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Author
Sauer, Kenneth.

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Kenneth Sauer

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OPTICAL ROTATORY DISPERSION OF CHLOROPHYLL IN SOLUTION AND IN CHLOROPLAST SUBUNITS*

By Kenneth Sauer

Department of Chemistry and Lawrence Radiation Laboratory,
University of California, Berkeley, California

Preparations of isolated pigmented lamellar fragments from spinach chloroplasts exhibit many of the photochemical activites associated with photosynthesis.[1-3] In the electron microscope these lamellae appear to be made up of a highly regular two-dimensional array of subunits which have been called quantasomes.[1,4] Within the quantasomes, which correspond to a molecular weight of 2×10^6, is a complex complement of pigments, redox agents, colorless lipid and structural protein. Although little is known about the environment and interrelationships of the pigment molecules and other cofactors, there is evidence that a small fraction of the chlorophyll is oriented in a way that produces a dichroic absorption maximum at 690 to 700 mμ.[5,6] Other spectrophotometric and photochemical evidence suggests that the immediate environment of the pigment molecules in quantasomes is very similar, if not identical, to that present in intact chloroplasts or in whole cells.[2]

Several recent studies have shown that the optical rotatory dispersion (ORD) can, in favorable circumstances, be a useful measure of the interaction among pigment molecules or chromophores in an optically-active environment. Blout and Stryer found that symmetric dye molecules bound to helical polypeptides exhibit pronounced Cotton effects in the regions of the dye absorption bands.[7,8] Furthermore, it has been shown theoretically and experimentally that chromophoric molecules with intrinsic optical activity can undergo profound changes in the observed Cotton effects

when the absorbing species are brought into close association.[9,10] In order to obtain further information on the environment of chlorophyll and other pigment molecules in photosynthetic systems we have examined the ORD of suspensions of spinach quantasome aggregates (lamellar fragments). To aid in the interpretation of the complex Cotton effects observed, we have also measured the ORD spectra of chlorophyll a in two different solvents and over a wide range of concentrations.

Experimental.—Quantasome aggregates were prepared from sonicated spinach chloroplasts according to the method of Park and Pon.[1,11] A fraction sedimenting between 45,000 x g (10 min) and 145,000 x g (20 min) in a Spinco Model L Ultracentrifuge was washed with 0.02 M (K) PO_4 buffer, pH 6.8, and the final precipitate at 145,000 x g (20 min) was resuspended in the same buffer for the ORD and absorption measurements. Chlorophyll a from spinach was separated chromatographically according to the method of Anderson and Calvin.[12] Following the chromatography on sugar, the isooctane was evaporated from the eluate fraction containing chlorophyll a. The solid residue was then redissolved in acetone, transferred to isooctane, washed free of acetone using water, and finally evaporated to give solid chlorophyll a. Acetone, carbon tetrachloride (both Baker and Adamson, Reagent Grade) and absolute ethanol were used without further purification. ORD measurements were made at room temperature using a Cary Model 60 Spectropolarimeter with cells of 0.02, 0.025, 0.10, 1.00 and 10.0 cm path lengths. Absorption and difference spectra of chlorophyll solutions were measured using a Cary Model 14 Spectrophotometer adapted with a red-sensitive photomultiplier (Hamamatsu R-136). Quantasome absorption spectra were measured using the Cary Model 1462 Scattered-Transmission Accessory, as described previously.[2]

Results.--Suspensions of quantasome aggregates show a complex ORD spectrum with many Cotton effects throughout the visible and ultraviolet regions of the spectrum (Figs. 1 and 2). In the visible and near ultraviolet regions these Cotton effects are in the regions of strong absorption of chlorophylls a and b and carotenoids. A pronounced trough at 234 mμ in the ORD spectra is presumably associated with protein in the quantasome matrix. Samples obtained at different times in the growing season of spinach show distinct differences in their ORD spectra, and these differences appear to correlate with small differences in their absorption spectra, especially in the region from 450 to 500 mμ. Those samples obtained in mid-autumn (Fig. 1) appear to contain less carotenoid relative to chlorophyll compared with those harvested in early winter (Fig. 2).[13]

The pigments absorbing in the visible region of the spectrum can be almost completely extracted with a mixture of 80% acetone-20% water. The ORD of such an extract (Fig. 3), with nearly the same concentration of chlorophyll as was present in the quantasome suspensions, shows virtually none of the pronounced Cotton effects present in the quantasomes.

Solutions of purified chlorophyll a in acetone (Fig. 4) exhibit weak Cotton effects at 431 mμ (positive) and at 662 mμ (negative), centered precisely at the corresponding absorption maxima. This ORD spectrum is very similar both in general shape and in the amplitudes of the Cotton effects (in molecular rotation units) to that reported by Ke and Miller in ether solution.[14] As seen in Fig. 4, the amplitudes of the Cotton effects in terms of molecular rotation [ϕ], are little altered over the concentration range from 1×10^{-6} to 5×10^{-4} moles/liter, in the acetone solutions.

