Title
LIGHT SCATTERING EFFECTS ON THE CIRCULAR DICHROISM OF CHLOROPLASTS

Authors
Philipson, Kenneth D.
Sauer, Kenneth.

Publication Date
1973-04-01

Submitted to Biochemistry

LBL-1771

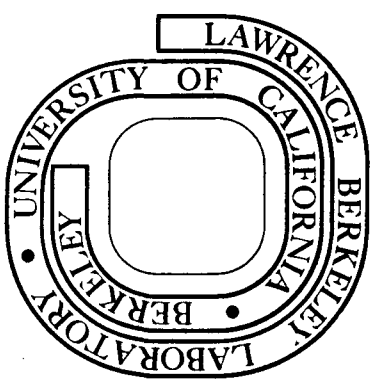
c.1

LIGHT SCATTERING EFFECTS ON THE CIRCULAR DICHROISM OF CHLOROPLASTS

Kenneth D. Philipson and Kenneth Sauer

April 1973

Prepared for the U.S. Atomic Energy Commission
under Contract W-7405-ENG-48



LBL-1771

c.1

DISCLAIMER

Light Scattering Effects on the Circular Dichroism of Chloroplasts†

Kenneth D. Philipson and Kenneth Sauer*

Running Title: Light Scatter and CD of Chloroplasts

†From the Department of Chemistry and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720. Received . The research described in this report was supported, in part, by a grant from the National Science Foundation (GB-24317) and, in part, by the U. S. Atomic Energy Commission.

ABSTRACT: A study is made of the effect of differential scattering of left and right circularly polarized light on the CD spectra of intact and broken chloroplasts. Dramatic differential light scattering effects on the spectrum of the intact chloroplasts are observed by varying the fraction of scattered light which is collected by the detector of the CD spectrometer. Evidence is presented which implies that the intrinsic CD (i.e., after elimination of scattering distortions) of intact chloroplasts is similar to that of non-scattering, sonicated chloroplasts. It is shown that it is not scattering per se, but rather scattering dependent upon the specific ordered arrangement of internal membranes of chloroplasts which produces the large differential scattering effect.

The technique of circular dichroism (CD) spectrometry has achieved notable success in the study of biological molecules in solution for several years. More recently, this method has been extended to include the study of particulate biological matter in suspensions exhibiting pronounced light scattering. For example, this approach has been used in experiments on mitochondria (Urry et al., 1967; Steim and Fleischer, 1967), virus particles (Maestre and Tinoco, 1967; Maestre et al., 1971), chromosomes (Cantor and Hearst, 1969), and membrane bound proteins (Schneider et al., 1970; Glaser and Singer, 1971). [Further references on these studies can be found in Urry (1972) and Dorman and Maestre (1973).] In most of the earlier studies the effect of light scattering on the resultant CD spectrum was either neglected or considered to be negligible (Wallach and Zahler, 1966; Lenard and Singer, 1966; Hammes and Schullery, 1968).

In 1968, Urry and Ji proposed the presence of distortions in the CD of particulate systems. One of the sources of these distortions, the differential scatter of left and right circularly polarized light, has received recent attention in the literature from both experimental and theoretical viewpoints (Urry and Krivacic, 1970; Ottaway and Wetlaufer, 1970; Schneider, 1971; Gordon and Holzwarth, 1971; Gordon, 1972; Dorman and Maestre, 1973; Dorman et al., 1973; Schneider, 1973). Despite this attention the effect of differential scatter on CD spectra is still far from being completely understood or widely recognized. We report here some striking effects of differential scattering of circularly polarized light on the CD spectrum of spinach chloroplasts.

Gregory et al. (1972) have reported large changes in the CD of intact chloroplasts upon fragmentation. They interpret this result in terms of a disaggregation and rearrangement of the membrane-bound chlorophyll molecules accompanying comminution of the chloroplasts, without considering the possible consequences of effects arising from a change in the light scattering properties of their preparations. We have found that the observed CD spectrum of whole chloroplasts is critically dependent on the fraction of scattered light detected by the photomultiplier of the spectrometer. This dependence implies that different quantities of left and right circularly polarized light are being scattered from the measuring beam of the instrument. This differential scattering alters the measurement of differential absorption which constitutes normal CD.

The dependence of the CD on detector geometry is, in fact, so dramatic for whole chloroplasts as to make interpretation of this measurement (with our current understanding of differential scatter) totally unreliable. Results are presented which suggest, however, that, if all scattering effects could be eliminated, the CD of intact chloroplasts would indeed resemble that of fragmented chloroplasts. We also present data which support the concept that it is not particulateness per se which is responsible for the large differential scatter of chloroplasts. Rather it is scattering from the complex, highly ordered stacking of lamellar membranes into grana structures within the chloroplasts which produces these large distorting effects on the CD.

Materials and Methods

Intact chloroplasts were prepared from growth chamber spinach (Spinacea

oleracea var. early hybrid No. 7) by grinding leaves in a Waring Blendor for 1 min in .35 M NaCl, .04 M NaH_2PO_4 buffer at pH 7.4, followed by straining through 8 layers of cheesecloth. This suspension was centrifuged at 400 x g for 2 min and the supernatant from this spin was centrifuged at 1200 x g for 10 min. The intact chloroplasts were then obtained by resuspending the final precipitate in the grinding buffer. Fragmented chloroplasts were made by sonicating these intact chloroplasts with a Sonifier Cell Disruptor (Ultrasonics, Inc., Model W185) for 90 sec at 50 W. The sonicated chloroplasts were centrifuged for 30 min at 40,000 x g and the supernatant was used in the experiments.

