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INTRODUCTION

Isolated chlorophyll molecules in solution are unable to carry out the energy-converting photoreactions characteristic of photosynthetic organisms. These organisms provide lipoprotein matrices (chloroplast grana or lamellae; bacterial chromatophores) in which the chlorophylls, carotenoids and some important oxidation-reduction cofactors are brought together in a highly specific relationship to one another. Any attempt that the experimenter makes to alter this relationship almost invariably leads to complete loss of the photosynthetic activity. This applies to solvent extraction, treatment with most detergents, mild heating, etc. On the other hand, chloroplasts from leaves which have been fixed chemically with glutaraldehyde are found to retain a substantial fraction of their ability to carry out quantum conversion *via* the Hill reaction.[1]

The evidence at hand points to an important role for the particular way in which the pigments and cofactors are arranged spatially. The pigment molecules, for example, cannot be considered to be isolated from one another even as a good first approximation. Typical lamellar fractions contain 6-8% chlorophyll by weight and pigment concentrations run in excess of 0.1 mole-l^{-1}.

Evidence from absorption spectra of chloroplasts from higher plants and algae as well as of photosynthetic bacteria suggests that *in vivo* the chlorophyll is at least partially aggregated. For example, low temperature spectroscopy of plant material resolves several absorption maxima in the long wavelength region, but the extracted chlorophyll *a* has only a single peak.[2] Fluorescence excitation spectra of

these samples suggest that a part of the chlorophyll a, absorbing on the short wavelength side of the main band, is unaggregated and highly fluorescent, while the chlorophyll a absorbing on the long wavelength side of the main band is weakly fluorescent and is supposed to be aggregated.[2]

Studies aimed at elucidating the nature of the pigment associations and their environments have met with only partial success. Oriented chloroplasts or fragments from them show little or no dichroism for most of the pigment absorption.[3-5] The fluorescence of the chlorophyll in these organelles is found to be almost completely depolarized. A long wavelength form of chlorophyll a which is dichroic and gives rise to polarized fluorescence involves only a small fraction of the total chlorophyll in chloroplasts.[4-6] This fraction may, however, be of utmost importance to the energy conversion process.

Studies of nuclear magnetic resonance, infrared and visible absorption spectra and of apparent molecular weights of purified chlorophylls in solution show that dimers and higher aggregates form readily under some conditions.[7-10] These aggregates have greatly enhanced optical activity compared to the monomers and have been studied by optical rotatory dispersion (ORD) in the case of chlorophyll a.[11] The chlorophyll in chloroplast subunits also shows optical activity which is large compared to that of the extracted chlorophyll,[11,12] and this has been used as additional evidence for the presence of aggregated chlorophyll *in vivo*. It has been recognized, however, that the large optical activity of the chloroplast lamellar fragments (quantasomes) could be due to chlorophyll-protein or chlorophyll-lipid interactions, as well as to chlorophyll-chlorophyll interactions. The circular dichroism (CD) measurements reported here are particularly

useful, because they reflect a specific kind of chlorophyll-chlorophyll interaction, the degenerate exciton interaction, in a much more sensitive way than ORD or absorption measurements. The degenerate interaction causes a splitting of the monomer absorption band in the aggregate, and results in a double circular dichroism that crosses zero in the region of the absorption maximum. In the presence of a large amount of other non-degenerate interactions the crossing point may be shifted, but it is still clearly seen. The degenerate interaction is important because it cannot be caused by chlorophyll-protein or chlorophyll-lipid interactions. It can only be due to chlorophyll-chlorophyll interactions. Furthermore, the degenerate interaction carries geometrical information about the aggregate which one has some hope of interpreting in a detailed way. Small exciton splittings that are not directly resolvable in the absorption spectra result in a double CD under most circumstances. The CD spectra of the chlorophylls in quantasomes and chromatophores contain large degenerate contributions which are strong evidence for chlorophyll-chlorophyll interactions in these systems.

EXPERIMENTAL

Materials

The preparation of chlorophyll a, chlorophyll b and bacteriochlorophyll are described elsewhere.[10] Carbon tetrachloride was reagent grade and was used without purification.

The suspension of microcrystalline chlorophyll a was prepared by adding isooctane to a small sample of the solid chlorophyll. After vigorous stirring, the suspension was spun in a clinical centrifuge at

top speed for 5 min, and the supernatant suspension was used directly. No noticeable settling occurred during the half hour period of measurement.

Chromatophores from Rhodospirillum rubrum and Rhodopseudomonas speroides were obtained by washing 5-day old cultures free of growth medium, followed by sonication for 3 min at 0°C with a Biosonik oscillator. Fragments sedimenting between 40,000 g (30 min) and 180,000 g (50 min) were washed and resuspended in 0.05 M phosphate buffer, pH 7.5.

Barley was grown from seed in a phytotron under controlled illumination and temperature, and was harvested about 3 weeks after germination. The normal (Lyon) and the mutant strain (Chlorina 2), which is missing chlorophyll b,[13] were grown under identical conditions and harvested at the same time. Chloroplasts were isolated from the homogenized leaves essentially by the procedure of Park and Pon.[14] Sonication of the chloroplast suspension was followed by isolation of a fraction sedimenting between 9000 g (10 min) and 110,000 g (30 min), resuspension in 10^{-3} M phosphate buffer, pH 7.5, and clarification at 9000 g (10 min).

Methods

Absorption spectra were recorded using a Cary 14 spectrophotometer. In the case of scattering samples the Model 1462 Scattered-Transmission Accessory was used. Optical rotatory dispersion spectra were obtained using a Cary 60 instrument modified with a special red-sensitive photomultiplier.

The circular dichroism instrument is one of our own design. A detailed description of the apparatus will appear in the near future.[15] A few general features of the instrument are worth mentioning here. The apparatus employs a Cary 14 monochromator and a pockels cell polarization modulator driven at 400 Hz.[16] Energy variations are compensated

by controlling the PM voltage. Most measurements are made at a band width of about 1 mμ. The noise level of the instrument varies with wavelength at fixed resolution but is approximately $\pm$ 1x10^{-5} O.D. units at 1 mμ band width over much of the wavelength range of 0.21 μ to 1.2 μ. The sensitivity of the intrument is further increased by averaging many scans of the spectrum using a Nuclear Data ND800 Enhancetron. Under this mode of operation, the monochromator scan is controlled by the Enhancetron time base. Multiple scans reduce the noise level to $\pm$ 1x10^{-6} O.D. units in a reasonable period of time (ca. 2 hr). A Dumont 6911 PM tube is used for near infrared work, and an EMI 9558Q/A is used from 210 mμ to about 800 mμ.

RESULTS AND DISCUSSION

Circular Dichroism Spectra of Chlorophyll Dimers

In vitro measurements of the CD of chlorophyll a, chlorophyll b, and bacteriochlorophyll in most solvents show extremely small optical activity (nearly unmeasurable by CD methods). In carbon tetrachloride, however, increasing concentration results in spectral changes and enhanced circular dichroism. The circular dichroism and absorption spectra of chlorophyll a, chlorophyll b and bacteriochlorophyll solutions containing about 85% dimer are given in Figures 1, 2 and 3. We can determine the molar circular dichroism of the dimers from measurements on these concentrated solutions, since the solutions are stable and we know the extent of dimerization from the equilibrium constants measured using absorption spectra.[10] Precise CD measurements on dilute solutions, free of aggregates, have not yet been possible in carbon

tetrachloride because of decomposition of the chlorophyll during the prolonged time of measurement. Measurements on dilute solutions in the same solvent can be made rapidly, to avoid decomposition, but with a resulting decrease in signal to noise. The dilute solutions show essentially no dichroism, with an upper limit of 5-10% of the concentrated signals.