Solutions of chlorophyll a in carbon tetrachloride, a solvent which is known to favor the formation of chlorophyll a dimers at high concentrations,[15] give ORD spectra which exhibit a very pronounced concentration dependence (Fig. 5). At high concentrations (up to 5×10^{-4} moles/liter) of chlorophyll a in carbon tetrachloride the amplitudes of the Cotton effects in terms of molecular rotation are greatly enhanced over those occurring in dilute (1×10^{-6} moles/liter) solutions in the same solvent or in acetone. The signs of the rotations for the two principal Cotton effects in the blue and in the red appear to be reversed by concentration in carbon tetrachloride, and the center of the Cotton effect in the red is shifted from 660 mμ to 675 mμ with the increase in concentration. Noticeable changes in the absorption spectra occur at the same time. Increasing concentration in carbon tetrachloride leads to the formation of shoulders on the long wavelength sides of both the principal blue and red absorption bands. Difference spectra show maxima at 445 and 680 mμ and minima at 431 and 663 mμ (Fig. 5). These are much more pronounced than those occurring in the difference spectra of acetone solutions of chlorophyll a over the same concentration span (Fig. 4).

The addition of a small amount (0.5% v/v) of ethanol to a concentrated solution of chlorophyll a in carbon tetrachloride produces a complete alteration of both the ORD and the absorption spectra. With added ethanol both these curves appear now to be characteristic of the undissociated species, and Katz, et al. have shown that this addition does apparently lead to the breakup of the chlorophyll dimers.[15]

In the model proposed for chlorophyll dimer formation, the planes of the porphyrin rings are thought to be oriented in a nearly parallel arrangement. The interaction arises primarily between the magnesium

atom of one molecule and the carbonyl group of ring V of the other.[16,17] The close juxtaposition of the two chromophores would seem to provide the origin of the pronounced alteration of the ORD spectrum from that of the monomers. A theoretical justification of such effects has been presented by Tinoco, Woody and Bradley.[9] The situation is complicated for chlorophyll a by the fact that there are two nearly degenerate electronic transitions in the red and two in the blue region of the spectrum.[18] A detailed analysis of the ORD of the dimers must await further studies of the optical properties of chlorophylls and related compounds.

There is much evidence from studies of electronic energy transfer, fluorescence spectra and absorption spectra to suggest that chlorophyll occurs in an aggregated state *in vivo*. The precise nature of this aggregation is not yet known. Comparison of the ORD spectrum of quantasome suspensions with that of chlorophyll a dimers suggests that strong pigment-pigment interaction is a sufficient explanation for the large Cotton effects observed in quantasomes. The quantasome ORD spectrum has additional Cotton effects resulting from the presence of chlorophyll b and carotenoids, which further complicate its analysis. An alternative explanation would account for the enhanced Cotton effects in the quantasome ORD spectrum in terms of interactions between the individual pigment molecules and colorless, optically-active components of the lipoprotein matrix. Although this latter explanation can by no means be ruled out, the former one seems more likely on the basis of the known interactions among the pigment molecules and from the similarity in amplitudes of the Cotton effects based on chlorophyll molar rotation for quantasomes (see legends of Figs. 1 and 2) and for the chlorophyll dimers.

Summary.--The ORD spectrum of pigmented lamellar fragments (quantasome aggregates) from spinach chloroplasts shows a number of strong Cotton effects associated with absorption bands in the red and blue regions of the spectrum. In the corresponding spectrum of a pigment extract these Cotton effects are strongly attenuated and reversed in sign. Purified chlorophyll a in solutions in carbon tetrachloride, an aggregating solvent, exhibits profound changes in its optical rotatory dispersion spectrum with changing concentration, and large Cotton effects are observed to be present at high concentrations where chlorophyll dimers are known to be stable. The observed dispersions in quantasomes are interpreted to arise from strong interactions among aggregated pigment molecules in their lipoprotein matrix.

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[1] Park, R. B., and N. G. Pon, J. Mol. Biol., 3, 1 (1961).

[2] Sauer, K., and R. B. Park, Biochim. Biophys. Acta, 79, 476 (1964).

[3] Sauer, K., and J. Biggins, Biochim. Biophys. Acta, in press, 1965.

[4] Park, R. B., and J. Biggins, Science, 144, 1009 (1964).

[5] Sauer, K., and M. Calvin, J. Mol. Biol., 4, 451 (1962).

[6] Sauer, K., Biophys. J., in press, 1965.

[7] Blout, E. R., and L. Stryer, Proc. Nat. Acad. Sci., 45, 1591 (1959).

[8] Stryer, L., and E. R. Blout, J. Am. Chem. Soc., 83, 1411 (1961).

[9] Tinoco, I., Jr., R. W. Woody and D. F. Bradley, J. Chem. Phys., 38, 1317 (1963).

