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LINEAR DICHROISM OF ELECTRIC FIELD ORIENTED BACTERIOCHLOROPHYLL *a*-PROTEIN FROM GREEN PHOTOSYNTHETIC BACTERIA

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Summary

Bacteriochlorophyll *a*-protein from *Prosthecochloris aestuarii* strain 2K was oriented in a pulsed electric field. The room temperature linear dichroism spectrum of the oriented protein in the Q_y region of the bacteriochlorophyll *a* absorption exhibits a single asymmetrical peak at 813 nm with a shoulder extending to the blue. The $\approx$12 nm fullwidth of the linear dichroism peak is only about half that of the 300 K absorption spectrum. The linear dichroism at 813 nm was not saturated at field strengths of up to 15 kV/cm. The time dependence of the linear dichroism suggests that the orienting particles are aggregates of at least some tens of bacteriochlorophyll *a*-protein trimers. The linear dichroism peak coincides in wavelength with the 813-nm peak of the 300 K, 4th derivative absorption spectrum of the protein and is therefore attributed to the bacteriochlorophyll *a* Q_y exciton transition observed in absorption at the same wavelength.

Introduction

Linear dichroism (LD) spectra have been obtained for a large number of photosynthetic pigment complexes, ranging from whole chloroplasts to purified reaction centers [1—6]. LD spectra provide information on the orientation of certain electronic transition moments within these complexes. In some cases these are the transition moments of individual chromophores, in others

Abbreviations: Bchl, bacteriochlorophyll; CD, circular dichroism; LD, linear dichroism

the moments of collective excitations, i.e. exciton states. In general, an electronic excitation in a complex of interacting pigment molecules is neither purely localized nor purely delocalized but is some mixture of the two. What mixture is determined by the relative magnitudes of the energy of the resonant or exciton interaction between pigments and the spread in transition energies of the pigments due either to chemical or environmental differences. In the complexes whose LD spectra have been studied, not enough details of the arrangement of pigment molecules are known to allow calculation of the magnitude of exciton interaction energies. This complicates the interpretation of these LD spectra. We therefore undertook the measurement of the LD spectrum of a Bchl *a* complex for which it is established that the peak separations observed in absorption and CD spectra are accounted for by calculated exciton interaction energies. In this case the transition moments involved ought to be those of the exciton states rather than those of the individual chromophores.

The Bchl *a*-protein isolated from the green photosynthetic bacterium, *Prosthecochloris aestuarii* strain 2K, is so far the only chlorophyll-protein whose three-dimensional structure has been determined by single crystal X-ray diffraction analysis [7—9]. The protein consists of three identical subunits each containing seven Bchl *a* molecules with the three subunits arranged around a 3-fold symmetry axis. The positions and orientations of the chlorophylls within the Bchl *a*-protein are known [10], have been used to calculate the exciton interaction energies [9], and can, in principle, be used to calculate [11] the exciton-state energy levels of the Bchl *a* core [8,9,12]. Experimental evidence for the existence of exciton states in the Bchl *a*-protein comes from the absorption and CD spectra [12—15]. The 300 K absorption spectrum shows only a single broad, unresolved peak at 809 nm for the Bchl *a* Q_y transition [7,12]. The 300 K 4th derivative absorption spectrum reveals three peaks, at 824, 813, and 801.5 nm [15], which correspond to the 825, 815, and merged 805 and 800 nm peaks of the 5 K spectrum [15]. The latter peaks, together with other features of the low temperature absorption and CD spectra that extend from about 790 to 825 nm [12—15], have been interpreted as corresponding to exciton levels. The large magnitude of the essentially conservative CD features relative to the CD of Bchl *a* in solution is most simply interpreted as the result of exciton interactions among the Bchl *a* molecules in the protein [12].

We report here the measurement of the LD spectrum of the Bchl *a*-protein from *P. aestuarii* oriented by a pulsed electric field. Although it has been reported that microcrystals of the Bchl *a*-protein exhibit LD at the specific wavelengths 605 and 809 nm [16], no wavelength dependence of the effect in Bchl *a*-protein oriented by any means has yet been determined. Our measurements are confined to the spectral region corresponding to the Bchl *a* Q_y transition in the Bchl *a*-protein, that is from about 770 to about 840 nm, because this is the physiologically most important transition (to the lowest excited singlet state of the Bchl *a*) and the one best characterized in earlier spectroscopic work [7,12—15]. We also measured the electric field and time dependences of the LD. We interpret the LD spectral results in terms of the assignment of exciton levels in the Bchl *a*-protein.