The dimers give large circular dichroism signals in the relatively isolated far red band, as well as in the more complex Soret band, which is a composite of two nearby electronic transitions. We shall concentrate, initially, on interpreting the CD of the far red bands of these compounds. All three chlorophyll dimers show very similar CD spectra. This is consistent with all the evidence from NMR, IR, and absorption measurements,[7-10] that the three dimers have very similar structure. All three chlorophylls show a double circular dichroism spectrum that reverses sign close to the center of the absorption band. This behavior is due to the degenerate exciton interaction of the long wavelength transition moments of the two monomers in the dimer.[17] The same interaction leads to the observed splitting of the absorption band in the dimer. The degenerate exciton interaction observed in the CD indicates that the monomer transition moments are not parallel nor are the chlorophyll rings coplanar in the dimer. Furthermore, the magnitude and sign of this degenerate CD component is related to the detailed geometry of the dimer in a relatively simple way. The CD spectrum is not symmetrical in amplitude above and below zero because of interactions of each chlorophyll with the electro-static field and polarizabilities (that result from higher energy transitions) presented by the other.[18] This is the same interaction that gives rise to the observed hyperchromism

in the dimer.[10,18,19] It is difficult to assign geometrical significance to the CD asymmetry effect because it originates in the interactions of the transition of interest with very many higher energy transitions as well as a complicated static field interaction.[18]

The degenerate (double CD) contribution can be extracted from the experimental CD spectrum by a straightforward procedure. We fit the CD spectrum to a linear combination of a degenerate component (double CD, as in I in Figure 4) and a non-degenerate component (single CD curve, as in II in Figure 4) whose shape is assumed to be that of the monomer absorption. The degenerate component is recognized in the CD by a change of sign near the absorption maximum, while in the ORD the degenerate component merely gives an assymetry to the apparent single Cotton effect unless the degenerate component is larger in amplitude than the non-degenerate part. The above decomposition is done with a linear least-squares computer program.[20] In the calculation ν_o, the mean frequency of the exciton components, is obtained from an analysis of the absorption spectra.[10]

Geometry of Dimers

A double CD spectrum that is caused by dimerization demonstrates that there is a degenerate interaction between the monomers in the dimer. The most general theory tells us that the chromophores are not coplanar and that the monomer transition moments are not parallel or exactly perpendicular in the dimer. Furthermore, with the aid of a particular theoretical model we may relate the degenerate CD to the detailed geometry of the dimer in a relatively simple way. The interaction may be treated as between point dipoles as a first approximation.[17] This approximation is not exact, but is useful as a start because it is

simple, tractable, and may work satisfactorily.

For the point dipole approximation the dimer dipole strengths ($D_\pm$), rotational strengths ($R_\pm$) and absorption frequencies ($\nu_\pm$ in cm^{-1}) of the two exciton components resulting from monomer transitions at ν_o are given by Equations 1-4:[17]

$$D_\pm = \mu^2 \pm \vec{\mu}_1 \cdot \vec{\mu}_2 = D_o \pm \vec{\mu}_1 \cdot \vec{\mu}_2 \tag{1}$$

$$R_\pm = \mp \frac{\pi}{2} \nu_o (\vec{R}_{12} \cdot \vec{\mu}_1 \times \vec{\mu}_2) \tag{2}$$

$$\nu_\pm = \nu_o \pm V_{12}/hc \tag{3}$$

$$V_{12} = \frac{1}{R_{12}^3} \left[\vec{\mu}_1 \cdot \vec{\mu}_2 - \frac{3(\vec{R}_{12} \cdot \vec{\mu}_1)(\vec{R}_{12} \cdot \vec{\mu}_2)}{R_{12}^2} \right] \tag{4}$$

where $\vec{\mu}_1$ is the electric dipole transition moment of monomer one and $\vec{R}_{12}$ is a vector connecting the centers of the two monomers in the dimer. If the exciton splitting, V_{12}, is smaller than the band width, most of the rotational strength cancels, owing to the strong overlap of components with opposite signs of rotation, as illustrated in Figure 5. In order to get the theoretically useful quantity, the degenerate rotational strength ($R_\pm$), from the experimental values, we must know the exciton splitting of the transition. This is the most difficult and uncertain part of the calculation, but fortunately, in the chlorophyll dimers, we can get experimental values for the exciton splitting from the absorption spectrum. The exciton splittings and the degenerate rotational strengths of the three chlorophylls are given in Table 1.[10] The exciton splitting

Table 1. Spectral Properties of the Exciton Split Components of the Dimers of Three Chlorophylls in Carbon Tetrachloride. (λ_o and ν_o refer to the average of the two exciton band positions.)

Molecule	λ_o (mμ)	ν_o (cm^{-1})	$\Delta\nu$ (cm^{-1})	D_+/D_-	$R_\pm \cdot 10^{40}$ cgs	$\frac{R_\pm}{D_o\nu_o} \cdot 10^8$ cgs
Chlorophyll a	674	14848	363	1.48	ca. ± 20	± 0.74
Chlorophyll b	655	15259	398	1.81	ca. ± 18	± 0.79
Bacteriochlorophyll	796	12565	490	1.32	± 44.5	± 0.92
	590	16940	470	0.9	± 6.9	

$$\Delta\nu = \nu_+ - \nu_- = \frac{2V_{12}}{hc}$$

is assumed to be positive for the purposes of Table 1. The quantities D_+/D_- and $R_z/D_o\nu_o$ depend only on the geometry of the dimers and not on the intensities or positions of the absorption bands. The similarities of these quantities for the three chlorophylls supports the conclusion that their dimer structures are similar.[10] The absorption measurements imply an angle of about 80 degrees between the red transition moments of the two chlorophylls in the dimers for the point dipole model.[10] The angle between the transition moments derived from the CD spectra is about 12 degrees if we assume that the monomer planes are parallel in the dimer. Within the limitations of the point dipole model, the angle between the transition moments of 80 degrees found from the absorption spectra is not dependent on any assumption about the parallelism of the monomer planes. The large discrepancy between the intertransition moment angle found from the absorption spectra (80 degrees) and the angle found from the CD spectra assuming parallel planes (12 degrees) seems much too large to ascribe completely to inadequacies in the point dipole model, since the exciton splitting is experimentally observed. We are forced to the conclusion that the chlorophyll planes are not parallel in the dimer.

If we attach a coordinate system to one monomer, two angles and a distance are needed to specify a vector from the center of the fixed monomer to the center of the other monomer. Three additional angles are sufficient to specify the orientation of the second monomer in this coordinate system. Six parameters, 5 angles and a distance, are needed to specify the dimer geometry in the general case. The observable parameters available for each absorption band are the degenerate rotational

strength, $R_\pm$, the ratio of the dipole strengths, D_+/D_-, and the exciton splitting, V. Thus, there are three interaction parameters for each absorption band that exhibits exciton interaction. We can specify the geometry of the dimer from the interaction parameters for two independent transitions polarized along different directions in the molecule.

Bacteriochlorophyll has a distinct electronic transition at 590 mμ polarized in the molecular plane, but polarized perpendicular to the previously discussed 780 mμ transition.[21,22] The 590 mμ transition is fairly strong (oscillator strength, f=0.11) and shows a double CD component. The six interaction parameters for the two bands of bacteriochlorophyll are given in Table 1, and the analysis of these data is in progress. The available evidence indicates that chlorophyll a, chlorophyll b and bacteriochlorophyll form nearly identical dimers.[7-10] If a structure of the bacteriochlorophyll dimer can be determined, we can propose this structure for the other chlorophyll dimers with some confidence and test it against the CD and absorption data.

Crystalline Chlorophyll a

Figure 6 shows the absorption and CD spectra for chlorophyll a microcrystals in suspension. The crystal structure is not yet known for any of the chlorophylls; however, the CD measurement has definite qualitative interest in relation to the observed quantasome chlorophyll CD. There is a slight solubility of chlorophyll a in isooctane, the suspension medium, so there is a small monomer peak at 666 mμ. In the crystal absorption spectrum this peak is shifted to 745 mμ. This large red shift of the crystal over the monomer is probably due to the enormous polarizability of the crystalline environment compared to the

solvent surrounding the monomer. It is a general observation that π-π^* transitions of molecules in polarizable solvents are red-shifted relative to those in solvents of low polarizability.[23] These red shifts are experimentally observed for the chlorophyll transitions. Increasing chlorophyll aggregation leads to increasing red shift of the absorption. A 10-15 mμ red shift for the dimer relative to the monomer is observed in carbon tetrachloride. At very high concentrations in carbon tetrachloride the bacteriochlorophyll absorption and CD show evidence of higher aggregates, red shifted 30 mμ relative to the monomer. The molecule in the crystal is essentially dissolved in chlorophyll, a very polarizable medium, and the large red shift (80 mμ for chlorophyll a) results.

An analysis of the crystal CD shows that the degenerate component crosses zero within 1 mμ of the observed absorption peak. Therefore, the weak short wavelength shoulder observed in the crystal absorption spectrum (ca. 720 mμ) is not an exciton split peak and must be vibrational in origin. The gaussian half width is only about 25 cm^{-1} larger than for the monomer in CCl_4, so the exciton splitting in the crystal must be quite small.