[10] Holcomb, D. N., and I. Tinoco, Jr., Biopolymers, in press, 1965.

[11] Park, R. B., and N. G. Pon, J. Mol. Biol., 6, 105 (1963).

[12] Anderson, A.F.H., and M. Calvin, Nature, 194, 285 (1962).

[13] Sauer, K., and M. Calvin, Biochim. Biophys. Acta, 64, 324 (1962).

[14] Ke, B., and R. M. Miller, Naturwissenschaften, 51, 436 (1964).

[15] Katz, J. J., G. L. Closs, F. C. Pennington, M. R. Thomas and H. H. Strain, J. Am. Chem. Soc., 85, 3801 (1963).

[16] Closs, G. L., J. J. Katz, F. C. Pennington, M. R. Thomas and H. H. Strain, ibid., 85, 3809 (1963).

[17] Anderson, A.F.H., and M. Calvin, Arch. Biochem. Biophys., 107, 251 (1964).

[18] Gouterman, M., J. Mol. Spectr., 6, 138 (1961).

Figs. 1 and 2. Absorption spectrum (upper curve) and ORD spectrum (lower curve) of buffered aqueous suspensions of quantasome aggregates prepared from sonicated spinach chloroplasts. Commercial spinach harvested in mid-autumn (Fig. 1) and early winter (Fig. 2) were used. Path lengths were 1.00 cm. Optical rotations of 10 millidegrees correspond to molecular rotations [ϕ], based on estimates of chlorophyll a content, of 93×10^3 (Fig. 1) and 120×10^3 (Fig. 2) in degrees-dm^{-1}-$(moles/cc)^{-1} \times 10^{-2}$.

Fig. 3. The absorption spectrum and ORD spectrum of a total pigment extract of spinach quantasomes in 80% acetone-20% water. Path length 1.00 cm. An optical rotation of 10 millidegrees corresponds to a molecular rotation, [ϕ], based on chlorophyll a content, of 112×10^3 in degrees-dm^{-1}-$(moles/cc)^{-1} \times 10^{-2}$.

Fig. 4. Absorption spectra, difference spectrum and ORD spectra of chlorophyll a in acetone at two concentrations. Concentrations and path lengths as indicated. The difference spectrum (lower curve) shows the absorbance of the concentrated solution (5.10×10^{-4} M; 0.20 cm path length) minus that of the dilute solution (1.02×10^{-6} M, 10.0 cm path length) measured directly in a double beam spectrophotometer.

Fig. 5. Absorption spectra, difference spectra and ORD spectra of chlorophyll a in carbon tetrachloride at four concentrations. Concentrations and path lengths as indicated. The difference spectra show the absorbance of the more concentrated solutions minus that of the most dilute solution (1.14×10^{-6} M, 10.0 cm path length) measured directly in a double-beam spectrophotometer.

Fig. 6. Absorption spectra and ORD spectra of chlorophyll a in carbon tetrachloride (5.10×10^{-4} M, path length 0.025 cm) and of the same solution to which 0.5% (v/v) ethanol is added.

