LINEAR DICHROISM OF CHLOROSOMES FROM *CHLOROFLEXUS AURANTIACUS* IN COMPRESSED GELS AND ELECTRIC FIELDS

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ABSTRACT The linear dichroism of chlorosomes from *Chloroflexus aurantiacus* was measured between 250 and 800 nm. To orient the chlorosomes we used a new way of compressing polyacrylamide gels, where the dimension of the gel along the measuring light-beam is kept constant. The press required for such a way of compressing is relatively easy to construct. A theoretical description is given to interpret the measured linear dichroism in terms of the orientation of the transition moments. The results obtained with the polyacrylamide gels are compared with the linear dichroism measurements for chlorosomes oriented in electric fields. Both the spectral features as well as the absolute size of the linear dichroism signals are in reasonable agreement. We find that the transition moment corresponding to the 741 nm bacteriochlorophyll *c* (Bchl *c*) absorption band makes an angle of 20° with the long axis of the chlorosome. For the 461 nm Bchl *c* band an angle of 30° is found. Both angles are significantly lower than the values reported so far in literature and they imply that Bchl *c* is highly organized in the chlorosomes.

INTRODUCTION

An important method to study the structure of complex light-absorbing macromolecules is the measurement of linear dichroism (LD)[1] (Fredericq and Houssier, 1973; Norden, 1978). This technique is widely used in the study of pigment organization in photosynthetic particles, the orientation of chromophoric groups in proteins, and also in the study of DNA conformation. To perform LD measurements requires a method that can create macroscopic anisotropy. Orientation in electric fields (Fredericq and Houssier, 1973) has been used for instance in the study of DNA-conformation (Charney et al., 1982) in solutions. A serious drawback of this approach is the fact that these experiments are limited to low salt conditions (<15 mM NaCl).

Abdourakhmanov et al. (1979) presented a new orienting method. Reaction centers from *Rhodopseudomonas sphaeroides* R-26 were oriented in compressed polyacrylamide (PAA) gels, containing 15% wt/vol acrylamide. An important advantage of this method is the fact that the particles are in an aqueous environment with pH values and salt concentrations that can be selectively varied in a broad range. The orientation of rodlike and disclike particles and the corresponding linear dichroism in the case of biaxial compressing of the gels was mathematically described by Ganago et al. (1980). This model leads to reasonable fits with experimental results (Ganago et al., 1980; Fetisova et al., 1986) for cases where less than ~50% orientation is expected. Measurements beyond that point do not seem to be possible with this method, so the predicted amount of LD at perfect orientation cannot be tested in a direct way. Comparison with results obtained from other orientation methods may lead to an independent test for the validity of the theoretical description. For several photosynthetic particles the orientation in gels has been compared with orientation in electric fields by Gagliano et al. (1985) but only in a qualitative way, i.e., the amount of orientation and therefore orientation angles of transition moments have not been determined.

To gain more insight in the degree of orientation in compressed gels and therefore in the interpretation of LD results, we compared in a quantitative way the LD of rodlike chlorosomes from *Chloroflexus aurantiacus* in orienting electric fields and in compressed gels of different compositions. To orient particles in a gel we used a somewhat different way of compressing the gel than the one described by Abdourakhmanov et al. (1979). We compressed a gel in one direction, keeping the dimensions

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[1] *Abbreviations used in this paper: BChl a*, bacteriochlorophyll *a*; BChl *c*, bacteriochlorophyll *c*; CD, circular dichroism; LD, linear dichroism; PAA, polyacrylamide.

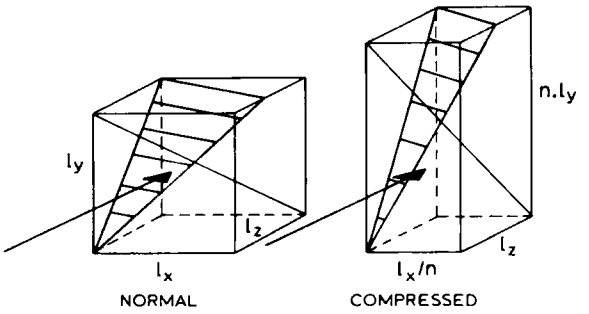

FIGURE 1 Compression of a polyacrylamide gel along the x-axis with a compression factor n. The volume $l_x \cdot l_y \cdot l_z$ remains constant. Light is incident along the z-axis. Orientation of a line and a plane in the gel before and after compression are indicated.

along the light-beam constant and allowing the gel to expand only in one perpendicular direction (see Fig. 1). The advantage of this way of compressing is that a press for these measurements is much easier to construct than a press for biaxial pressing. Moreover, a gel can be used with a small dimension along the measuring light-beam for all degrees of compression, thereby decreasing the amount of background absorption and scattering of the gel in the UV, which is useful in the study of DNA and proteins.

In our way of compressing there is less symmetry in the gel than in the case of biaxial pressing. However, using the same assumptions as Ganago et al. (1980) for rotationally symmetric rodlike and disclike particles, we were able to derive a formula that relates the amount of LD at a certain degree of compression to an average angle between transition moments and the orientational axis of rodlike and disclike particles.

The LD spectra we obtained in electric fields and compressed gels are very similar, and also the obtained angles with the long axis of the chlorosomes for the bacteriochlorophyll *c* (Bchl *c*) transition moments are in reasonable agreement.

Chlorosomes from *Chloroflexus aurantiacus*

Chloroflexus aurantiacus is a green gliding filamentous bacterium that lives in hot spring effluents at temperatures from 50 to 70°C (Pierson and Castenholz, 1974). Its light-harvesting chlorosomes containing mainly BChl *c* are flat elongated sacs with rounded ends and measure ~100 × 30 × 10 nm (Staehelin et al., 1978) as determined by electron microscopy. Recently Golecki and Oelze (1987) reported that the length of the chlorosomes ranges in fact from ~80 to 200 nm, depending somewhat on the growing conditions for the bacteria, and peaking at ~110 nm. The dimensions of chlorosomes can vary from strain to strain (Pierson and Castenholz, 1974) and the thickness is also dependent on the growing conditions (Sprague et al., 1981; Golecki and Oelze, 1987). Chlorosomes consist of a core and an envelope, which is ~2 nm thick (Staehelin et al., 1978) and they are appressed to the inside of the cytoplasmic membrane, to which they are connected via a special crystalline baseplate (Staehelin et al., 1978). The chlorosomes also contain β- and γ-carotene (Staehelin et al., 1978; Schmidt et al., 1980) and a small amount of BChl *a* absorbing at ~790 nm (Schmidt, 1980). It has been suggested that this BChl *a* functions as an intermediate during energy transfer from BChl *c* to BChl *a* in the membrane (Betti et al., 1982) and it is probably located in the baseplate.

