# Macroorganization of Chlorophyll *a*/*b* Light-Harvesting Complex in Thylakoids and Aggregates: Information from Circular Differential Scattering†

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ABSTRACT: Circular dichroism (CD) and magnetic circular dichroism (MCD) spectra were recorded for spinach thylakoids and for isolated, aggregated chlorophyll *a*/*b* light-harvesting pigment–protein complex, in random and magnetically aligned states of orientation at room and low temperatures. The shape and magnitude of the CD signal of most bands strongly depended on the orientation of the thylakoid membranes or the aggregated pigment–protein complex. In both thylakoids and aggregated light-harvesting complexes, however, the MCD spectra of the two different orientations were almost identical. Random and magnetically aligned samples exhibited anomalous, large CD signals outside the bands of pigment absorbance. Lack of similarity between the corresponding MCD and CD spectra showed that the large CD signals are not produced as a distortion of CD of absorbance by light scattering. Instead, these anomalous spectral features are believed to originate in differential selective scattering of circularly polarized light. Our results lead to the conclusion that the light-harvesting pigment–protein complex in thylakoid grana forms a helical macroarray with dimensions commensurate with the wavelengths of the anomalous circular dichroism signals. A hypothesis is put forward suggesting a role for these macrodomains in granal organization.

Circular dichroism (CD)[1] is a powerful technique, yielding structural information on biological systems. CD of absorbance (CDA), an intrinsic property of chiral molecules, may also arise from short- or long-range coupling between chromophores of macromolecules, aggregates, or complexes (Moscowitz, 1962; Tinoco, 1962; Pearlstein, 1982; Keller & Bustamante, 1986). In larger particles such as DNA and viruses, an additional signal, circular differential scattering (CDS), emerges which is characteristic of the long-range chiral organization in the particle (Bustamante et al., 1983). CDS and CDA carry important but distinct information concerning the molecular architecture of the specimen. However, they can significantly overlap, and in a regular dichrograph, the two signals are combined together into a net CD signal, the deconvolution of which may prove difficult. The CD signal from turbid samples containing macroscopic scatterers can also be influenced by nonpolarized light scattering; distortions from this source must be taken into account in the interpretation of spectral data.

Chloroplasts and thylakoids exhibit an anomalously intense CD signal which cannot be recomposed from the CD spectra of constituent photosynthetic pigment–protein complexes [for a recent review, see Garab et al. (1987)]. An involvement of light scattering in this CD signal was first recognized by Philipson and Sauer (1973) and investigated in more detail by Gregory and Raps (1974) and Faludi-Daniel et al. (1978). The data, however, do not allow firm statements to be made concerning the significance of the light-scattering component in defining the overall CD signal.

In recent years, progress has been made in understanding the physical nature and characteristics of CDS in large biological particles. Circular intensity differential scattering was shown to occur in microscopic particles (Maestre et al., 1982), and the theory of this phenomenon has been developed (Bustamante et al., 1980, 1984; Patterson et al., 1986). It seemed timely, therefore, to reinvestigate the question of possible scattering distortions in the CD signal and the putative contribution of CDS to the dichroism of chloroplasts. Because of a correlation between the large CD signal of chloroplasts and their content of LHC (Gregory et al., 1980; Faludi-Daniel & Mustardy, 1983), this question must also be addressed in the case of the large CD signal of isolated, aggregated LHC.

The experimental approach adopted here was to compare the effects of sample orientation on signals from the fringes or "tails" of the major CD bands and from the bands themselves. CD outside the absorption bands was found to change sign upon magnetically orienting a suspension of chloroplasts or LHC aggregates. The CD signal in the tails is CDS (Keller et al., 1985). CDS also contributes to the CD signal inside the absorption bands where it may be accompanied by a psi-type CD signal (Keller & Bustamante, 1986). These anomalous CD signals are believed to originate in helically organized domains. CD changes driven by photochemistry can also be correlated with the CDS component of the CD signal, as will be discussed in the following paper (Garab et al., 1988).

## Experimental Procedures

Chloroplasts were isolated as described by Slovacek and Hind (1977) in medium containing 0.4 M sorbitol, 3 mM

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[1] Abbreviations: CD, circular dichroism; CDA, circular dichroism of absorbance; CDS, circular differential scattering, $(I_L - I_R)/(I_L + I_R)$, where $I_L$ and $I_R$ are the intensities scattered upon illumination with left and right circularly polarized light, respectively; Chl, chlorophyll; LHC, chlorophyll *a*/*b* light-harvesting pigment–protein complex of photosystem II; MCD, magnetic circular dichroism; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine.