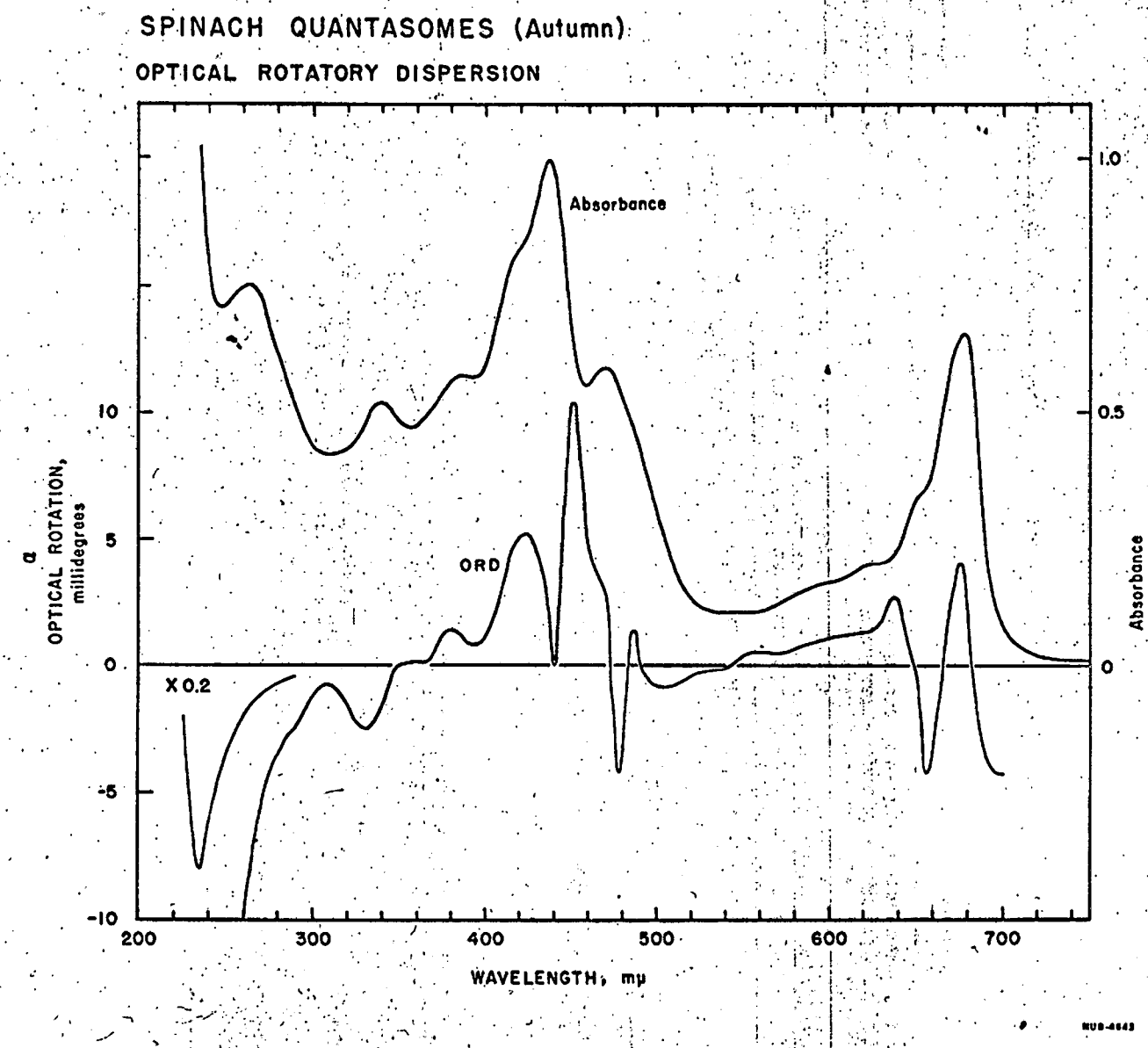
SPINACH QUANTASOMES (Autumn)
OPTICAL ROTATORY DISPERSION
Absorbance
ORD
X 0.2
α
OPTICAL ROTATION, millidegrees
Absorbance
WAVELENGTH, mμ
10
5
0
-5
-10
1.0
0.5
0
200
300
400
500
600
700

Fig. 1.

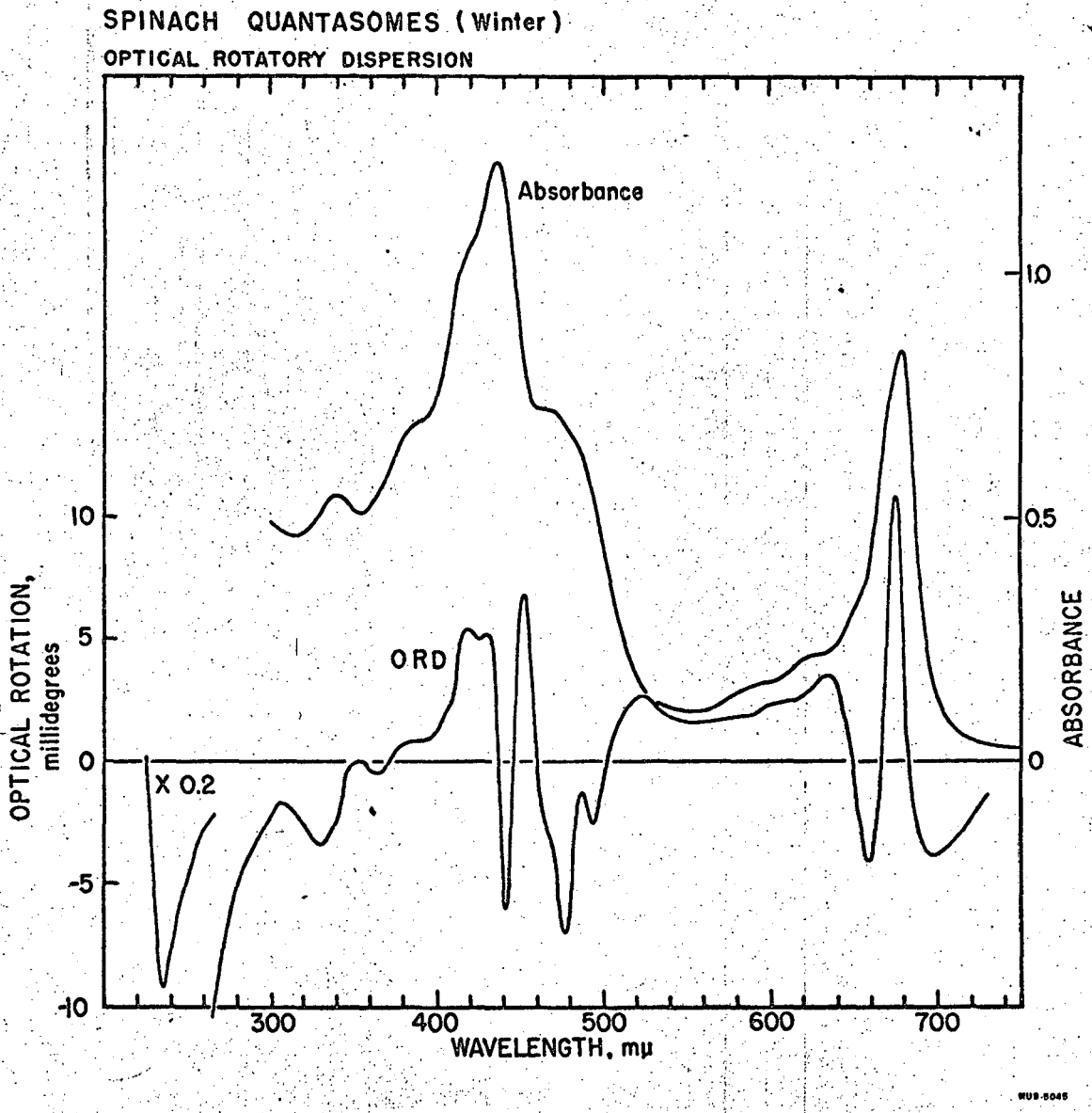
SPINACH QUANTASOMES (Winter)
OPTICAL ROTATORY DISPERSION
Absorbance
ORD
X 0.2
OPTICAL ROTATION, millidegrees
ABSORBANCE
WAVELENGTH, mμ
10
5
0
-5
-10
1.0
0.5
0
300
400
500
600
700
MUB-5045