Quantasomes from Barley Chloroplasts and from a Mutant Deficient in Chlorophyll b

Analysis of the optical properties of a preparation from higher plant chloroplasts is complicated by the presence of chlorophyll a, chiefly responsible for the absorption maxima at 678 and 436 mμ, and chlorophyll b, which gives rise to the distinct shoulder near 650 mμ and another at about 470 mμ. The carotenoids present have absorption maxima at 485, 455, and 428 mμ.[24] Figure 7 shows the ORD and absorption

spectra of suspensions of lamellar fragments (quantasomes) of chloroplasts from normal barley as well as from a mutant that is completely missing chlorophyll b.[13] In the visible region of the spectrum, the mutant and normal quantasomes have identical ORD and absorption spectra except for the chlorophyll b regions near 650 and 470 mμ, where there are large differences. Figure 8 shows the CD spectra of these same materials. The chlorophyll a part of the CD spectrum in the mutant appears to be identical to the chlorophyll a region in the normal barley.

The chlorophyll a in the quantasomes shows a large double CD component as we saw in the dimers and crystals. Presence of the double CD component in the chlorophyll a absorption region is good direct evidence for chlorophyll a-chlorophyll a interaction in quantasomes. Chlorophyll-protein or chlorophyll-lipid interactions would lead only to single CD bands and, if present, would only increase or decrease the asymmetry of the observed double CD component. The presence of a double CD implies that the interacting chlorophylls are not coplanar nor are the red chlorophyll a transition moments parallel or exactly perpendicular in the quantasomes. The double CD component crosses zero at about 685 mμ. This is the average frequency of the exciton bands $\nu_o = (\nu_+ + \nu_-)/2$ that give rise to the double CD. Thus, the interacting chlorophyll a molecules absorb on the long wavelength side of the quantasome absorption peak at 678 mμ. The chlorophyll that absorbs on the short wavelength side of the main peak is thought to be unaggregated, because it has relatively high fluorescence efficiency.[2]

The shape of the chlorophyll CD in quantasomes is reminiscent of the shape of the crystalline chlorophyll CD, which suggests that the chlorophyll a molecules in quantasomes and in the crystal have similar geometries. However, the interacting chlorophyll a in quantasomes is

not aggregated exactly like a three-dimensional crystal because the absorption is not red shifted as far as is the three-dimensional crystal. Chlorophyll a monolayers have their absorption shifted to 680 mμ.[25] The red shift of the chlorophyll that gives rise to the double CD in the quantasomes is about the same as the red shift in the monolayer. It may be, therefore, that the aggregated chlorophyll in the quantasomes is only one molecule thick, corresponding to a geometry like a two-dimensional chlorophyll a crystal.

The CD amplitude is small for quantasomes compared to that for a crystal suspension with equal peak absorption. A decrease in CD amplitude in a one- or two-dimensional crystal is expected relative to a three-dimensional crystal of otherwise identical geometry. The exciton forces giving rise to the CD effects are relatively long range,[26] and therefore depend on the extent of the aggregate.

The quantasome chlorophyll a CD (Figure 8) is about the same amplitude as that of the chlorophyll a dimer CD (Figure 1) for equivalent total red absorption. It would be useful to subtract the absorption of the nonaggregated chlorophyll in the quantasome to determine the aggregate CD amplitude/unit of aggregate absorption. However, we have no direct evidence on the fraction of the total chlorophyll a that is aggregated in the quantasome. The exciton splitting evidenced by the CD is not resolved in the crystal or quantasome absorption at normal temperatures. However, the liquid nitrogen temperature derivative spectra of plant material resolves peaks at 673 mμ, 683 mμ and about 695 mμ.[2] The double CD component crosses zero at about 685 mμ, and the observed absorption peak in the 673 mμ region is too far away to contribute to the double CD of the aggregate. The 673 mμ peak, estimated

by Brown and French[27] to be about 50% of the total chlorophyll a absorption, is a reasonable approximation to the amount of unaggregated chlorophyll a. It is consistent with the CD evidence to propose that both the chlorophyll a 683 and 695 mμ are exciton peaks resulting from the same aggregate. If this were true, about one half of the chlorophyll a is in the aggregate. Assuming that at most one half of the chlorophyll a in the quantasome is aggregated,[29] the CD amplitude of the aggregated chlorophyll a is at least twice that of the solution dimer.

The chlorophyll a dimer CD has a shape similar to the quantasome chlorophyll a CD, although the dimer CD has opposite sign. The opposite sign is not an indication of any great difference in the two geometrical structures, for it could be given by a mirror image relationship. We take the similarity in shape of the dimer and quantasome CD to mean that the two geometrical structures are similar. A long wavelength shoulder is clearly seen in the dimer spectrum, while the spectrum of plant material does not show a shoulder except at low temperature. The plant material shoulder must be somewhat obscured by absorption from unaggregated chlorophyll. The large amplitude of the quantasome chlorophyll a CD suggests a more extensive aggregate than a dimer. Helices, which are analogues of a one-dimensional crystal, are predicted to have a relatively large dependence of the rotational strength on chain length.[28] Two-dimensional systems might be expected to have an even stronger dependence on aggregate size. It is possible that the quantasome aggregates are dimers with a geometry that leads to larger rotational strength than the solution dimer. The aggregated chlorophyll a in the quantasomes is at least a dimer, and is most probably a more extensive aggregate in one or two dimensions.

Plants can be grown with altered amounts of the different chlorophyll components. From the CD of these materials we may be able to determine the absorption of the aggregated components, find out how much of the chlorophyll a is aggregated and, in principle, determine the geometry of the aggregate.

The predominant interaction of chlorophyll a is with itself, since the CD in the chlorophyll a region is identical in the normal and chlorophyll b-free mutant. The chlorophyll b region also has a degenerate component in the quantasome CD that is seen in the difference between the normal and the b-free mutant CD curves. This double CD in the chlorophyll b region indicates that in the normal barley at least some of the chlorophyll b is interacting with other chlorophyll b molecules.

One must not ignore the possibility that the observed CD in quantasomes is not due to degenerate interaction between chlorophylls, but rather due to more than one type of independent non-interacting chlorophyll. This explanation would require two types of chlorophyll environment, one absorbing at long wavelength with negative CD and the other at short wavelength with smaller positive CD. The close relation between the chlorophyll a dimer and the crystal CD, where only chlorophyll-chlorophyll interactions are present, and the quantasome chlorophyll a tends to favor the chlorophyll-chlorophyll interaction origin of the CD in quantasomes. Experimental investigation of plant material having altered amounts of the different chlorophyll components should answer this question directly.

Chromatophores from Photosynthetic Bacteria

Figure 9 shows the CD spectrum and absorption spectrum of Rhodospirillum rubrum chromatophores, and Figure 10 shows the absorption

spectrum of chromatophores of Rhodopseudomonas spheroides, together with portions of the ORD and CD spectra. The near infrared peaks of chromatophores from both species of bacteria show pronounced double CD components, indicating strong bacteriochlorophyll interactions. The double CD shows that the interacting bacteriochlorophylls are not coplanar and that their transition moments are not perpendicular to one another.

R. rubrum chromatophores exhibit a double CD band (Figure 9) that crosses zero very close to the main absorption peak at 880 mμ. This indicates that at least some of the bacteriochlorophyll with absorption centered at the main peak is aggregated with an exciton splitting small compared to the band width. Little unaggregated bacteriochlorophyll seems to be present, as this peak in the chromatophore spectrum is sharp and not spread out, as it would have to be if isolated and aggregated molecules were present together. The bacteriochlorophyll long wavelength peak is slightly wider for the chromatophores than for the monomer absorption in carbon tetrachloride. If we assume a band shape like that in the monomer spectrum, the exciton splitting is of the order of 30-60 cm^{-1}.

In R. rubrum, the 590 mμ bacteriochlorophyll CD peak has no obvious double CD component. However, the CD peak is red shifted from the absorption peak. Since this is an allowed electronic transition, one would not expect vibrational effects to shift the CD maximum from the absorption maximum.[29] A negative component of the bacteriochlorophyll CD on the short wavelength side of the 590 mμ peak may be obscured by the positive carotenoid CD. This could be the origin of the 2.5 mμ red shift of the positive bacteriochlorophyll CD peak. The CD curve

at the bottom of Figure 5 shows an example of the shift of a single CD peak centered at the absorption maximum by the addition of a double CD component. R. rubrum can be grown carotenoidless under the proper conditions.[30] Carotenoidless chromatophores would allow us to see the 590 mμ bacteriochlorophyll CD and absorption free of interference.

R. spheroides chromatophores (Figure 10) show a particularly strong double CD in the longest wavelength absorption band, indicating aggregation of the bacteriochlorophyll. The sharp peak on the short wavelength side at 799 mμ seems to have a comparatively weak double CD component, judged by the shift to 793 mμ in the CD spectrum, with a magnitude approximately equal to that of the solution dimer. The 799 mμ band absorbs about where the solution dimers do, but shows no obvious splitting in the chromatophore absorption spectrum. The positions of the double CD and absorption indicate that the band may be due to dimers of bacteriochlorophyll. The small exciton splitting requires that the dimers have a different geometry from that of the solution dimers.

