The decrease in the applied voltage with increasing time in Fig. 5 is due to the finite conductivity of the samples.

The electric field-induced ΔA signal for a CP I solution is shown in Fig. 5B. The electric field dependence of the ΔA signal for CP I particles is shown in Fig. 6. Saturation, i.e. maximum orientation, is obtained at electric fields of approximately 7000 $V \cdot cm^{-1}$.

For the CP I particles, two peaks of negative sign for the ΔA are observed around 685 and 440 nm. A positive signal around 675 nm has also been found, but variations in its amplitude and relaxation kinetics (sometimes different from the relaxation times of the ΔA signals at 440 and at 685 nm) might reflect contamination of our CP I preparations by other types of chlorophyll · proteins and/or chlorophyll · detergent complexes. The peak at 685 nm is considerably red-shifted with respect to the absorption maximum around 675 nm. Even though ΔA for CP I particles is negative both at 440 and at 685 nm (as in whole or broken chloroplasts), the magnitude of ΔA at 440 nm is larger by about 50 % than ΔA at 685 nm. In whole chloroplasts on the other hand, ΔA is smaller in the Soret region than in the red region (Fig. 2). This quantitative difference between CP I chlorophyll · protein complexes and whole chloroplasts is indicative of a difference in the orientation of the chlorophyll molecules with respect to the applied electric field for CP I particles and for chloroplasts. Experiments to elucidate this orientation are presently under way (in collaboration with Dr. Acker).

In the case of the bacterial chromatophores there are several inversions in the sign of ΔA as a function of wavelengths (two examples of ΔA at 375 and at 480 nm induced by a voltage pulse are shown in Figs. 7C and 7D) between 350 and 820 nm, corresponding to different absorption bands. Thus, ΔA is negative at 820 and at 375 nm, and is positive in the wavelength region of 400–610 nm. Comparison of this data with the linear dichroism spectrum of Breton [26] (obtained by orientation of the chromatophores by air-drying on a glass surface) shows similar sign inversions in the wavelength dependence of ΔA. This indicates that the electric orientation technique and the air-drying technique [26] give qualitatively similar results, even though the chromatophores are dry in one case and are in an aqueous environment in the electric field experiment.

On the mechanism of orientation

The orientation of particles in electric fields is due to either or both of the following effects [15]:

(1) The presence of a permanent dipole moment which tends to align itself with the applied electric field.

(2) An anisotropic polarizability which gives rise to an induced dipole moment when an external electric field is applied.

In general it is possible to estimate the relative contributions of these two effects by techniques discussed by Benoit [27], O'Konski et al. [28] and by Frederic and Houssier [15].

These techniques include a detailed analysis of the rise time and the decay time of the orientation when a square wave voltage is first applied and then removed. If the rise time is slower than the fall time of the orientation, the contribution of a permanent

dipole moment is indicated. This appears actually to be the case in Fig. 5A and we conclude in a qualitative manner that a permanent dipole moment contributes to the orientation of the chloroplasts.

Furthermore, plots of the quantity

$$\varphi = (\Delta A/E^2)\,(\Delta A/E^2)^{\theta 1}_{E\to 0} \quad \text{vs.} \quad \log E^2 \tag{3}$$

have characteristic shapes depending upon the mechanism of the orientation (see for example ref. 15, pp. 106–110). Theoretical curves plotted according to Eqn. 3 for the cases when either the permanent dipole moment only, or the polarizability term only are responsible for the orientation are shown in Fig. 7. The experimental data for whole chloroplasts appear to resemble the $\beta = 0$ curve, indicating an important contribution of an induced dipole moment to the mechanism of orientation. Quantitative measurements of these parameters are presently under way.

Another question which may arise is whether the strong applied electric fields are also capable of re-orienting some particular component within the membranes. Such a possibility cannot be excluded a priori. A similar possibility has been considered in the magnetic field orientation case [3], but no evidence was found for such a mechanism. In the electric field orientation case we first note that the particles themselves are oriented, since the relaxation time of the induced orientation is strongly dependent on the external viscosity of the suspension of the solution. Similar effects are observed in the case of the magnetic field orientation [1, 3].

Furthermore, the shapes of the linear dichroism spectra for chloroplasts oriented either electrically or magnetically or by air-drying, exhibit the same ΔA wavelength dependence. Thus, all methods of orientation reveal the same intrinsic chlorophyll orientation. Even bacterial chromatophores oriented either by electric fields or by air-drying show similar ΔA spectra.