A BChl *c* binding protein has been identified (Wechsler et al., 1985) consisting of 51 amino acids, which presumably binds seven BChl *c* molecules. The protein forms dimers, which are probably further organized into subunits of six dimers (Wechsler et al., 1985). These structures may be the repeating units in the rod-shaped elements (diameter, 5.2 nm; length, 6.0 nm), that extend the full length of the chlorosome (Staehelin et al., 1978) with an orientation parallel to the long axis of the vesicle. The composition of the protein suggests an α- helical structure, where BChl *c* can bind to one side via the central MG-atom, with the porphyrin-ring laying flat to the helix (Wechsler et al., 1985). Linear dichroism spectra were measured (Betti et al., 1982; Van Dorssen et al., 1986*a*). where especially the 460- and 740-nm BChl *c* bands lead to clear dichroism signals. Betti et al. (1982) oriented the chlorosomes by stretching films, but the chlorosomes were probably not perfectly oriented. Therefore the reported angle of 40° for the 740-nm transition moment and the long axis should be considered as an upper limit. Van Dorssen et al. (1986*a*) oriented chlorosomes in biaxially compressed gels. They find that the chlorosomes orient very differently than expected from the theory of Ganago et al. (1980) and perfect orientation seems to occur at degrees of compression where one would expect only about 50% orientation when applying the theory of Ganago et al. Assuming perfect orientation Van Dorssen et al. (1986*a*) calculate an average angle of 37° with the long axis for the 740-nm transition moment and 39° for the 460-nm transition moment. There are some clear differences between the LD results reported in both studies. Van Dorssen et al. report that the LD between 550 and 630 nm is negative or nearly zero and find a negative band near 800 nm, whereas Betti et al. find a clearly positive LD signal between 550 and 625 nm and the LD around 800 nm is about zero. To resolve these differences and to estimate the angles that the Bchl *c* and Bchl *a* transition moments make with the long axis of the chlorosome we studied the LD of the chlorosome using two independent methods of orientation in compressed polyacrylamide gels and electric fields. The results show that the angles for the Bchl *c* 740- and 460-nm transition moments are much smaller than those reported earlier.

THEORY

Linear Dichroism in Compressed PAA Gels

In Fig. 1 it is shown how a PAA gel is compressed. The gel is compressed in the x-direction with a factor n and

expands along the y-axis with the same factor n. The measuring light-beam is incident along the z-axis. For rodlike rotationally symmetric particles with a long axis a and two short axes b and c, with $a \gg b = c$, and disclike rotationally symmetric particles with $a \ll b = c$, we have the following relation (see Appendix):

$$\frac{\Delta A}{3A} = \frac{A_y - A_x}{3A} = \pm \left\langle \frac{1}{2}(3\cos^2\xi - 1) \right\rangle \Phi(n) \qquad (1)$$

$$\Phi(n) = \frac{n^4 + 1}{n^4 - 1}\left[1 - \frac{n(n^2-1)^2}{(n^4+1)|n^4-1|} \cdot \int_0^1 dx \frac{x^2 + 2[n/(n^2-1)]^2}{\{-x^4 + x^2 + [n/(n^2-1)]^2\}^{1/2}}\right]. \qquad (2)$$

$+$ *rod*

$-$ *disc*

A is the absorbance of the particles under study in an uncompressed gel. $A_{x,y}$ is the absorbance for light polarized along the x- and y-axis, respectively, ξ is the angle between the transition moment and the long axis of a rod or the normal to the plane through the disc; symbols $\langle \ldots \rangle$ denote an average over all values of ξ. $\Phi(n)$ is an orientation function, which is derived in Appendix. It is 0 for $n = 1$ and 1 for $n = \infty$. It can be evaluated numerically. It can be shown that

$$\Phi(n) = \frac{4}{5}\frac{n^2 - 1}{n^2 + 1}, \qquad (3)$$

when $n \to 1$. In the following we will call $\Delta A/A$ the dichroic ratio P. If for a certain degree of compression $P/\Phi(n) < -\frac{3}{2}$ the particle must be disclike and if $P/\Phi(n) > \frac{3}{2}$ it must be rodlike (see Appendix).

In deriving Eq. 1 we made the assumption that rodlike or disclike particles orient in the same way as a line or a plane in the gel, respectively, having the same orientation (see Fig. 1), while the particles are not deformed. The particles must be larger than the dimensions of the pores of the gel. In addition, it is assumed that the volume of the gel is constant during compression. These assumptions shall be discussed below. As mentioned above the assumptions made in deriving the formula given above are the same as made by Ganago et al. (1980) and we only have a different orientation function because we use a different way of compressing.

Linear Dichroism in Electric Fields

Particles in solution can often be oriented by an electric field, usually applied as a rectangular voltage pulse across two electrodes. This orientation is due to the interaction of the electric field (E) with an electric dipole moment of the particle, which may be permanent in nature and/or which may be induced by the electric field. This can lead to a linear dichroism ΔA, where $\Delta A = A_\parallel - A_\perp$, and $A_\parallel$ and $A_\perp$ are the absorbances parallel and perpendicular, respectively, to the electric field. It takes some time after the onset of the voltage pulse before an equilibrium value ΔA_s is reached. We can write a similar relation as in the case of linear dichroism in gels, which is valid for both disclike and rodlike particles:

$$\frac{\Delta A_s}{3A_{iso}} = \frac{(A_\parallel - A_\perp)_s}{3A_{iso}} = \left\langle \frac{1}{2}(3\cos^2\delta - 1) \right\rangle \Phi(E), \qquad (4)$$

where $\Phi = 1$ when $E = \infty$. δ is the angle between an optical transition moment and the orientation axis of the particles in the electric field. A_{iso} is the absorbance in the absence of the electric field E. Again the symbols $\langle \ldots \rangle$ denote an average over all values of δ.

Apart from E the degree of orientation depends also on the absolute magnitudes of the electric dipole moments. In the case of rotationally symmetric particles an expression for Φ has been given by O'Konski et al. (1959), expressed in terms of the electric field strength E, the permanent electric dipole moment and the electric dipole moment, which is induced on the particles by the electric field.

The shape of the curve log (ΔA_s) vs. log (E^2) depends on the ratio of the permanent and induced dipole moments (O'Konski et al., 1959; Charney and Yamaoka, 1982) and can be calculated from the expression given by O'Konski et al. (1959) in the case of rotationally symmetric particles. In real experiments saturation ($\Phi \approx 1$) can usually not be reached, and therefore the relative contribution of permanent and induced dipole moments must be determined to make a reliable estimation of the value of δ. One way to make such an estimation is to fit log (ΔA_s) vs. log (E^2), searching for the best values of the permanent and induced dipole moments. However, when the sample is not completely homodisperse, a single value for the permanent and induced dipole moments will not suffice and the estimation becomes more difficult, although it is still possible to have a good indication of the degree of orientation. In some cases an easy extrapolation to $E = \infty$, i.e., to perfect orientation, is possible. From O'Konski et al. (1959) it follows that in the case of a pure permanent dipole moment plotting ΔA_s vs. $1/E$ gives a straight line within very good approximation when $\Phi > 0.8$. Then the intercept of this line with the ΔA_s-axis is the value of ΔA_s for $E = \infty$. In the case of an induced dipole moment ΔA_s vs. $1/E^2$ is linear when $\Phi > 0.6$. When both kinds of dipole moments are present neither extrapolation is correct.

