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Kenneth D. Philipson and Kenneth Sauer

December 1971

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Exciton Interaction in a Bacteriochlorophyll-Protein from

Chloropseudomonas ethylica: Absorption and Circular

Dichroism at 77K*

Kenneth D. Philipson and Kenneth Sauer

Running Title: Exciton Interaction in a Bacteriochlorophyll-Protein

ABSTRACT: A water soluble bacteriochlorophyll-protein complex from the green bacterium <u>Chloropseudomonas</u> <u>ethylica</u> has previously been shown to consist of four subunits, each of which contains five bacteriochlorophyll <u>a</u> molecules. Interaction among the bacteriochlorophyll <u>a</u> molecules produces exciton splittings observable at 77K in the long wavelength (809 nm) absorption band of the bacteriochlorophyll-protein. An exciton contribution to the rotational strength results in multiple components in the circular dichroism spectrum in this region. Four absorption and five circular dichroism components are observed, indicating the interaction of at least five bacteriochlorophyll <u>a</u> molecules in the complex. The total exciton band splitting is 490 cm^{-1}. The circular dichroism of a second electronic transition at 603 nm exhibits three resolved exciton components. The separation of bacteriochlorophyll <u>a</u> molecules is estimated to be 12-15 $\overset{o}{A}$ in one subunit of the protein.

Photosynthetic organisms typically contain one or more types of chlorophyll-related molecules incorporated into a protein or a lipoprotein matrix. The nature of the binding of the chlorophyll and the relative orientations and distances between chlorophylls inside the protein are largely unknown. Questions closely related to these relationships are the strength of the interaction between chlorophylls and the mode of electronic excitation energy transfer *in vivo*. Since the detailed structure of no chlorophyll-protein is known, models for the pigment-lipoproteins have been constructed primarily on the basis of theoretical considerations (Weier et al., 1966; Kreutz, 1970).

The chlorophyll-protein which has been studied in most detail and which has been best characterized is the bacteriochlorophyll-protein (BChl-P)[1] complex from the green photosynthetic bacteria, *Chloropseudomonas ethylica* (Cps. ethylica) (Olson, 1966; Thornber and Olson, 1968; Olson, 1971). This BChl-P is crystallizable and has four subunits, each containing five bacteriochlorophyll a (BChl a) molecules. The complex is blue-green in color and has a molecular weight of 152,000. *In vivo* the BChl a in the protein accepts excitation energy from chlorobium chlorophyll (which forms the bulk of the antenna pigment in green bacteria) and transfers this energy to the reaction center bacteriochlorophyll, P840.

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[1]Abbreviations used are: BChl-P, bacteriochlorophyll-protein; Cps. ethylica, Chloropseudomonas ethylica; Bchl a, bacteriochlorophyll a; CD, circular dichroism.

The BChl-P from <u>Cps. ethylica</u> is water soluble and, unlike most chlorophyll-containing proteins, can be isolated without the use of solubilizing detergents, which might alter its structure. Electron microscopy of BChl-P crystals (Labaw and Olson, 1970) suggests that the shape of the protein is a noncompact sphere of 81.5 Å. The resistance of the BChl-P to pheophytinization at low pH implies that the BChl $\underline{a}$ molecules are imbedded within the protein (Ghosh <u>et al.</u>, 1968) rather than located on its surface. The BChl $\underline{a}$ molecules are apparently bound by peptide chain folding and not by covalent bond linkages (Thornber and Olson, 1968).

In this paper we present the results of optical studies on the BChl-P. Absorption and circular dichroism (CD) spectra at room temperature and at liquid nitrogen temperature have been measured for the long wavelength Q_y and Q_x transitions of the BChl $\underline{a}$ chromophores. The fact that BChl-P has been relatively well characterized enables us to interpret the optical spectra with some confidence. The resolving power of CD, especially at liquid nitrogen temperature, gives direct evidence for the presence of five components in the Q_y (809 nm) transition of the BChl-P. The five transitions are interpreted as the result primarily of exciton interactions among the five BChl $\underline{a}$ molecules located within each of the subunits of the protein. From the magnitude of the splitting between component peaks of the absorption band we estimate the distances between BChl $\underline{a}$ molecules to be 12-15 Å. These results imply that, at least within a subunit of the BChl-P, energy transfer is by exciton coupling rather than by a Förster resonance transfer mechanism (Kasha, 1963)

Materials and Methods

The BChl-P from Cps. ethylica was prepared (Olson, 1971) in the laboratory of J. M. Olson and was stored as a solid precipitate in an ammonium sulfate solution. Before use, the sample was resuspended in .01 M tris buffer, pH 7.5, with a salt concentration of .2 M NaCl. The absorbance ratio A_{809}/A_{371} has been used as a criterion for the integrity of the complex (Olson, 1971), and for all spectra shown here this ratio was 2.15 or larger. Concentrations were determined by using 154 $mM^{-1}cm^{-1}$ as the extinction coefficient of the 809 nm peak (Olson, 1966).

