Partial Analysis of the Bands Seen in Circular Dichroism Spectra of Green and Blue-Green Algal Cells and Thylakoids

B. Nathanson and J. E. White
Department of Biological Sciences, Hunter College of the City University of New York, New York 10021 and Department of Biological Sciences, New York City Community College of the City University of New York, New York

ABSTRACT

A comparison was made of the circular dichroism (C.D.) spectra of Chlorella, Euglena, and Anacystis cells and thylakoids. Analyses of the spectra reveal that these C.D. bands are similar to those observed previously in whole spinach chloroplasts and subchloroplast particles. C.D. spectra of Euglena chloroplasts show bands at longer wavelengths than previously reported. From comparisons of circular dichroism spectra and fine structure, it was concluded that: (a) bands seen in circular dichroism spectra were not the result of light scattering from thylakoid membranes; and (b) bands seen in the C.D. spectra of nonmembranous systems (previously reported) could account for circular dichroism of algae. We also concluded that comparisons would have to be made with model systems in order to correct for effects of absorption flattening, concentration obscuring, and differential light scattering of membranous systems.

Studies have been reported using circular dichroism techniques to elucidate chloroplast structure by comparison of the C.D. spectra of Chl a in solution and as organized into membranes (particulate Chl). Dratz et al. (10) and Houssier and Sauer (16) observed that the dimeric form of Chl a in CCl₄ gave rise to a C.D. doublet with bands at 656 and 678 nm. There was only one band (with maximum at 662 nm) seen in the C.D. of Chl a monomers (16). Dratz et al. (10) concluded that the bands seen in the C.D. of broken spinach chloroplasts could be interpreted as being due to Chl-Chl interactions in the Chl lipid proteins of the chloroplast membrane. This was based on a comparison with the C.D. bands of Chl a dimers in CCl₄. In an extension of these works, Brody and Nathanson (1) and Nathanson (21) examined the C.D. spectra of Chl a in CCl₄ and in hexane, Chl proteins in solution, and Chl in membranous systems (subchloroplast particles and whole spinach chloroplasts). These authors concluded that the bands seen in the C.D. spectra of nonparticulate Chl could account for all of the bands seen in the spectra of subchloroplast particles isolated from spinach leaves. The C.D. bands attributable to Chl a in the subchloroplast particles consisted of a positive band at 665 nm and a negative band at 679 nm. These two bands were postulated to be the bands of a Chl a dimer, analogous to the in vitro situation of Chl a in CCl₄. A C.D. band additional to that observed in subchloroplast particles was seen in intact spinach chloroplasts (1, 15, 21): this band (positive at 683 nm) was correlated with a shift in the negative band from 679 nm in subchloroplast particles to 672 nm in the intact chloroplasts (1, 21). Addition of linolenic acid to a suspension of intact spinach chloroplasts (1, 21) resulted in the disappearance of the 683 nm band and a shift in the negative band from 672 nm back to 679 nm. Brody and Nathanson (1) and Nathanson (21) concluded that the 683 and 672 nm bands could be correlated with one species of Chl a and the 679 nm and 665 bands with another.

In all of the references mentioned above, the membranous fractions examined were derived from the chloroplasts of higher plants. However, as has been shown by French and co-workers (12) among others, there are a number of different absorbing forms of both Chl a and b in vivo. Among the green photosynthetic organisms, Euglena has been shown to have a higher proportion of the long wavelength-absorbing forms of Chl a and a lower Chl b content than do other green photosynthetic organisms such as Chlorella and higher plants (12). A further complication is encountered in the blue-green algae. In these organisms (such as Anacystis), phycobilins, which are easily leached out of the cells upon disruption of the thylakoid membranes, are found in place of Chl b. All of these pigments overlap to some extent in their absorption in the red region of the spectrum. We have, in the present work, examined differences in C.D. spectra between a blue-green alga (Anacystis nidulans), a green alga (Chlorella pyrenoidosa), and an euglenoid (Euglena gracilis) in an attempt to clarify the relationships between the observed C.D. and known photosynthetic pigments. Both whole cells and thylakoids of the aforementioned organisms were used in our investigation. The changes in C.D. patterns can be related to differences in pigment content. Therefore, a comparison among the cells and thylakoids of Euglenoids, green algae, and blue-green algae and between the cells and thylakoids of each of these organisms can furnish information concerning the C.D. bands associated with Chl a and b and with phycocyanin.