For monodisperse axially symmetric particles the field-free decay time τ of the dichroism signal is related to the transverse rotational diffusion coefficient D_r by

$$\tau = 1/6D_r. \qquad (5)$$

For a rigid rod D_r can be expressed as (Broersma, 1960):

$$D_r = \frac{3kT}{\eta\pi L^3}[\ln(2L/d) - \gamma_r]. \qquad (6)$$

k is the Boltzman constant, T is the absolute temperature, η is the solvent viscosity, L is the length of the particle, and

d is its diameter. γ_r is a frictional parameter which accounts for end effects. Several expressions for γ_r have been presented in the literature (Broersma, 1960; Mandelkern et al., 1981, Broersma, 1981; Tirado and Garcia de la Torre, 1980). The field free decay time τ is strongly dependent on the length of particles and can lead to accurate estimations of the length (see Elias and Eden, 1981).

Wegener et al. (1979) showed that the decay may consist of five exponentials in the case of arbitrarily shaped, monodisperse particles.

MATERIALS AND METHODS

Cells of *Chloroflexus aurantiacus* were grown as described by Van Dorssen et al. (1986*a*). The chlorosomes were prepared according to Feick et al. (1982). Using a 10-mM Tris-HCl buffer with pH 8.0, containing 0.05% Miranol (buffer I), we made gels containing 10%, 15%, and 20% acrylamide (wt/vol) resp., and the ratio of acrylamide:*N,N*-methylenebisacrylamide (BIS) was 29:1 in all cases. The percentage *N,N,N',N'*-tetramethyl-paraphenylenediamine (TEMED) was 0.5, 0.75, 1.0% resp. and the percentage of ammoniumpersulfate (APS) was 0.1, 0.15, 0.2%. Acrylamide from Serva (Heidelberg, FRG) was used. BIS and TEMED were from Merck-Schuchardt (Hohenbrunn, FRG), and the APS was from J. T. Baker Chemicals BV (Deventer, Holland). All reagents were used without further purification. For electric field dichroism experiments dilutions were made with the buffer I containing only 1 mM Tris. When concentrations of Tris lower than 1 mM were needed, the solution was diluted with distilled water.

All absorbances were measured on a Cary 219 spectrophotometer (Varian Associates, Inc., Palo Alto, CA) interfaced to a HP85 computer. Spectra were baseline corrected. Corrections were made for the changes in absorption going from solution to the gel phase.

The gels were compressed stepwise in a press with an optical pathlength of 5.0 mm from an initial width of 9.0 mm to a final width of ~5.5 mm. The gel appeared to be compressible in a reversible way.

Linear dichroism in gels was measured on a modified Cary 61 spectrapolarimeter (Varian Associates, Inc.) (Bokma et al., 1987). Calibration was done by comparing $A_\parallel - A_\perp$ with the values measured in the Cary 219 using a polarizer. LD spectra were recorded on a HP85 computer and they were corrected for the contribution of the gel, which was measured separately. For measurements at one wavelength the signals were averaged during ~4 min. Absorbance and gel dichroism spectra were recorded at 20°C.

Linear dichroism of chlorosomes oriented in an electric field was measured using a different experimental setup. A 150 W Xenon lamp, mounted in a universal monochromator illuminator model 7340 (Oriel Corp., Stratfort, CT) was used. An Oriel monochromator model 7240 selected the wavelengths with a bandwidth of ~3 nm. For measurements above 530 nm a high-pass filter KV520 was used to suppress second order light. 600 μl of chlorosome solution with a typical absorbance of ~0.25 at 741 nm was put in a quartz cell with an optical pathlength of 10 mm containing two platinum electrodes, 2.5 mm apart. A high-voltage pulser delivered a rectangular pulse between 0 and 2,000 V with a rise and decay time of ~0.5 μs (Greve, 1972). The cell is placed in a thermostated cell-holder.

A Glan-Thompson polarizer is placed in front of the cell with the polarization axis parallel or perpendicular to the electric field in the cell. The transmitted light is detected by an R376 photomultiplier tube (Hamamatsu Phototonics KK, Hamamatsu, Japan) in a PR-305 mounting (Products for Research, Inc., Danvers, MA). Using a current-voltage converter (10^4 V/A) with an RC time of several seconds the steady-state light level can be determined accurately. The converter also delivers an output signal with an RC time of several microseconds, which registrates the fast changes in the light intensity relative to the steady-state levels that occur due to linear dichroism induced by the electric field. This signal is amplified to a final ratio of 10^5 V/A and it is fed into an 8-bit transient digitizer (Biomation model 8100) with 2,048 points per trace, using a sample rate of 1 MHz. The applied voltage pulse and the corresponding change in transmitted light intensity can be registrated simultaneously. The time between the voltage pulses was at least 30 s. The registrated data were transmitted to a HP85 computer for calculation.

Instead of $A_\parallel - A_\perp$ we determined $A_\parallel - A_{iso}$ and/or $A_\perp - A_{iso}$ which are related by

$$A_\parallel - A_{iso} = -2(A_\perp - A_{iso}) = \tfrac{2}{3}(A_\parallel - A_\perp) \qquad (9)$$

in the case of an uniaxial field. So with the exception of a constant factor these expressions are the same. $(A_\parallel - A_{iso})_s$ is determined by log $(I_{iso}/I_\parallel)$ where $I_\parallel$ is the transmitted light intensity during a pulse when an equilibrium state has been reached (see Results). I_{iso} is the transmitted intensity after the pulse when the orientation has completely disappeared.

RESULTS

In Fig. 2 the absorbance spectrum of the chlorosomes in solution is given. It shows the Q_y band of BChl *c* around 741 nm and a band around 461 nm also resulting from BChl *c*. The Q_y band of BChl *a* is around 790 nm, where there is also a small contribution from the Q_y band of BChl *c*. The peak near 665 nm is somewhat more pronounced than in the spectrum given by Van Dorssen et al., 1986*a*, and it may be ascribed to a small amount of free BChl *c*, or perhaps bacteriopheophytin *c* (Van Dorssen et al., 1986*a* and *b*, Swarthoff et al., 1982). The same is probably also true for the band around 440 nm, which to some extent contributes to the absorbance at 461 nm. In the near UV the band around 330 nm stems from BChl *c* , the absorption around 280 nm must in part be due to the aromatic protein residues. Absorption due to the carotenoids can be observed near 510 nm.

Linear Dichroism in Compressed PAA Gels

When the chlorosomes were embedded in a PAA gel, the absorbance at 461 and 741 nm decreased by ~5%, differing somewhat from gel to gel, whereas the absorbance around 440 and 665 nm increases a little, indicating some additional degradation. The absorbance around 790 nm also decreases, but the percent decrease is more

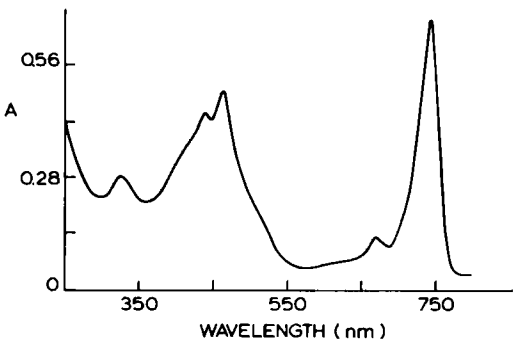

FIGURE 2 Absorbance spectrum of chlorosomes of *Chloroflexus aurantiacus* in solution (buffer I) at 20°C.

