Size Dependency of Circular Dichroism in Macroaggregates of Photosynthetic Pigment–Protein Complexes†

Virginijus Barzda, László Mustárdy, and Győző Garab*

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, P.O. Box 521, H-6701, Hungary

Received January 11, 1994; Revised Manuscript Received June 13, 1994®

ABSTRACT: Large molecular aggregates, condensed biological macromolecules, intact membrane systems, and cell organelles often exhibit intense anomalous circular dichroism (CD) bands which are absent in systems of lower structural complexity. Theory predicts that in dense, chirally organized macroaggregates the size of the aggregate controls the magnitude of the anomalous CD bands [Keller, D., & Bustamante, C. (1986) *J. Chem. Phys. 84,* 2972–2979]. Photosynthetic pigment–protein complexes in their native thylakoid membranes and *in vitro* were used to provide direct experimental evidence of the size dependency of CD in macroaggregates.

Circular dichroism (CD)[1] spectroscopy is a powerful, noninvasive technique to obtain structural information in samples of biological origin. CD is the difference in absorption between left-hand and right-hand circularly polarized light. It arises from certain asymmetries (chirality) of structure, which can be intra- or intermolecular. Nearly all molecules and complexes that are synthesized in biological systems are chiral (Cantor & Schimmel, 1980).

The intrinsic CD of a small molecule, for a single electronic transition, has the same shape as the absorption, and its sign is determined by the handedness of the molecule (Cantor & Schimmel, 1980). In molecular complexes or small aggregates, CD is generally induced by short-range, excitonic coupling between chromophores (Tinoco, 1962; DeVoe, 1963). Excitonic interactions give rise to a conservative band structure (i.e., the positive and negative bands of the split spectrum are represented with equal areas). In DNA aggregates (Reich et al., 1991), sperm heads (Maestre et al., 1982), and condensed chromatins (Livolant & Maestre, 1988), much stronger CD signals have been observed, with nonconservative, anomalously shaped bands accompanied by long tails outside the absorbance. The CD in these macroaggregates has been attributed to long-range chiral organization of the chromophores.

Theory predicts that in polymer- and salt-induced (psi) aggregates the magnitude of the anomalous CD, at a constant density of chromophores and a constant pitch of the macrohelix, is controlled by the volume of the aggregate (Keller & Bustamante, 1986). A very strong size dependency of CD has been demonstrated in a mathematical model of a twisted cubic lattice of polarizable groups (Kim et al., 1986). However, to our knowledge, no direct experimental evidence has been presented to test this theoretical prediction.

In chlorophyll-containing systems, the structural parameters can be varied in a broad interval, between solutions of monomeric chlorophylls (Chls) and large arrays of pigment dipoles in macroaggregates of pigment–protein complexes. In photosynthetic membranes, Chls are noncovalently bound to proteins. In all pigment–protein complexes the Chls are present at high density and fixed stoichiometry (Zuber, 1987). The molecular architecture of many complexes is well characterized, and all transition dipoles of Chls posses a well-defined orientation angle with respect to the protein axis and the plane of their native membrane (Breton & Vermeglio, 1982; Garab et al., 1987). The structure of the bacterial reaction center complex is known at atomic resolution (Deisenhofer et al., 1985). This has opened up new theoretical vistas in the field of Chl organization *in vivo,* and the possibility of interpreting spectroscopic data in relation to exact structural parameters.

The structure of the light-harvesting Chl *a*/*b* pigment–protein complex (LHCII), which is one of the most abundant proteins in the biosphere, has been determined at 3.4-Å resolution (Kühlbrandt et al., 1994). In LHCII, (i) the chromophore density is high, and the transition dipoles are coupled to each other via energy transfer, which can extend to long distances, (ii) the Chl dipoles are aligned with respect to the protein axis and the sheets of macroaggregates (Kiss et al., 1986; Garab et al., 1987), and (iii) in the macroaggregate, the trimeric organization (Butler & Kühlbrandt, 1988) and/or asymmetric adhesion of trimers may introduce long-range chirality. Hence, three-dimensional aggregates of LHCII larger than one-quarter of the wavelength of the incident light (Kim et al., 1986) are expected to exhibit psi-type CD.