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$MgCl_2$, and 20 mM Tricine, pH 7.8; the same medium was used for resuspension and storage until use. Thylakoids were prepared from chloroplasts by osmotic shock in distilled water. The reaction medium consisted of 100 mM sucrose, 20 mM NaCl, 10 mM $MgCl_2$, 30 mM Tricine, pH 7.7, chloroplasts or thylakoids at the desired Chl concentration, and 60% glycerol when the samples were prepared for low-temperature measurements. No marked difference was seen in data from chloroplasts and thylakoids.

Some nonlinearities were observed in the dependence of different CD bands on Chl concentration. Typically, less than 30% deviation from linearity occurred between 5 and 60 $\mu$g of Chl/mL for a 1-cm path length. The major characteristics of the CD spectra, namely, the position and sign of the bands as well as the presence of the long tails outside the absorbance bands, persist at a Chl concentration of 2 $\mu$g/mL in both randomly and magnetically aligned samples.

Isolation and purification of LHC were carried out by the method of Burke et al. (1978). The $Mg^{2+}$-precipitated LHC aggregate (>1 mg of Chl/mL) was gently dispersed in a sonic cleaning bath in order to remove macroscopic aggregates. The suspension was diluted to the desired Chl concentration and gently sonicated in a medium containing 10 mM Tricine, pH 8.0, 25 mM KCl, 10 mM $MgCl_2$, 0.1% Triton X-100, and 60% glycerol for low-temperature samples; it was incubated in this medium for 10 min at 4 °C and filtered through a nylon mesh.

CD and MCD spectra at room and low temperatures were recorded in the apparatus previously described (Sutherland et al., 1976; Sutherland, 1981). CD and MCD signals are expressed in absorbance units. The magnetic field, with vector parallel to the measuring beam, was used to orient both the chloroplasts (Geacintov et al., 1972) and the aggregated LHC (Kiss et al., 1986) and to generate MCD in the samples. MCD spectra at low temperatures were recorded for random and magnetically aligned chloroplasts, but at room temperature, the MCD for randomly oriented chloroplasts was not measurable since the particles quickly underwent orientation in the field. In fact, care was required even at low temperatures to achieve proper rigidity of the sample before application of the magnetic field.

The dichrograph was operated under the control of a Tektronix 4051 computer. Spectra were smoothed by a 7-point moving polynomial function (Savitzky & Golay, 1964).

Relative changes of the transmitted light intensity upon magnetically aligning the chloroplasts at room temperature were measured by bypassing the 50-kHz circuit carrying the CD signal and recording the anode current from the dichrograph operating at constant photomultiplier voltage.

## Results and Discussion

### *Isolated Chloroplasts*

*Dependence of CD on the Orientation of Chloroplasts.* Figure 1 shows that aligned and randomly oriented chloroplasts exhibit very different net CD spectra. The spectrum of the randomly oriented sample is essentially identical with those in the literature [e.g., see Gregory et al. (1980)]. In comparison, the CD spectrum of the magnetically oriented sample is inverted in sign outside the absorption bands, between 520 and 600 nm as well as above 700 nm.

A small contribution of MCD to the spectrum of the magnetically aligned sample was removed by the following procedure. As judged from the magnetic field dependence of the transmission of the suspension, about 0.5 T field strength is sufficient to align chloroplasts (Garab et al., 1986); MCD, on the other hand, is linearly proportional to the magnetic field

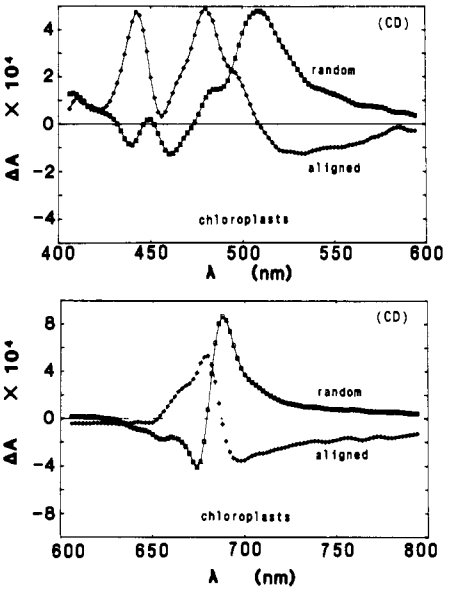


FIGURE 1: Net CD spectra of isolated chloroplasts at room temperature in a random (□) or magnetically aligned (♦) state of orientation. The latter spectra are corrected for MCD as described in the text. Chlorophyll concentration, 20 $\mu$g/mL; optical path length, 1 cm.