Fig. 2.

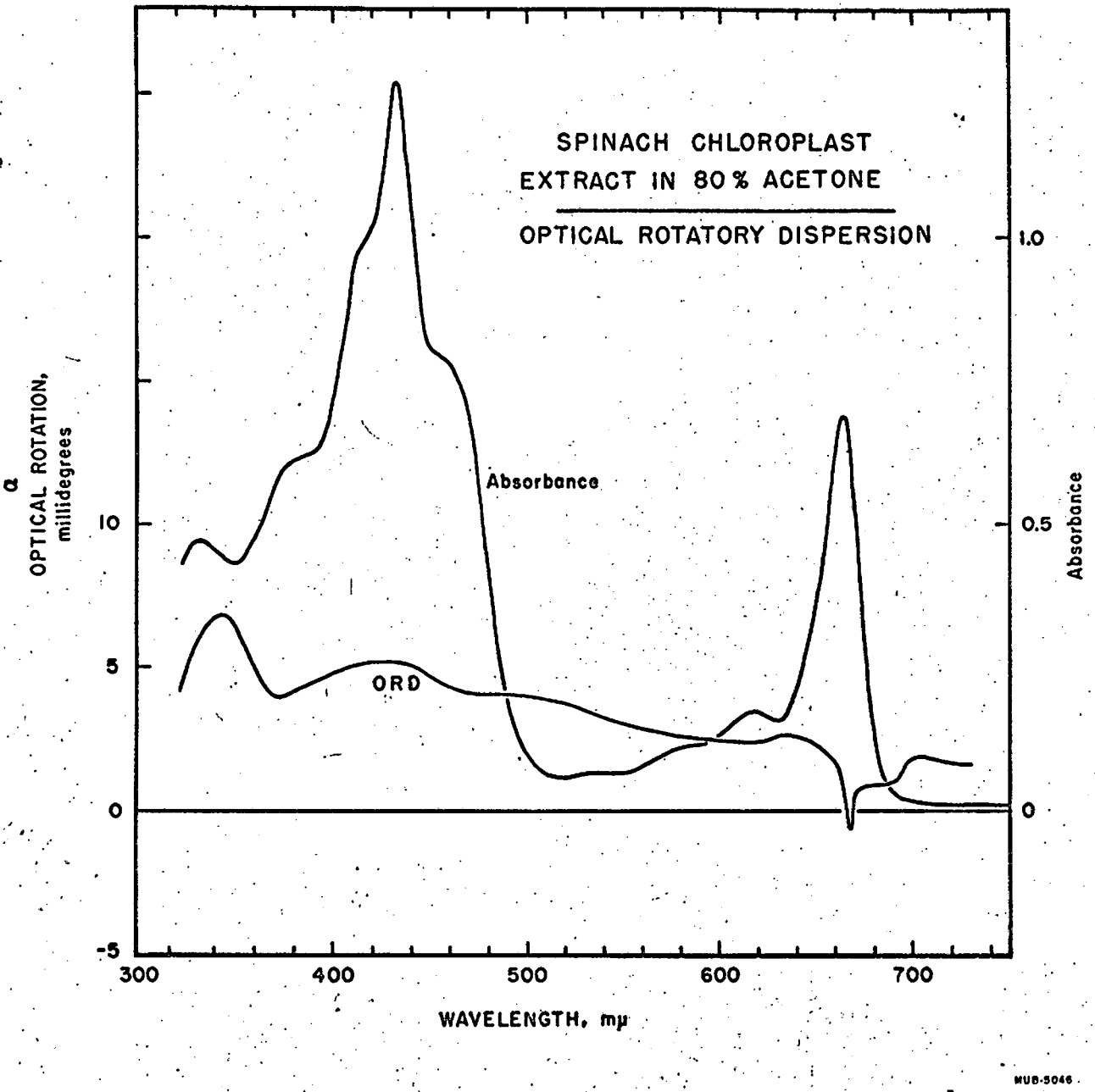
SPINACH CHLOROPLAST
EXTRACT IN 80% ACETONE
OPTICAL ROTATORY DISPERSION
Absorbance
ORD
α
OPTICAL ROTATION,
millidegrees
10
5
0
-5
1.0
0.5
0
Absorbance
300
400
500
600
700
WAVELENGTH, mμ
MUB-5046