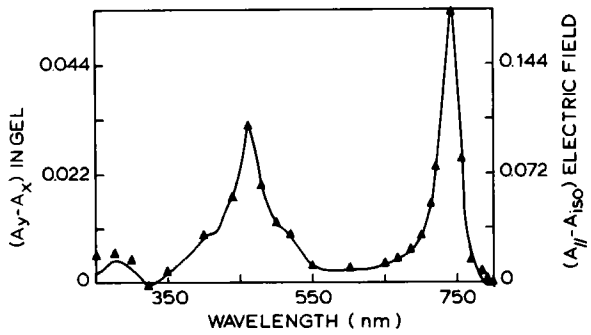

FIGURE 3 Linear dichroism of chlorosomes of *Chloroflexus aurantiacus*. The drawn spectrum is measured in a 15% polyacrylamide gel, containing 10 mM Tris-HCl with pH 8.0 (OD[741 nm] = 0.09), using a compression factor n = 1.59 at 20°C. The points marked with ▲ are measured in an electric field of 5.6 kV/cm in buffer I diluted with distilled water to [Tris] = 0.67 mM (OD[741 nm] = 0.20). The spectra are normalized in the 741-nm peak.

difficult to determine because of the low absorbance and some variation in the baseline correction of a blanco gel. A crude estimation indicates that the amount of decrease can be ~30–50%.

In Fig. 3 the LD spectrum of the chlorosomes in a 15% PAA gel is given. The linear dichroism at 741 nm was high at this degree of compression (n = 1.59), corresponding to a value of ξ of ~17°. We note that the bands around 440 and 665 nm, clearly visible in the absorption spectrum, are almost absent. $(A_y - A_x)$ is slightly negative around 790 nm but not so pronounced as reported by Van Dorssen et al. (1986*a*), who performed their measurements at 77 K. In contrast to Van Dorssen et al. (1986*a*), we see a significant amount of positive dichroism in the wavelength range from 550 to 630 nm.

The reduced dichroism P at 461 and 741 nm in a 15% PAA gel as a function of the compressing factor n is given in Fig. 4 for one measurement. The curve can be described very well with the orientation formula for rods (formula 1)

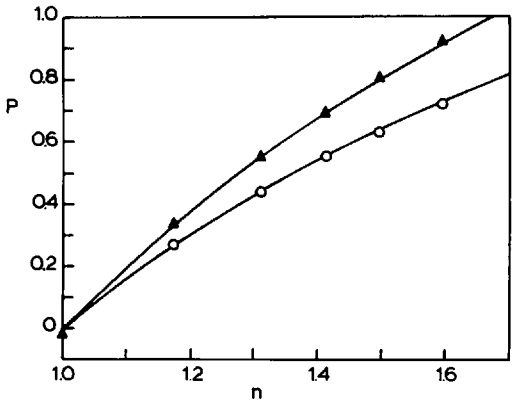

FIGURE 4 The dichroic ratio P of chlorosomes of *Chloroflexus aurantiacus* in a 15% polyacrylamide gel containing buffer I at 20°C, measured at 741 nm (▲) and 461 nm (○) plotted vs. the compression factor n. The drawn lines are theoretical fits assuming a rodlike shape with ζ = 17.3° at 741 nm and 27.0° at 461 nm.

using ξ = 17.3° at 741 nm and ξ = 27.0° at 461 nm in this particular case.

The measurements appeared to be very reproducible leading to ξ = 17 ± 4° at 741 nm and ξ = 27 ± 3° at 461 nm for one particular chlorosome preparation. The errors mainly reflect uncertainty in the amount of dichroism due to the calibration of the Cary 61.

For the other transition moments we can not directly estimate their orientation because of the overlap of the bands and small uncertainties in the baseline correction. However it is obvious that for the fraction shown in Fig. 3 the angles must be larger than 54.7° for the 330 and 790 nm bands because of the negative values of $A_y - A_x$ (see Discussion). Assuming no contribution from BChl *c* at 795 nm, the calculated angle for the Q_y transition moment is between 64 and 76° in case the absorption at 790 nm in the gel has decreased betwene 30 and 50%. Other chlorosome fractions gave angles for the 795-nm band close to 60° (not shown).

During compression, the shape of the LD spectrum is the same for all values of n (not shown), suggesting that the chlorosomes are not deformed.

In the orientation model the assumption is made that the pores of the gels are small compared with the dimensions of the particles. To check this assumption we performed the same measurements using a 10% and a 20% gel, which must have different mean pore sizes. For a 10% gel somewhat lower dichroism signals were obtained, whereas in a 20% gel the signals were the same as is in a 15% gel, suggesting that the pores are small enough in a 15% gel.

Another assumption that is made is that the volume of the gel remains constant during compression. This was tested using a gel containing cytochrome *c*, which did not show linear dichroism. The absorbance at 407 nm due to the cytochrome *c* Soret peak did not change during compression, indicating that the volume of the gel remains constant.

Several experiments were done with different batches of chlorosomes. The amount of LD does not vary much over

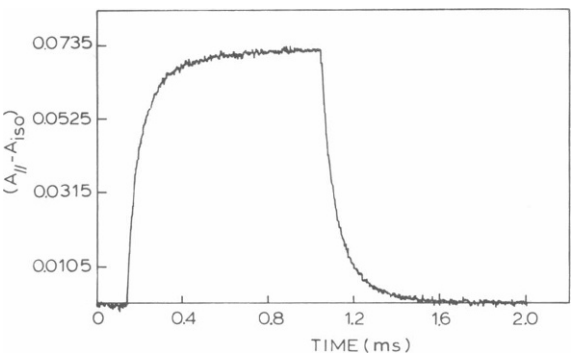

FIGURE 5 The time course of the electric field induced linear dichroism signal $A_{\parallel} - A_{\infty}$ of chlorosomes of *Chloroflexus aurantiacus* at 461 nm (OD = 0.15). The temperature is 12.2°C. A voltage pulse of 4 kV/cm is applied. [Tris] = 0.67 mM.

the various batches, and in general angles in the range 17–27° are found. For one particular sample angles of 37 and 40° were observed for the 741 and 461 nm bands, respectively. A possibility is that the variation is due to a variable degree of aggregation of the chlorosomes: the more aggregation, the lower the LD. The alternative possibility, namely that the amount of dichroism is a function of the structure of the chlorosomes and therefore dependent on the growing conditons is currently under investigation. However, preliminary results indicate that this is not the case (van Amerongen et al., to be published).

Linear Dichroism in Electric Fields

Chlorosomes of *Chloroflexus aurantiacus* could be very well oriented by an electric field pulse. In Fig. 5 a typical trace of $A_{\parallel} - A_{iso}$ measured at 461 nm is shown. A saturation level ΔA_s is reached and this value appears to be very reproducible when measuring with the same sample. After about 30 pulses of ~1 ms above 5.6 kV/cm, the signals started to decrease somewhat and fresh samples were taken. At all wavelengths where measurements were performed the ratio $(A_{\parallel} - A_{iso})/(A_{\perp} - A_{iso})$ was always −2.0 within a few percent, indicating that the electric field is uniaxial.

In Fig. 3 the linear dichroism spectrum is shown, measured with pulses of 5.6 kV/cm. It is normalized to the spectrum measured in a 15% gel in the 741-nm peak. The spectra are almost identical apart from some deviation below 300 nm probably due to some uncertainty in the baseline correction of the gel which starts to absorb significantly in this region. In addition, there is a small but significant difference around 790 nm where ΔA measured in electric fields is low but not negative as with the PAA gel.