The long wavelength absorption band at 852 mμ is red shifted much farther than the solution dimer and, since the double CD indicates an aggregate, the bacteriochlorophyll absorbing here is undoubtedly a higher aggregate than a dimer. There is an obvious long wavelength shoulder (ca. 880 mμ) on the main absorption band (852 mμ). The double CD component crosses zero slightly to the long wavelength side of the main absorption peak. Similar behavior is observed in all of the chlorophyll dimers in solution. The long wavelength shoulder may well be an exciton component split off from the main peak. Many photosynthetic bacteria show this long wavelength shoulder, sometimes to an extent that varies with growth conditions.[31] Under these conditions,

either the bacteriochlorophyll aggregate geometry is altered or two different aggregated forms are present in variable amounts. Low temperature absorption and CD measurements may distinguish between these alternatives.

SUMMARY

The circular dichroism (CD) and absorption spectra of dimers of chlorophylls a, b and bacteriochlorophyll in carbon tetrachloride solution and of suspended crystalline chlorophyll a are presented. The dimers of all three chlorophylls seem to have a very similar structure by the criterion of these measurements. The chlorophyll-chlorophyll interactions in the dimer give rise to very large optical activity relative to the monomer. Our analysis of the dimer structure is not yet complete, but we can conclude with some confidence that the molecular planes are not parallel or coplanar in the dimer, nor are the transition moments parallel. The CD and absorption spectra of photosynthetic particles -- barley quantasomes containing chlorophylls a and b, quantasomes prepared from a barley mutant that lacks chlorophyll b, and R. rubrum and R. spheroides chromatophores that contain bacteriochlorophyll -- are also presented. The CD measurements give strong evidence for chlorophyll-chlorophyll interaction in all of the photosynthetic particles examined. We conclude that some of the chlorophyll a absorbing on the long wavelength side of the main quantasome absorption band is aggregated. The aggregate is at least a dimer and may be a one- or two-dimensional analogue to the chlorophyll a crystal. The chlorophyll a aggregate has the midpoint of exciton components at 685 mμ. We suggest that the chlorophyll a bands observed at 683 and

695 mμ observed in low temperature derivative spectra may result from a single type of aggregate. The chlorophyll b shows evidence of interactions with other chlorophyll b in the quantasome. We open the possibility of finding the geometrical relationship of the interacting chlorophylls in the quantasome from further experiments. Bacteriochlorophyll appears to be aggregated in the two species of photosynthetic bacteria that were examined; however, the detailed structure of the aggregates is apparently different in these two cases.

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FIGURE CAPTIONS

Figure 1. Absorption and CD spectra of chlorophyll a dimers in carbon tetrachloride.

Figure 2. Absorption and CD spectra of chlorophyll b dimers in carbon tetrachloride. The CD spectrum shown at wavelengths longer than 530 mμ has been multiplied by 4.0.

Figure 3. Absorption and CD spectra of bacteriochlorophyll dimers in carbon tetrachloride. The CD spectrum shown at wavelengths longer than 700 mμ has been multiplied by 0.5.

Figure 4. Typical CD and ORD curves resulting from degenerate (I) and non-degenerate (II) interactions. These curves are obtained by decomposing the corresponding lower curves (I + II)/2, which approximate those observed for chlorophyll dimers.

Figure 5. Typical double CD component for an exciton split transition where the splitting is small compared to the band width. The cancellation of rotational strength in the center of the band is illustrated. Both R_+ and R_- have gaussian shapes with halfwidth Θ, and they are separated by $\Delta\nu$.

Figure 6. Absorption and CD spectra of a suspension of chlorophyll a microcrystals in isooctane. Path length, 1.0 cm.

Figure 7. Absorption and ORD spectra of quantasomes from normal barley (solid curves) and from a mutant lacking chlorophyll b (dashed curves).

Figure 8. Absorption and CD spectra of quantasomes from normal barley (solid curves) and CD spectrum of quantasomes from a mutant lacking chlorophyll b (dashed curve).

Figure 9. Absorption and CD spectra of chromatophores from Rhodospirillum rubrum.

Figure 10. Absorption, CD and ORD spectra of chromatophores from Rhodopseudomonas spheroides.

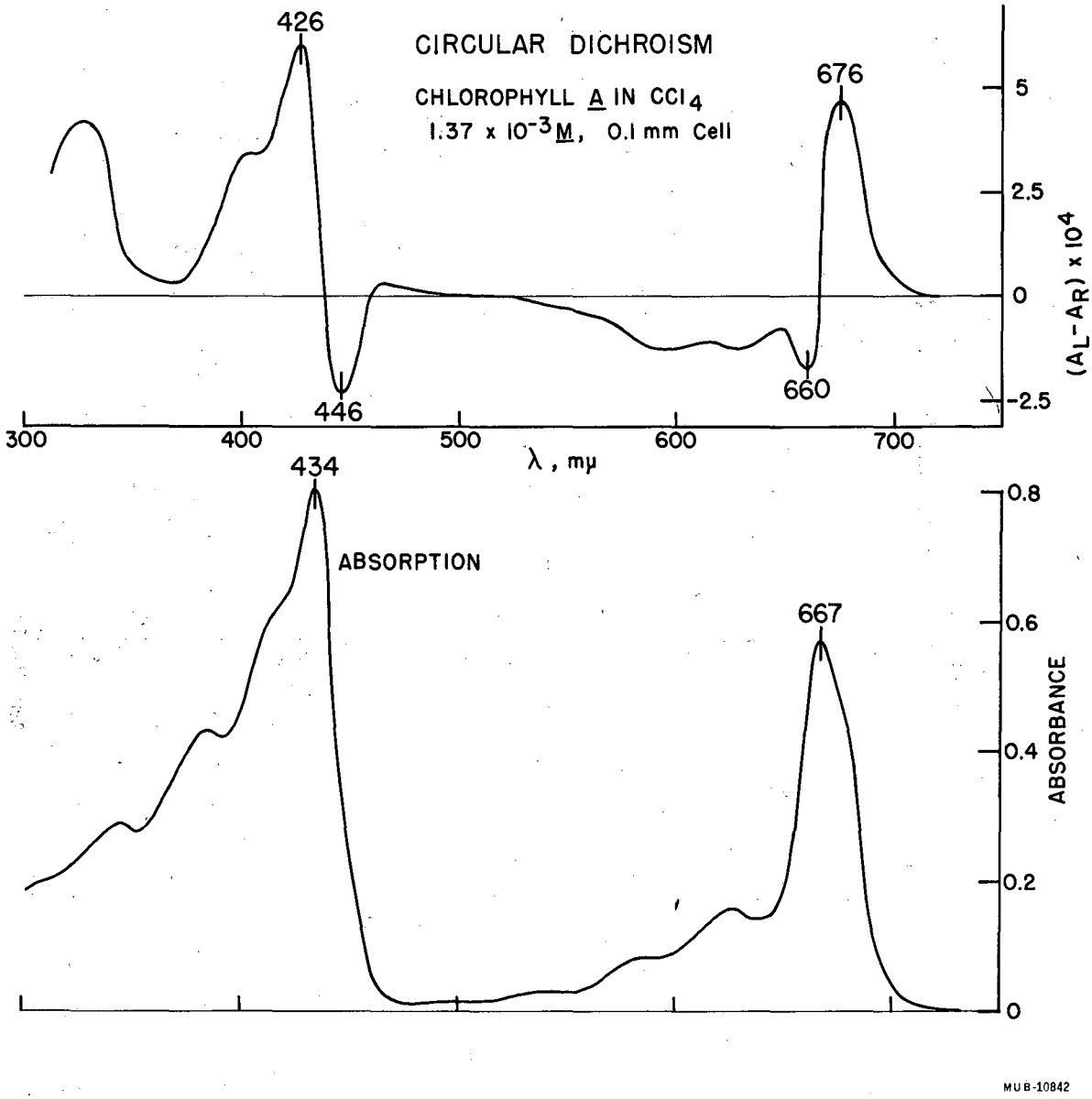
CIRCULAR DICHROISM
CHLOROPHYLL A IN CCl4
1.37 x 10-3 M, 0.1 mm Cell
426
446
660
676
5
2.5
0
-2.5
(AL-AR) x 10^4
300
400
500
600
700
λ, mμ
434
ABSORPTION
667
0.8
0.6
0.4
0.2
0
ABSORBANCE
MUB-10842