Fig. 3.

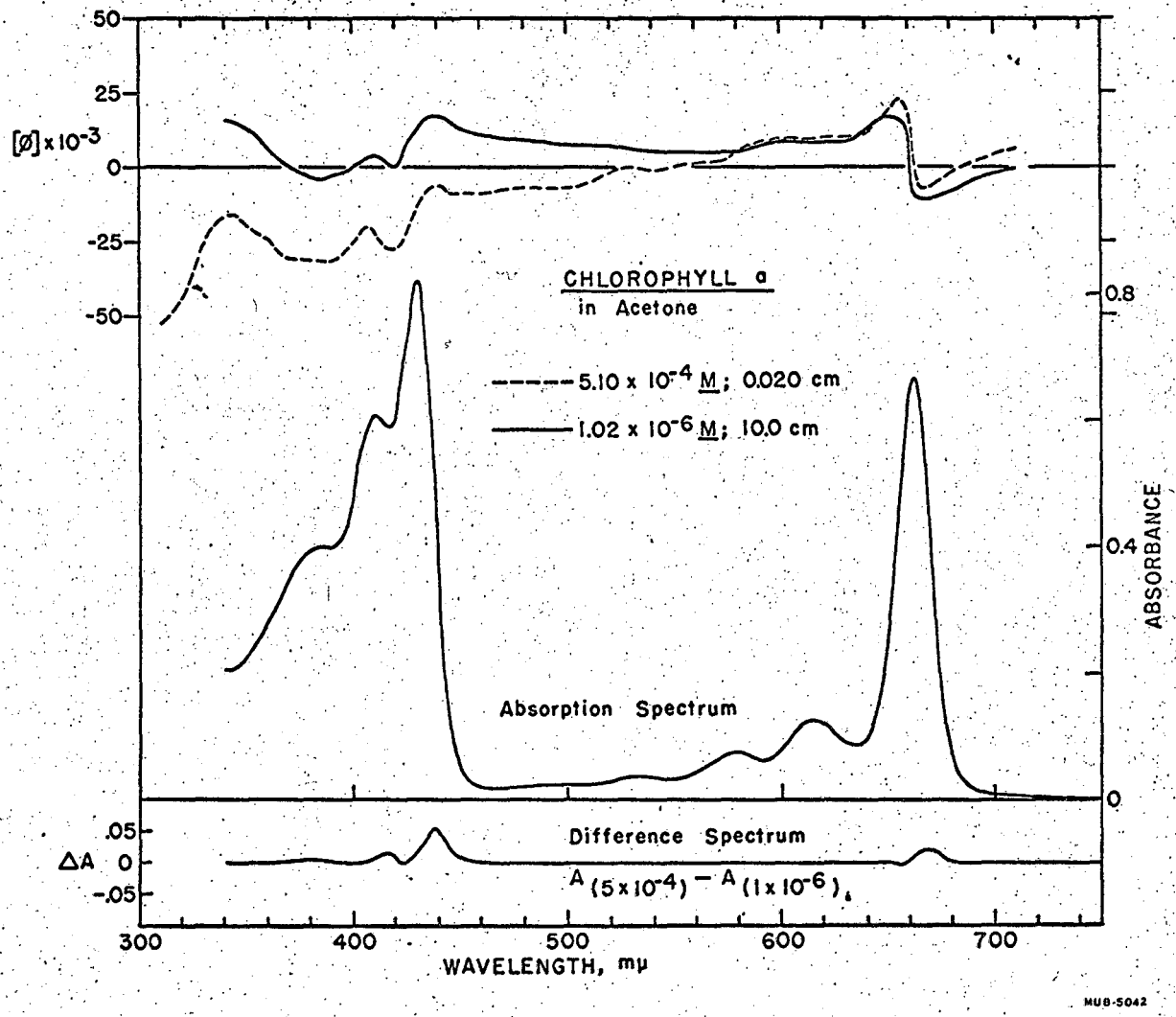
50
25
[ø]x10-3
0
-25
-50
CHLOROPHYLL a
in Acetone
5.10 x 10-4 M; 0.020 cm
1.02 x 10-6 M; 10.0 cm
0.8
0.4
0
ABSORBANCE
Absorption Spectrum
.05
ΔA 0
-.05
Difference Spectrum
A (5x10-4) − A (1x10-6)
300
400
500
600
700
WAVELENGTH, mμ
MUB-5042