In Chl-containing systems, three levels of molecular organization can be distinguished, which possess strikingly distinct CD characteristics and physical origin. (i) In monomeric solutions of these planar tetrapyrrole molecules the intrinsic CD is very weak (Dratz et al., 1966). (ii) In pigment–protein complexes, Chls typically exhibit CD with a conservative band structure. This CD is about 1 order of magnitude stronger than the intrinsic CD of Chls and arises from excitonic interactions (Pearlstein, 1982). (iii) In general thylakoid membranes (Gregory et al., 1972) and in macroaggregates of LHCII (Gregory et al., 1980; Garab et al., 1988a), nonconservative CD signals with extremely large amplitudes have been observed. CD in chloroplasts has been shown to be combined with differential scattering of left and right circularly polarized light (Philipson & Sauer, 1973). Differential scattering has been shown to be superimposed on

† This work was supported by Grants OTKA III/2999 and III/887 from the Hungarian Research Fund. CD and absorbance measurements were carried out on the dichrograph and spectrophotometer of the OTKA Regional Center for Scientific Instruments.

* To whom correspondence should be addressed.

® Abstract published in *Advance ACS Abstracts,* August 1, 1994.

[1] Abbreviations: CD, circular dichroism; Chl, chlorophyll; LHCII, light-harvesting chlorophyll *a*/*b* pigment–protein complex; psi, polymer- and salt-induced.

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the "classical" CD bands and the anomalous CD has been shown to originate from long-range coupling of chromophores of chirally organized macrodomains (Garab et al., 1988b; Finzi et al., 1989).

In the work presented here, we have investigated the CD of pigment–protein complexes in different aggregation states both in thylakoid membranes and in isolated LHCII. We provide direct experimental evidence, to our knowledge for the first time in a biological system, that the magnitude of the major CD bands increases with the size of the aggregates.

EXPERIMENTAL PROCEDURES

Chloroplast thylakoid membranes were isolated from 2-week-old dwarf pea (*Pisum sativum* L. cv. Rajnai törpe) leaves grown in the greenhouse, with an isolation procedure described earlier (Garab et al., 1988a). LHCII was prepared from pea chloroplasts (Burke et al., 1978).

Thylakoid membranes were cross-linked with 8 mM disuccinimidyl tartrate (DST) (Pierce) at 4 °C for 10 min; the reaction was stopped with 15 mM ammonium acetate. The density of samples was adjusted to $A_{680-730} = 0.8$ in a cell of 0.1 cm optical path length. The membranes were solubilized with 0.25% (v/v) Triton X-100, and the suspension was stirred for 30 min at room temperature, then loaded onto a 0.1–2.0 M linear sucrose gradient containing 0.025% (v/v) Triton X-100 and centrifuged for 30 min at 70000*g*. Fractions (0.5 mL) were collected, and absorbances characteristic of Chl *a* and *b* were measured at 672 and 652 nm, respectively.

LHCII aggregates were cross-linked with 0.5 mM dithiobis-(succinimidyl propionate, DSP) (Pierce) for 20 min at 4 °C. The reaction was stopped with 1.5 mM *N*-ethylmaleimide and the suspension was loaded onto a 0.1–1.6 M linear sucrose gradient containing 0.025% (v/v) Triton X-100 and centrifuged at 70000*g* for 20 min.

The sizes of the aggregates were determined in an Opton 902 electron microscope with negative staining technique and in a Leitz Laborlux S fluorescent and light microscope. Aliquots of LHCII were taken from the suspension used in the CD measurement or from different fractions collected after cross-linking and centrifugation. The sizes of the aggregates in the thylakoids were determined by the same methods, in samples after the cross-linking and solubilization of membranes.

The diamagnetic susceptibility anisotropy of LHCII aggregates was determined as described earlier (Papp & Meszéna, 1982; Kiss et al., 1986). The membranes have been shown to align in a magnetic field due to the net torque on a nonrandom distribution of the diamagnetically anisotropic molecules (Knox & Davidovich, 1978). Because of the nonrandom orientation of the Q_y transition dipoles with respect to the membrane plane, the intensities of the fluorescence emission polarized parallel to or perpendicular to the membrane plane differ from each other (Geacintov et al., 1972). Hence, the diamagnetic susceptibility anisotropy can be calculated on the basis of the dependence of the dichroism of the fluorescence emission on the strength of the magnetic field (Knox & Davidovich, 1978).