Fig. 1

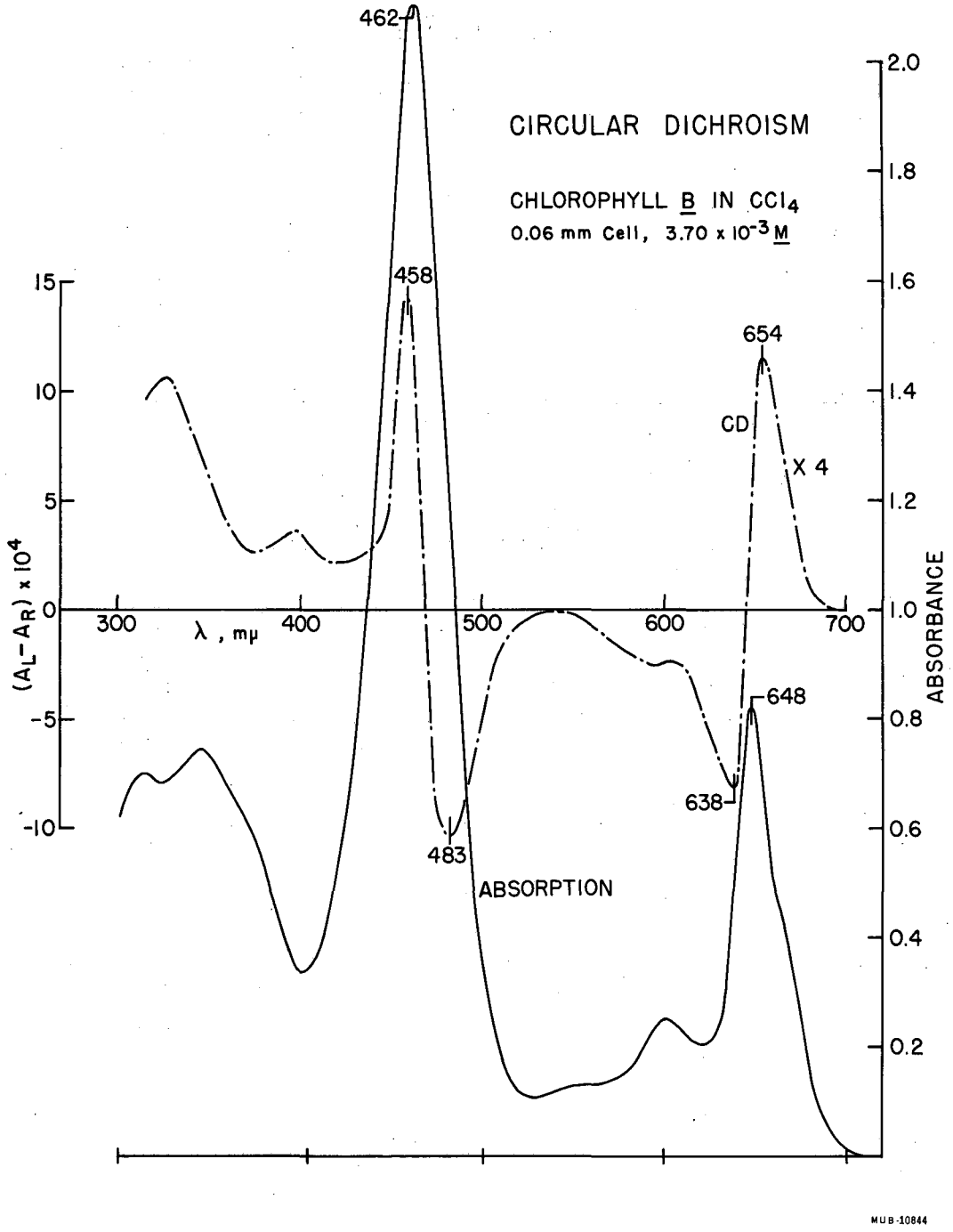
CIRCULAR DICHROISM
CHLOROPHYLL B IN CCl4
0.06 mm Cell, 3.70 x 10-3 M
462
458
654
CD
X 4
483
ABSORPTION
638
648
λ , mμ
300
400
500
600
700
15
10
5
0
-5
-10
(AL-AR) x 10^4
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
ABSORBANCE
MUB-10844

Fig. 2

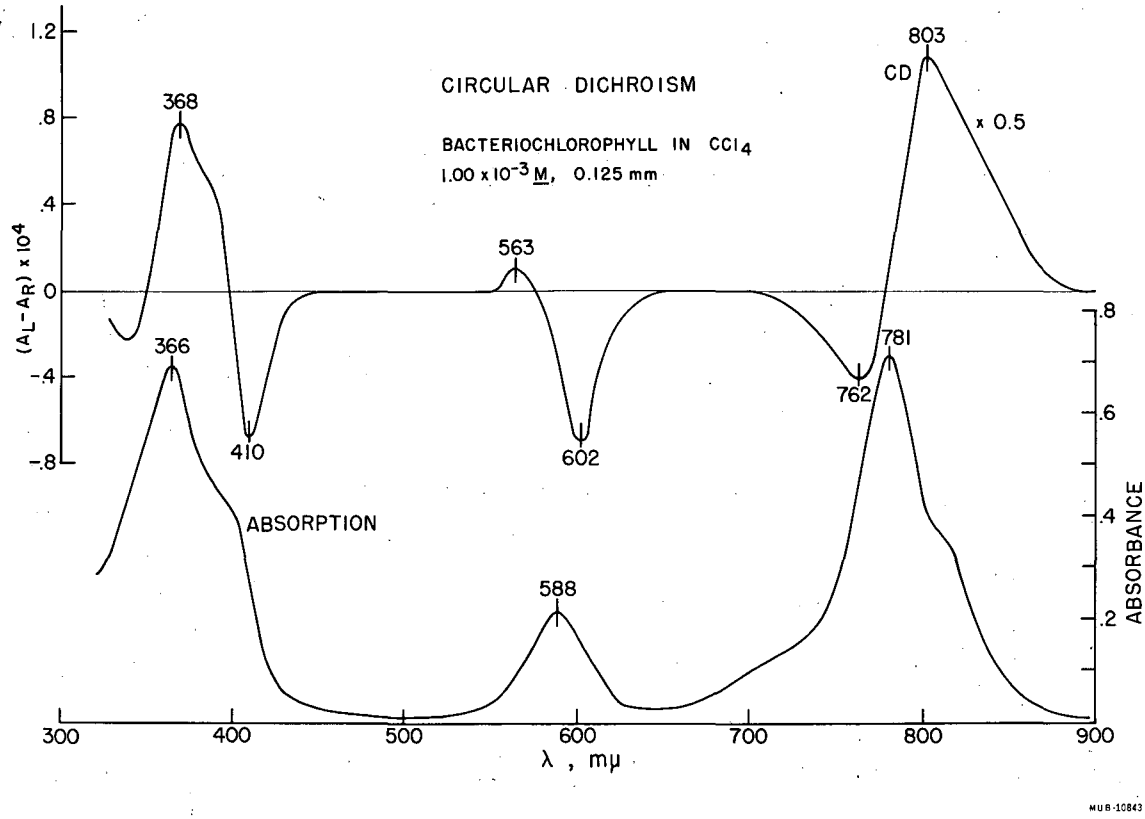
CIRCULAR DICHROISM
BACTERIOCHLOROPHYLL IN CCl_4
1.00×10^{-3} M, 0.125 mm
CD
x 0.5
ABSORPTION
$(A_L - A_R) \times 10^4$
ABSORBANCE
λ , mμ
368
366
410
563
602
588
762
781
803
1.2
.8
.4
0
-.4
-.8
.8
.6
.4
.2
300
400
500
600
700
800
900
MUB-10843