MATERIALS AND METHODS

Chlorella pyrenoidosa Emerson’s strain 3 was supplied by S. S. Brody, New York University; Anacystis nidulans was obtained from T. E. Jensen, Lehman College; Euglena gracilis Klebs strain Z Pringsheim was kindly supplied by S. H. Hunter of the Haskins Laboratory, New York, N. Y.

The algae were cultured for 4 days at 25 C and aerated by vigorous agitation in air with a Burrell wrist-action shaker. Chlorella was cultivated in the medium of Pinson and Ruppel (24), Anacystis was grown in medium C prepared according to Kratz and Myers (18), and Euglena was cultured in “Euglena Broth” purchased from Difco Laboratory. The algae were cultured under conditions in which the light intensity falling on the flasks was 1.9 × 10⁹ erg cm⁻² sec⁻¹ (weak light culture) as measured with a Yellow Springs Radiometer. Prior to experiments, algal cells in the log phase of growth (determined by cell counts) were collected by centrifugation at...
Anacystis isolated thylakoids

Plant anin thylakoids (c) unfixed

Unin Chl gradient according isolation of phycocyanin band

The method of Ludlow and Park (19) involving pre-fixation of phycobilins to the lamellar membranes of cells was used prior to isolation of lamellar fragments from A. nidulans. Lamellar fragments of Chlorella were prepared only from unfixed cells. In the case of Anacystis, both fixed and unfixed cells served as sources and were prepared according to the method of Murano and Fujita (20).

Euglena cells in suspension were broken by grinding with Ottawa sand (1:3 volume of cells to sand) for 30 sec at 4 C. Thylakoid vesicles of Euglena were isolated on a sucrose density gradient according to the procedure of Tolbert (29).

Absorption spectra were obtained using a Cary 14R recording spectrophotometer. Circular dichroism spectra were obtained using the Durrum-Jasco recording spectropolarimeter, model J-20 (Durrum Instrument Corp., Palo Alto, Calif.) as previously described (1). Fluorescence spectra were determined using the apparatus of Brody et al. (3).

Algal pellets and thylakoid vesicles were obtained for fine structure studies by gentle centrifugation at 500g for 5 min, followed by fixation for 2 hr at 4 C with 2.5% glutaraldehyde in 50 mM cacodylate buffer. Specimens were dehydrated in ethanol, passed through propylene glycol, and embedded in Epon 812. Thin sections were stained with uranyl acetate followed by lead citrate. They were then viewed in an RCA EMU 3-H electron microscope.

RESULTS

The shapes of the C.D. spectra of both Chlorella cells and thylakoids are similar to the spectra of whole spinach chloroplasts reported earlier (1, 15, 21; Fig. 1). In the case of Chlorella cells, the positive C.D. band (corresponding to Chl a) has its maximum at 694 nm rather than the 683 to 688 nm, as seen in the C.D. of intact spinach chloroplasts (1, 15, 21). There is a large negative band with a maximum at 684 nm, as well as positive bands at 660 nm and 640 nm. The C.D. spectrum of isolated Chlorella thylakoids is similar to that of whole Chlorella cells, with blue shifts of 6 nm and 8 nm occurring in the 693 and 684 nm bands, respectively. We also observed a disappearance of the 640 nm band (as seen in Chlorella cells)—this band corresponding to absorption by chlorophyll b, and the 660 nm band—this latter band corresponding to the positive portion of the suggested 660-679 nm doublet (1, 21). The disappearance of the 640 and 660 nm bands is accompanied by the appearance of a 650 nm band. This band is due to a shift of the Chl a band upon isolation of the thylakoids (and consequent merging of the shifted band with the 640 nm band).