CD spectra were recorded in a Jobin-Yvon CD6 dichrograph. CD is expressed in absorbance units. The distortion of CD by linear dichroism has been shown to be small (Garab et al., 1991a). Absorption spectra were determined in a Shimadzu UV3000 spectrophotometer.

In spectroscopic measurements of LHCII dilute, homogeneous suspensions were always used. In experiments starting from purified LHCII, microcrystals were dissolved with detergents or by mild sonication. When cations were added, special care was taken to avoid the inhomogeneity of the samples and the appearance of microcrystals.

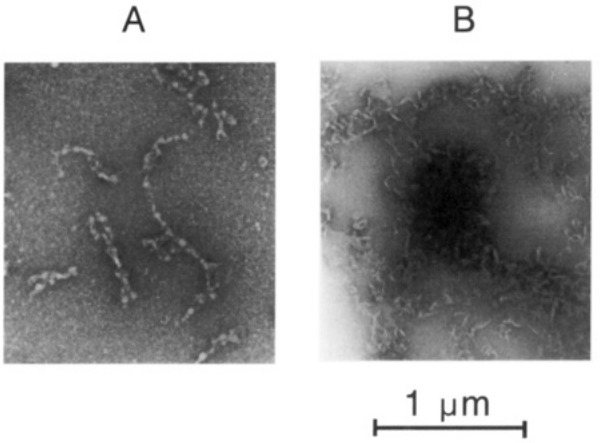

FIGURE 1: Electron micrographs of negatively stained preparations obtained after cross-linking and partial solubilization of thylakoid membranes. Before cross-linking, the membranes were suspended in 10 mM tricine (pH = 7.8) in the absence (**A**) or presence (**B**) of 5 mM $MgCl_2$. (For further details, see Experimental Procedures).

RESULTS AND DISCUSSION

Aggregates in Chloroplast Thylakoid Membranes. As shown in Figure 1A, for a cross-linked and solubilized sample, membranes suspended in low-salt buffer contain isolated small particles and short filaments. In contrast, in preparations in which thylakoid membranes were suspended in the presence of Mg ions, we observed large densely stained areas which indicate the presence of macroaggregates whose size approximated the diameter of the granum membrane sheets (Figure 1B). Since the density of negatively stained electron micrographs is sensitive to the thickness of the sample on the grid, the dense areas suggest that in these regions the macroaggregates possess three-dimensional structure.

These findings are in good agreement with freeze–fracture electron microscopic results. In chloroplasts, two types of particles with diameters of about 8–12 nm and 15–18 nm have been shown to be embedded in the thylakoid membranes (Armond et al., 1977). In the absence of cations, the two types of particles are uniformly distributed on the freeze–fracture surfaces (Staehelin, 1976). Chloroplasts suspended in hypotonic, low-salt buffer lose their granal ultrastructure but retain their Chl content and photochemical activity (data not shown). In these membranes, no macroaggregates of particles could be detected with the cross-linker, which spans 0.65 nm (Figure 1A). In the presence of Mg ions, the partition region of stacked membranes is enriched in large particles (Staehelin, 1976) containing a photosystem 2 reaction center surrounded by LHCII and other antenna complexes. LHCII, which is on the periphery of the photosystem 2 particle, mediates stacking of adjacent membranes via electrostatic interactions (Barber, 1982). The finding that in the presence of Mg ions the membranes contain macroaggregates (Figure 1B) indicates that the particles in the stacked region are coupled to each other in a three-dimensional network. Large, crystallinelike two-dimensional arrays of pigment–protein complexes were earlier observed in membranes of photosynthetic purple bacteria (Miller, 1982).