To estimate the relative contribution of permanent and induced dipole moments to the orientation mechanism log (ΔA_s) was plotted vs. log (E^2) (not shown) (Charney et al., 1982; O'Konski et al., 1959). The shape of the thus obtained curve can only be explained if we assume that the chlorosomes are mainly oriented due to an induced dipole moment with probably a small contribution from a permanent dipole moment. This is further confirmed by an analysis of the rise and decay of the $A_{\parallel} - A_{iso}$ curve. A comparison shows that the rise time is only somewhat larger than the decay time at low fields (below 10% orientation) again indicating a predominant contribution from an induced dipole moment (Fredericq and Houssier, 1973).

To obtain the values of $A_{\parallel} - A_{iso}$ at complete orientation we extrapolated to infinite field strengths by plotting $A_{\parallel} - A_{iso}$ vs. $1/E^2$ (O'Konski et al., 1959), which must be appropriate in the case of an induced dipole moment. This gives probably a small underestimation of the dichroism at perfect orientation because of a small contribution of a permanenet dipole moment. In Fig. 6 two typical extrapolations are shown for 461 and 741 nm in 0.67 mM Tris at 12.2°C. There is no significant deviation from a linear dependence as expected from the theory of O'Konski et al. (1959), in the case of an induced dipole moment. The obtained values of δ in this particular case are 27.1° at 741 nm and 34.2° at 461 nm. The standard errors in these angles in one series of experiments with one sample were always <1° and typically 0.5°. The results appeared to be very reproducible from one sample to another (see Table I).

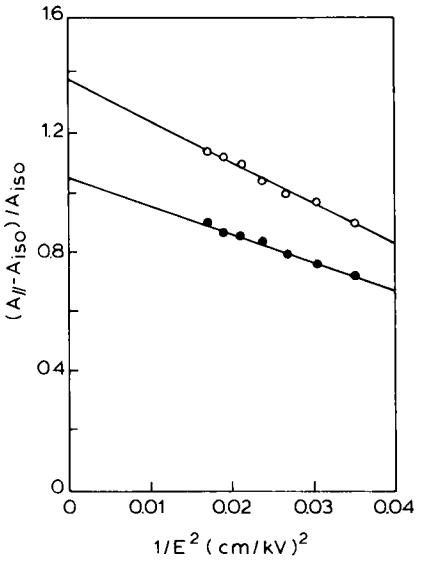

FIGURE 6 Linear dichroism of chlorosomes of *Chloroflexus aurantiacus* at 12.2°C plotted vs. $1/E^2$ to extrapolate to infinite field strengths. (○) 741 nm. (●) 461 nm. [Tris] = 0.67 mM. The drawn lines are the best linear fits.

To test whether a small contribution of a permanent dipole moment may lead to an underestimation of the extrapolated values we varied the concentration of Tris to change the relative contribution of the permanant dipole moment (Fig. 7 and Table I). In 10 mM Tris the signals were considerably smaller and a linear region for extrapolation could not be reached. For the different solutions with

TABLE I
AVERAGE ANGLES δ BETWEEN TRANSITION MOMENTS CORRESPONDING TO ABSORBANCE AT 741 AND 461 nm AND ORIENTATION AXIS AT DIFFERENT Tris CONCENTRATIONS

[Tris]*	δ (741 nm)	δ (461 nm)
mM	*degrees*	*degrees*
0.48	25.8 ± 1.9	33.9
0.67	26.5 ± 0.7	34.1 ± 1.4
1.9	27.9 ± 0.6	35.9 ± 0.8

*As determined by an $1/E^2$ extrapolation. Standard errors are given when measurements on different samples have been done under the same conditions. The standard errors in a single experiment have not been taken into account.

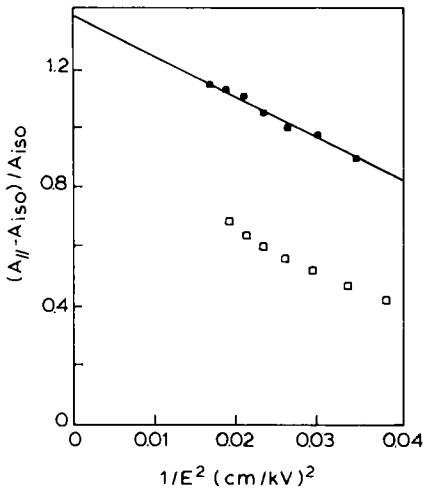

FIGURE 7 Electric field induced linear dichroism of chlorosomes of *Chloroflexus aurantiacus* at 12.2°C in solutions containing different concentrations of Tris. (□) 10 mM Tris (■) 0.67 mM Tris.

a low concentration of Tris a linear dependence is seen in all cases and similar results are obtained in all experiments. There seems to be a tendency to somewhat smaller angles when less Tris is used, suggesting a lower contribution of a permanent dipole moment in lower salt, as was also found in the case of double stranded DNA (Charney et al., 1982). The angles obtained in the lowest Tris-concentration was ~26 and 34° for the 741 and 461 nm bands, respectively. Standard errors are given in the table in the cases where several measurements have been performed. The errors in the individual measurements have not been taken into account.

The decay of the electric field–induced linear dichroism after the field is switched off is governed by the rotational diffusion coefficients of the chlorosomes around their principal rotational axes. The decay (not shown) appeared to be multiexponential, as one would expect when there is a distribution of lengths. Moreover, asymmetry of particles of the same length may also give rise to a multiexponential decay. As a first approximation a one-exponential fit is taken over the time region from the end of the electric field pulse until the signal has decreased 85%. We find that the decay time τ decreases from ~87 μs at 2 kV/cm to ~70 μs at ~8 kV/cm (T = 12.2°C), indicating that at low fields the larger particles are oriented better. Taking 70 μs, this leads to an average length of ~130 nm for the chlorosomes, assuming a diameter of ~25 nm and applying the models for rodlike molecules previously mentioned. The length must be smaller when the diameter is larger. Note that the large chlorosomes are oriented better on the average, and therefore they contribute more to the linear dichroism, increasing the average length measured. Even when we estimate the slowest decay time contributing to the decay curve, this decay time corresponds to a length of ~150 nm. Because the detergent will probably lead to an increase of the effective length of the chlorosomes, this measurement yields an upper limit for the average length of these chlorosomes. Our results support the conclusion by Golecki and Oelze (1987) that chlorosomes in fact contain a rather wide distribution of lengths. Moreover, we can conclude that for this particular batch of chlorosomes no aggregation has occurred.

DISCUSSION

As was shown in Results, the amount of dichroism in a compressed PAA gel as a function of the compression factor n can be described very accurately with the orientation function $\Phi(n)$ as derived in the Appendix. The calculated functional dependence is similar for rotationally symmetric rods and discs. Nonspherical rigid particles with another shape will probably show a similar orientational behavior.