In order to obtain satisfactory spectra at 77K, a solvent must be used which forms a clear glass and does not denature the protein or otherwise introduce artifacts into the CD spectrum. A solvent which meets these criteria is a mixture of 2 parts 75% potassium glycero-phosphate in water (K and K Laboratories), 1 part aqueous solution containing the sample of interest, and 1/2 part glycerol (Baker analyzed Reagent) (Vredenberg, 1965). For the 77K spectra reported here, samples were immersed rapidly in liquid nitrogen.

Unfortunately, the BChl-P is of limited solubility in this solvent and CD spectra of the weak Q_x band of the BChl-P could not be obtained at 77K. A 50% mixture of glycerol and water (a common solvent for low temperature absorption measurements on biological materials) was unsuitable for CD measurements, as it produced a large baseline shift and excessive noise as the temperature approached 77K. The CD spectrum of the Q_x band of the BChl-P at -120°C was taken in glycerol-water. At this temperature the baseline shift did not occur.

All absorption spectra were recorded on a Cary 14R spectrophotometer. The CD spectra in Figures 1, 2, and 3 were recorded on an instrument built in this laboratory (Dratz, 1966). The CD spectrum in Figure 4 was recorded on a Durrum-Jasco J-20 CD spectrometer with far red sensitivity extended to 1000 nm. The magnitude of the CD signals was calibrated with an aqueous (1 mg/ml) solution of d_{10}-camphorsulfonic acid. For all spectra the spectral bandwidth was 3 nm or less.

Results

The CD and absorption spectra of BChl a in its monomeric form in ether are shown in Figure 1. The long wavelength Q_y transition at 769 nm and the Q_x transition at 573 nm are electrically allowed and are polarized nearly perpendicularly to one another. The band assignments are from fluorescence polarization measurements (Goedheer, 1966) and from theoretical considerations (Gouterman, 1961). The short wavelength shoulder on each band is due to higher vibrational components. The separation of these electronic transitions from other absorption bands enables the effects of perturbations to be studied without the complications of band overlap. The CD band in the Q_y region of the BChl a spectrum is a weak positive peak barely above the noise level of the measurement. The Q_x CD is a small negative peak. The small rotational strengths observed are the result of perturbations supplied by interactions of the asymmetrically-placed side groups with the inherently symmetric porphyrin chromophore (Houssier and Sauer, 1970; Philipson et al., 1971).

In Figures 2 and 3 we show the absorption and CD of the Q_y transition of BChl-P at room and liquid nitrogen temperatures, respectively. A detailed interpretation of these spectra is reserved for a later section but several features are of note. The absorption peak in Figure 2 is shifted to longer wavelength (809 nm) compared with that of BChl $\underline{a}$ in solution (Figure 1), but the shape of the absorption band at room temperature is little altered by the change in environment. The CD, however, has changed drastically; its magnitude is greater in the BChl-P by more than an order of magnitude, and three components are clearly visible (at 822, 808, and 778 nm). A similar spectrum has previously been reported (Kim and Ke, 1970). At 77K (Figure 3) increased resolution uncovers more structure. Absorption reveals four resolved components (at 789, 804.5, 813.5, and 824.5 nm) (Olson, 1971); the Q_y transition of monomeric BChl $\underline{a}$ in solution shows no hint of having more than one peak at 77K (Olson, 1966). At 77K the CD spectrum exhibits four components (786, 799.5, 811.5, and 823 nm) and a shoulder (817 nm) implying the existence of a fifth component. The magnitude of the CD for BChl-P at 77K is more than double that at room temperature. This is mostly due to band narrowing which increases the amplitudes and decreases the overlap of neighboring positive and negative peaks.

Similar effects are seen in the weaker Q_x band (603 nm) of the BChl-P at room temperature (Figure 4). The single small negative CD peak of BChl $\underline{a}$ in ether (Figure 1) is replaced by a complex band of three components (593, 605, and 616 nm). The effects of temperature lowering are not so drastic in this case as with the Q_x band. At temperatures down to -120°C the CD shows no evidence of additional peaks. The absorption at

liquid nitrogen temperature shows the main peak with weak shoulders on both its long and short wavelength sides (Olson, 1971).

Comparison of the spectral properties of the BChl-P with the BChl $\underline{a}$ monomers makes it clear that specific interactions within the protein matrix are perturbing the BChl $\underline{a}$ chromophores.

The CD of the BChl-P shows small variations from one sample to another with respect to exact position and magnitude of its component peaks. We attribute this to small conformational differences of the protein (see Discussion).

The absorption and CD spectra of the Q_y band of the BChl-P at 77K (Figure 3) were computer resolved into component peaks (Figure 5 and Table I). Since the absorption peak of monomeric solution BChl $\underline{a}$ is asymmetric (the half-width of the short wavelength side of the peak is significantly larger than the long wavelength side), gaussian curves of asymmetric shape were used for the fitting. The computer program did simultaneous fitting of the absorption and CD. Initially we required that each absorption component have a orresponding CD component at the same wavelength and of the same half-width. With these restrictions we were unable to obtain a precise fit of the experimental data. The requirement that the centers of the absorption and CD components occur at the same wavelength was then relaxed, and it was required only that corresponding absorption and CD components have the same half-width and skew (a measure of the asymmetry of the gaussian curve used for the fit). In this manner the computer fit shown in Figure 5 was obtained. The long sloping line in the upper half of Figure 5, which is the tail of a gaussian curve centered at 770 nm, is intended to represent the