In order to understand better the contribution of Chl a and b to the C.D. spectra seen above, we examined Anacystis, an alga lacking Chl b. Figure 2 shows the absorption spectra for: (a) whole Anacystis cells; (b) thylakoids from fixed and unfixed Anacystis cells; and (c) the extract resulting from the isolation of thylakoids from unfixed Anacystis cells. Although the spectrum for fixed cells (not shown) is similar to that of unfixed cells, the spectra for cells and thylakoids from unfixed cells differ markedly in the regions of phycocyanin absorption. Note that the extract resulting from the isolation of thylakoids from unfixed cells has bands corresponding to Chl a absorption as well as to phycocyanin absorption. The long sonication time utilized in our preparation could have resulted in the formation of very small particles of Chl-containing components, as well as soluble phycocyanin in the supernatant. The ratio of absorption of phycocyanin to Chl (618 nm to 678 nm) is 1.33, 0.88, 0.83, and 0.37 for (a) the phycocyanin extract; (b) whole Anacystis cells (fixed or unfixed); (c) thylakoids from fixed Anacystis cells; and (d) thylakoids from unfixed Anacystis cells, respectively. That the thylakoids isolated from fixed cells have a similar Chl-phycocyanin composition to these cells can be seen from the above.

Therefore, both Anacystis cells and thylakoids from fixed cells have approximately the same ratio of phycocyanin to Chl, while thylakoids from unfixed cells have a relatively increased amount of Chl a.

The C.D. spectra for fixed and unfixed whole Anacystis cells and the phycocyanin liberated from unfixed cells upon sonication are shown in Figure 3A. The C.D. of fixed cells shows a negative ellipticity compared to unfixed cells. This may be due to the increase in light scattering caused by the fixation process (8). There is no appreciable shift in the wavelength of C.D. absorption of fixed compared to unfixed cells. The Chl a bands in the red region of the spectrum are similar to those seen in Chlorella, except for the presence of the long wavelength band at 693 nm in the latter organism.

The C.D. spectra of isolated thylakoids (Fig. 3B) are essentially similar to that of whole cells. The main difference is found in the 600 to 630 nm region of thylakoids from unfixed cells, Figure 1. Circular dichroism spectra (ellipticity in millidegrees) of Chlorella Cells and thylakoids. Chlorella cells (—); thylakoids isolated from Chlorella cells (— — —). Chl concentration of cells and thylakoids = 1.5 × 10⁻⁵ M. Cuvette path length = 1 cm.

Figure 2. Absorption spectra of Anacystis cells and fractions from Anacystis cells. Whole Anacystis cells (unfixed) (——); thylakoids isolated from formaldehyde-fixed Anacystis cells (——); thylakoids isolated from unfixed Anacystis cells (——); phycocyanin-containing extract from unfixed Anacystis cells (——). Chl concentration of cells and thylakoids = 1.5 × 10⁻⁵ M. Cuvette path length = 1 cm. Chl concentration of phycocyanin-containing extract = 4.5 × 10⁻⁴ M. Cuvette path length = 5 cm.
The absorption spectra for whole *Euglena* chloroplasts and chloroplast fragments isolated from a discontinuous sucrose density gradient are shown in Figure 4. Both preparations contain equal concentrations of Chl. There is considerable difference in absorption due to scattering (as seen at 800 nm). In the inset to this figure are seen the absorption spectra resulting from normalization at 800 nm. The C.D. spectra are shown in Figure 5; they are similar but not identical. In conjunction with this, there is noted increased absorption and band broadening in the blue region of the spectrum of whole *Euglena* chloroplasts as compared with that of chloroplast fragments (inset to Fig. 4). As in the case of *Chlorella* and *Anacystis*, fragmentation of the *Euglena* chloroplasts is demonstrated by the decreased Chl absorbance at 680 nm with increased absorbance at 693 nm, as shown in the inset to Figure 4.

Figure 3. A: Circular dichroism spectra (ellipticity in millidegrees) of *Anacystis* cells and a fraction from unfixed *Anacystis* cells. Unfixed *Anacystis* cells (---); formaldehyde-fixed *Anacystis* cells (····); phycocyanin-containing extract from unfixed cells (----). Conditions as in Figure 2. B: Circular dichroism spectra (ellipticity in millidegrees) of *Anacystis* thylakoids. Thylakoids isolated from formaldehyde-fixed *Anacystis* cells (····); thylakoids isolated from unfixed *Anacystis* cells.