Fragmented samples of the blue-green alga, Aphanocapsa sp. 6714, were obtained by passing whole cells of the algae through the French pressure cell at an applied pressure of 3000 psi and centrifuging at 10,000 x g for 10 min. About 80% of the phycocyanin pigments remained in the supernatant. The precipitate was resuspended in .01 M Tris at pH 7.4 and sonicated for 90 sec at 50 W. After centrifuging for 10 min at 10,000 x g, the supernatant was taken for use in measurements.

Chromatophores were obtained from the purple bacterium, Rhodopseudomonas spheroides, by sonicating whole cells for 2 min at 50 W and then centrifuging for 10 min at 10,000 x g. Chromatophores were obtained as the supernatant fraction of this spin.

All CD spectra were recorded using a Durrum-Jasco J-20 spectropolarimeter with sensitivity extended to 1000 nm. A Dumont 6911 phototube with S-1 response was used to record the spectra of R. spheroides and a Dumont 2703 phototube with S-20 response was used for all other spectra. Both phototubes have photocathode surfaces with 1.7-inch diameters. The instrument is provided with a cell holder for normal operation which is 53 cm from

the face of the end window photomultiplier tube. There is also a cell holder which places the sample 15 cm from the phototube. For the purpose of these experiments, a cell holder was constructed which would hold a sample cuvette only .2 cm from the phototube. All spectra were obtained with fixed slitwidths such that spectral resolution was 2-4 nm.

Absorption measurements were made using a Cary 14 spectrometer equipped with a Model 1462 scattered transmission accessory. Absorption at the peak maximum is given in each figure legend. Also reported is the measured absorption at a long wavelength where the samples should have practically no true absorption. This number can be used as a measure of the turbidity of the suspensions.

Results

CD spectra of whole spinach chloroplasts in the long wavelength region taken with the sample cuvette at a far, intermediate, and close distance (53, 15, and .2 cm clearance between cuvette and detector outer glass surface) from the end window photomultiplier are presented in Figure 1. At the far distance (the normal cell position for the instrument) the spectrum is dominated by a single large negative component centered at 682 nm. This spectrum varied from sample to sample (e.g., see Figure 2, solid curve) but always retained this general feature. When the cuvette is placed in a position close to the detector so as to collect a large fraction of the scattered light, the CD (Figure 1) is dominated by a large positive peak at 688 nm. In this close configuration it can also be seen that the CD at long wavelengths (~720 nm), where the chloroplasts have little absorption (and hence should exhibit little CD), is close to the instrument baseline. With the sample cuvette at an intermediate distance from the detector, a

spectrum (Figure 1) which resembles a superposition of the far and close distance spectra is obtained. This spectrum has a positive component at 691 nm and a negative one at 678 nm, and is similar to what has been reported as the CD of intact spinach chloroplasts by other workers (Gregory et al., 1972; Brody and Nathanson, 1972)..

When chloroplasts are fragmented, either by sonication (Dratz et al., 1966) or detergent treatment (Gregory et al., 1972), so as to reduce scattering, the CD is dramatically altered. The magnitude of the CD is reduced by an order of magnitude and a spectrum with three components is seen. The CD spectrum of sonicated chloroplast fragments is shown in Figure 2 compared with that of intact chloroplasts, both in the "normal" cuvette position (53 cm). Note that the fragmented chloroplast CD spectrum has been multiplied by a factor of ten although the chlorophyll absorption of the two preparations is nearly identical. The CD spectrum of the sonicated chloroplasts was found to be independent of the position of sample cuvette with respect to the instrument detector. The sonicated chloroplasts exhibit components at 686(-), 670(+), and 654(-) nm, as reported previously (Dratz et al., 1966). The 654 nm negative component has been shown (Dratz et al., 1966) to be due to chlorophyll b and is absent in the spectrum of sonicated mutant barley chloroplasts lacking this pigment.

These results and literature discussions of CD scattering artifacts (e.g., Gordon, 1972; Dorman and Maestre, 1973) led us to suspect that the large differential scattering effect seen in intact chloroplast might be due primarily to scattering from the ordered arrangement of internal membranes within the chloroplast. These ordered membrane structures contain the chlorophyll molecules responsible for the absorption and CD in this

spectral region. The distance between successive units within these stacked membranes, called grana, is estimated to be about 25 nm in iso-osmotic medium (Steinmann and Sjöstrand, 1955; Heath, 1969). In order to explore this possibility we examined the effect of light scattering on the CD of other photosynthetic materials. In Figure 3 it can be seen that scattering causes effects on the CD of whole cells of the unicellular green alga Chlorella pyrenoidosa similar to those observed in spinach chloroplasts. Chlorella, which has a chloroplast structure much like that found in spinach, exhibits the large single negative CD component with sample cuvette far from the photomultiplier tube and develops a large positive peak to longer wavelengths as the cuvette-to-detector distance is decreased.

In the procaryotic blue-green algae the disposition of the lamellar membranes within the cell varies considerably from species to species (Cohen-Bazire, 1971). The ordering of these photosynthetic membranes into the grana stacks found in chloroplasts, however, is never observed. With whole cells of the unicellular blue-green alga, Aphanocapsa sp. 6714, we observed (Figure 4, upper and lower curves) only relatively small changes in the CD as the distance was varied between phototube and cuvette. In both far and near positions from the detector these algae exhibit a negative (691 and 688 nm, respectively) and two positive (676, 644 and 673, 638 nm) CD components. The peak near 640 nm is due to the phycocyanin accessory pigments of blue-green algae. The baseline displacement for these spectra is real and unexplained. When these algae are fragmented and have most of their phycocyanin removed the two long wavelength CD components (Figure 4, middle curve) exhibited by the whole algae cells remain.