contributions from the absorption of higher vibrational components and from a small amount of scattering. It is needed to improve the fit in the short wavelength region of the absorption spectrum. The CD spectrum also exhibits its poorest fit in the short wavelength region. As seen in Table I, the gaussian curves used to fit the shortest wavelength absorption and CD components are of a much larger half-width (13.2 nm) than needed to fit any of the other components. This is probably due to the effect of the higher vibrational components. For all five of the major components the CD component was placed by the computer a few nanometers (from 1 to 5 nm) to shorter wavelength than the corresponding absorption component. This is outside the range of likely discrepancies in the wavelength calibration of the spectrometers used. It has been found in this laboratory that the position of absorption and CD bands for monomeric chlorophylls, where there is only one component to the electronic transition band, do not always correspond; the direction of the discrepancy varies from case to case (see the spectra in Houssier and Sauer, 1970, for examples).

Discussion

The multiple components in the absorption and CD spectra of the BChl-P are most readily interpreted in terms of an exciton model. We define an exciton state as the collective excitation of an assembly of molecules, by contrast with the localized excitation of each individual member of the assembly. This is the molecular exciton model of Kasha (Kasha et al., 1965), first formally developed by Davydov (Davydov, 1948). The like molecules are resonantly coupled to one another so strongly that it is not possible to excite one molecule individually.

The source of this coupling is in electrostatic interactions between transition charge distributions usually approximated as transition dipole-transition dipole interactions. These interactions result in the splitting of energy levels, where the number of new levels equals the number of molecules interacting. Each of these energy levels will have a characteristic transition probability determined by the geometrical relationships among the molecules.

The signs and rotational strengths of the CD band components resulting from exciton interaction are determined by the specific geometric arrangement of the interacting molecules, rather than by the inherent asymmetry of the individual molecules. The exciton contribution to the rotational strength is conservative (Tinoco, 1962), meaning that its contribution to the rotational strength should sum to zero over the exciton band. Thus, it is characteristic of exciton interactions that the different components within the one electronic band will have both positive and negative signs.

Other contributions, besides exciton interaction, to the rotational strength of the BChl a transitions within the protein include the asymmetry of the individual molecules (roughly of the same magnitude and sign as seen in Figure 1) and asymmetric interactions of each of the BChl a molecules with transitions in the protein matrix and with non-degenerate transitions in the other BChl a molecules. The magnitudes and signs of these last contributions are difficult to estimate (Hsu and Woody, 1971a; Hsu and Woody, 1971b) without knowing the detailed molecular structure of the BChl-P, but evidence is given below which implies that they are not the major source of the BChl-P rotational strength.

The molecular exciton model provides a consistent explanation of the band splittings observed in the BChl-P spectra. Five resolved

components are visible in the CD of Figure 3. The CD for both the Q_y and Q_x bands is greatly enhanced as compared with the BChl a solution monomers (Figure 1) and the characteristic positive and negative components of a conservative exciton contribution to rotational strength are present.

In the past, multiple components in absorption spectra of photosynthetic materials have been assigned to chlorophylls in different environments (Vredenberg and Amesz, 1966; Clayton, 1966). We feel that our data is better explained by exciton interaction than by BChl a molecules being in different local protein environments. If the primary perturbation on the BChl a molecules which causes the band splitting at 77K (Figure 3) is the specific protein environment of each BChl a, then it would be expected that each of the component absorption peaks would be of approximately the same area. Studies of various chlorophylls in solution show that the dipole strengths (absorption intensities) are virtually invariant in different solvents, although the band maxima may differ in position by 10-20 nm (Seely and Jensen, 1965; Sauer, Lindsay Smith, and Schultz, 1966). Although multiple protein environments are undoubtedly present for the differently situated BChl a molecules in the BChl-P, the exciton model provides the only satisfactory explanation for the widely different intensities (band areas) found for each of the absorption components shown in Figure 3 and listed in Table I.

Equations for calculating exciton spectral properties have been developed by Tinoco (1963). The wave function for an exciton state is written as a linear combination of states in which the excitation is localized on

each of the molecules of the aggregate. These linear-combination wave functions and the energy of the transition to each of the exciton states are obtained as the eigenvalues and eigenvectors of the perturbation matrix whose elements are the transition interaction energies between each of the pairs of like molecules. The interaction energies have roughly an inverse cube dependence on distance. Using this procedure, calculations were undertaken to see if the distances between BChl a molecules could be estimated. In these calculations the elements of the perturbation matrix were obtained by using electric transition monopoles located at each of the conjugated atoms of the porphyrin ring (Philipson et al., 1971), rather than by the more common point dipole approach. This method better takes into account the effect of delocalization on the interaction potential.