Fig. 3

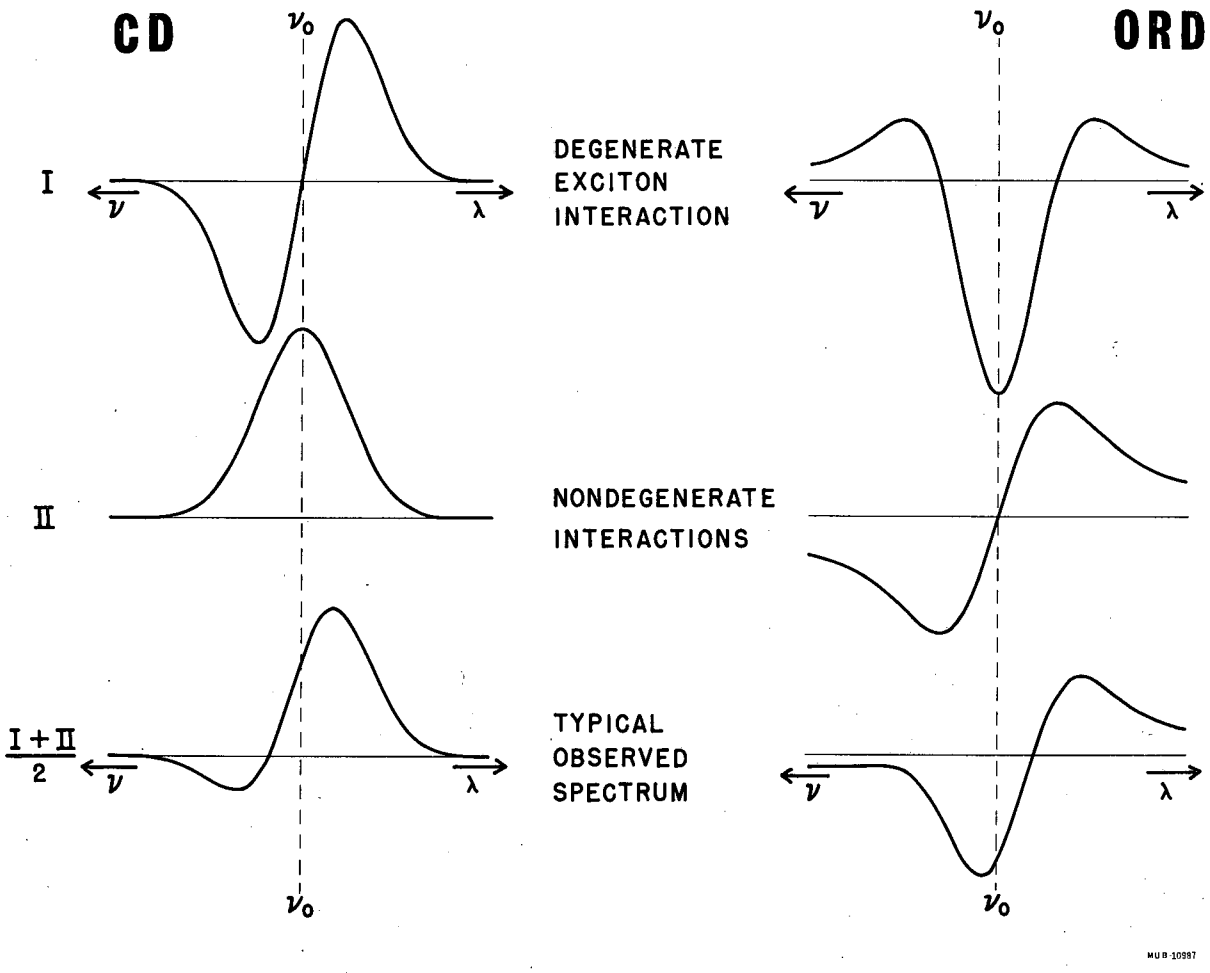
CD
ORD
ν_0
I
II
$\frac{I+II}{2}$
ν
λ
DEGENERATE
EXCITON
INTERACTION
NONDEGENERATE
INTERACTIONS
TYPICAL
OBSERVED
SPECTRUM
MUB-10987

Fig. 4

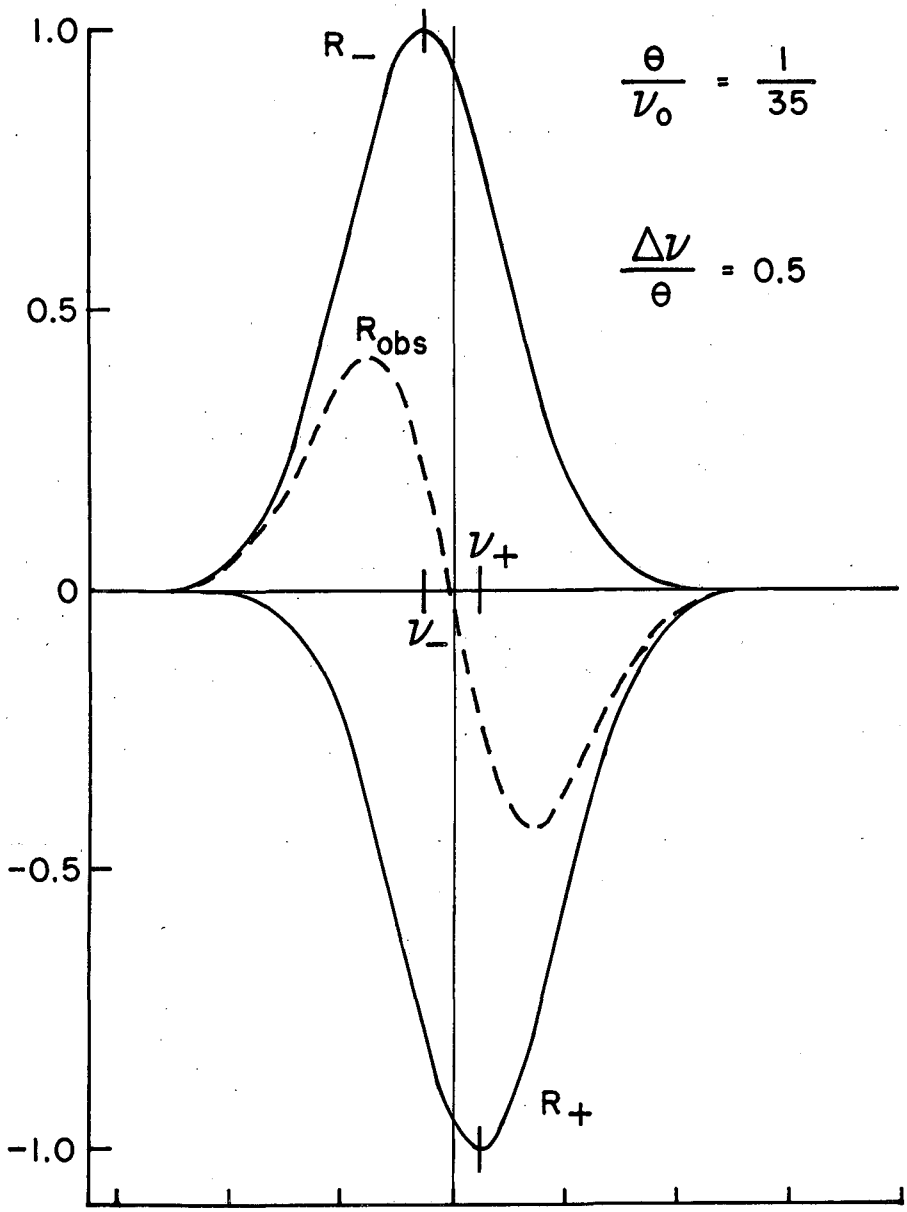

Fig. 5

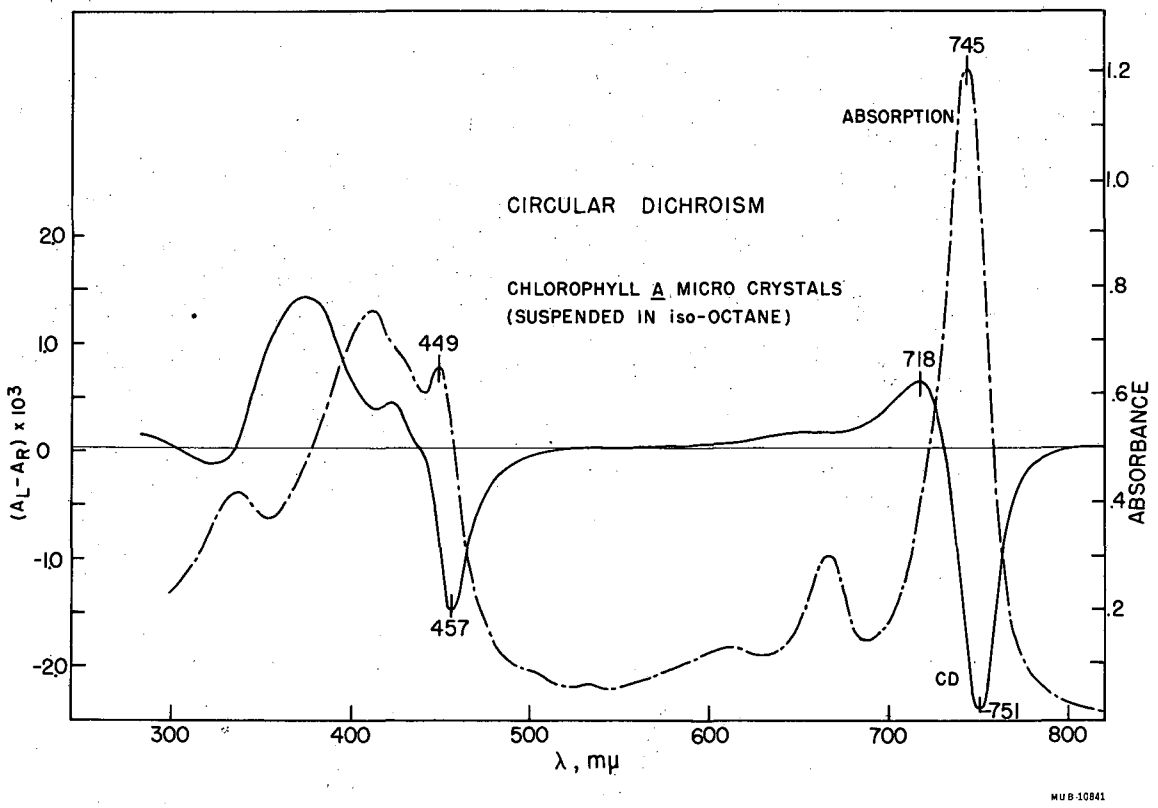
CIRCULAR DICHROISM
CHLOROPHYLL A MICRO CRYSTALS
(SUSPENDED IN iso-OCTANE)
ABSORPTION
745
718
449
457
751
CD
$(A_L - A_R) \times 10^3$
ABSORBANCE
λ, mμ
MUB-10841