The size distribution of cross-linked particles revealed two major populations (Figure 2). Small aggregates of particles which hardly penetrated into the gradient could be seen in both samples. In contrast, large aggregates were found only

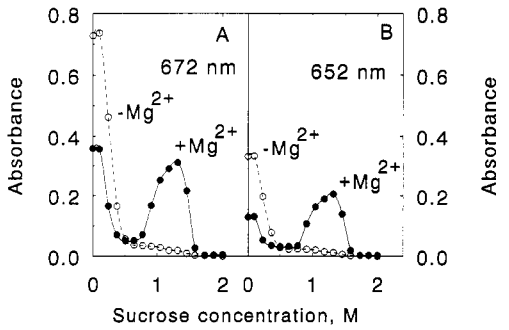

FIGURE 2: Size distribution of cross-linked aggregates prepared from thylakoid membranes suspended in the absence (---) or presence (—) of 5 mM $MgCl_2$. The absorbances of fractions collected after sucrose gradient centrifugation were measured at 672 nm (**A**) and 652 nm (**B**), respectively.

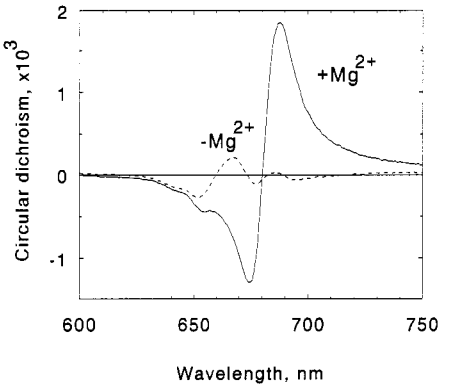

FIGURE 3: CD spectra of thylakoid membranes in the absence (---) or presence (—) of 5 mM $MgCl_2$. Concentration of Chl (a+b), 20 μg/mL; optical path length, 1 cm.

in preparations from thylakoids suspended in the presence of Mg ions. The average diameter of the macroaggregates which form the packed area was found to be 300–600 nm. It is important to note that the fractions containing macroaggregates of particles were enriched in Chl *b*. This is evident from a comparison of the absorbances measured near the absorbance maxima of Chl a (672 nm) and Chl b (652 nm), respectively (compare the relative amplitudes at low and high sucrose concentrations in Figure 2, panels A and B). (The Chl *b* enrichment in the macroaggregates was confirmed in acetonic extracts of the pigments.) Since LHCII contain about half of the Chl *a* and almost all of the Chl *b*, this finding is consistent with the central role of LHCII in the stacking of adjacent membranes via electrostatic interactions (Barber, 1982).

CD spectra of membranes in the presence and absence of Mg ions are shown in Figure 3. In the sample in which the particles were not aggregated, the CD was small and its band structure appeared conservative. The spectrum was dominated by bands originating from excitonic interactions in LHCII (Van Metter, 1977; Hemelrijk et al., 1992), which is the most abundant Chl-containing complex in chloroplasts. The CD spectra in other pigment–protein complexes are similar in magnitude and spectral features to that in LHCII (Bassi et al., 1985). Hence, the CD of thylakoid membranes in the absence of Mg ions can be interpreted as the weighted sum of the CD spectra of the complexes.

Upon the formation of grana in the presence of Mg ions, the magnitude of the CD increased dramatically (Figure 3). Very intense positive and negative bands emerged around 685 and 674 nm, respectively. The membranes also showed a long-tail anomaly, i.e., an intense CD above 700 nm [and also between 500 and 600 nm (not shown)]. This long tail indicates the occurrence of the differential scattering of left and right circularly polarized light (Keller & Bustamante, 1986; Philipson & Sauer, 1973; Garab et al., 1988a,b). In contrast with these large changes, the shape and intensity of the band at around (–)650 nm, which is the "fingerprint" of excitonic interactions between Chl *b* molecules in LHCII (Van Metter, 1977), appeared unchanged.