The good fits for the 461- and 741-nm bands indicate that the gel deformation on a microscopic scale at the level of the chlorosome dimensions is similar to that on a macroscopic scale, i.e., $x' = x/n$ and $y' = n \cdot y$, and that the chlorosome orientation follows this deformation. Because 15 and 20% PAA gels with different average pore sizes lead to the same dichroism signals, it is reasonable to assume that the pores in the gel are small compared with the particle dimensions. The forces that the chlorosomes experience under influence of gel compression can be split into two contributions: a force that causes a rotation of the chlorosomes resulting in an average orientation, which is described by the orientation function $\Phi(n)$ and a force that tends to stretch the particle and which is discarded in the orientation model by assuming that the particles remain undeformed during compression. The experimental orientation curve can indeed be described assuming only rotation. Moreover the shape of the LD spectra remained unchanged during compression, indicating that no internal structural changes occur. For these reasons we believe that the chlorosomes remain undeformed.

The assumption of the volume being constant appeared to be correct because of the constantness of the cytochrome *c* absorption during compression. The 15 and 20% gels after compression with a factor 1.7 take their original shape when the pressure is released.

The formula presented is derived for very long rods and very flat discs. The chlorosomes with their axial ratios *a*:*b*:*c* of ~10:3:1 do not fulfill these requirements. They are mainly rodlike but they can also be considered as elongated discs. However, the ratio P(741nm)/$\Phi(n)$ is ~2.8, whereas it can be maximally 1.5 in the case of disclike particles, indicating that the chlorosomes are mainly oriented with their long axis along the direction of gel expansion.

It is assumed in calculating the angle ξ that the particles are rotationally symmetric, which is probably not true. Assuming that the *b*-axis is being oriented perpendicular to the direction of compression and of course perpendicular to the direction of expansion (orientation of *a*-axis) then in the limit of $n \rightarrow \infty$ dichroism signals will be obtained that

are different from those obtained with rotationally symmetric particles. Assuming all transition moments oriented in the plane through the *a*- and *b*-axis, the calculated angle of 17° at 741 nm would really be 14.7° whereas it would be 20.9° when all transition moments would be perpendicular to the *b*-axis. Similarly the determined angle of 27° at 461 nm could vary from 23.1 to 33.9°. However it seems very unlikely that all transition moments lie exactly in a plane through two main axes of the chlorosome and the error made by assuming a rotationally symmetric particle is probably much smaller. The interpretation of the calculated angles will be dealt with below.

Also orientation in electric fields led to clear dichroism signals with very reproducible amplitude and wavelength dependencies. The orientation is predominantly due to an induced dipole moment as could be concluded from the degree of dichroism P as a function of the electric field strength E in the entire region of E, in particular the linear dependence of P on $1/E^2$ at high fields and the shape of the dichroism pulses at low fields. This induced dipole moment is most likely largest along the long axis of the chlorosome. Therefore, the chlorosomes are expected to orient in a similar way as in compressed gels. Indeed, the LD spectra have a similar shape in the wavelength region that was measured, i.e., from 250 to 800 nm. Below 300 nm there is some deviation which must possibly be ascribed to some uncertainty in the base-line correction of the gel, the contribution of which starts to increase significantly in this region. However, the deviation near 800 nm cannot be ascribed to such an uncertainty and it is either due to a somewhat different orientation of the chlorosomes using both methods of a different structure of the chlorosomes in the gel (see below).

The calculated angles 26 ± 2° and 34 ± 2° for the 741- and 461-nm bands are larger than 17 ± 4° and 27 ± 3° obtained from the gel measurements. Aggregation of chlorosomes into less organized structures could lower the amount of dichroism, thereby leading to an increase of the corresponding angles. However, as shown in the results, from the calculated lengths we must conclude that no significant aggregation has taken place. Other chlorosome fractions with slower field free decay times, indicating aggregation, also showed lower LD (van Amerongen, unpublished results). The different values may be due partially to the contribution of a permanent dipole moment not necessarily oriented along the long axis of the chlorosomes, resulting in an orientation of the long axis not perfectly parallel to the field direction. Moreover, the presence of a small permanent dipole contribution can lead to a small curvature in the $A_\parallel - A_{iso}$ vs. $1/E^2$ curves. In that case the extrapolated values of the dichroism are an underestimation of the true values and therefore the real angles should be slightly smaller. The contribution of an induced dipole moment is expected to be larger as compared with the contribution of the permanent dipole moment when the salt concentration is lowered and indeed there is a tendency to smaller values of δ when the concentration of Tris is lowered.

In the gels the chlorosomes probably lose some Bchl *c*. The absorbance at 461 and 741 nm decreases a few percent, whereas the absorbance near 440 and 667 nm increases upon gel formation, possibly indicating an increase of free Bchl *c*. This effect most likely concerns BChl *c* molecules which are easily accessible to the reagents added when the gel is made. They are probably on the outside of the chlorosomes or free in solution with no preferred orientation, which is in agreement with the fact that there is no dichroism near 440 and 667 nm. However these BChl *c* molecules do contribute to the dichroic ratio in the electric field measurements thereby possibly lowering it a few percent as compared with the gel measurements and again leading to somewhat larger angles. We believe that for these reasons the average angles for the 461- and 741-nm transition moments are somewhat smaller than the values obtained with the electric field measurements. In light of these uncertainties the results of both methods are in reasonable agreement. For 741 nm the angle between the transition moment and the long axis of the chlorosome will be ~20° and for 461 nm it will be ~30°. Finally, these results indicate that the orientation of rod-like particles in gels can be described very reasonably with the formalism given originally by Ganago et al. and modified by us for this particular case.

Structure of the Chlorosomes

In this study we have concentrated mainly on two absorption bands namely those around 461 and 741 nm. The 741 nm band corresponds to the Q_y transition moment of BChl *c* lying in the plane of the porphyrin-ring system, in the ring I to ring III direction (Breton and Vermeglio, 1982). The 461-nm band corresponds to a higher excited state of BChl *c*, but other pigments (e.g., carotenoids) contribute to the absorption at this wavelength. The obtained angles should be considered as "average" orientations of all the transition moments absorbing at that particular wavelength in all the chlorosomes with respect to the long axis of the particles. These angles are ~20 and 30° for the 741- and 461-nm transition, respectively. These angles are significantly lower than the values reported earlier by Betti et al. (1982) for the 741-nm band, i.e., 40°, and also much lower than those given by Van Dorssen et al. (1986*a*), i.e., 37° for the 741-nm band and 39° for the 461-nm band measured in biaxially compressed gels, whereas the difference between the angles corresponding to both transitions is much higher in our case. For several reasons we do not believe that these differences reflect "normal" variations between chlorosome preparations and we shall compare the results now in more detail.

(*a*) As was already mentioned in the introduction, the angle of 40° for the 741-nm transition by Betti et al. (1982) can only be considered as an upper limit. Perfect orienta-

tion was assumed for the calculation, but as these authors already mention, this assumption is not necessarily true.

(*b*) Van Dorssen et al. (1986*a*) calculate their angles assuming perfect orientation at a relatively low degree of compression. The authors are supported in their idea of perfect orientation by the fact that they observed a maximum dichroic ratio in the Bchl *a* absorption band. However, this result is remarkably different from ours and from that obtained by Betti et al. (1982). In our gel measurement we find an angle of almost 70° and often a lower value. In the electric field measurements where no loss of Bchl *a* absorption occurs, the dichroism near 790 nm was close to zero, indicating an average orientation of the Bchl *a* Q_ys around the magic angle. Similarly, Betti et al. (1982) report no dichroism in the 790-nm band upon orientation in polyvinyl alcohol films.