Fig. 6

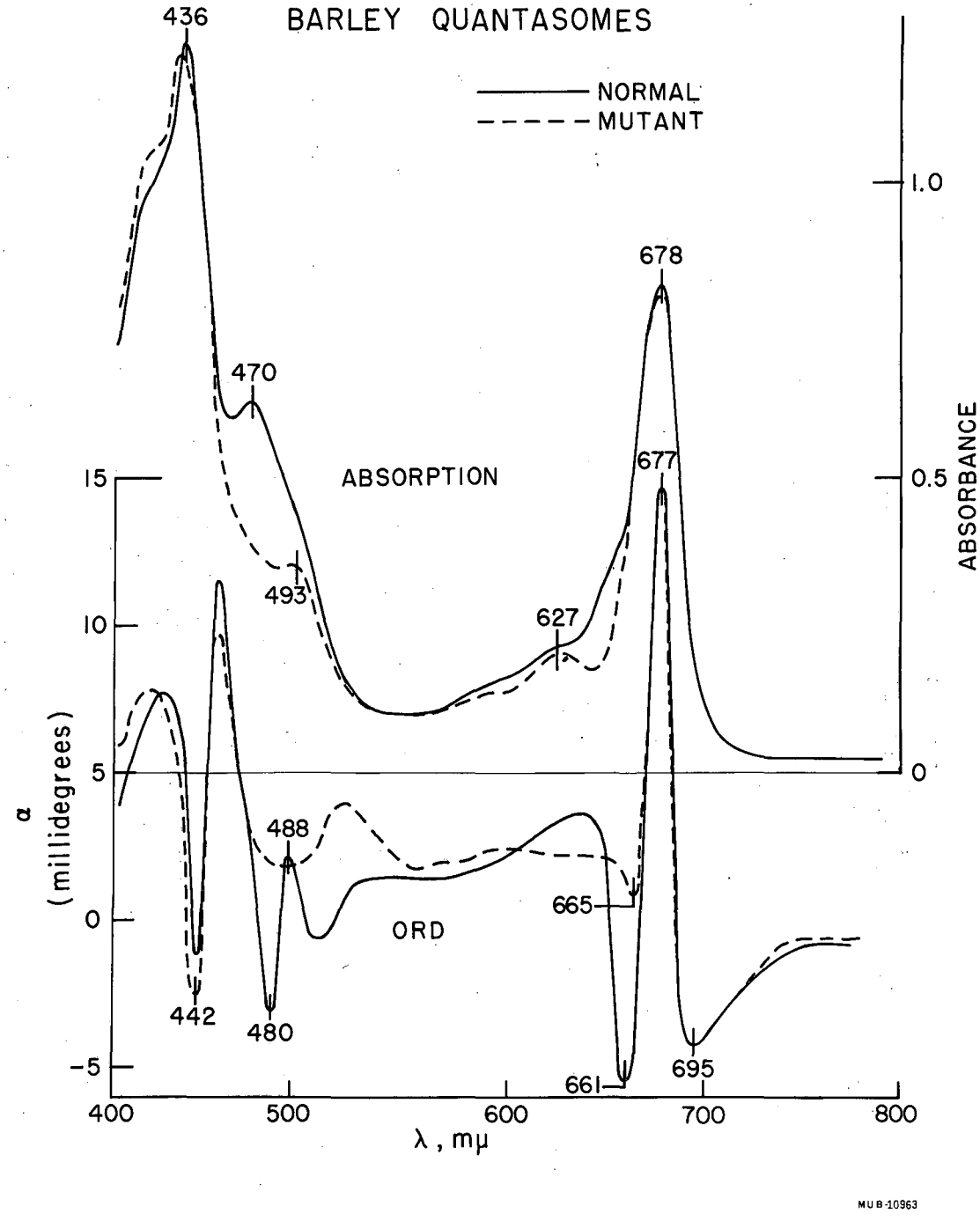
BARLEY QUANTASOMES
NORMAL
MUTANT
436
470
493
627
678
677
665
661
695
442
480
488
ABSORPTION
ORD
α (millidegrees)
ABSORBANCE
15
10
5
0
-5
1.0
0.5
0
400
500
600
700
800
λ, mμ
MUB-10963

Fig. 7

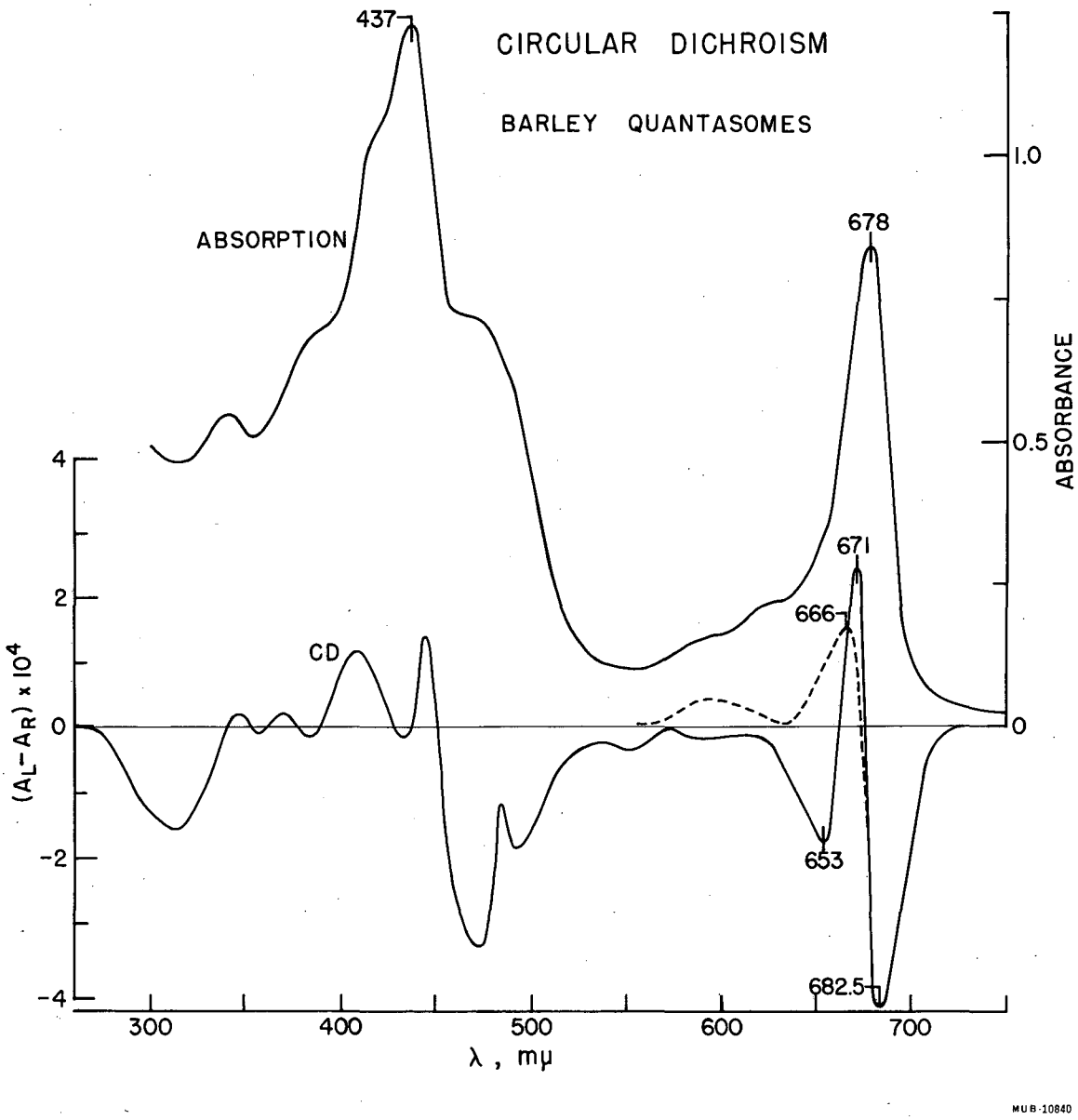
CIRCULAR DICHROISM
BARLEY QUANTASOMES
ABSORPTION
437
678
671
666
653
682.5
CD
1.0
0.5
0
ABSORBANCE
4
2
0
-2
-4
$(A_L - A_R) \times 10^4$
300
400
500
600
700
λ , mμ
MUB-10840