The CD of Aphanocapsa (which does not contain chlorophyll b) in both the whole and fragmented state strongly resembles that of sonicated chloroplasts from the barley mutant lacking chlorophyll b (Dratz et al., 1966). We have obtained almost identical CD spectra for the unicellular blue-green alga, Anacystis nidulans, using both whole and fragmented cells.

The CD spectrum (Figure 5), using the "normal" cuvette position, for whole cells of Rhodopseudomonas spheroides, a purple photosynthetic bacterium, changes only slightly upon sonication and separation of chromatophore particles. The whole cells exhibit a negative component at 867 nm and a positive one at 848 nm. The chromatophores have similar components at 865 and 845 nm. In these organisms the major photosynthetic pigment is bacteriochlorophyll a, which is located in spherical vesicles of about 50 nm diameter (Cohen-Bazire, 1971). It is noteworthy that the CD spectra of a scattering suspension of intact cells and/non-scattering chromatophores from R. spheroides are quite similar.

An attempt was made to see if the large differential light scattering effect could be reintroduced in sonicated chloroplasts by forcing them to aggregate. Suspensions with increased light scattering were made from sonicated chloroplasts by addition of either cupric ions or glutaraldehyde. In both cases little or no CD change was observed as scattering increased by more than a factor of 10.

Electron microscopy studies (Izawa and Good, 1966; Murakami and Packer, 1971) have shown that the grana stacking of photosynthetic membranes within a chloroplast is sensitive to the ionic strength of the medium. With this in mind, the effect of ionic strength on the CD of chloroplasts was examined. Two sets of experiments were carried out in which the CD spectrum of chloroplasts, under conditions of low salt concentration, was

compared with the CD spectrum of intact chloroplasts in isoosmotic suspension. In one case only a relatively small CD change was seen. In the second experiment the predominantly negative CD of whole chloroplasts was changed into a spectrum with two components similar to that observed for whole chloroplasts (Figure 1) when the sample is 15 cm from the phototube. This is in contrast to the results of Gregory et al. (1972) and L. Vickery (personal communication), both of whom observed a four-banded spectrum under conditions of low osmotic strength. These different results may be an expression of the sensitivity of grana structure to different experimental conditions such as method of preparation, suspending medium, and exact salt concentration.

Discussion

We consider this research to be relevant to two active areas of research. The first of these concerns the structure of chloroplasts and the arrangement of chlorophyll molecules within the chloroplast membranes. The other area involves the more general problem of th origin and significance of the differential scatter of circularly polarized light and its effect on the apparent CD of suspensions of biological materials. These two questions will be considered in order.

Chloroplast structure. In order to help elucidate chloroplast structure, photosynthetic membranes have, in recent years, been subjected to a wide variety of fractionation procedures. Methods of cell disruption (French press, sonication, detergent treatment) followed by separation techniques (column chromatography, ultracentrifugation) have been used in an attempt to break the membranes into representative fragments or

their component parts. Such approaches have resulted in the isolation and characterization of several chlorophyll-proteins. The question always remains, however, of whether alterations in the internal molecular arrangements have been introduced into the system by the initial fractionation procedure. That is, do the subchloroplast particles retain their in vivo organization? Addressing this problem, Gregory et al. (1972) observed the large change in the CD of intact chloroplasts upon addition of the detergent, digitonin. They proposed that the more intense spectrum indicated stronger interactions among aggregated chlorophyll molecules in intact chloroplasts, and that upon fragmentation some of the chlorophyll redistributes itself with consequent reduction in aggregation and CD amplitude. Our results on the CD of intact chloroplasts (Figure 1) show that the observed spectrum is a strong function of the fraction of scattered light detected by the instrument. This distorted spectrum is so sensitive to differential light scatter as to preclude knowledge of the intrinsic CD of intact chloroplasts without more detailed investigation. For this reason conclusions about relative amounts of aggregated chlorophyll based on comparisons of the uncorrected CD spectra (Figure 2) of whole and fragmented chloroplasts are, in our view, essentially meaningless.

Some indication, however, of the intrinsic CD of intact chloroplasts may be found in the experiments done with the blue-green algae, Aphanocapsa sp. 6714 and Anacystis nidulans, which are approximately the same size as chloroplasts. Turbid suspensions of these organisms (which, biochemically, have a photosynthetic apparatus very much like that of higher plants) do not exhibit the dramatic differential light scattering seen in chloroplasts. The CD spectra (Figure 4) of whole cells of the blue-green algae in the far and close distances to the detector and of fragmented algae are all

qualitatively alike (after taking into account that phycocyanin has been removed from the fragmented sample--see Results). These spectra are, in turn, rather similar to that of sonicated chloroplasts (Figure 2) (after taking into account, in this case, that the blue-green algae contain phycocyanin and also that the sonicated chloroplasts contain chlorophyll b which gives rise to the 654 nm(-) component in Figure 2). The only known significant difference between the arrangement of photosynthetic apparatus of higher plants and blue-green algae is that in higher plants the lamellar membranes which contain chlorophyll are mostly located in highly ordered stacks called grana inside the chloroplast (Jensen and Park, 1967), while in blue-green algae the lamellae are unstacked and are distributed throughout the cell (Cohen-Bazire, 1971). Theoretical analysis of the differential scatter of left and right circularly polarized light (Schneider, 1971) shows it to be a function of the structure of the scattering particles. It is likely that the ordered arrangement of membranes profoundly influences the large differential light scatter from chloroplasts. This tentative conclusion is supported by the fact that the in vivo photosynthetic apparatus of blue-green algae, which has a less ordered macrostructure, does not exhibit the impressively large CD scattering effects of chloroplasts. Furthermore, since the CD spectrum of whole blue-green algae is similar to that of sonicated chloroplasts, it is entirely feasible that the intrinsic CD of intact chloroplasts is like that of fragmented chloroplasts.