intensity [cf. Sutherland and Holmquist (1980)]. MCD-free CD spectra were thus obtained by subtracting the spectrum recorded at 1 T from twice the amplitude of that obtained at 0.5 T. The "new" bands in the CD spectrum of magnetically aligned chloroplasts (Figure 1) were shown in this way not to originate in MCD.

The origin of the anomalous long-wavelength tails in the CD signal from suspensions containing scattering particles is now well understood. Distortions from macroscopic linear dichroism are not involved, since neither the random sample nor that magnetically aligned with the field vector parallel to the measuring beam shows detectable linear dichroism. Instead, these tails originate in CDS produced by large chiral aggregates (Bustamante et al., 1983; Keller & Bustamante, 1986). Our observation that the sign of the CD signal is changed upon magnetic orientation of the chloroplasts is in harmony with the result of a theoretical analysis (Keller et al., 1985) describing the behavior of a CDS signal upon reorientation of a scattering helical array. Inversion of the CD signal is not confined to these tails but can clearly be recognized in different regions within the absorbance bands, e.g., between 660 and 680 nm as well as between 690 and 700 nm. Similar changes occur in the Soret region.

CD changes induced by the magnetic field could not be attributed to field-induced microstructural rearrangements within the thylakoid membrane for the following reason. The time course of relaxation of the CD changes (not shown) after turning off the magnetic field was identical with that of the nonpolarized transmission changes. The relaxation half-time, typically about 20 s, was similar to the value determined from the relaxation of polarized fluorescence emission (unpublished observation) and is indicative of rotational randomization of macroscopic particles of several micrometers radius [cf. also Geacintov et al. (1972) and Garab et al. (1981)]. Because of the preferentially in-plane orientation of Chl *a* $Q_y$ dipoles between 660 and 700 nm (Haworth et al., 1982), the absorbance cross section around 680 nm increases by 20–25% upon alignment. However, the observed radical changes in the CD spectrum (Figure 1) including band shifts and inversions are qualitatively different and could not arise from alteration in the absorbance cross section.

On the other hand, the relative intensity of scattered light can change with orientation, due to the lamellar structure and intrinsic anisotropy of chloroplasts (Swenberg & Geacintov, 1976). Upon magnetic alignment, large variations in the efficiency of scattering in the forward direction were observed (not shown). Fractional changes in transmission were largest outside the absorbance bands where interference from the dipole orientation does not occur, exceeding 50% between 700 and 800 nm. However, as with the CD changes, the transmission changes upon magnetic alignment of chloroplasts were not confined to the spectral regions outside the principal absorbance bands. The transmission around 690 nm, i.e., around the positive peak in the randomly oriented suspension, increases despite the fact that the absorbing Chl *a* dipoles are in-plane-oriented. A significant increase of transmission was also detected between 660 and 670 nm where the expected increase in the absorbance cross section due to the orientation of dipoles was small relative to the increase in the transmitted light due to the variations in the scattering efficiency. Similar transmission changes occurring in parallel with the CD changes were observed in the green spectral regions.

Changes in scattering efficiency would be expected to influence the proportion of CDS signal in the total apparent CD signal. It must be emphasized, however, that the CDS component is not simply modulated upon magnetic alignment but actually changes sign (as seen in the long-wavelength tail of Figure 1). The angular distribution of CDS must be markedly different from that of nonpolarized scattering (Bustamante et al., 1980). Nonpolarized scattering data thus provide no simple prediction concerning the CDS changes that accompany chloroplast orientation.