Materials and Methods

The green photosynthetic bacterium, *P. aestuarii* strain 2K, was raised in a mixed culture (ref. 17 and Shaw, E.K. and Olson, J.M., personal communication) from an inoculum kindly furnished by J.M. Olson. The cells were harvested and the Bchl *a*-protein extracted and purified ($A_{209}/A_{371} > 2.0$, $A_{267}/A_{371} < 0.7$) as described previously [7]. The purified protein in 0.2 M NaCl and 10 mM Tris · HCl (pH 8.0) was concentrated by ultrafiltration to approx. 1 g/l and stored in a refrigerator in the dark until use.

Because of design limitations of the pulser and the necessity to avoid overheating the sample, it was not possible to measure LD on samples in the high ionic strength solutions in which the protein is most stable. A procedure that appears to satisfy both the requirements of protein stability and electric field orientation consists of dialyzing overnight the concentrated high-salt solution of Bchl *a*-protein against a solution of 0.1 mM NaCl and 5 mM Tris in distilled water, then diluting the dialysate with a 2.5 mM Tris solution to produce a sample of desired absorbance at 809 nm. Final concentrations are about 0.01 mM NaCl, 2.5 mM Tris (pH approx. 9) and 0.5 μM Bchl *a*-protein. Samples prepared in this way give current densities of 1—2 A/cm^2 for an applied field of 13 kV/cm, depending on the precise final NaCl concentration and the amount of dissolved CO_2. Absorption spectra (360—900 nm) of these low ionic strength preparations at ambient temperature are indistinguishable from those taken with high-salt preparations. CD spectra in the Q_y region show no significant change when the salt concentration is reduced essentially to zero, which suggests that the Bchl *a*-protein conformation is insensitive to salt concentration. All LD measurements were performed on the diluted, low-salt dialysates.

Absorption spectra were measured on a Cary 14 spectrophotometer in its visible light mode with a standard sample holding arrangement. LD measurements were done with the same instrument, but modified in several ways. A new sample cell holder was constructed to fit in the sample chamber. The sample cells, 1 cm quartz cuvettes, were held by phosphor bronze springs in a water-cooled copper assembly. The field electrodes were cut from 0.051 cm thick platinum sheet. They were held 0.15 cm apart by Teflon spacers at the top and bottom and had an effective area of 1.00 × 1.05 cm. The sample beam was masked so that only light passing between the electrodes was measured. A Glan-Foucault polarizing prism through which the sample beam passed could be electrically rotated to polarize the light parallel or perpendicular to the electric field direction. The photomultiplier in the spectrometer was replaced by a light pipe leading to an external RCA C31034 photomultiplier (GaAs cathode) cooled by dry ice and methanol. Timing signals indicating when the light was directed through the sample and reference chambers were taken from the grids of the relay drivers in the Cary 14. From these signals, gate pulses were derived which triggered the high-voltage pulser and gated on various scalers counting the photons passing through the sample, with and without the electric field, and through the reference chamber.

Pulsed high voltage to the platinum electrodes was supplied by an Instrument Research Co. laboratory pulse generator. The pulser furnished about

2 A at 2.3 kV for pulses of 250 μs duration. Voltage across the electrodes was monitored with a voltage divider and oscilloscope. The source within the pulser is a charged 8.3 μF capacitor, so for low sample resistance the pulse waveform was not perfectly rectangular (see Results). Pulse repetition rates were limited by sample heating and the pulser duty cycle. The sample was pulsed at a rate of one pulse every 8.5 s (1/256 of the Cary 30 Hz chopping rate) in order to allow the energy of up to 1 J deposited by each pulse to dissipate. The transient temperature changes tend to cancel for the measurement of LD, which depends on the difference of two absorbances (see below). On the other hand, the time-dependent measurements, which involve only a single absorbance, may show some temperature effects.

The linear dichroism is defined * as the difference in absorbance of light polarized parallel and perpendicular to the direction of the field,

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

where either absorbance is given by

$$A = -0.4343 \ln(I/I_0) \tag{2}$$

In Eqn. 2, I is the transmitted and I_0 the incident light intensity. If $I_{\parallel}$ and $I_{\perp}$ are the transmitted intensities for light polarized parallel and perpendicular to the field direction, and if the intensity changes induced by the field are small, one can write

$$\Delta A = (A_{\parallel} - A) - (A_{\perp} - A)$$

$$\simeq -0.4343\left[\frac{I_{\parallel}(\epsilon)}{I_{\parallel}(0)} - \frac{I_{\perp}(\epsilon)}{I_{\perp}(0)}\right] \tag{3}$$

where ϵ denotes electric field strength and we have expanded the logarithms for small absorbance changes. If the incident intensity is independent of the direction of polarization the expression can be further simplified. However, the output from our monochromator is partly polarized so that the transmitted intensity without field is different for the two directions of polarization. As long as the incident intensity does not change with field, knowledge of the reference intensity I_0 is not required for LD calculations.