The possibility, of course, exists that in addition to inducing a large differential scattering effect the stacking of photosynthetic membranes causes some rearrangement of chlorophyll molecules which changes the CD spectrum. There is no current evidence that this occurs, and the

authors consider it to be unlikely. Our CD results indicate that fragmented chloroplasts can be used as model systems for studies on the *in vivo* environment of chlorophyll.

The CD experiments done with other organisms are consistent with the conclusions above. *Chlorella pyrenoidosa*, a green alga with a spinach-like chloroplast, showed the large differential scatter effect (Figure 3) while *R. spheroides*, a purple bacterium without highly ordered photosynthetic membranes, did not (Figure 5). The experiment in which the aggregated sonicated chloroplasts did not exhibit different light scatter (see Results) implies that it is not scattering *per se* but the specific internal structure of the scattering particle which is responsible for the CD effects. The varied results obtained with chloroplasts in low salt buffers (see Results) suggest that the effect of differential light scatter on CD is sensitive to the precise configuration of the chloroplast membranes.

Differential light scattering. Experimental techniques for correcting CD spectra for distortions due to light scattering have, until recently, been noticeably lacking. This is in contrast to absorption measurements where many instrumental techniques have been developed to eliminate scattering distortions by enlarging the solid angle of detection (Shibata, 1958; Butler, 1964). The only exception to this has been the recent work of Dorman and Maestre (1973), who detected differential light scatter by observing variations in the CD spectrum of T2 phage with changes in the light collection geometry of their instrument. This technique is general and has been used in the research presented here.

Two sources of distortion of CD spectra of particulate suspensions have been recognized. The first of these is a flattening effect analogous

to that present in absorption measurements of particulate matter discussed in detail by Duysens (1956) and Rabinowitch (1956). This effect has its origin in "absorption statistics". That is, the suspension will have regions (the particles themselves) where absorption is very high and other regions (between suspended particles) where absorption is negligible. Flattening effects on optical activity have been analyzed by Gordon and Holzwarth (1971). Since this effect is inherent in the particulate materials, it cannot be corrected for instrumentally.

The second source of distortion is the differential scatter of left and right circularly polarized light. This is the principal effect responsible for the gross distortion of the CD of intact chloroplasts reported here. The CD scattering effects of intact chloroplasts are the largest and most dramatic reported to date. We attribute these large effects to the complex internal structuring of membranes within the chloroplast.

The most sophisticated calculations of differential light scattering have been done by Gordon (1972) and Gordon and Holzwarth (1971), using Mie scattering theory. These calculations, however, were done using symmetrical model systems such as a solid sphere or spherical shell, and their direct application to complex structures such as the grana stacks of chloroplasts is not valid. In addition, the large effects which we have observed may be a manifestation of differential scattering from an asymmetrically ordered membrane structure as proposed by Dorman and Maestre (1973). This proposal, however, needs to be placed on a firmer theoretical basis.

It is clear that both experimental and theoretical research must be extended before the effect of differential light scattering on CD is

understood. Chloroplasts, which show an unusually large effect, are an excellent material for further, more extensive experimental studies. For example, the measurements made here could be extended down into the UV region of the spectrum in order to examine effects on the CD attributable to the protein components. The ratio of the wavelength of light to the size of the scattering structures would then be different from that in the visible region, and different scattering properties might be expected. A more systematic study on the dependence of the CD of chloroplasts on ionic strength should yield interesting results. Also, relevant information could be obtained by direct measurement of the differential scattering; that is, by measuring, at various angles, the differential circular polarization of the scattered light.

In closing, we note that we have refrained from referring to differential light scattering as an artifact, as most other workers have. This is because that
we feel/there is much information potentially to be gained from measurements of this effect. Further research should find it to be a valuable tool in the study of macrostructures.

Acknowledgments

The authors wish to thank Dr. B. P. Dorman for many enlightening discussions. Preliminary measurements were made by Dr. L. Vickery. Samples were provided to us by Dr. R. Pelroy (Aphanocapsa sp. 6714), Mr. H. Yokota (Chlorella pyrenoidosa, Anacystis nidulans), and Mr. L. Austin (Rhodopseudomonas spheroides).

References

Brody, M., and Nathanson, B. (1972), Biophys. J. 12, 774.

Butler, W. L. (1964), Ann. Rev. Plant Physiol. 15, 451.

Cantor, K. P., and Hearst, J. E. (1969), J. Mol. Biol. 49, 213.

Cohen-Bazier, G. (1971), in Biological Ultrastructure; The Origin of Cell Organelles, Harris, P. J., Ed., Corvallis, Oregon, Oregon State University Press, p. 65.

Dorman, B. P., Hearst, J. E., and Maestre, M. F. (1973), Methods Enzymol. 27, in press.