A second point is the positive dichroism in the region 550–630 nm that we find with two orientation methods in accordance with the results of Betti et al. (1982) but in contrast to the dichroism measured by Van Dorssen et al. (1986*a*) at 77 K, which is negative or about zero. It seems unlikely that a sharpening of absorption bands upon cooling to 77 K leads to a sign reversal of the LD and a large change in the dichroic ratio over such a broad wavelength range. Maybe an uncertainty must be introduced concerning the exact position of their baseline. However, this would also imply that the dichroism of the 790-nm band was not maximal and that perfect orientation need not be achieved. Roughly applying the theory of Ganago et al. (1980) to the results of Van Dorssen et al. (1986*a*) up to a reduction of 30% of the gel thickness and assuming that the chlorosomes start to orient after a reduction of 3% of the gel thickness, leads to an angle of 19° for the 741-nm transition moment, in good agreement with our results.

(*c*) Our work suggests a different average angle with the long axis of the chlorosome for the 741-nm transition moment as compared with the 461-nm transition moment, where the difference is ~10°. In our case the reduced dichroism of the 461-nm band may be slightly contaminated by the lower dichroism of other bands. When we reinterpret the results of Van Dorssen et al. at 77 K where a better spectral resolution is obtained, using the theory of Ganago et al. (1980) we find indeed a smaller difference, i.e., about 5° (where it would only be 2° assuming perfect orientation of the chlorosomes in the gel), again indicating that the transition moments are not perfectly parallel to each other. This is also in agreement with the fluorescence depolarization measurements of Van Dorssen et al. (1986*a*). It should be noticed that the difference between the angles measured for the 461 nm and the 741 nm transition moments is not necessarily equal to the angle between the transition moments in one Bchl *c* molecule. It only reflects the difference in average angles with respect to the orientation axis of the chlorosome.

We propose that the small angles observed by us for the BChl *c* Q_y transition moments most directly reflect the local pigment organization. A model for the structure of the chlorosomes was suggested by Wechsler et al. (1985). The average angle in their model between the 741-nm transition moments (in the ring I to ring III direction (Breton and Vermeglio, 1982) and the long axis of the α-helical polypeptide is ~20°. The fact that we find a similar angle for the transition moments with the long axis of the chlorosome implies that these α-helical polypeptides must lie perfectly parallel to each other along the long axis of the chlorosome. This conclusion is also in accordance with the proposal of Wechsler et al. (1985) that these polypeptides form regular subunits of six dimers that correspond to the repeating subunits seen on electronmicrographs (Staehelin et al., 1978) and which are highly organized and extend the full length of the chlorosome.

APPENDIX

Orientation Model for Particles in a Compressed Gel

We shall present a model for the orientation of rodlike and disclike particles in a PAA gel, which is compressed in one direction and allowed to expand in one perpendicular direction. Given an arbitrarily chosen orientation of a transition moment within the particle, the linear dichroism is calculated for all degrees of compression. First, the calculation will be done for rodlike particles. Once this result is known, the calculation for disclike particles is relatively simple and is presented afterwards. The model and corresponding calculations are essentially the same as given by

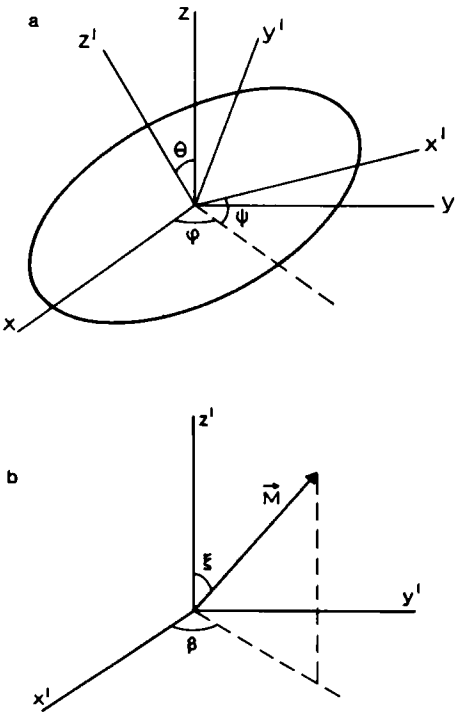

FIGURE 8 (*a*) Euler angles θ, ϕ, and ψ defining the orientation of a chlorosome coordinate system (x', y', z') with respect to the laboratorium coordinate system (x, y, z). (*b*) Orientation of a normalized transition moment **M** within the chlorosome coordinate system given by the angles ξ and β.

Ganago et al. (1980) for gel compression in two perpendicular directions, but the mathematics is more complicated.

A laboratory frame is defined with the x, y, and z-axis along the edges of the gel, which is a rectangular parallelopiped (see also Fig. 1). The measuring light-beam is incident along the z-axis. The gel is compressed in the x direction with a compression factor n and expands in the y direction with the same factor n, so the dimensions of the gel change in the same way: $l'_x = l_x/n$, $l'_y = l_y n$, $l'_z = l_z$.

Now consider a rodlike particle with a local coordinate system with a x'-, y'-, and z'-axis. The z'-axis is defined along the long axis of the particle. The x'- and y'-axis are defined perpendicular. They are chosen arbitrarily but are the same for all particles, which are assumed to be identical. The orientation of this coordiante system in the laboratory frame can be decribed using the Euler angles θ, ϕ, and ψ (see Fig. 8 *a*). The orientation of the transition moment μ in a rotational symmetric rodlike particle is given in Fig. 8 *b*, using the angles ξ and β, where **M** is the unit vector $\mu/|\mu|$.

Next we take a line in the gel through an arbitrarily chosen origin and a point (x_0, y_0, z_0), which has a distance of unit length from the origin and we shall describe how this line reorients in the gel upon compression. For comparison purposes we shall choose this line along the z'-axis in Fig. 8 *a* and we assume that upon compression a rodlike particle reorients in the same way as the line along the z'-axis and, in addition, that the particle is not deformed. Because the particle is rotational symmetric around the long axis, the disribution of ψ remains equiprobable. The angle between this line and the z-axis is given by $\tan\theta = \sqrt{x_0^2 + y_0^2}/z_0$. The line also determines the angle ϕ : $\tan\phi = -x_0/y_0$.