For these model calculations the centers of the five BChl a molecules were arbitrarily fixed at the coordinates of a regular array (either a trigonal bipyramid or a linear array). The distance between molecules was allowed to vary and for each different distance, five orientations of each BChl a molecule were supplied in a random manner by the computer program. Using these geometries and the monopoles for the Q_y transition (Philipson et al., 1971), the calculated exciton band width (the energy difference between the highest and lowest components) was then compared with that found experimentally (490 cm^{-1}) for the BChl-P. Agreement between the calculated and experimental band splitting was found only when the molecules were between 12 and 15 Å apart, regardless of their orientation. On this basis we estimate the distance between BChl a centers to be between 12 and 15 Å. We consider this estimate to be

reliable unless the BChl $\underline{a}$ molecules are in an unusual geometrical arrangement (e.g., all transition oscillators oriented approximately parallel to one another) such as to cause inordinately large or small band splittings. The CD and absorption are inconsistent with highly symmetrical molecular arrangements, however.

The diameter of this non-compact, spherical protein, as mentioned earlier, is 81.5 Å and its measured specific volume is 193,000 Å³ (Olson and Thornber, 1971). The calculated diameter of one subunit (assuming a spherical shape) of the protein is, therefore, 45 Å. These dimensions for the subunit will suffice to hold five molecules of BChl $\underline{a}$ with centers 12 to 15 Å from one another. Thus, the distances estimated from the exciton calculations above are consistent with what is known about the protein structure. Because of the dependence of the interaction potential on the inverse cube of the distance, exciton interactions between BChl $\underline{a}$ molecules in adjacent subunits are unlikely to result in additional observable optical effects.

The magnitude of the observed splitting indicates that we are dealing with strong coupling according to the criteria of Simpson and Peterson (Simpson and Peterson, 1957); this case is designated weak exciton coupling by Förster (Förster, 1965). Qualitatively, this requires that the exciton band width be greater than the band width for an individual molecule and implies that excitation is delocalized over the array. Additional mechanisms for energy transfer (e.g., localized exciton migration or Förster's inductive resonance) must exist in vivo, where some of the intermolecular distances are greater and the interactions are not so strong as within the BChl-P subunits.

In most photosynthetic organelles there is probably a heterogeneous arrangement of the photosynthetic pigments, with strong exciton delocalization predominant among chlorophylls in close contact and Förster resonance transfer occurring among the more widely separated subunits.

In order to fit the shoulder on the long wavelength negative component in the CD (Figure 3), it was necessary to use two large gaussian curves of opposite sign almost on top of one another. These are the components in Figure 5 (located at 812 and 814 nm) which largely cancel one another. This feature is interesting in that it would seem to explain the variability (see Results) which is sometimes found in the CD spectra of different samples of the BChl-P. That is, if there are large components in the spectrum which largely cancel each other, a small shift in protein conformation which slightly alters transition energies would result in relatively large changes in this sensitive region of the CD spectrum. The computer curve resolution implies that two of the CD components may be only 2 nm apart. This small separation leads to an observable inflection in the 77K CD spectrum (the 817 nm feature in Figure 3) and is an impressive demonstration of the superior ability of CD to resolve components which are undetectable in absorption measurements.

The narrowness of the gaussian curves (Table I) used in the resolution of the spectrum is unusual. The half-widths of these components (as small as 6.6 nm) are less than would be expected on the basis of studies of the spectrum of monomeric BChl $\underline{a}$ as a function of temperature. The half-widths of the Q_y band of BChl $\underline{a}$ in ether at 26° and -93°C are 44.0 and 36.5 nm, respectively (J. Lindsay Smith and K. Sauer, 1966,

unpublished data). The very narrow components in the BChl-P may be the
result of the rigid protein matrix or an exciton effect (Kasha, 1963).
In cases of strong coupling, where the excitation is spread over several
molecules, the internuclear distances will be little affected by excitation
into vibrationally excited components of the upper electronic state. By
the Franck-Condon principle, this will mean that the 0-0 vibrationless
electronic transition will tend to predominate and a characteristic band
narrowing will be observed. Thus, the surprisingly small band widths found
for the BChl-P may be another manifestation of the strong exciton inter-
actions present in this system.

The possibility of electronic excitation energy transfer by an
exciton mechanism and its implications within the photosynthetic unit
have been discussed in a theoretical paper by Robinson (Robinson, 1966).
Recent work on various chlorophyll-proteins provides evidence that exciton
interactions play an important role in vivo. In particular, the absorption
and CD of reaction center preparations from the purple bacteria R. spheroides
(Sauer et al., 1968) and R. viridis (L. Coyne, 1968, unpublished data)
have been interpreted in terms of exciton interactions among three BChl
molecules in the reduced form and between two molecules in the oxidized
form. Spectral changes accompanying the protochlorophyllide to chloro-
phyllide transformation in holochromes indicate exciton effects (Schultz
and Sauer, 1972).

CD has proved to be a useful tool in detecting exciton interaction,
owing both to its sensitivity to intermolecular interaction and to its
ability to resolve closely spaced components. Since CD closely reflects
the geometric arrangement of molecules, it should also prove useful in

detecting similarities between chlorophyll-proteins from different organisms. Using this method, reaction centers from R. spheroides and R. viridis appear to be similar and a BChl-P from Chlorobium thiosulfato-philum has been found to be closely related to the BChl-P from Cps. ethylica (J. M. Olson, K. D. Philipson, and K. Sauer, 1972, in preparation) discussed in this paper. Thus, a CD study can reveal more detailed similarities than would be possible through biochemical techniques.