For LD spectra we measured the four quantities $I_{\parallel}(\epsilon)$, $I_{\parallel}(0)$, $I_{\perp}(\epsilon)$, and $I_{\perp}(0)$ at each of a number of wavelengths. The Cary monochromator was stopped at each of these wavelengths and the electric field pulsed a number of times, typically 4, at 8.5-s intervals. At each wavelength the monochromator slit width was adjusted manually to give a predetermined photon counting rate for $I_{\parallel}(0)$. The reference intensity was found to be quite stable so no reference correction was required. Upon completion of the pulsing sequence, counts accumulated in the scalers were printed out on a Teletype unit. The Cary monochromator was then set to a new wavelength and the process repeated.

* Gagliano et al. [6] define $A_{\parallel}$ as absorbance parallel to the electrodes rather than the field in order to ease comparison with magnetic field alignment results. This definition merely changes the sign of ΔA in Eqn. (1), and hence of the LD.

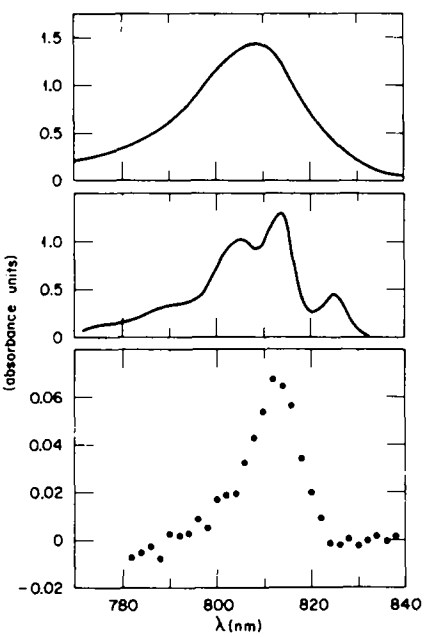
A number of scans were made in this way with alternate polarizations of the exciting light on successive scans. After each complete scan the electrodes were raised and lowered in the cuvette several times to agitate the sample and thereby at least partially reverse the effect of plating out the Bchl *a*-protein on the electrodes. Reversing the direction of the electric field on alternate pulses was ineffective in this regard. Except for a slight loss of signal after each scan, the LD spectrum was otherwise quite reproducible.

The modified Cary 14 instrument was tested with fragments of chloroplasts isolated from Good King Henry (*Chenopodium Bonus-Henricus* L.) [18]. The subchloroplast fragments were prepared by the method of Gagliano et al. [6]. The LD spectrum of these particles obtained with our instrument was essentially identical to the one they reported.

Measurements of LD time dependence were made with a Nuclear Data model 2400 multichannel analyzer operated in its multiscaling mode with 10 μs per channel. Alternate sweeps with and without the field pulse were stored in separate memory sections. The multiscale start signal was provided by the photon counting gate circuit.

Results

The LD spectrum of the Bchl *a*-protein is shown in Fig. 1 along with the absorption spectrum of the same sample, both taken at ≈300 K. The LD spectrum displays a single asymmetrical peak at 813 nm with a shoulder extending to the blue; the absorption maximum is at 809 nm. In addition,



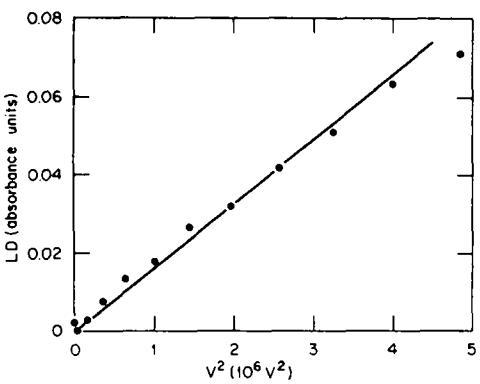


Fig. 1. LD spectrum (lower curve) of the Bchl *a*-protein at 300 K. The standard deviation at each wavelength for Poisson photon-counting statistics is 0.004 absorbance unit. The upper curve is the 300 K absorption spectrum of the same sample, and the middle curve is the 77 K absorption from ref. 14.