Fig. 8

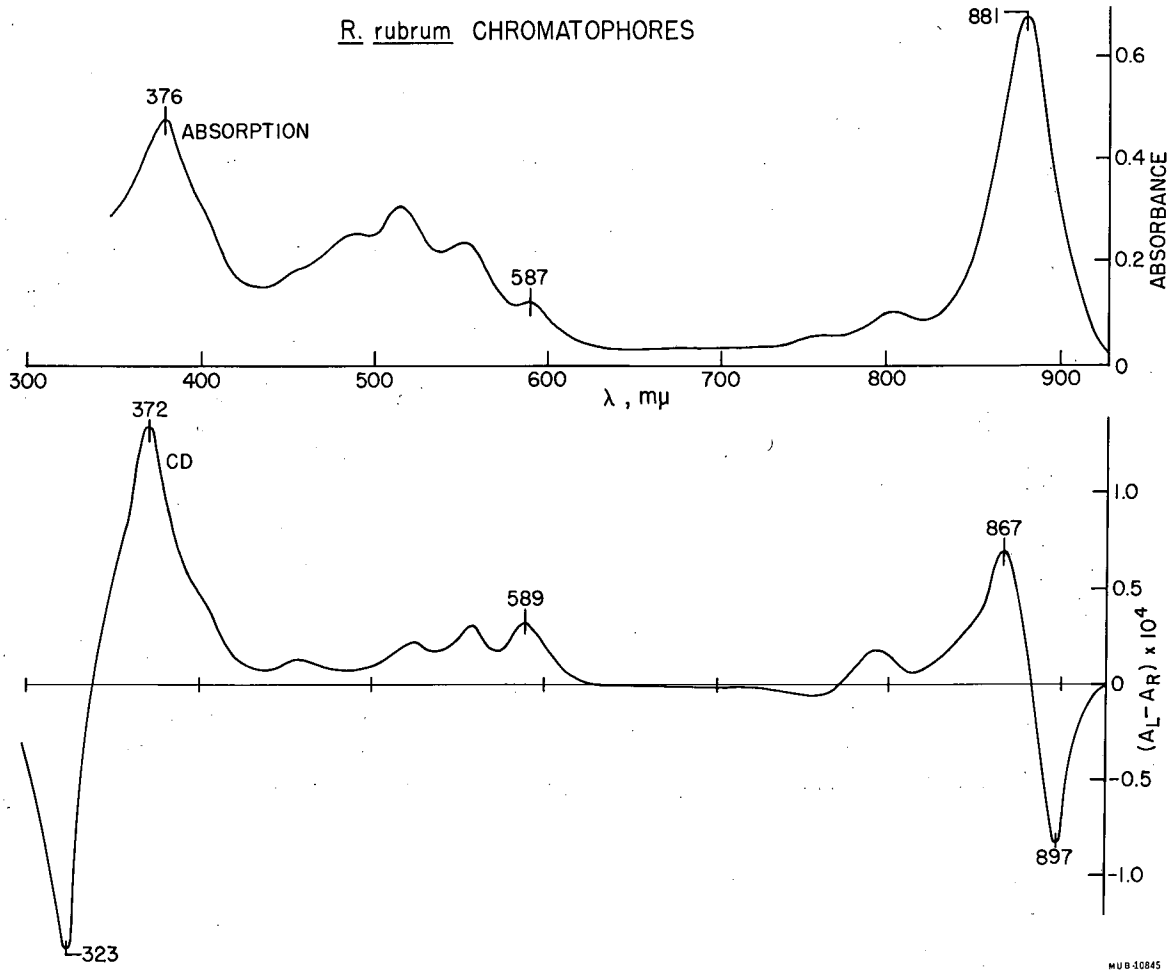
R. rubrum CHROMATOPHORES
376
ABSORPTION
587
881
ABSORBANCE
0.6
0.4
0.2
0
300
400
500
600
700
800
900
λ , mμ
372
CD
589
867
897
323
1.0
0.5
0
-0.5
-1.0
(A_L - A_R) x 10^4
MUB-10845

Fig. 9

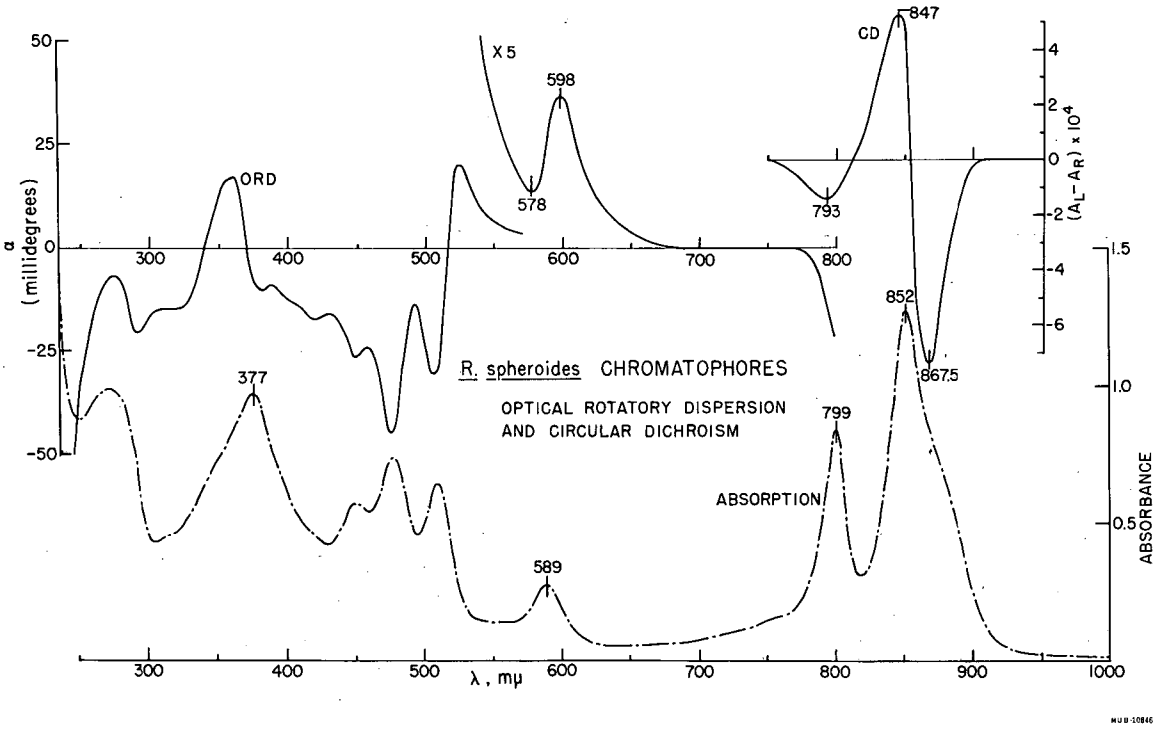
50
25
0
-25
-50
α (millidegrees)
ORD
X5
598
578
CD
847
793
852
867.5
377
589
799
ABSORPTION
R. spheroides CHROMATOPHORES
OPTICAL ROTATORY DISPERSION
AND CIRCULAR DICHROISM
300
400
500
600
700
800
900
1000
λ, mμ
4
2
0
-2
-4
-6
(A_L - A_R) x 10^4
1.5
1.0
0.5
ABSORBANCE

Fig. 10