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TABLE I: Parameters of the Computer-resolved Components of the Absorption and CD Spectra of the Q_y Band of the BChl-P at 77K (see Figure 5) and Data on the Q_y Band of BChl <u>a</u> in Acetone at 299 and 180K.

λ (nm)		Dipole strength (debye^2)	Rotational strength (debye magneton)	Full-width at half maximum (nm)	Skew[a]
Abs	CD				
BChl-P at 77K					
792	787	9	-.6	13.2	.65
804	800	26	1.3	9.8	.65
813	812	25	2.5	6.6	.75
817	814	2	-1.6	6.6	.65
824	823	9	-1.2	8.3	.82
BChl <u>a</u> in Acetone at 299K[b]					
770		49	.06	44.0	.69
BChl <u>a</u> in Acetone at 180K[b]					
771		50		36.5	.74

[a] Half-width of long wavelength side of gaussian = (Skew) x (Half-width of short wavelength side).

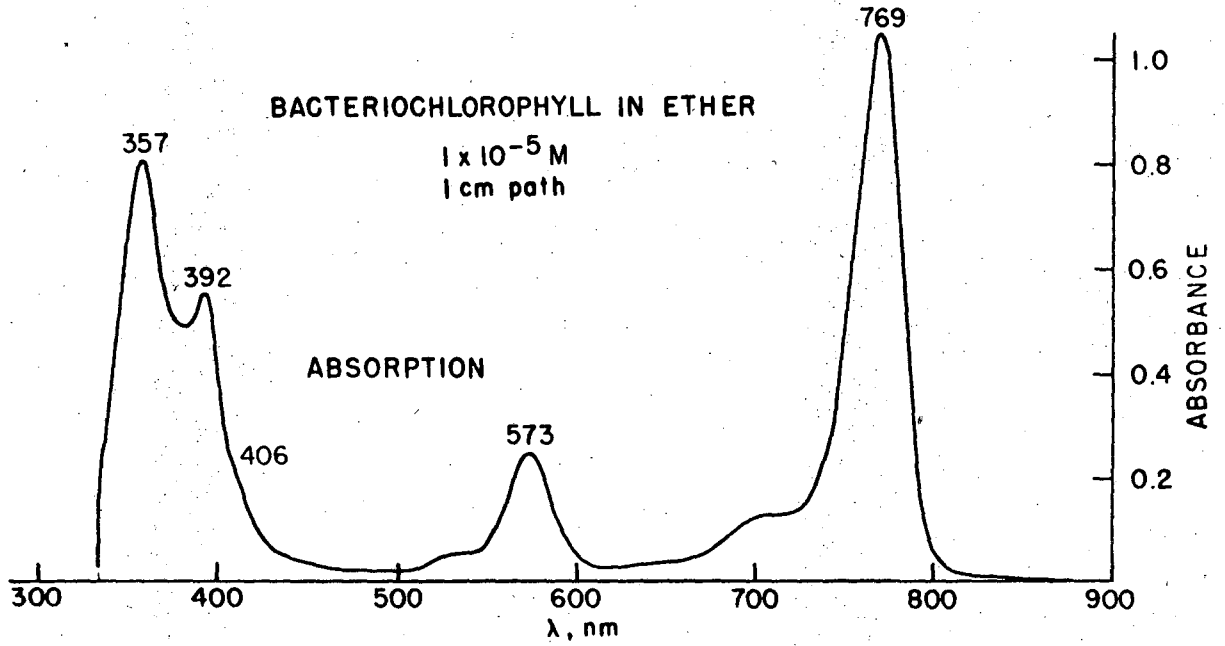[b] J. R. Lindsay Smith and K. Sauer, 1966, unpublished data.