Fig. 2. LD at 813 nm versus the square of the applied voltage. Electrode separation was 0.15 cm.

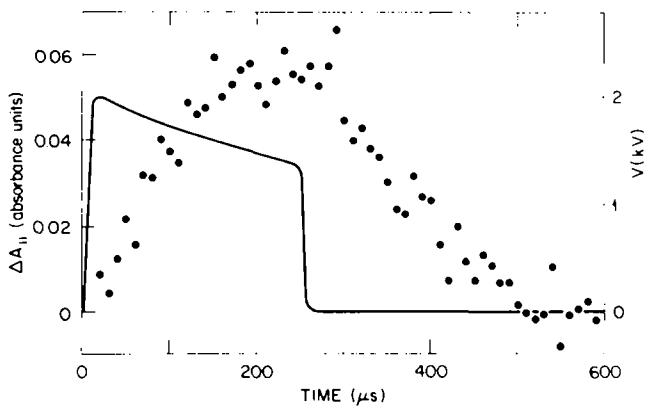


Fig. 3. Time dependence of $\Delta A_{\parallel} \equiv A_{\parallel} - A$ (see text) at 813 nm (left scale) and of the high voltage pulse (right scale).

the LD peak has only about half the full width at half maximum of the absorption peak (≈12 nm versus 26 nm). The significance of these aspects of the LD spectrum is made clearer by comparison with the 77 K absorption spectrum of the Bchl *a*-protein, also shown (after Olson et al. [14]) in Fig. 1. It can be seen that the LD peak coincides in wavelength with the highest peak in the 77 K absorption peak, and that the width of the former is nearly twice that of the latter. The LD peak also coincides in wavelength with the most prominent peak in the 300 K 4th derivative absorption spectrum [15]. We interpret these results in the next section.

The electric field dependence of the LD at 813 nm (Fig. 2) is observed to be approximately quadratic, which is characteristic of orientation due either to permanent or induced dipole moments [19]. There may be a slight tendency away from quadratic field dependence at the higher field strengths.

Additional information about the size of the particles can be obtained from the time dependence of the dichroism signal. Because of the large number of sweeps required to obtain good statistics, only $I_{\parallel}(\epsilon,t)$ and $I_{\parallel}(0,t)$ were measured as functions of time. The results are shown in Fig. 3 along with the time dependence of the field pulse, for a measurement at 813 nm. The rise of the dichroism signal appears to be somewhat faster than the decay, which has a time constant of about 140 μs (obtained from the time to decay to half the initial value, divided by ln 2). In addition to the effects shown, there was a much slower and smaller effect on $I_{\parallel}(\epsilon,t)$ having the opposite sign, a duration of more than 10 ms and an unknown origin. Since only $I_{\parallel}$ was measured as a function of time, it is possible the slow signal resulted from a change in the total absorption, for example due to transient sample heating, which would normally affect the parallel and perpendicular components in such a way as to cancel from the LD itself.

Discussion

Our results demonstrate, we believe, that only one of the seven Q_y exciton states of a Bchl *a*-protein subunit contributes appreciably to the 300 K LD

spectrum. This conclusion is based on the fact that the LD peak coincides in position with the 813 nm exciton peak of the 300 K 4th derivative absorption spectrum [15] and the most prominent peak in the low temperature absorption spectra [12–15]. Moreover, the LD peak is much narrower than the 300 K absorption band. The latter point requires some elaboration.

The full width at half maximum of the LD peak is about 180 cm^{-1}, which is somewhat less than the value of kT (equivalent to 208 cm^{-1}) at 300 K. If one assumes that for a molecule as large as chlorophyll the electronic ground state has a quasi-continuum of vibrational sublevels, then the width of a transition from the ground state to a single Q_y exciton level must be $\gtrsim kT$. The width of the 77 K absorption line at 813.5 nm is about 100 cm^{-1}; at 77 K, kT is about 50 cm^{-1}. If the 300 K LD peak is primarily a temperature-broadened representation of the 77 K, 813.5-nm absorption line, one expects the width of the LD peak to be $\gtrsim kT \simeq 200$ cm^{-1}. That the actual width of the LD peak is a little narrower than this may be due to some partial overlap on the 813-nm state of an adjacent exciton state of opposite dichroic sign. Such an overlapping state could account in part for the particularly rapid fall on the long wavelength side of the LD peak. On the other hand, the much more slowly falling short wavelength side may be broadened by an overlapping state of like dichroic sign. In either case, it seems clear that the LD peak is intrinsically as narrow as the 77 K, 813.5-nm absorption line, except for temperature broadening. Since the narrowness of the 77 K absorption lines is thought to be at least partially the result of exciton narrowing [12,20], the same may be said of the 300 K LD peak.