The contribution of scattering is much larger on the long-wavelength slope of absorbance bands than outside the bands as a consequence of the presence of selective light scattering, which in chloroplasts peaks around 690 nm and around 510 nm (Latimer & Rabinowitch, 1959; Bialek et al., 1977). Thus, in these regions, i.e., around the major CD peaks, a significant contribution of CDS to the CD signal can be expected. The data in Figure 1 clearly show that the CD signal within the principal bands experiences inversion in sign along with the sign inversion of the CDS signal outside the absorption bands. This strongly implies that CDS, originating in a macrohelical array, has a predominant role in defining the major CD bands around 690 and 510 nm in chloroplasts. The CD signals from within and outside of the principal absorption bands were also seen to be interrelated when CD changes were driven by photochemistry [see Garab et al. (1988)] rather than by orientation.

An alternative interpretation of the phenomena discussed above is that the CD changes result both from CDS and from a correlated psi-type CD signal within the absorption bands. Keller and Bustamante (1986) suggest that such a psi-type CD anomaly would depend on the presence of a larger macrohelical array ($>\lambda/4$ rather than $>\lambda/20$) and densely packed chromophores ($\sim 1/nm^3$). Because of the high density of pigments in the thylakoid and the importance of energy-transfer interactions (the range of which is extended by the intrinsic anisotropy of dipoles with respect to the membrane plane), it is probable that psi-type CD bands make some, as yet undetermined, contribution to the CD signal in chloroplasts.

The CD spectrum at −55 °C (Figure 2) shows that the long tails above 700 nm, and between 520 and 600 nm, are considerably diminished both in random chloroplasts and in chloroplasts frozen in a magnetically aligned state. However,

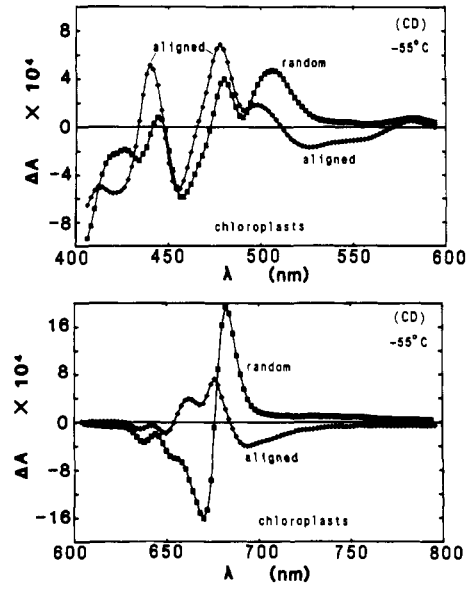


FIGURE 2: Net CD spectra of isolated chloroplasts frozen in a random (■) or aligned (♦) state of orientation. Chlorophyll concentration, 180 μg/mL; optical path length, 2 mm. Alignment of chloroplasts was performed in a magnetic field of 1 T applied before and during freezing; spectra were recorded at 0 T field and at −55 °C.

the pronounced differences between the spectra of the two different orientational states remain essentially unaffected by low temperature (cf. Figure 1). Minor differences between the spectra in Figures 1 and 2 are attributable to the presence of glycerol (data not shown) and may be due to a decrease in the intensity of scattering or of CDS.

The low-temperature CD spectra (Figure 2) also reveal the presence of certain bands which do not correlate with CDS changes observed upon alignment of the sample, and thus presumably originate from CDA. Bands around 640 and 650 nm, for example, are closely similar to those observed in isolated LHC (Gregory et al., 1980; see also below) and are believed to originate from excitonic interaction of Chl *b* molecules arranged as a trimer (Van Metter, 1977; Shepanski & Knox, 1981). The negative and positive bands around 460 and 480 nm are also only marginally affected by alignment and may be correlated with the 640- and 650-nm bands.

Chloroplasts should theoretically exhibit more CDA bands, corresponding to those observed in the isolated complexes. Although these bands are not resolved in the present work, we show that deconvolution of CDA bands from the complex CD signal is at least theoretically possible.

*MCD of Randomly and Magnetically Oriented Chloroplasts.* Low-temperature MCD spectra of chloroplasts with different orientational states provide firm evidence that the large CD signal of chloroplasts does not result from distortion of CDA bands by light scattering. MCD is a CDA signal whose shape can be reliably predicted on the basis of in vitro Chl *a* and *b* MCD spectra (Houssier & Sauer, 1970; Breton & Hilaire, 1972). Figure 3 shows that the MCD signals generated in the two samples of different orientational states, with radically different apparent CD spectra, are nonetheless similar. Both spectra contain characteristic bands: the split bands of Chl *a* and *b* around 435 and 470 nm, respectively, and the Chl *a* bands at 580, 620, and above 660 nm. Small differences between the two MCD spectra could not be correlated with differences between the CD spectra of the corresponding samples; for instance, the MCD band assignable to the Chl *a* $Q_y(0-0)$ transition [cf. Houssier and Sauer (1970)] is a more intense positive band in the magnetically aligned than in the random state, even though the CD of the