The large aggregation-induced CD could, in principle, be explained by short-range interactions, e.g., due to excitonic interactions between molecules on neighboring particles, and a distortion by differential scattering. However, these assumptions seem quite unlikely: (i) There is no indication for the occurrence of intense CD due to excitonic interactions between molecules on neighboring complexes even inside the particles. The complexes in the particle are coupled to each other and capable of supplying the reaction center with excitation energy with good efficiency. This suggests that most of the short-range interactions are present within the individual particles. Furthermore, the particles are stable aggregates of more than a dozen complexes, whereas the adhesion between the particles is evidently weaker. Thus, it is unlikely that aggregation of particles would induce strong Chl–Chl interactions which could account for the big CD. (ii) Differential scattering has been shown not to distort the CD substantially but to be superimposed on the signal (Garab et al., 1988b).

Indeed, recent experimental evidence ruled out the possibility that the big CD originates from short-range excitonic interactions. In a differential polarization microscope, the positive and negative CD signals were found to be spatially separated (Finzi et al., 1989). Microspectropolarimetry revealed the existence of giant broad-banded positive and negative signals of psi-type origin. More recently, the large positive and negative CD bands have been separated in macroscopic measurements (Garab et al., 1991b).

It is important to point out that, although the particles themselves are already relatively large aggregates of pigment–protein complexes, which constitute a densely packed array of interacting dipoles, they do not exhibit anomalous CD. In fact, this is in accordance with expectations, since the size of the particles (15–18 nm) is too small for psi-type CD. The threshold value is about $\lambda/4$, λ being the wavelength where the anomalous CD band peaks (Keller & Bustamante, 1986).

Another interesting feature of this system is that, in contrast with other condensed proteins and/or DNA aggregates, the "classical" CD bands remain clearly discernible and appear unperturbed by the macroaggregation [see also Garab et al. (1988a, 1991b)].

Aggregates of the Light-Harvesting Chl a/b Complex. Systematic investigations showed that, in homogeneous suspensions of aggregated LHCII, cations increased while detergents decreased the amplitudes of the major anomalous bands. This is in agreement with an earlier suggestion that the anomaly is linked to the state of aggregation of the complexes (Gregory et al., 1980).

Figure 4 shows absorbance and CD spectra of LHCII in the presence of different concentrations of detergent in a given sample of isolated complexes. No major changes could be observed in the absorbance (Figure 4A). In contrast, large, asymmetric intensity changes could be observed at around 680 and 490 nm in the CD. The magnitude of the aggregation-induced CD spectrum of LHCII was similar to that of the aggregation-induced CD in thylakoids. However, the sign of the major bands was different for the two aggregates—probably because of differences in the macroorganization of LHCII complexes *in vitro* and *in vivo*.

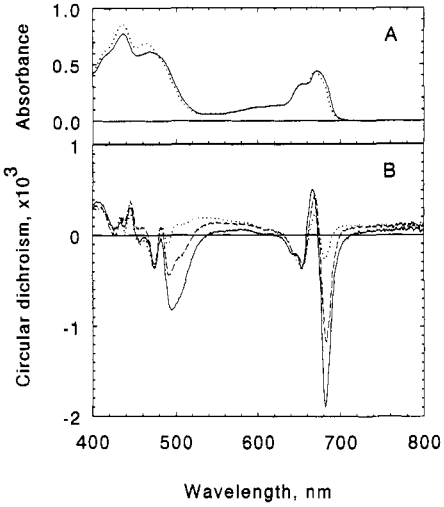

FIGURE 4: Absorption (**A**) and CD (**B**) spectra of LHCII in aggregated (—), intermediate (---), and desaggregated (···) states; LHCII was suspended in 10 mM tricine (pH = 7.8) buffer containing 0, 0.01%, and 0.025% Triton X-100, respectively. Concentration of Chl (*a*+*b*), 20 μg/mL; optical path length, 1 cm. (For clarity, the absorbance of the intermediate case is not shown.)

The intense negative CD signals which peaked at 682 and 493 nm were accompanied by tails outside the absorbance bands. On the other hand, both the intensity and the shape of the (–)652-nm excitonic band and the bands between 400 and 480 nm remained essentially unchanged. In samples containing a high concentration of detergent, the excitonic bands [cf. Van Metter, (1977)] and Hemelrijk et al. (1992)] dominated the spectrum. The (+)665-nm band appeared to be variable, evidently in consequence of the superposition of a positive signal onto the (+) band of the excitonic couplet.