The time dependence of the LD effect indicates that the aligned particles are substantially larger than individual protein trimers. We applied Benoit's method of analysis (based on Perrin's equation) [19] to the decay of the LD, assuming that the aligned particles are highly elongated aggregates. For a 140 μs decay time this analysis shows that the particles must contain at least 36 trimers. If the aggregates are not highly elongated they must each contain substantially more trimers. The nonexponentiality of the decay suggests that these aggregates are poly-disperse. The rise of the LD signal depends on the orientation mechanism as well as on the time dependence of the electric field. Benoit showed in the small field approximation that for rectangular field pulses induced dipoles align as rapidly as they decay, while permanent dipoles align more slowly. We solved Benoit's equations for the time dependence of our field pulses, which are not rectangular. The time dependence of the LD effect (Fig. 3) is more closely fit with Benoit's induced dipole model. The absence of saturation of the LD with field strength (up to 15 kV/cm) would yield an upper limit to the size of the orienting particles if their shape were known [21,22]. We can only say, therefore, that the particles we have aligned in the electric field are aggregates of at least some tens of Bchl *a*-protein trimers.

We have presented evidence that the LD peak we observe at 300 K arises from one of the seven exciton states in the Bchl *a* Q_y electronic transition of the Bchl *a*-protein. The interpretation of LD in terms of exciton transitions, rather than the transitions of individual chromophores, is relatively straightforward for this chlorophyll complex in which the exciton splittings are observable and in agreement with theory. We conclude that for other, less

well characterized, chlorophyll complexes one should be cautious in deciding what mixture of localized or collective excitations determines observed LD features.

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References

1 Sauer, K. and Calvin, M. (1962) J. Mol. Biol. 4, 451—466
2 Geacintov, N.E., van Nostrand, F., Becker, J.F. and Tinkel, J.B. (1972) Biochim. Biophys. Acta 267, 65—79
3 Geacintov, N.E., van Nostrand, F. and Becker, J.F. (1974) Biochim. Biophys. Acta 347, 443—463
4 Breton, J., Michel-Villaz, M. and Paillotin, G. (1973) Biochim. Biophys. Acta 314, 42—56
5 Vermeglio, A. and Clayton, R.K. (1976) Biochim. Biophys. Acta 449, 500—515
6 Gagliano, A., Geacintov, N.E. and Breton, J. (1977) Biochim. Biophys. Acta 461, 460—474
7 Olson, J.M. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), Plenum Press, New York, in press
8 Fenna, R.E. and Matthews, B.W. (1975) Nature 258, 573—577
9 Fenna, R.E. and Matthews, B.W. (1977) Brookhaven Symp. Biol. 28, 170—182
10 Fenna, R.E., Ten Eyck, L.F. and Matthews, B.W. (1977) Biochem. Biophys. Res. Commun. 75, 751—755
11 Hemenger, R.P. (1977) J. Chem. Phys. 66, 1795—1801
12 Philipson, K.D. and Sauer, K. (1972) Biochemistry 11, 1880—1885
13 Olson, J.M., Philipson, K.D. and Sauer, K. (1973) Biochim. Biophys. Acta 292, 206—217
14 Olson, J.M., Ke, B. and Thompson, K.H. (1976) Biochim. Biophys. Acta 430, 524—537
15 Whitten, W.B., Nairn, J.A. and Pearlstein, R.M. (1978) Biochim. Biophys. Acta 503, 251—262
16 Olson, R.A., Jennings, W.H. and Olson, J.M. (1969) Arch. Biochem. Biophys. 129, 30—41
17 Bose, S.K. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L.P., eds.), pp. 501—510, Antioch Press, Yellow Springs, Ohio
18 Arnold, W.A. and Azzi, J.R. (1977) Plant Physiol. 60, 449—451
19 Benoit, H. (1951) Ann. Phys. (Paris) 6, 561—609
20 Hemenger, R.P. (1977) J. Chem. Phys. 67, 262—264
21 O'Konski, C.T., Yoshioka, K. and Orttung, W.H. (1959) J. Phys. Chem. 63, 1558—1563
22 Stratton, J.A. (1941) Electromagnetic Theory, p. 211, McGraw-Hill, New York