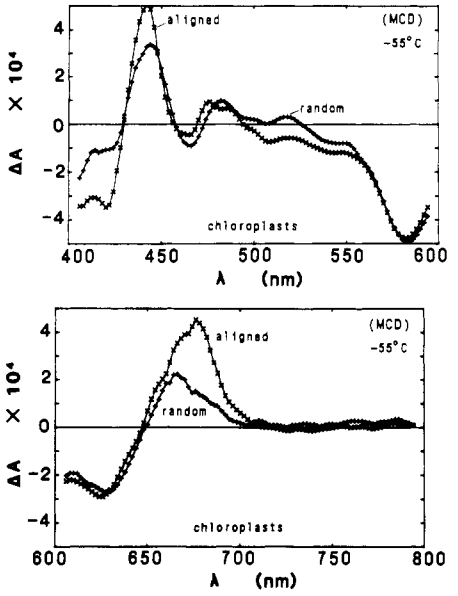


FIGURE 3: MCD spectra of a chloroplast suspension frozen in random (+) or magnetically aligned (×) states of orientation. The spectra were obtained as the difference between the corresponding spectra recorded at 1.0 and 0 T magnetic field strengths. Spectra at 0 T are shown in Figure 2.

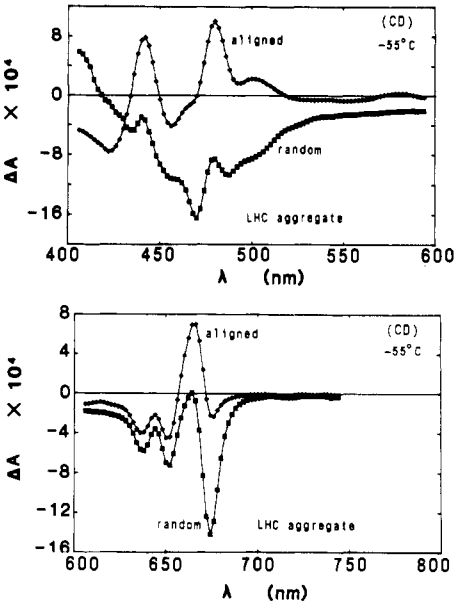


FIGURE 4: Net CD spectra of aggregated LHC frozen in random (□) or aligned (♦) states of orientation. Chlorophyll concentration and conditions for alignment and measurement are as described for Figure 2.

oriented sample becomes negative above 680 nm.

MCD would be expected to show some sensitivity to magnetic alignment owing to the orientational anisotropy of thylakoid pigments with respect to the membrane plane and consequent changes in the absorbance cross section and in the orientation of the porphyrin planes with respect to the field vector [cf. Sutherland (1978)]. The observed changes in MCD spectra upon alignment are explicable by the assumption that the porphyrin heads (not just the $Q_y$ dipoles) of Chl *a* molecules having higher wavelength red absorbance maxima are oriented at an angle closer to the membrane plane than are the heads of molecules absorbing at shorter red wavelengths, as is well established (Breton, 1985; Garab et al., 1986). MCD on oriented samples combined with linear dichroism studies may prove to be a suitable nondestructive method for obtaining information on the orientation of porphyrin heads of Chl *a* molecules responsible for different composite absorbance bands above 660 nm.

*Aggregated LHC*

Good correlation has been found between the LHC content and the characteristic large CD signal of thylakoids (Faludi-Daniel & Mustardy, 1983). The amplitude of the CD of aggregated LHC is almost as large as that of the chloroplasts (Gregory et al., 1980). This complex is known to form a three-dimensional liquid-crystalline structure on aggregation (Kuhlbrandt, 1984; Li, 1985) which may give rise to CDS and/or psi-type CD (Keller & Bustamante, 1986) indicative of long-range supermolecular chiral order. Given the correlation between the LHC content of chloroplasts and the large CD signal, the question arises as to how much of the CDS signal identifiable in chloroplasts can be assigned to the LHC aggregate. Again, the effect of particle orientation coupled with investigation of the long tails provides an experimental approach to the problem.