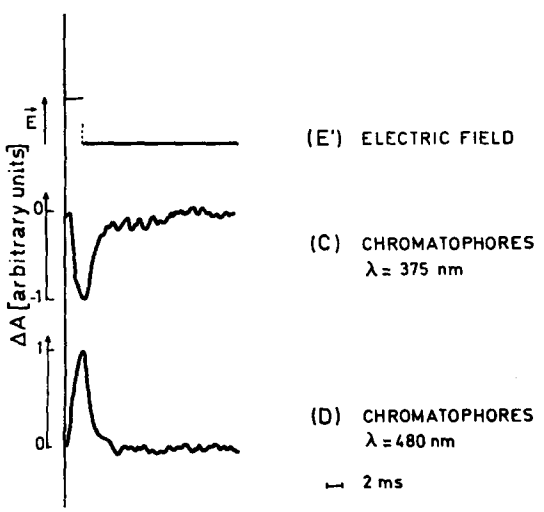

Fig. 7. Linear dichroism (ΔA) signals induced by rectangular voltage pulses with chromatophores. (C) $A_{376\,nm} = 1.26$; 2 M sucrose; 11 000 V · cm^{-1}, 7670 Ω · cm^{-1}. (D), same as (C) with $A_{480\,nm} = 1.05$.

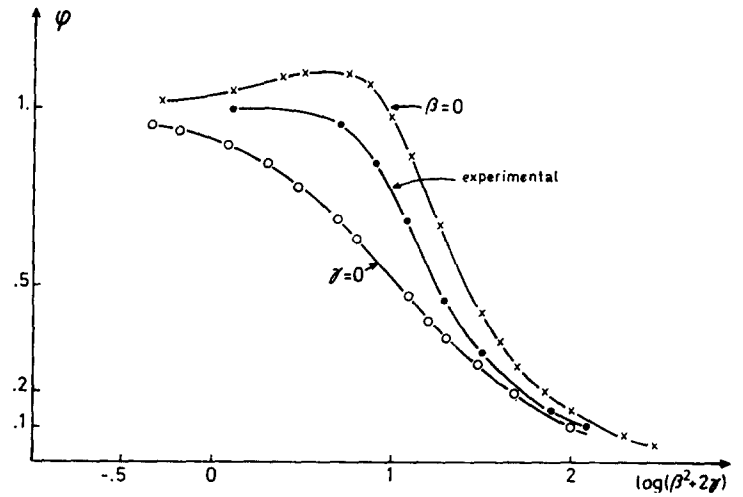

Fig. 8. Theoretical and experimentally observed (for whole chlororoplasts) orientation function φ (defined in Eqn. 3 and ref. 15, pp. 106–110. $\beta = C\mu E$ and $\gamma = C'\Delta\alpha E^2$, where C and C' are, for a fixed temperature, appropriate numerical constants, μ, the apparent permanent dipole moment, $\Delta\alpha$, the anisotropy in the polarizability, and E is the applied electric field strength. The quantity $\log(\beta^2 + 2\gamma)$ is proportional to $\log E^2$. The two theoretical plots correspond to the two cases when the orientation is due to either a permanent dipole moment only ($\gamma = 0$), or to an induced dipole moment only ($\beta = 0$). Experimental conditions: $A_{680\ nm} = 0.16$; 0.4 M sucrose; 36 700 $\Omega \cdot cm^{-1}$.

Finally, single voltage pulses of several thousand volts applied to a suspension of whole chloroplasts do not produce any linear dichroism signal, indicating that no anisotropy can be induced within the chloroplasts on a time scale of 10 ms or less. Therefore, there is no detectable reorientation of any of the pigments embedded within the membranes on these time scales. Since the isolated chlorophyll · protein complexes in solutions do orient in pulsed electric fields, the lack of any rapid orientation when they are embedded in the membranes indicates that the local viscosity is too high to respond to the applied electric fields within such a short time. The overall orientation of chloroplasts may be due to the cooperative effect of all of the protein complexes within the membrane which gives rise to a torque sufficiently large to orient the whole chloroplast. Since the sign of ΔA for CP I complexes and whole chloroplasts is the same, this result indicates that the chlorophyll · protein complexes may provide the dominant contribution to the electrical anisotropy of whole chloroplasts which causes their orientation in electric fields.

CONCLUSIONS

The results described in this paper indicate that electric fields can be utilized for the orientation of intact and broken chloroplasts. The mode of orientation of the lamellae in electric fields has been elucidated. Small subchloroplast fragments, which

cannot be oriented in magnetic fields of 10–15 kG, but can be readily oriented in electric fields, display linear dichroism spectra similar to those of intact chloroplasts. This observation indicates that form dichroism does not contribute significantly to the linear dichroism signals. Since reorientation of pigments within the membranes is highly unlikely in the presence of applied electric fields, the strong electric linear dichroism signals can be related to the intrinsic orientation of chlorophyll in vivo.

In this study, we have demonstrated the applicability of the pulsed electric field technique to orient bacterial chromatophores and subchloroplast particles such as the CP I chlorophyll · protein complexes.

An analysis of the electric properties such as the electric moments and the orientation of pigment molecules within the protein complexes, together with these same properties obtained with whole chloroplasts and subchloroplast fragments, may yield further information on the structural properties of photosynthetic membranes.

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