In Figure 3A, it is seen that the C.D. of the phycocyanin extract liberated from the thylakoids of unfixed cells differs from that of the cells in the following: (a) there is a very broad band from around 530 to 630 nm (due to phycobilins); and (b) there is a shift in the negative band of the red region of the spectrum from 686 nm (in cells and thylakoids) to 682 nm. In thylakoids from unfixed cells, the negative band in the red region of the spectrum has its maximum at 678 nm. However, it is fairly broad and has a shoulder at 693 nm. As is the case with *Chlorella* cells and thylakoids, the blue shifts, seen in the C.D. spectra of thylakoids of *Anacystis* compared with the cells, can be interpreted as being due to both decrease in light scattering and deaggregation of Chl a upon isolation of these thylakoids. Evidence for deaggregation has been given in the work of Nathanson (unpublished results) in which comparison of the low temperature (77 K) 735 fluorescence excitation spectra of *Anacystis* thylakoids (from both fixed and unfixed cells) with the fluorescence excitation of the cells reveals that in the former there is a shift in the maximum wavelength of Chl a from 684 nm to 682 nm. This decrease in contribution of long wavelength Chl a in low temperature fluorescence excitation is also seen in the spectrum of *Chlorella* thylakoids compared to *Chlorella* cells (Nathanson, unpublished results). Additionally, the shift in C.D. maximum of the phycocyanin extract to 620 nm from the maximum seen in both intact cells and in thylakoids at 630 nm is further evidence for the deaggregating effect caused by the treatment used to obtain thylakoids.

Figure 4. Absorption spectra of whole *Euglena* chloroplasts and *Euglena* fragments isolated from a sucrose density gradient. Whole *Euglena* chloroplasts (---); *Euglena* chloroplasts isolated from a discontinuous sucrose density gradient (---). Chl concentration = 1.8 x 10^-5 M. Cuvette path length = 1 cm. Inset: same as above except that curves were normalized at 800 nm.

Figure 5. A: Circular dichroism spectra (ellipticity in millidegrees) of *Euglena* chloroplasts. Whole chloroplasts (---); whole chloroplasts + 2 x 10^-4 M linolenic acid (····); whole chloroplasts + 2 x 10^-3 M linolenic acid (----); chloroplasts from discontinuous sucrose density gradient (---). Chl concentration = 1.8 x 10^-5 M. Cuvette path length = 1 cm. B: Fluorescence emission spectra of *Euglena* chloroplasts. Conditions and samples as in Figure 5A.
Euglena chloroplast results in a hypsochromic shift in the C.D. band and a narrowing of the absorption bands in the red region. A positive band is not observed at 694 in the spectrum of whole chloroplasts. A broad band occurs in the region at 705 nm, which may include the 694 nm band under its envelope. This 705 nm band has also been seen by Geacintov et al. (13) in a linear dichroism study of suspensions of whole Euglena cells oriented in a magnetic field. In our C.D., a broad negative band occurs at 679 nm.

Although in Chlorella and Anacystis a positive C.D. band with maximum at 660 nm is evident, this is not seen in the C.D. of either whole or broken Euglena chloroplasts. One possible explanation is that the 660 nm band is low in intensity in the C.D. spectra of Chlorella cells and in whole spinach chloroplasts (1, 15, 21). This may be obscured due to the relatively larger amount of scattering by Euglena thylakoids compared to either spinach or Chlorella. In relation to this, note that the C.D. of Euglena exhibits very low intensity in the blue region of the spectrum in relation to the intensity of the red region (Fig. 5). The relative intensity of the bands in the blue compared to the red region of either Chlorella or spinach chloroplasts is much greater than that observed in Euglena. Although the 679 nm band has been postulated to be part of a 660 to 679 nm dimer doublet (1, 10, 21), the 660 nm band is not in evidence in the preparations of Euglena thylakoids. An alternative explanation was also considered for the presence of this 679 nm band (in the absence of the 660 nm band). Since a positive band at longer wavelengths (697 or 705 nm) is seen in the C.D. spectra of Euglena chloroplasts, these longer wavelength bands could be associated with the 679 nm band, since they correspond to absorption by aggregated forms of Chl (3). However, that the 679 nm band is not associated with the 705 or 697 nm bands can be deduced from the following: (a) while the isolation of thylakoids on a discontinuous sucrose density gradient results in a shift of the positive red band (in whole Euglena chloroplasts) from 705 to 697 nm, there is no corresponding shift in the negative band at 679 nm; (b) treatment with linolenic acid (linolenic acid/Chl = 10/1; see Fig. 5A) results in a shift of 5 nm in the positive band (from 705 to 700 nm) but only 2 nm (from 679 to 677) in the negative band of whole chloroplasts. The shift of both bands is due to the effect of deaggregation by linolenic acid of aggregated Chl into less aggregated components (1, 7, 21). This is further demonstrated by the corresponding fluorescence emission spectra monitored at 77 K (Fig. 5B). The spectrum of control thylakoids is essentially similar to whole chloroplasts. Upon treatment of thylakoids with linolenic acid (at fatty acid to Chl molar ratios of 10:1), there results a change in aggregate to monomer ratio (F 720/F 700) from 1:6 to 3:1 which represents a deaggregation.