The increment in the positive band peaking between 662 and 665 nm may be indicative of an additional excitonic interaction induced by aggregation. It has been proposed that an excitonic couplet in this region disappears upon the segregation of aggregates to monomers (Gülen et al., 1986). The change in the 77 K fluorescence spectrum upon the aggregation of LHCII (Horton et al., 1991) is in line with this assumption. The negative counterpart of the putative (+)-662-nm excitonic band may be contained in the broad (–)-682-nm band. However, this mechanism cannot be responsible for the intense, asymmetric (–)682-nm band. [The same holds true for the (–)493-nm band.]

A mechanism, similar to that proposed by Scherz and Rosenbach-Belkin (1989), for the generation of asymmetric CD through interactions between Chls on neighboring complexes cannot be excluded. However, these interactions, if they occur, are likely to take place in the trimers, rather than in the macroaggregates. The trimers are the building blocks both *in vitro* and *in vivo* (Butler & Kühlbrandt, 1988). In samples containing trimers of LHCII, no large asymmetric negative band was observed (Hemelrijk et al., 1992). Hence, the anomalous (–)493- and (–)682-nm bands with the extremely nonconservative band shapes are attributed to psi-type CD signals.

The distribution of LHCII on gradient centrifugation after cross-linking of the aggregates (Figure 5) showed a positive correlation between the size of the aggregates and the amplitude of the psi-type CD (Figure 4). Indeed, negative staining electron microscopy of samples taken from fractions at 1.4, 0.7, and 0.3 M sucrose concentrations revealed the presence of large (4–6 μm in average diameter), medium-sized (0.5–3 μm), and very small (<100–200 nm) aggregates,

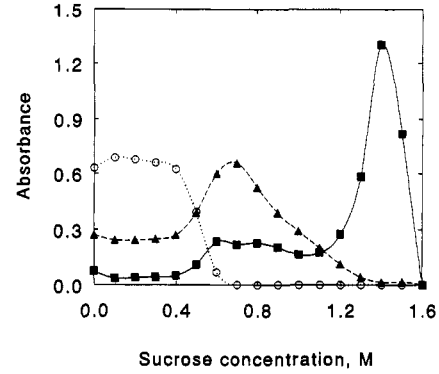

FIGURE 5: Size distribution of LHCII in different aggregation states. The CD results on these samples are shown in Figure 4; the same line symbols were used in the two figures. The absorbance of fractions was measured at 672 nm.

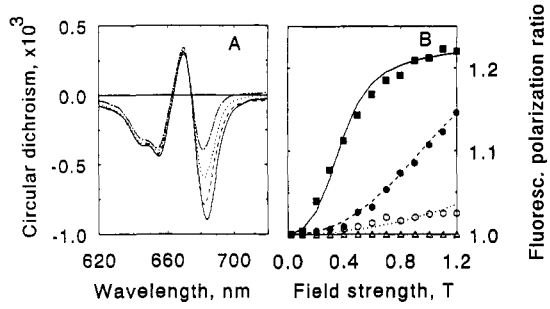

FIGURE 6: CD spectra of LHCII aggregates (**A**) and fluorescence polarization ratio as a function of the external magnetic field strength (**B**). Fluorescence intensities of linear polarization perpendicular to and parallel to the field vector were measured (data points), and curves were fitted with the diamagnetic susceptibility anisotropy values (joules/tesla^2): 2×10^{-19} (—), 4×10^{-20} (---), 7×10^{-21} (···), or $< 6 \times 10^{-22}$ (– · –, Δ). (Identical line symbols were used in panels **A** and **B** for identical preparations.) Concentration of Chl (*a*+*b*), 20 μg/mL; optical path length, 1 cm. The size of aggregates was gradually decreased by the gradual addition of 0.002–0.02% (v/v) Triton X-100 to the suspension; the sample was not changed between the measurements.

respectively. Similar aggregate sizes were demonstrated in aliquots taken from the suspensions used in the CD measurements, i.e., before the addition of the cross-linker. Furthermore, the density of the negatively stained samples suggested a multilayer type of aggregation rather than a two-dimensional organization of macroaggregates (data not shown).