Dorman, B. P., and Maestre, M. F. (1973), Proc. Nat. Acad. Sci. U.S. 56, 1552.

Dratz, E. A., Schultz, A. J., and Sauer, K. (1966), Brookhaven Symp. Biol. 19, 303.

Duysens, L. N. M. (1956), Biochim. Biophys. Acta 19, 1.

Glaser, M., and Singer, S. J. (1971), Biochemistry 10, 1780.

Gordon, D. J. (1972), Biochemistry 11, 413.

Gordon, D. J., and Holzwarth, G. (1971), Arch. Biochem. Biophys. 142, 481.

Gordon, D. J., and Holzwarth, G. (1971), Proc. Nat. Acad. Sci. U.S. 68, 2365.

Gregory, R. P. F., Raps, S., Thornber, J. P., and Bertsch, W. F. (1972), in Proceedings of the IInd International Congress on Photosynthetic Research, Fiorti, G., Avron, M., and Melandri, A., Eds., The Hague, Dr. W. Junk N.V., p. 1503.

Heath, O. V. S. (1969), The Physiological Aspects of Photosynthesis, Stanford, California, Stanford University Press, p. 4.

Izawa, S., and Good, N. E. (1966), Plant Physiol. 41, 544.

Jensen, W. A., and Park R. B. (1967), Cell Ultrastructure, Belmont, California, Wadsworth Publishing Co., p. 16.

Lenard, J., and Singer, S. J. (1966), Proc. Nat. Acad. Sci. U.S. 56, 1828.

Maestre, M. F., Gray, D. M., and Cook, R. B. (1971), Biopolymers 10, 2537.

Maestre, M. F., and Tinoco, I., Jr. (1967), J. Mol. Biol. 23, 323.

Murakami, S., and Packer, L. (1971), Arch. Biochem. Biophys. 146, 337.

Ottaway, C. A., and Wetlaufer, D. B. (1970), Arch. Biochem. Biophys. 139, 257.

Rabinowitch, E. I. (1956), Photosynthesis, New York, Wiley, p. 1863.

Schneider, A. S. (1971), Chem. Phys. Lett. 139, 257.

Schneider, A. S. (1973), Methods Enzymol. 27, in press.

Schneider, A. S., Schneider, M. T., and Rosenheck, K. (1970), Proc. Nat. Acad. Sci. U.S. 66, 793.

Shibata, K. (1958), J. Biochem. 45, 599.

Steim, J. M., and Fleischer, S. (1967), Proc. Nat. Acad. Sci. U.S. 58, 1292.

Steinmann, E., and Sjöstrand, F. S. (1955), Exp. Cell Res. 8, 15.

Urry, D. W. (1972), Biochim. Biophys. Acta 265, 115.

Urry, D. W., and Ji, T. H. (1968), Arch. Biochem. Biophys. 128, 802.

Urry, D. W., and Krivacic, J. (1970), Proc. Nat. Acad. Sci. U.S. 65, 845.

Urry, D. W., Mednicko, N., and Bejnarowicz, E. (1967), Proc. Nat. Acad. Sci. U.S. 57, 1043.

Wallach, D. F., and Zahler, P. H. (1966), Proc. Nat. Acad. Sci. U.S. 56, 1552.

Figure Legends

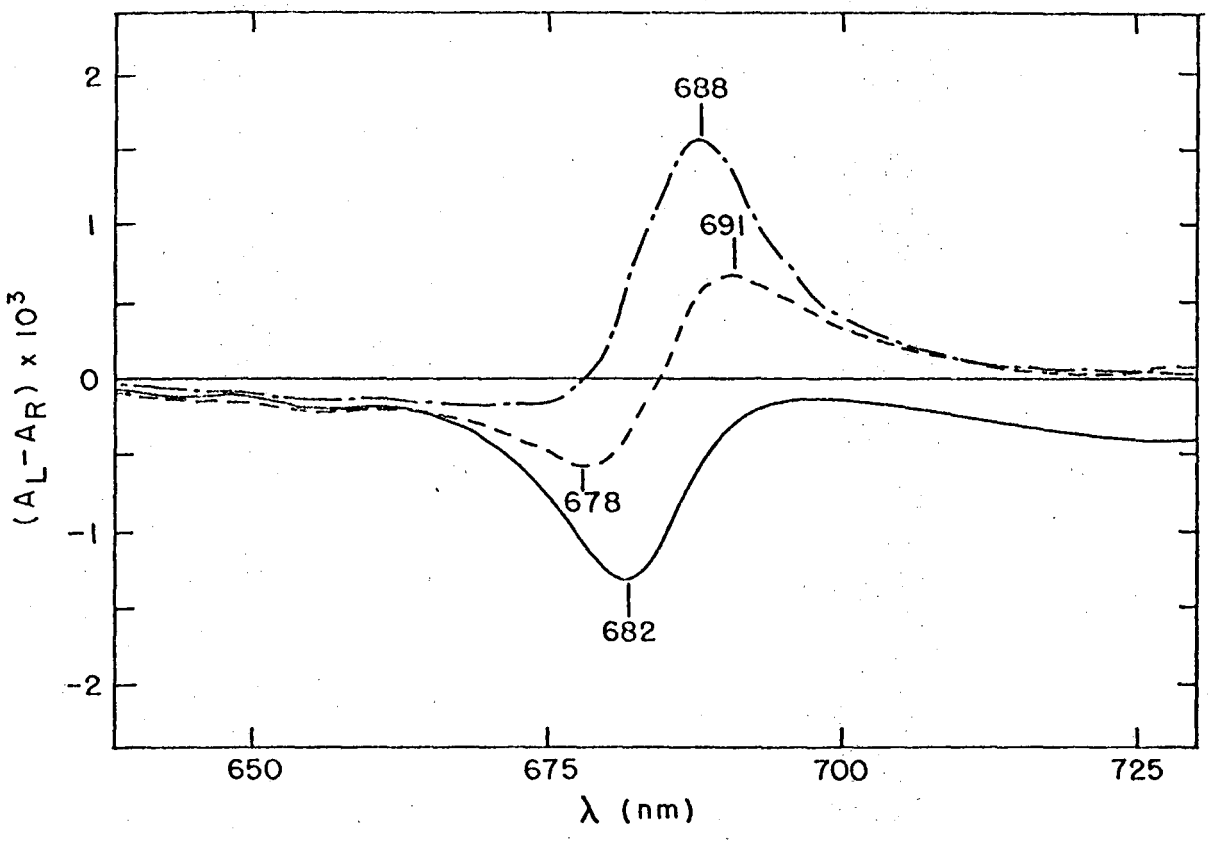
Figure 1. CD spectra of intact spinach chloroplasts taken at three distances between cuvette and photomultiplier tube: 53 cm (——), 15 cm (- - -), .2 cm (— - —). A_{679} = .54, A_{750} = .06. 40 mM phosphate buffer, pH 7.4, .35 M NaCl, 1 cm pathlength, room temperature.