LHC aggregates are known to be oriented with their lamellar planes perpendicular to the magnetic field (Kiss et al., 1986), yielding samples free of linear dichroism in the geometry employed here. Because of the somewhat lower diamagnetic susceptibility of these aggregates (Kiss et al., 1986) compared to the chloroplasts, it was not possible to record MCD-free spectra of the LHC aggregate at room temperature. The presence of CDS outside the absorbance, however, was clearly revealed by a long tail above 700 nm (not shown). The sign of this tail, though initially opposite to that found in chloroplasts, could be inverted upon magnetic alignment of the aggregate. The relative amplitude of the CD signal at 700 nm was about 10% of the negative peak of the random suspension. Upon alignment, the main CD band around 678 nm was strikingly diminished. Similar observations were made by Ganago et al. (1983) with the LHC aggregate embedded in a polyacrylamide gel. Upon squeezing the gel and measuring the CD in a geometry giving no interference from linear dichroism, the intense negative peak near 680 nm disappeared, and the positive band broadened.

The low-temperature spectra shown in Figure 4 for the two orientation states of aggregated LHC are markedly different. As in the case of room temperature data, the main CD band at around 675 nm is the most influenced by orientation. CD spectral changes induced by alignment are extensive and more complex in the Soret region. As in the case of chloroplasts, CDS, alone or in accompaniment with psi-type CD, is presumed to play an important role in defining the CD spectrum of the LHC aggregate. Gregory et al. (1980) were first to note that the most intense bands in the red and around 510 nm are of opposite sign in chloroplasts and in the aggregated LHC. The suggestion was made that such sign inversion reflects an opposite helicity. While our data do not exclude this possibility, the sensitivity of the CDS component to parameters such as acceptance angle and pitch/wavelength ratio (Bustamante et al., 1985; Hall et al., 1985) must be considered as a likely cause, especially at a relatively wide acceptance angle. Measurements aimed at characterizing the angular distribution of CDS would be required to resolve this issue and are in progress.

Aggregated LHC clearly possesses a number of bands assignable to CDA on the basis of orientation independence (this interpretation resting, as before, on the assumption that CDS within spectral bands and in spectral tails will be comparably responsive to orientation). Bands at around 460, 475, 640, and 650 nm (Figure 4) were also identified in the chloroplast (Figure 2) as CDA bands. A band at 662 nm in LHC is

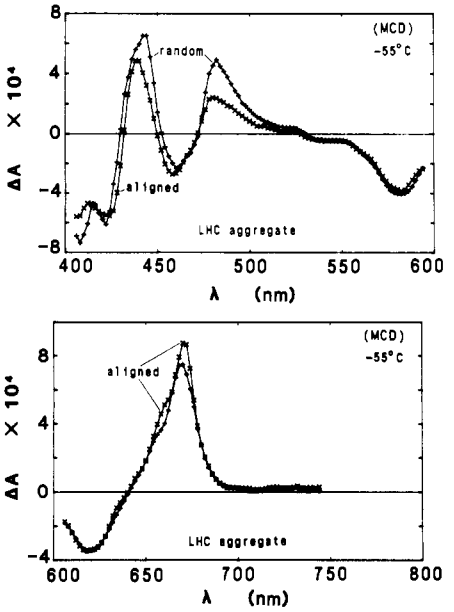


FIGURE 5: MCD spectra of aggregated LHC frozen in a random (+) or magnetically aligned (×) state of orientation. The spectra for MCD were recorded at 1.0 and 0 T field strengths and substracted. Corresponding spectra at 0 T are shown in Figure 4.

overlapped by the major negative 675-nm band in the random state and may itself be independent of orientation. It could be one of the bands suggested by Van Metter (1977) to originate from a Chl *b* trimer. The occurrence of further CDA bands above 660 nm, e.g., from excitonic interactions between Chl *a* and Chl *b* [cf. Gulen et al. (1986)], cannot be ruled out.

The MCD bands (Figure 5) were quite insensitive to the actual shape of the CD spectrum and were very similar to those in chloroplasts. Notable differences between the two were the absence of long-wavelength MCD in LHC, owing to a lack of photosystem I Chl *a* molecules, and the relative enrichment of LHC in the 480-nm Chl *b* band. As noted above, the independence of MCD from the CD spectra reaffirms our views that CDA bands are not distorted significantly by light scattering and can be extracted from the observed CD spectra even in the presence of intense CDS bands.