Fig. 4.

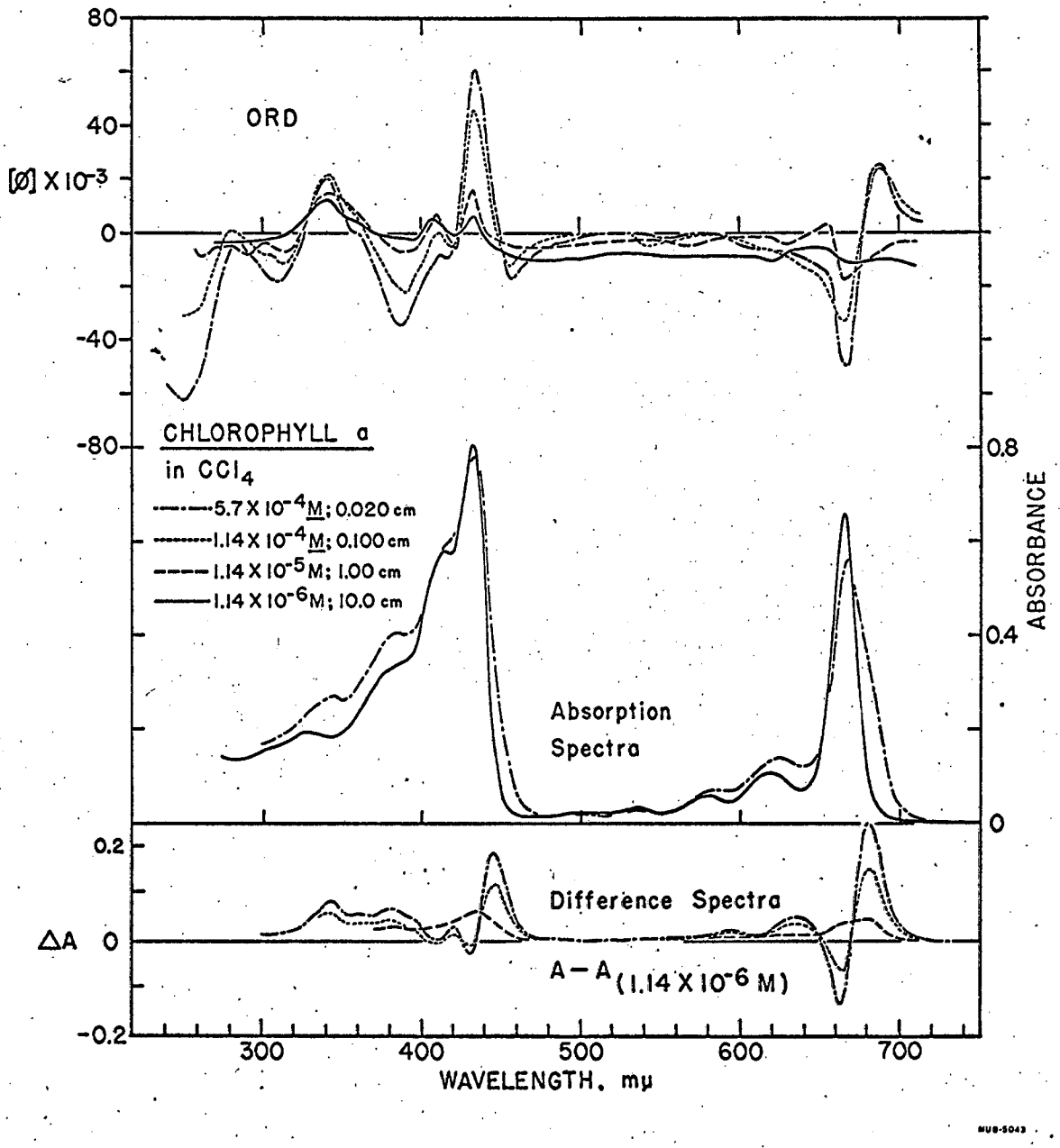
80
40
[ø] X10⁻³
0
-40
-80
ORD
CHLOROPHYLL a
in CCl4
5.7 X 10⁻⁴ M; 0.020 cm
1.14 X 10⁻⁴ M; 0.100 cm
1.14 X 10⁻⁵ M; 1.00 cm
1.14 X 10⁻⁶ M; 10.0 cm
0.8
0.4
0
ABSORBANCE
Absorption
Spectra
0.2
ΔA 0
-0.2
Difference Spectra
A − A(1.14 X 10⁻⁶ M)
300
400
500
600
700
WAVELENGTH, mμ
MUB-5043

Fig. 5.