Figure 2. CD spectra of intact (——) and sonicated (- - -) spinach chloroplasts. Cuvette-to-photomultiplier distance equals 53 cm. Note that the sonicated chloroplast spectrum has been multiplied by a factor of 10. Intact chloroplasts: A_{679} = .34, A_{750} = .06; sonicated chloroplasts: A_{679} = .34, A_{750} = .01. 40 mM phosphate buffer, pH 7.4, .35 M NaCl, 1 cm pathlength, room temperature.

Figure 3. CD spectra of Chlorella pyrenoidosa, a green alga, taken at two distances between cuvette and photomultiplier tube: 53 cm (——) and .2 cm (- - -). A_{680} = .56, A_{750} = .18. 40 mM phosphate buffer, pH 7.4, .35 M NaCl, 1 cm pathlength, room temperature.

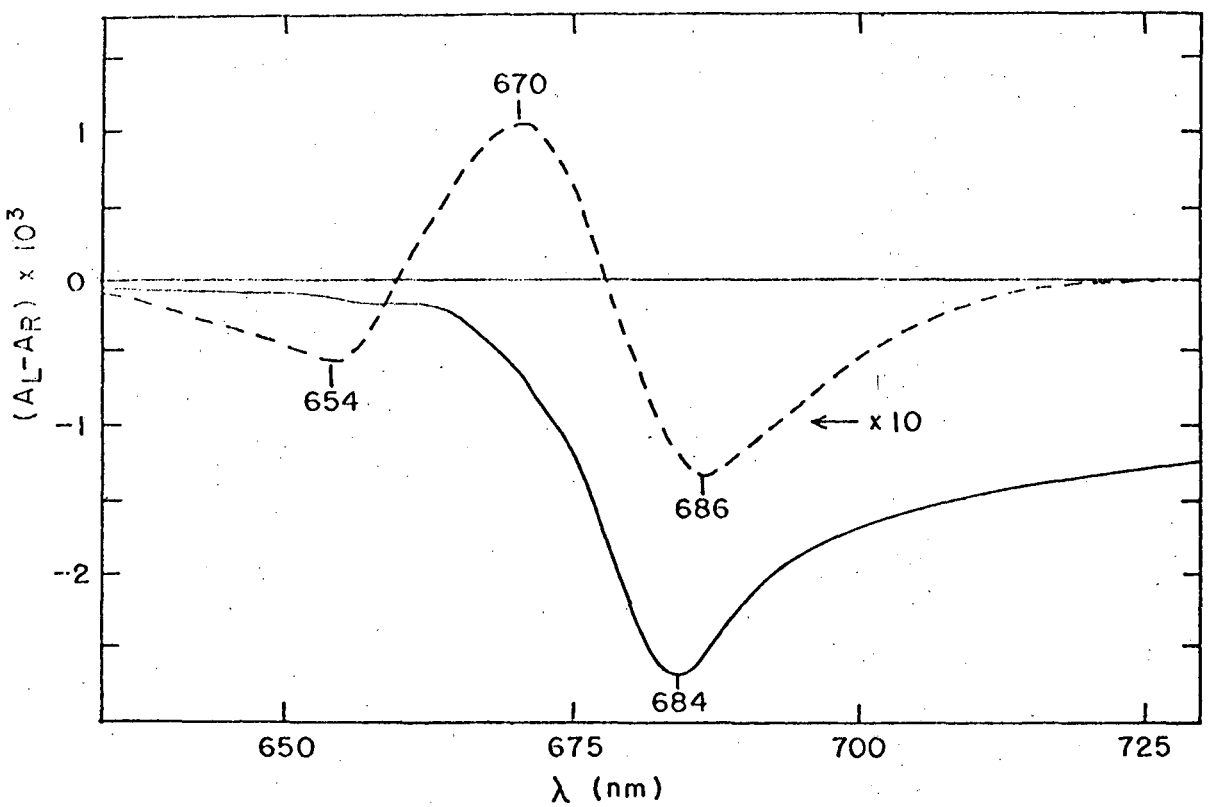
Figure 4. CD spectra of Aphanocapsa sp. 6714, a blue-green alga, taken at two distances between cuvette and photomultiplier tube: 53 cm (——) and .2 cm (- - -), and in the fragmented state (— - —) at 53 cm. The fragmented algae have had most of their phycocyanin removed (see Results). Whole cells: A_{678} = .55, A_{750} = .21; fragmented cells: A_{672} = .33, A_{750} = .01. Whole cells are in culture medium. Fragmented algae are in 10 mM Tris, pH 7.5. 1 cm pathlength, room temperature.