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0 0 0 0 5 7 0 9 0 8

Figure Legends

FIGURE 1: Absorption and CD spectra of BChl $\underline{a}$ in ether at room temperature; conc. 1×10^{-5} M and path length 1 cm (L. Coyne, 1968, unpublished data).
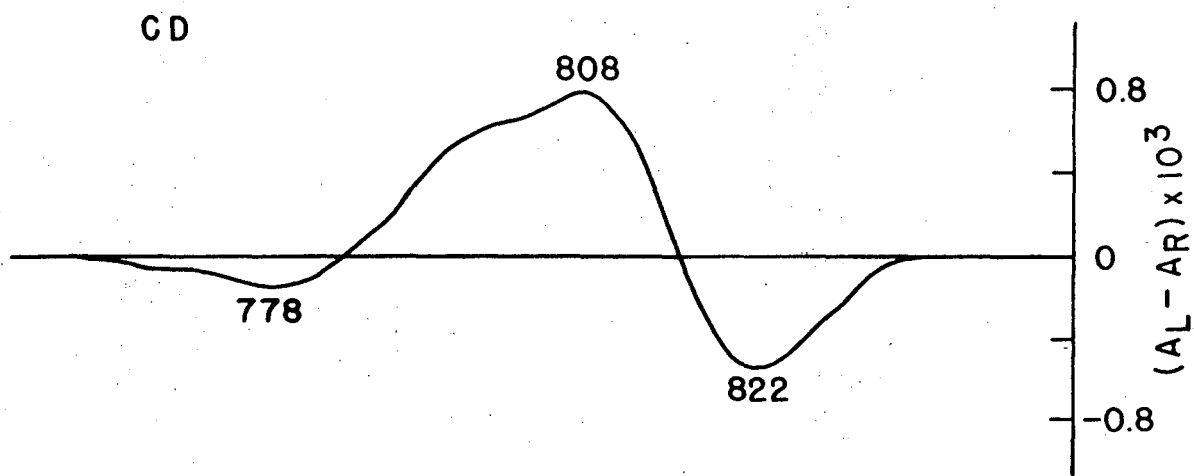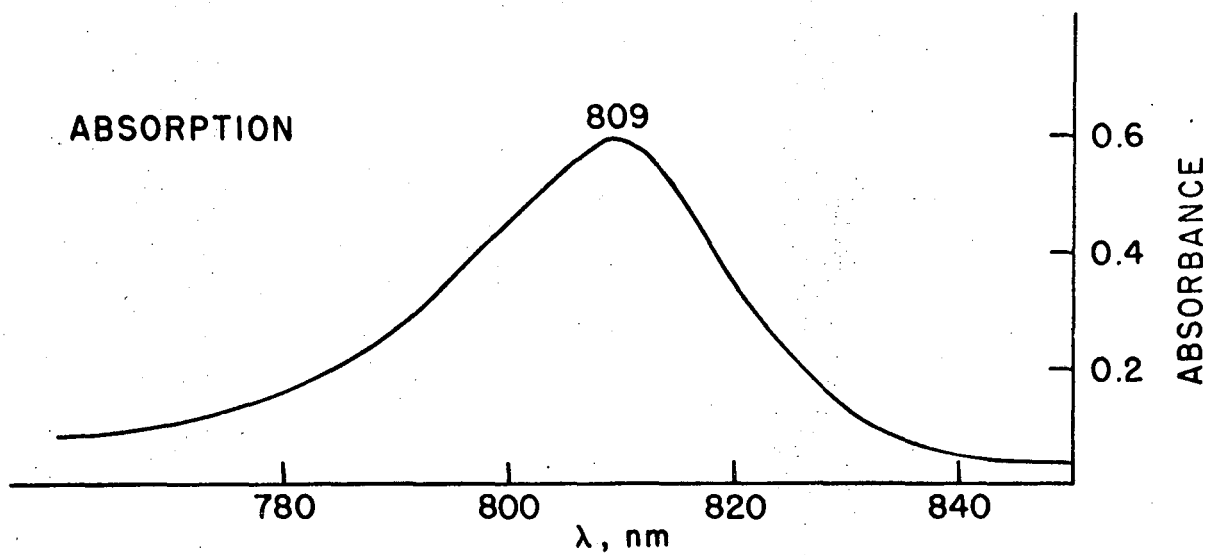
FIGURE 2: Absorption and CD spectra of Q_y band of BChl-P at room temperature in potassium glycerophosphate, tris buffer (pH 7.5, .2 M NaCl), glycerol; BChl $\underline{a}$ conc. 1.3×10^{-5} M and pathlength 3 mm.

FIGURE 3: Absorption and CD spectra of Q_y band of BChl-P at 77K in potassium glycerophosphate, tris buffer (pH 7.5, .2 M NaCl), glycerol; BChl $\underline{a}$ conc. 1.3×10^{-5} M (uncorrected for solvent shrinkage) and path length 3 mm.