As an independent approach, we compared the intensity of the (–)682-nm psi-type CD band and the diamagnetic susceptibility anisotropy values of LHCII aggregates. The magnitude of the diamagnetic susceptibility anisotropy depends on the molecular architecture of the aggregates of pigment–protein complexes (Knox & Davidovich, 1978). It is small in small aggregates. It is also small in macroaggregates in which the components are situated at random and thus the vectors cancel each other. On the other hand, the diamagnetic susceptibility anisotropy is large in macroaggregates possessing long-range order. Thus, it provides information on the "effective", rather than the geometric size of the macrodomains. In a series of measurements on the same sample, the magnitude of the psi-type CD gradually diminished on gradual increase of the concentration of detergent. Typical data are presented in Figure 6. It is interesting to note that in this sample the CD did not change between 600 and 670 nm. This example shows that, in accordance with the theory, the psi-type anomaly is confined to transition dipoles coupled to each other in long-range chiral order (Keller & Bustamante, 1986).

In parallel with the increase in the amplitude of the psi-type CD by a factor of about 2, the diamagnetic susceptibility anisotropy of the sample increased by more than an order of magnitude. Although CD increase for large aggregates in most preparations lagged somewhat behind the increase in the diamagnetic susceptibility anisotropy, probably because of a breakdown of the long-range coupling between the chromophores, the CD in homogeneous suspensions always increased monotonously with the "effective" size. Thus, we conclude that, in accordance with the theoretical prediction (Kim et al., 1986), the psi-type CD increases with the domain size of the aggregate.

In summary, our data show that, for both chloroplasts and isolated LHCII, the magnitude of the psi-type CD bands increases with the size of the macroaggregates. To our knowledge, this is the first direct experimental evidence of the size dependency of CD, a novel CD feature which was earlier predicted theoretically for psi-type aggregates (Keller & Bustamante, 1986).

We propose that macroaggregates of photosynthetic pigment–protein complexes are suitable for modeling the anomalous CD features of condensed macromolecules and other psi-type macroaggregates: (i) In Chl-containing macroaggregates, the size of aggregates can be varied in a broad interval. (ii) The chromophore density is high and the transition dipoles are aligned with respect to each other and the protein axis. Thus, as shown earlier and in this paper, psi-type CD bands with variable amplitudes can easily be generated. (iii) In contrast with nonpigmented macromolecular aggregates, in Chl-containing systems the measurements can be performed in the visible spectral range. (iv) The deconvolution of CD signals of different physical origins is possible, as indicated by the invariance of the excitonic bands in this hierarchically organized system. (v) The growing body of information available on photosynthetic pigment–protein complexes may provide a unique opportunity for the interpretation of spectral data in relation to the structure.

ACKNOWLEDGMENT

The authors are indebted to Dr. C. Bustamante (University of Oregon) for helpful discussions and to Zsuzsanna Tóth for skillful technical assistance.