Figure 5. CD spectra of R. spheroides, a purple bacterium. Whole cells (——) and chromatophores (- - -). Cuvette-to-photomultiplier distance equals 53 cm. Whole cells: A_{852} = .56, A_{950} = .13; chromatophores: A_{850} = .52, A_{950} = .005. Both suspensions in 10 mM Tris, pH 7.5. 1 cm pathlength, room temperature.



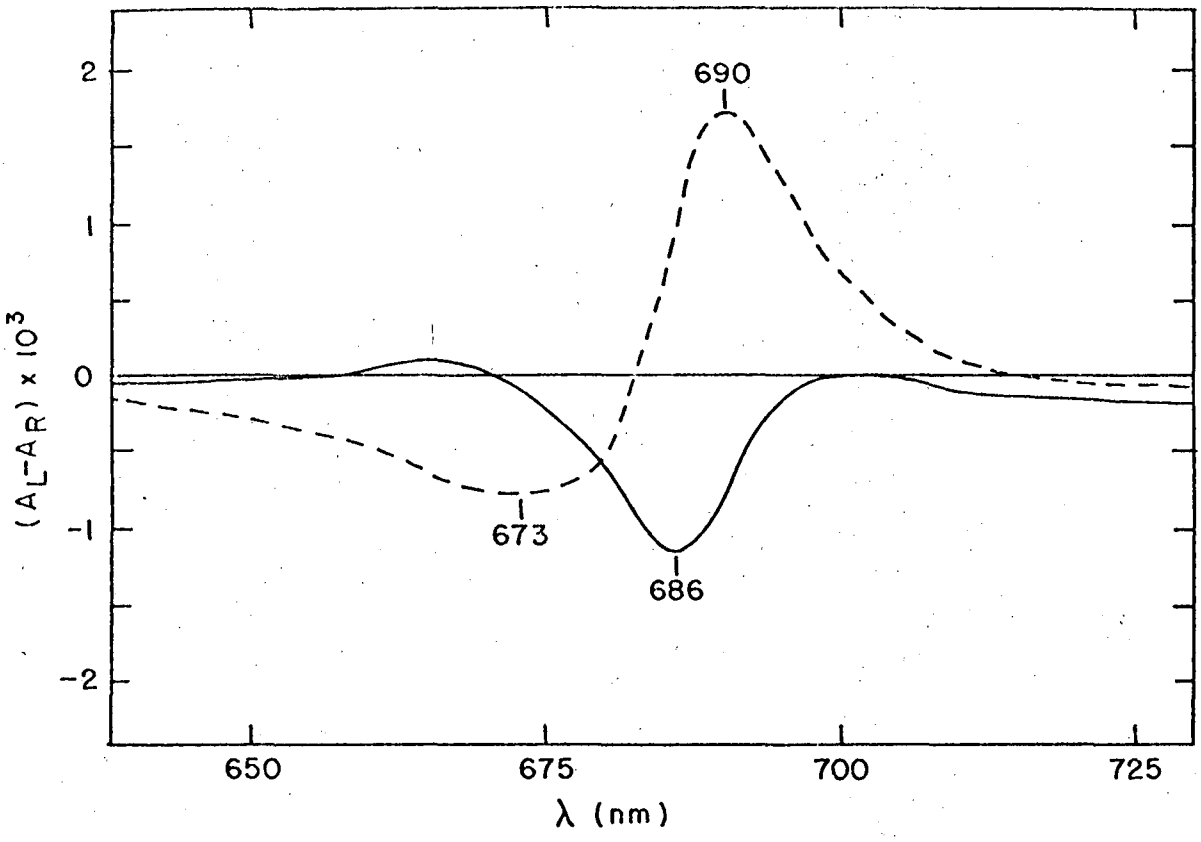
XBL733-4717

Fig. 1



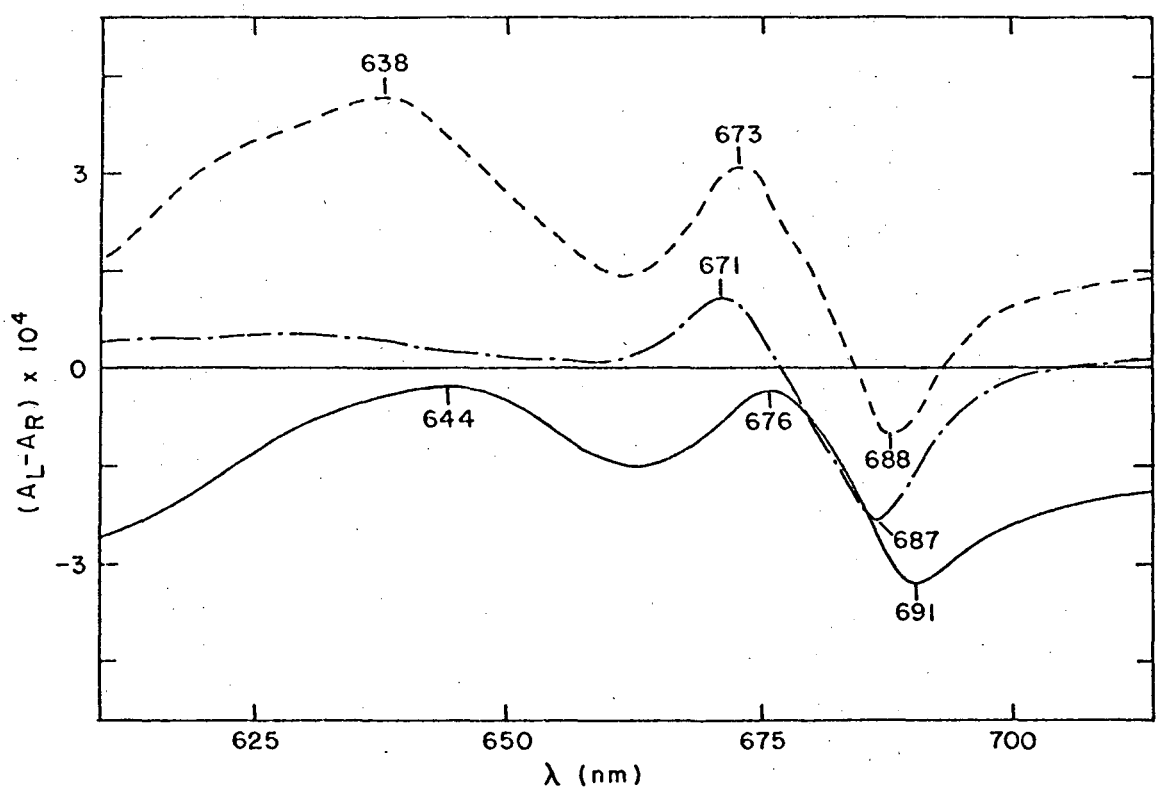
XBL733-4719

Fig. 2



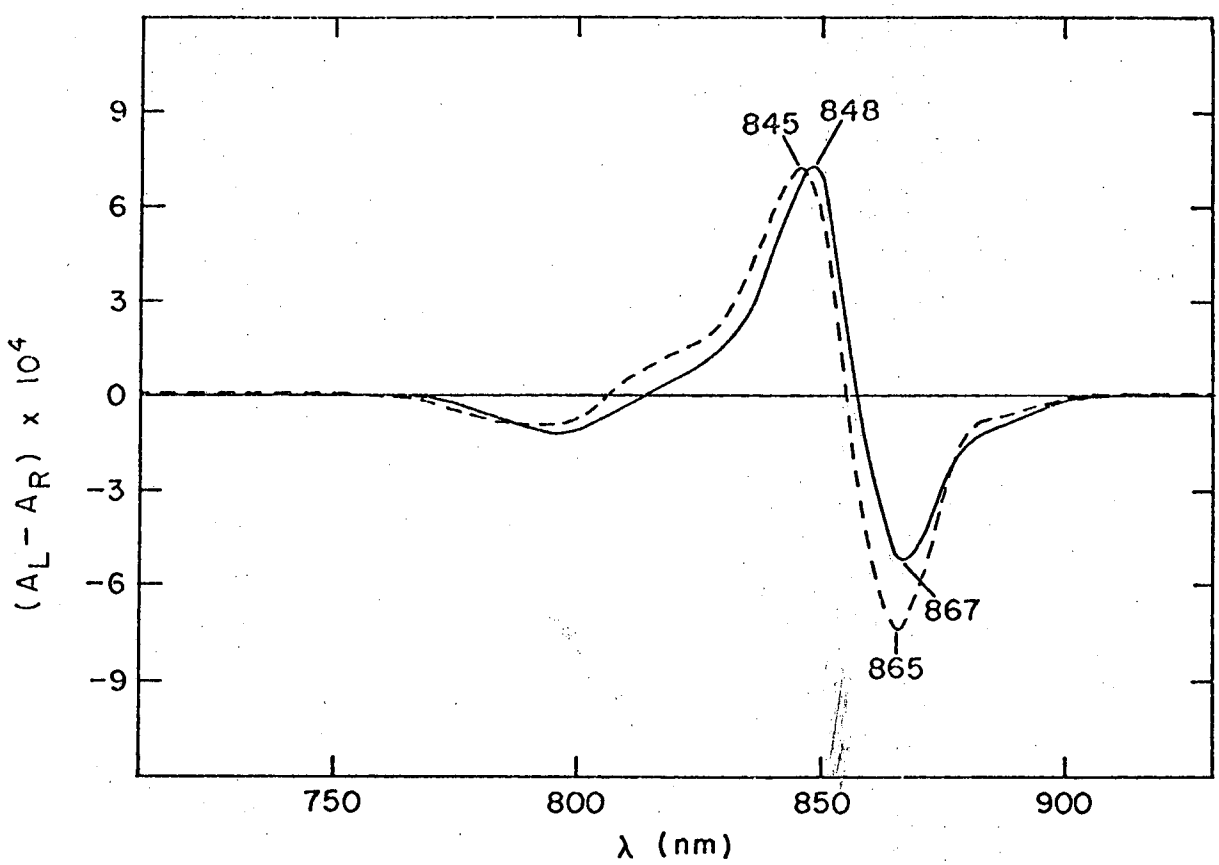
XBL733-4718

Fig. 3



XBL733-4720

Fig. 4



XBL733-4716

Fig. 5

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720