Physical parameters of the structural feature(s) giving rise to the intense CDS signal in the thylakoid membrane and the isolated LHC aggregate remain to be determined by study of the signal's angular dependence. A preliminary conclusion regarding these features is, however, that in thylakoids and in LHC aggregates there exists a long-range chiral array, the dimension of this array being comparable to the wavelength where CDS is detected, as theory predicts (Bustamante et al., 1984; Keller et al., 1985).

It is well established that aggregated LHC forms large domains where the adjacent "monomeric" complexes are arranged with respect to each other in an ordered fashion (Kuhlbrandt, 1984; Li, 1985). To understand the existence of a macrohelical array, we may speculate that it is generated in such a way that the well-defined orientation of the transition moment vectors and a nonrandom spatial arrangement of pigment binding sites play an equally important role. The former condition clearly applies (Breton, 1985; Garab et al., 1987) while the latter may be fulfilled by a liquid-crystal structure.

In thylakoids, a close correlation has been established between the large anomalous CD signal and the LHC content of the grana (Faludi-Daniel & Mustardy, 1983); thus, the "backbone" of the large domains can be identified as a macroarray of the LHC. This notion can be supported by diamagnetic susceptibility measurements of isolated LHC aggregates of differing dimensions, and thylakoids of differing LHC content. A clear correlation was evident between the magnetic susceptibility of the thylakoids and their LHC content (Kiss et al., 1986). Data from alignment of chloroplasts and LHC aggregates in an electrical field (J. G. Kiss, G. Garab, and A. Faludi-Daniel, unpublished results) also support the above conclusion regarding the existence of large LHC domains in the thylakoid membrane.

Studies on an LHC-deficient mutant of barley, and on the greening of plants rendered deficient in LHC by growth in intermittent light, have given strong circumstantial evidence of a direct role for LHC in granal organization (Arntzen et al., 1976). Its high self-assembly capacity presumably allows LHC to form a macroscopic network in which other complexes constituting the thylakoid grana are elastically embedded.

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# Reversible Changes in Macroorganization of the Light-Harvesting Chlorophyll *a*/*b* Pigment–Protein Complex Detected by Circular Dichroism†

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ABSTRACT: Light-induced changes in circular dichroism (CD) were studied in thylakoids isolated from spinach. The following features of CD responses occurring in the time range of 10 s to 1–3 min were noted: (i) The kinetics and relative amplitudes of the responses are similar over broad spectral ranges surrounding the major CD bands, i.e., between 670 and 760 nm and between 480 and 550 nm. This applies not only to randomly oriented samples but also to magnetically aligned membranes having markedly different CD spectra in the dark. (ii) Photosystem I is much more effective than photosystem II and can drive a 40–80% decrease in CD signal relative to the dark control level. (iii) Photosystem I driven changes are fully inhibited by nigericin or $NH_4Cl$ but are largely insensitive to gramicidin. CD changes driven by photosystem II, on the other hand, are sensitive to all of these reagents. (iv) The CD responses can be shown to originate in circular differential scattering rather than in circular differential absorbance. They can also be distinguished from light-induced, nonpolarized scattering changes. The data are qualitatively evaluated with respect to the theory of circular differential scattering of large helically organized macroaggregates, the size of which is commensurate with the wavelength of the measuring beam [Bustamante, C., Maestre, M. F., & Keller, D. (1985) *Biopolymers 24*, 1595–1612]. The observed decrease of the large CD signal is ascribed to a partial loss of macrohelicity in the light-harvesting chlorophyll *a*/*b* protein complex, in response to a proton gradient and/or surface electrical field generated most effectively by photosystem I.

Thylakoid membranes and their component complexes undergo conformational changes in response to illumination [see review by Barber (1982)]. Current understanding of these dynamic events is far from complete owing to a limited availability of techniques suited to the study of ultrastructural rearrangements within membranes.

In the preceding paper (Garab et al., 1988), we showed that the circular dichroism (CD)[1] of thylakoids is sensitive to the

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[1] Abbreviations: CD, circular dichroism; CDA, circular dichroism of absorbance; CDS, circular differential scattering; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC, light-harvesting chlorophyll *a*/*b* pigment–protein complex of photosystem II; PMS, *N*-methylphenazonium methosulfate; FCCP, carbonyl cyanide *p*-(trifluoromethyl)phenylhydrazone.

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