An important consideration in this study is the role of light scattering in the production of long wavelength C.D. bands. It has been suggested (23) that the long wavelength band seen at 688 to 690 nm in the C.D. spectra of spinach chloroplasts and Chlorella cells is the result of light scattering from the granal membranes of these systems. That this is not so can be deduced from the following. In Figure 6A is presented an electron micrograph of whole Euglena chloroplasts in a continuously light-grown cell. The chloroplasts contain lamellae but no grana. Figure 6B is an electron micrograph of light-grown Euglena thylakoid fragments isolated from the boundary between the 1.50 and 1.25 bands of a discontinuous sucrose density gradient. In Figure 5C is seen an electron micrograph of grana from whole spinach chloroplasts. A comparison of these three electron micrographs demonstrates that: (a) in the spinach chloroplast, there is an association of eight or more lamellae continuous along the length of a granum; (b) in contrast to this, whole Euglena chloroplasts give evidence of association of perhaps three or four lamellae; and (c) in the Euglena thylakoids isolated from the sucrose density gradient, even less organization is seen. Nevertheless, it can be seen (Fig. 5) and has been reported (1) that not only is there a positive long wavelength band at 683 to 690 nm in spinach chloroplasts, there are also long wavelength bands in the C.D. of both whole Euglena chloroplasts and thylakoid fragments (at 705 and 697 nm, respectively). All three of these very different systems show long wavelength bands. This is presumptive evidence that these long wavelength bands are due to the presence of Chl aggregates in the chloroplast and not merely the result of light scattering from the granal membranes.

A summary is given in Table I of the bands in the red region of the spectrum seen in thylakoids and algal cells, as well as a listing of the bands of whole spinach chloroplasts.

**DISCUSSION AND CONCLUSIONS**

Due to the electronic structure of Chl, bands can be observed directly in the C.D. (1, 10, 15, 16, 21) which are predicted by derivative and computer analysis of absorption spectra (12). French and associates (12) have used computer analysis of the low temperature absorption spectra of various organisms to determine the different absorbing forms of Chl a in the spectra of algae and chloroplasts. The bands at 679 to 705, 683, 679, 664, and 650 nm, predicted from absorption spectra of membranous systems, are directly observed in C.D. of both membranous systems (in the present study) and in solution Chl (1, 10, 16, 21). Although there are observed shifts in Chl a bands from one organism to another in the membranous systems, this gradation of shifts is not observed in Chl in solution. In connection with this, it should be noted that Brody and Brody (3) applied an expression derived by McCae and Kasha (see 17) to an analysis of Chl a absorption bands associated with their respective fluorescence maxima and the size of the Chl a aggregate. They concluded that Chl having bands at 674 nm were in the monomeric form while those at higher wavelengths correspond to aggregated forms of Chl. Chlorophyll a with maximum absorption at 705 nm has an effective aggregate size of 2, i.e., dimeric Chl. However, there are a number of bands found in the C.D. spectra of membranous systems which are at wavelengths intermediate between the maxima at 674 and 705 nm. One possible explanation has been given by Brown and French (4). These authors attribute the shifts seen in membranous systems to binding of these Chl as to different proteins in the chloroplast. This interpretation is supported by the results of Quinlan’s study (25) of C.D. bands of Chl in aqueous formamide, a model for the Chl-protein system. He reported formation of Chl a aggregates upon the addition of aqueous formamide to an ethanolic solution of monomeric Chl a. This aggregate formation was attributed to the influence of the high dipole moment of the formamide peptide linkage resulting in dipole-dipole interaction between neighboring molecules. Moreover, Smitz et al. (27) have isolated three lamellar proteins on the basis of gel electrophoresis of a preparation from spinach chloroplasts.