REFERENCES

Armond, P. A., Staehelin, L. A., & Arntzen, C. J. (1977) *J. Cell. Biol. 73*, 400–418.
Barber, J. (1982) *Annu. Rev. Plant. Physiol. 33*, 261–295.
Bassi, R., Machold, O., & Simpson, D. (1985) *Carlsberg Res. Commun. 50*, 145–162.
Breton, J., & Vermeglio, A. (1982) in *Photosynthesis* (Govindjee, Ed.) pp 153–193, Academic Press, New York.
Burke, J. J., Ditto, C. L., & Arntzen, C. J. (1978) *Arch. Biochem. Biophys. 187*, 252–263.
Butler, P. J. G., & Kühlbrandt, W. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 3797–3801.
Cantor, C. R., & Schimmel, P. R. (1980) in *Biophysical Chemistry Part II: Techniques for the Study of Biological Structure and Function*, pp 409–433, W. H. Freeman and Company, San Francisco, CA.
Deisenhofer, J., Epp, O., Miki, K., Huber, R., & Michel, H. (1985) *Nature (London) 318*, 618–624.
DeVoe, H. (1965) *J. Chem. Phys. 43*, 3199–3208.
Dratz, E. A., Schultz, A. J., & Sauer, K. (1966) *Brookhaven Symp. Biol. 19*, 303–318.
Finzi, L., Bustamante, C., Garab, G., & Juang, C.-B. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 8748–8752.
Garab, G., Szitó, T., & Faludi-Dániel, Á. (1987) in *The Light Reactions* (Barber, J., Ed.) pp 305–339, Elsevier, Amsterdam, New York, Oxford.
Garab, G., Faludi-Dániel, Á., Sutherland, J. S., & Hind, G. (1988a) *Biochemistry 27*, 2425–2430.
Garab, G., Wells, K. S., Finzi, L., & Bustamante, C. (1988b) *Biochemistry 27*, 5839–5843.
Garab, G., Finzi, L., & Bustamante , C. (1991a) in *Light in Biology and Medicine* (Douglas, R. H., Moan, J., & Rontò, G., Eds.) pp 77–88, Plenum Press, New York, London.
Garab, G., Kieleczawa, J., Sutherland, C., Bustamante, C., & Hind, G. (1991b) *Photochem. Photobiol. 54*, 273–281.
Geacintov, N. E., Van Nostrand, F., Becker, J. F., & Tinkel, J. B. (1972) *Biochim. Biophys. Acta 267*, 65–79.
Gregory, R. P. F., Raps, S., Thornber, J. P., & Bertsch, W. (1972) in *Proceedings of the 2nd International Congress on Photosynthetic Research* (Forti, G., Avron, M., & Melandri, A., Eds.) pp 1503–1508, Dr. W. Junk N. V., The Hague, The Netherlands.
Gregory, R. P. F., Demeter, S., & Faludi-Dániel, Á. (1980) *Biochim. Biophys. Acta 591*, 356–360.
Gülen, D., Knox, R. S., & Breton, J. (1986) *Photosynth. Res. 9*, 13–20.
Hemelrijk, P. W., Kwa, S. L. S., Van Grondelle, R., & Dekker, J. P. (1992) *Biochim. Biophys. Acta 1098*, 159–166.
Horton, P., Ruban, A. V., Rees, D., Pascal, A. A., Noctor, G., & Young, A. J. (1991) *FEBS Lett. 292*, 1–4.
Keller, D., & Bustamante, C. (1986) *J. Chem. Phys. 84*, 2972–2979.
Kim, M., Ulibarri, L., Keller, D., Maestre, M. F., & Bustamante, C. (1986) *J. Chem. Phys. 84*, 2981–2989.
Kiss, J. G., Garab, G., Tóth, Zs., & Faludi-Dániel, Á. (1986) *Photosynth. Res. 10*, 217–222.
Knox, R. S., & Davidovich, M. A. (1978) *Biophys. J. 24*, 689–712.
Kühlbrandt, W., Wang, D. N., & Fujiyoshi, Y. (1994) *Nature (London) 367*, 614–621.
Livolant, F., & Maestre, M. F. (1988) *Biochemistry 27*, 3056–3068.
Maestre, M. F., Bustamante, C., Hayes, T. L., Subirana, J. A., & Tinoco, I., Jr. (1982) *Nature (London) 298*, 773–774.
Miller, K. R. (1982) *Nature (London) 300*, 53–55.
Papp, E., & Meszéna, G. (1982) *Biophys. J. 39*, 1–5.
Pearlstein, R. M. (1982) in *Photosynthesis* (Govindjee, Ed.) pp 294–327, Academic Press, New York.
Philipson, K. D., & Sauer, K. (1973) *Biochemistry 12*, 3454–3458.
Reich, Z., Ghirlando, R., & Minsky, A. (1991) *Biochemistry 30*, 7828–7836.
Scherz, A., & Rosenbach-Belkin, V. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 1505–1509.
Staehelin, L. A. (1976) *J. Cell Biol. 71*, 136–158.
Tinoco, I., Jr. (1962) *Adv. Chem. Phys. 4*, 113–160.
Van Metter, R. L. (1977) *Biochim. Biophys. Acta 462*, 642–658.
Zuber, H. (1987) in *The Light Reactions* (Barber, J., Ed.) pp 197–259, Elsevier, Amsterdam, New York, Oxford.