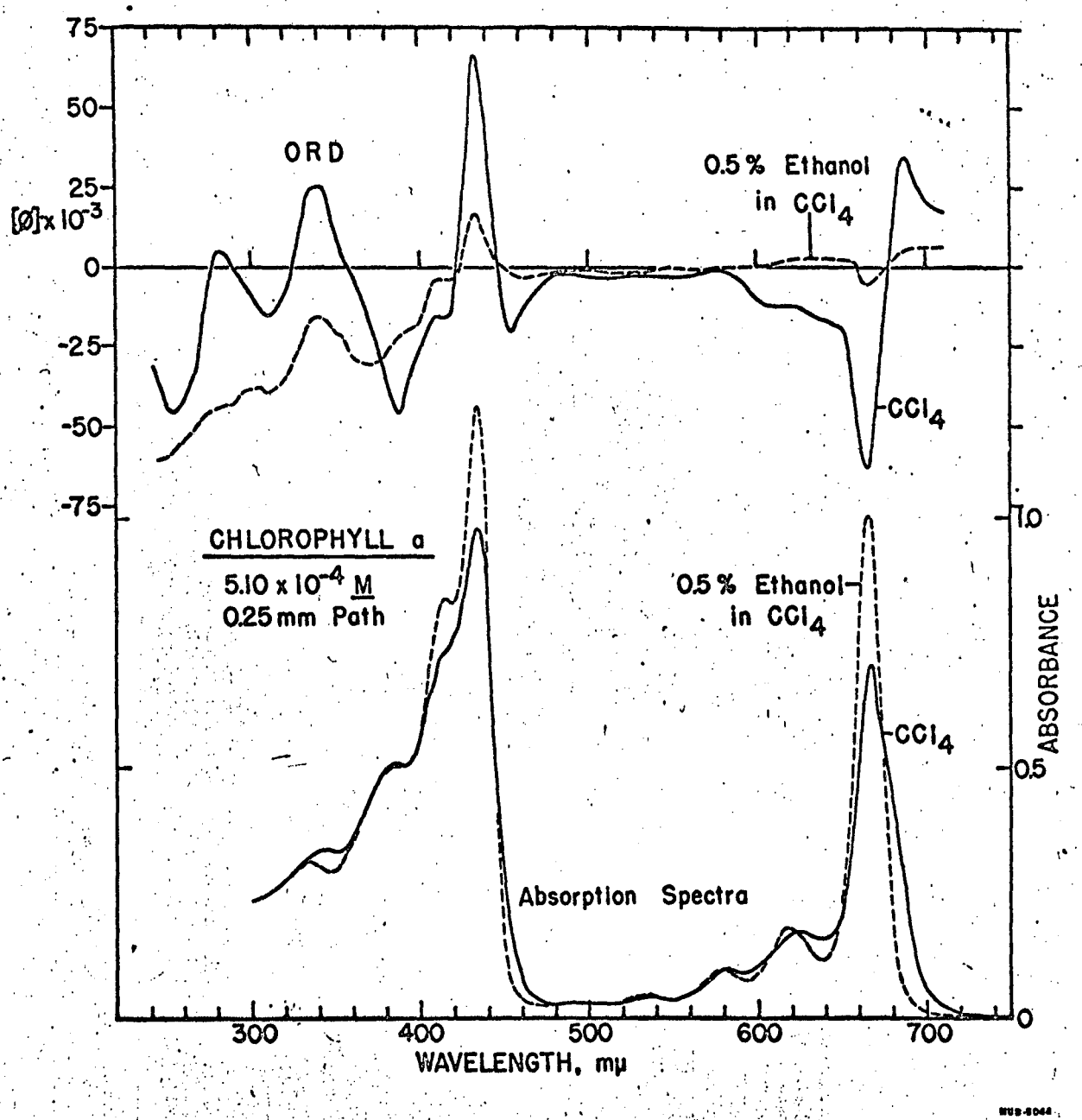
75
50
25
0
-25
-50
-75
[ø]x 10⁻³
ORD
0.5 % Ethanol
in CCl4
CCl4
CHLOROPHYLL a
5.10 x 10⁻⁴ M
0.25 mm Path
0.5 % Ethanol
in CCl4
CCl4
Absorption Spectra
1.0
0.5
0
ABSORBANCE
300
400
500
600
700
WAVELENGTH, mμ

Fig. 6.