We have seen here that hypsochromic shifts in C.D. maxima result upon isolation of thylakoid fragments from either cells of Chlorella and Anacystis or whole chloroplasts of Euglena. These changes can be explained by the occurrence of two processes—a decrease in light scattering upon isolation of the (smaller) thylakoids, and deaggregation of Chl as a consequence of the sonication treatment used in isolating the thylakoids. Both of these processes would result in blue shifts of the C.D. bands. Pecci and Fujimori (22) have shown that in c-phycocyanin from *A. nidula* the shift in C.D. maximum from 633 to 617 nm induced by mercurials is due to deaggregation of the protein portion of the phycocyanin. Earlier studies in this laboratory (1, 21) on the membranous Chl of spinach have shown that for digitonin-isolated particles containing systems I and II, blue shifts of 5 nm occur in the C.D. bands in the red region of the spectrum within...
minutes of addition of fatty acids. We also found that these fatty acids cause deaggregation of the Chl by a preliminary unwinding of the proteins to which the Chl are attached. We can consider that the bands seen in chloroplasts from leaves of higher plants and in algae may all correspond to dimeric forms of Chl, whether these bands are split (664–679 nm, 672–683 nm) or single (697 and 705 nm). The lack of split exciton interaction seen in the C.D. bands occurring at 697 and 705 nm does not negate the assignment of these bands to dimeric species of Chl a, since the appearance of a dimer doublet in the C.D. spectrum would be dependent upon the angle between the transition moments of the molecules comprising the dimer (see refs. 10 and 16). It would, therefore, be possible to have dimer formation and yet only have one band apparent in the C.D. spectrum of an aggregated molecule.

It would be possible to produce Chl dimers having different absorption maxima by changing the environment surrounding the Chl chromophores. Fenna and Matthews (11) have investigated the three-dimensional crystalline structure of a bacterio-Chl protein isolated from Chlorobium limicola. They proposed that the protein moiety forms a layer around bacterio-Chl aggregates, protecting them from an aqueous environment. Therefore, this protein would be the immediate environment of the bacterio-Chl. Additionally, Cogdell et al. (5) found that the circular dichroism of spheroidene (a carotenoid) was dependent upon its attachment to the proteins of reaction centers isolated.

Fig. 6. A: Electron micrograph of whole chloroplasts in a continuously light-grown cell of Euglena gracilis strain Z. × 40,000. B: Electron micrograph of thylakoid-containing fraction from a discontinuous sucrose density gradient of continuously light-grown Euglena gracilis strain Z. × 10,000. Band located at the interface between the 1.50 M and 1.25 M sucrose bands in the sucrose density gradient. C: Electron micrograph of grana from a whole spinach chloroplast. × 100,000.
from *R. sphaeroides*. All of this indicates that the role of protein may be one of the environment directly surrounding the Chl in whole chloroplasts and other photosynthetic membranes.

In contrast to the above, Gregory and Raps (14) attribute the C.D. changes caused by breakage whole chloroplasts to disruption of dimers due to Chl-Chl interaction in whole chloroplasts and revelation of less intense C.D. structure due to Chl-protein complexes seen in broken chloroplasts. This interpretation is based partly upon their comparison of whole chloroplasts to broken ones, in which the intensity of their C.D. in whole chloroplasts was 10 times that of fragments. We disagree with this interpretation. In our preparations (1, 21) we have found, at best, a 2:1 difference. One possible explanation is that adjustments to similar Chl concentrations were not taken into account in the work of Gregory et al. (15); another is the variability of their preparations of whole chloroplasts (both in ratio of peak heights in Chl a bands and in actual ellipticity). Even if their intensity differences are taken into account, however, the following should be noted: Brody et al. (2) have calculated that the concentration of Chl a on the lamellae for continuously light-grown *Euglena* is equivalent to a concentration of $3.3 \times 10^{-1}$ M in solution. Using their formulae, we have calculated that the equivalent molar concentration of Chl a in spinach chloroplasts is $3 \times 10^{-2}$ M. That the intensity of the C.D. bands seen in Chl-protein complexes is much less than that of Chl in whole chloroplasts is not surprising since the Chl concentration of these complexes is of the order of $10^{-3}$ M as calculated from the absorption coefficient given by Thornber (28). That the ellipticity of whole chloroplasts is not greater can be attributed to absorption flattening and concentration quenching in the whole chloroplasts. Isolation of disrupted chloroplasts could result in a decrease in intensity of the resultant C.D. due to release of Chl complexes into the medium.