The following relations hold: $x_0 = \sin\theta\sin\phi$, $y_0 = -\sin\theta\cos\phi$, $z_0 = \cos\theta$. Upon deformation of a homogeneous gel, which is assumed to be continuous, the new coordinates of the point (x_0, y_0, z_0) become $(x_0/n, ny_0, z_0)$. The line defined above remains a straight line after compression and defines new angles θ' and ϕ' by

$$\tan\theta' = \sqrt{x_0^2/n^2 + n^2 y_0^2}/z_0 \text{ and } \tan\phi' = -x_0/(y_0 n^2) \text{ or}$$

$$\tan\theta' = \tan\theta\,\sqrt{(\sin^2\phi/n^2 + n^2\cos^2\phi)} \quad \text{(A1)}$$

$$\tan\phi' = \tan\phi/n^2. \quad \text{(A2)}$$

Relations A1 and A2 can be written in another way which is more useful in the following calculations.

$$\cos^2\theta' = \frac{n^2(1 + (n^4 - 1)\cos^2\phi)^{-1}\cos^2\theta}{(n^2(1 + (n^4 - 1)\cos^2\phi)^{-1} - 1)\cos^2\theta + 1} \quad \text{(A3)}$$

$$\cos^2\phi' = \frac{n^4\cos^2\phi}{(n^4 - 1)\cos^2\phi + 1}. \quad \text{(A4)}$$

The amount of absorbance A of polarized light is proportional to $(\mu.\mathbf{E})^2$, where **E** is the electric field vector of the incident light. For the degree of dichroism the following relation holds.

$$\frac{\Delta A}{3A} = \frac{A_y - A_x}{3A} = \frac{\langle\mu_y^2\rangle - \langle\mu_x^2\rangle}{\mu^2} = \langle M_y^2\rangle - \langle M_x^2\rangle.$$

A is the absorbance of the particles in an unoriented sample. $A_{x,y}$ is the absorbance of light polarized along resp the x- and y-axis. $M_{x,y}$, $\mu_{x,y}$ are the projections of **M** and μ upon the x- and y-axis. The brackets indicate that the average is taken over all particles. Their orientations are arbitrary before compression. M_x and M_y can be expressed in terms of ξ, β, θ', ϕ', and ψ' as follows:

$$M_x = \sin\xi\cos\beta(\cos\psi'\cos\phi' - \sin\psi'\cos\theta'\sin\phi') + \sin\xi\sin\beta(-\sin\psi'\cos\phi' - \cos\psi'\cos\theta'\sin\phi') + \cos\xi\sin\theta'\sin\phi' \quad \text{(A5)}$$

$$M_y = \sin\xi\cos\beta(\cos\psi'\sin\phi' + \sin\psi'\cos\theta'\cos\phi') + \sin\xi\sin\beta(-\sin\psi'\sin\phi' + \cos\psi'\cos\theta'\cos\phi') - \cos\xi\sin\theta'\cos\phi' \quad \text{(A6)}$$

Now $\langle M_y^2\rangle - \langle M_x^2\rangle$ can be calculated.

$$\langle M_y^2\rangle - \langle M_x^2\rangle = \frac{1}{8\pi^2}\int_0^\pi \sin\theta\,d\theta\int_0^{2\pi} d\phi\int_0^{2\pi} d\psi(M_y^2 - M_x^2).$$

Using Eqs. A5 and A6 this leads to

$$\langle M_y^2\rangle - \langle M_x^2\rangle = \frac{(3\cos^2\xi - 1)}{8\pi}\int_0^\pi d\theta\sin\theta \cdot \int_0^{2\pi} d\phi(2\cos^2\phi' - 1)(1 - \cos^2\theta'). \quad \text{(A7)}$$

The integration over ψ has already been carried out, where ψ' has been replaced by ψ. This is allowed because the distribution with respect to ψ remains the same during compression. Substituting Eqs. A3 and A4 leads eventually to the following relation.

$$\frac{\Delta A}{3A} = \left\langle\frac{1}{2}(3\cos^2\xi - 1)\right\rangle\Phi(n). \quad \text{(A8)}$$

The brackets denote an average over all values of ξ. $\Phi(n)$ is the orientation function which is 0 when $n = 1$ and which is 1 for $n = \infty$. The expression for $\Phi(n)$ is

$$\Phi(n) = \frac{n^4 + 1}{n^4 - 1}\left[1 - \frac{n(n^2 - 1)^2}{(n^4 + 1)|n^4 - 1|}\int_0^1 dx \cdot \frac{x^2 + 2(n/(n^2 - 1))^2}{(-x^4 + x^2 + (n/(n^2 - 1))^2)^{1/2}}\right]. \quad \text{(A9)}$$

This expression can be evaluated numerically. It can be easily shown that

$$\Phi(n) = \frac{4}{5}\frac{n^2 - 1}{n^2 + 1}, \quad \text{(A10)}$$

when $n \to 1$. When pressing in the y-direction allowing the gel to expand in the x-direction n can be replaced by $1/n$ leading to the same expression for $\Delta A/3A$ with a minus sign, as expected.

From Eq. A8 it can be concluded that for rodlike particles,

$$-\frac{3}{2} \le \frac{P}{\Phi(n)} \le 3.$$

$P = \Delta A/A$. For the case of disclike particles the situation is somewhat different. Now we define the z'-axis along the normal to the plane through the disc. We shall now assume that a plane in the gel containing the disc will remain a plane during compression but its orientation changes. The reorientation of the disc upon compression of the gel is described by following the orientation of the normal. Note that this normal is not a line of the gel and its orientation is different from the orientation of a line of the gel as just described. Two perpendicular unit vectors ν_1 and ν_2 are chosen in the plane of the disc (x', y') in the following way.

$$\nu_1 = \begin{pmatrix}\cos\phi\\ \sin\phi\\ 0\end{pmatrix} \text{ and } \nu_2 = \begin{pmatrix}-\cos\theta\sin\phi\\ \cos\theta\cos\phi\\ \sin\theta\end{pmatrix}$$

The cross-product $\nu_1 \times \nu_2 = \begin{pmatrix} \sin\theta \sin\phi \\ -\sin\theta \cos\phi \\ \cos\theta \end{pmatrix}$ is a unit-vector along the z'-axis. For determining the orientation of the plane after compression we first determine the change in the vectors ν_1 and ν_2 to resp ν_1' and ν_2'. Again using $(x', y', z') = (x/n, ny, z)$ it follows that

$$\nu_1' = \begin{pmatrix} \frac{1}{n}\cos\phi \\ n\sin\phi \\ 0 \end{pmatrix} \text{ and } \nu_2' = \begin{pmatrix} \frac{-1}{n}\cos\theta\sin\phi \\ n\cos\theta\cos\phi \\ \sin\theta \end{pmatrix}$$

The direction of the normal to the plane is determined by the cross-product

$$\nu_1' \times \nu_2' = \begin{pmatrix} n\sin\phi\sin\theta \\ -\cos\phi\sin\theta/n \\ \cos\theta \end{pmatrix}.$$

Replacing n by $1/n$ we have again the same mathematics as in the case of rods and this leads to the following relation.

$$\frac{\Delta A}{3A} = -\left\langle \frac{1}{2}(3\cos^2\xi - 1) \right\rangle \Phi(n). \qquad \text{(A11)}$$

$\Phi(n)$ is given in expression A9. Now ξ is the angle between the transition moment and the normal to the disc. It follows that for disclike particles, $-3 \leq P/\Phi(n) \leq \frac{1}{2}$. This result is different from the result for rodlike particles. If $P/\Phi(n) < -\frac{1}{2}$ the particle must be disclike and if $P/\Phi(n) > \frac{1}{2}$ it must be rodlike.

The chlorosomes of *Chloroflexus aurantiacus* were expertly isolated by Mr. F. Zonneveld. The contribution of Mr. F. van Mourik to the construction of the gel press is gratefully acknowledged. Mr. C. Robertus and Mrs. M. Timmerman were involved in initiating and performing a large number of the experiments described in this paper. We thank Dr. J. Amesz for helpful comments on the first version of this manuscript.

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