FIGURE 4: Absorption and CD spectra of Q_x band of BChl-P at room temperature in glycerol, tris buffer (pH 7.5, .2 M NaCl); BChl $\underline{a}$ conc. 5.2×10^{-5} M and path length 5.7 mm.
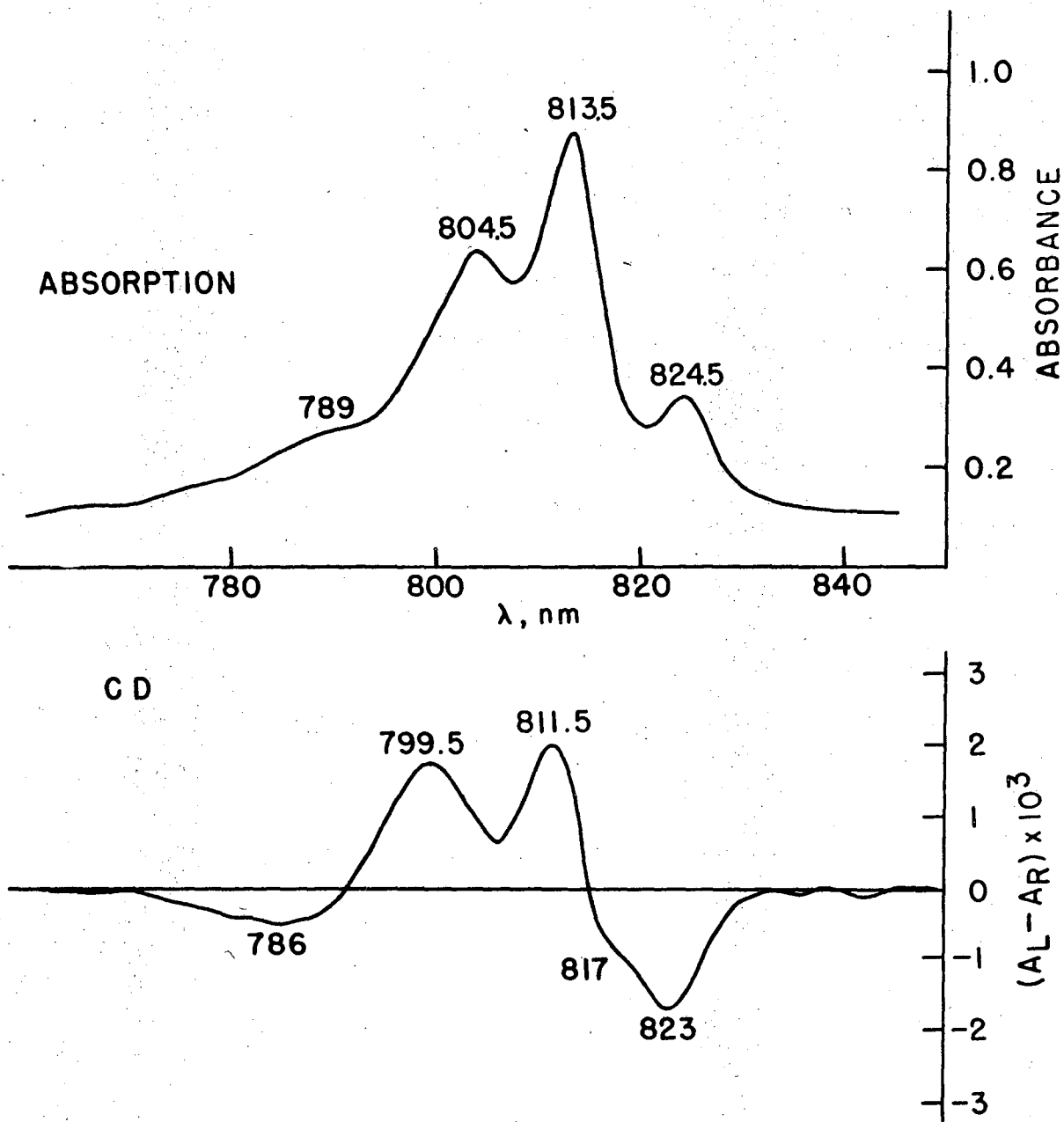
FIGURE 5: The absorption and CD of the Q_y band of the BChl-P at 77K resolved into asymmetric gaussians. See text and Table I. Experimental data, o; gaussian component, - - -; sum of gaussians, ——.
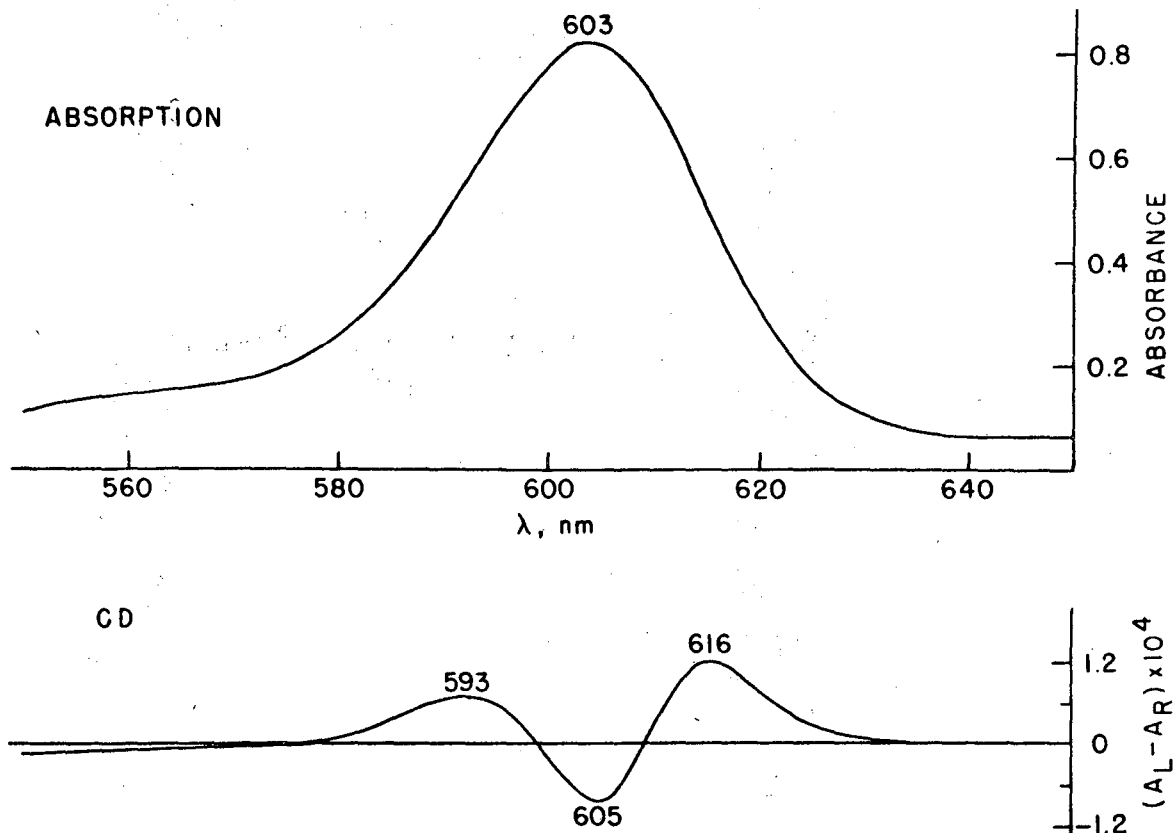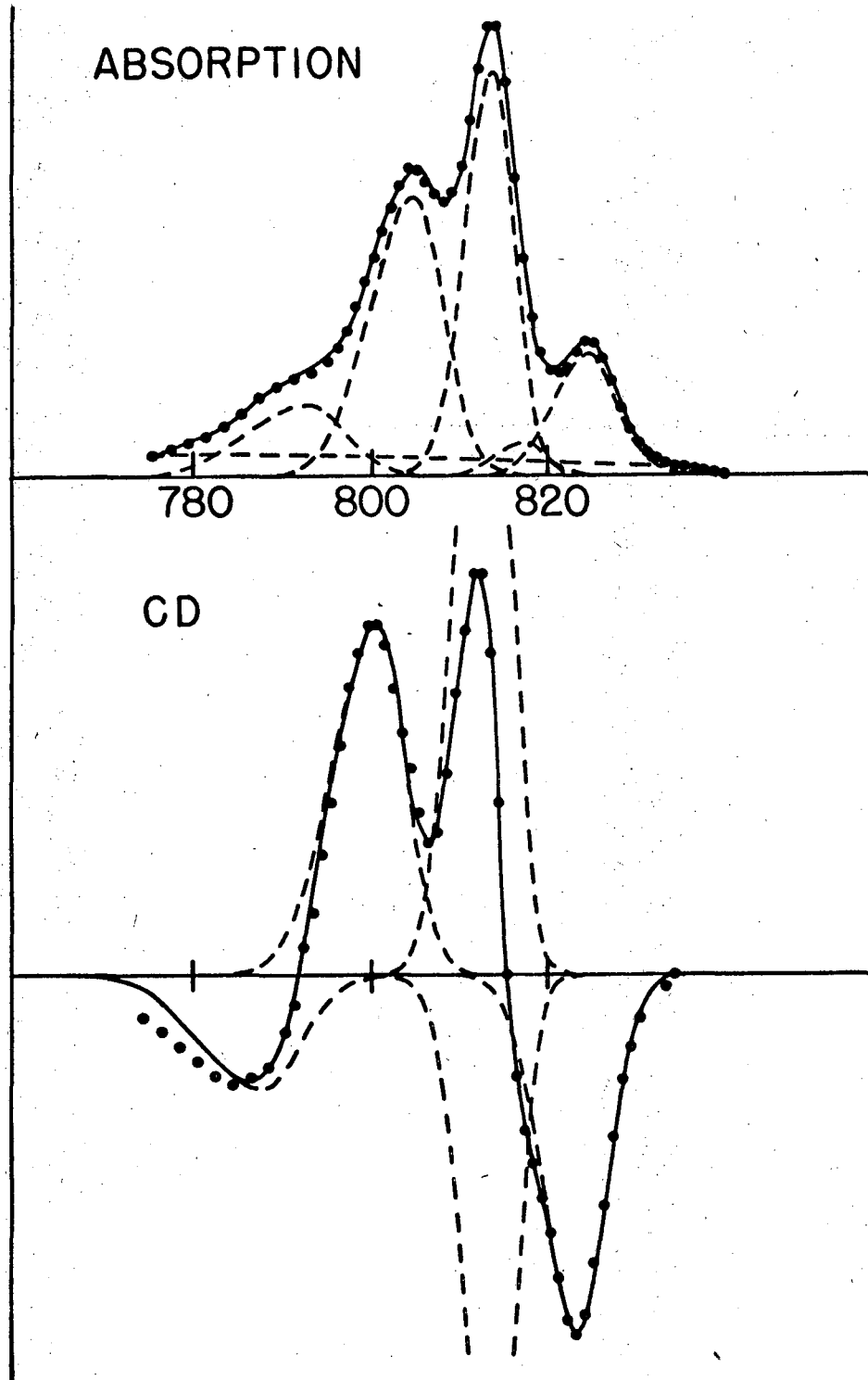
BACTERIOCHLOROPHYLL IN ETHER
1×10^{-5} M
1 cm path

ABSORPTION

CD

XBL 711-5021

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Fig. 1

ABSORPTION

809

0.6

0.4

0.2

ABSORBANCE

780 800 820 840

λ, nm

CD

808

0.8

0

778

822

-0.8

$(A_L - A_R) \times 10^3$

XBL711-5023

Philipson & Sauer
Fig. 2

XBL 711-5022

ABSORPTION

603

ABSORBANCE

λ, nm

CD

593

616

605

$(A_L - A_R) \times 10^4$

XBL 7110-5378

Philipson & Sauer
Fig. 4

ABSORPTION

CD

XBL 7110-5407

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Fig. 5