We would now like to consider the results obtained with blue-green algae. In the red region of the spectrum, bands occurred at 666 nm (positive) and 678 to 686 nm (negative). Dratz et al. (10), working with a Chl b-less mutant of barley, found that the C.D. of chloroplasts of the mutant had a positive band at 666 nm—different from the wild type. Our findings with blue-green algae also show a band with maximum at this wavelength. In addition, the negative bands seen at 678 to 686 nm in the various preparations of blue-green algae correspond well with the negative peak seen at 682.5 nm in the barley mutant (and in normal barley). A negative band with a peak at 678 and a shoulder at 693 nm occur in the thylakoids prepared from unfixed cells of blue-green algae. We feel that this two-handed form is analogous to bands observed in sonicated spinach chloroplasts (1, 21). The main peak corresponds to a 678 nm absorbing form, with a small absorption at 683 nm causing a positive inflection between the negative peak at 678 nm and the shoulder at 693 nm. We concluded that the negative bands seen at longer wavelengths in blue-green algae actually correspond to the 678 nm band in green algae and in chloroplasts from higher plants. Also, this negative band occurs at 681 nm in the spectrum of the phycoerythrin extract.

We would finally like to consider the effect of light scattering on the band peaks seen in the red region of the spectrum. We have shown that the large differences in the absorption and scattering of whole *Euglena* chloroplasts compared to thylakoid fragments are not reflected in the corresponding C.D. spectra. Although these thylakoid fragments are less highly organized than whole chloroplasts, they both have approximately the same degree of Chl aggregation (as seen from fluorescence emission spectra). In contrast to Philipson and Sauer (23), we feel that the C.D. bands in the red region of the spectrum are a true reflection of Chl organization and not due to intrinsic differences in light-scattering properties. A similar conclusion has been reached by Demeter *et al.* (9) in their investigation of the stacking capacity of mesophyll and bundle sheath chloroplasts of maize.

While Philipson and Sauer (23) found that increasing the distance between the samples and the photomultiplier of their C.D. apparatus resulted in change in shape of moderately and highly organized systems as seen in their preparations of spinach, *Euglena* and *Chlorella* cells, we have not found this effect in our studies with *Chlorella* cells. Although Philipson and Sauer (23) used a standard Durrum-Jasco J-20 spectropolarimeter, we used a prototype for this model. In our experiments, the only difference which resulted was a diminution in intensity of the signal transmitted to the photomultiplier, if we varied the distance of the sample to the photomultiplier from 1 cm (the usual position of the cuvette) to 11 cm (the limit of our sample compartment). Schooley and Govindjee (26), investigating the effect of cations on the C.D. of chloroplasts from lettuce and spinach, observed a decrease in intensity of the 676 nm band with decreasing distance from the photomultiplier. The 683 nm band of the chloroplasts did not change as a function of distance. It is possible that the effects seen by Philipson and Sauer (23) and Schooley and Govindjee (26) a function of the particular instrument and should therefore be determined for each type and model of spectropolarimeter.

We feel that a better estimate of the true C.D. patterns of membranous-containing systems as organized in the chloroplasts of plants or algal cells may be obtained by comparing these C.D. spectra with the C.D. spectra of Chl-proteins.

**Acknowledgments**—We would like to thank the Department of Chemistry of Hunter College of the City University of New York for use of their equipment. We are especially grateful to R. Ausbel for his help.

**LITERATURE CITED**


different grana content. Physiol. Plant. 32: 222-227.