*CIRCULAR DICHROISM SPECTRA AND THE MOLECULAR ARRANGEMENT OF BACTERIOCHLOROPHYLLS IN THE REACTION CENTERS OF PHOTOSYNTHETIC BACTERIA**

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Chromatophores, or membrane fragments, prepared from photosynthetic bacteria exhibit a variety of properties which demonstrate that they are able to carry out the quantum conversion steps characteristic of the intact bacterial cells.[1, 2] The pigment molecules consist of two distinct functional types: an antenna, which includes over 90 per cent of the bacteriochlorophyll molecules, and a smaller fraction incorporated into reaction centers. The primary function of the antenna is to absorb incident photons and to transfer the resulting electronic excitation energy to the reaction center bacteriochlorophyll molecules. It is in these reaction centers that the actual transformation to chemical energy is initiated.

Recently, Clayton and others have shown that it is possible to treat chromatophores of *Rhodopseudomonas spheroides* so as to remove the antenna bacteriochlorophylls without harming the reaction centers.[3–6] These preparations retain the ability to exhibit photoinduced, reversible absorption changes characteristic of bacteriochlorophyll in untreated chromatophores or in intact bacterial cells. In the reaction center preparations, an absorption band at 865 nm (P870) undergoes complete bleaching and a second, larger band at 803 nm (P800) exhibits a concomitant blue shift to 797 nm upon illumination. Added oxidizing agents, such as ferricyanide or chloroiridate, will induce the same absorption changes in the dark, which indicates that the bleaching of the P870 by illumination is a consequence of oxidation of bacteriochlorophyll.

We have examined the absorption and circular dichroism (CD) spectra of the reaction center preparations both in the reduced (dark) and in the oxidized (illuminated or chemically oxidized) state. The CD spectrum exhibits dramatic changes that accompany the absorption changes. Analysis of the spectral properties suggests that the reduced reaction center contains a trimer of bacteriochlorophyll molecules that are closely coupled and exhibit pronounced exciton interaction. Upon oxidation, one of these three molecules is converted to a form with an entirely different absorption spectrum, and the remaining two molecules no longer exhibit evidence of exciton interaction.

Experimental.—Preparations of reaction centers from *R. spheroides* were obtained from R. K. Clayton and D. W. Reed. Two types of preparations were studied: preliminary measurements were made using material obtained by oxidation of the antenna bacteriochlorophylls with excess chloroiridate;[7] the results reported in detail in this paper were obtained using materials where high concentrations of the nonionic detergent Triton X-100 served to remove the antenna pigments.[6] The latter preparation exhibits substantially less light-scattering and less absorption from the products of the antenna molecules; however, no significant differences were observed in the absorption or CD spectra at wavelengths longer than 780 nm. Absorption spectra were measured with a Cary 14R

spectrophotometer; the spectrum of the oxidized form was obtained using the IR No. 2 mode, where the undispersed spectrometer light source itself provided the illumination to convert the reaction center pigments. The CD spectrum was obtained using an instrument of our own design.[8, 9] To obtain the CD spectrum of the photoconverted form of the reaction center preparation, we illuminated the sample from the side with visible light from a tungsten lamp filtered by a Corning 4-94 glass. The photomultiplier detector of the CD spectrometer was protected from scattered exciting light by means of two 2-64 filters, which permitted the measuring beam at wavelengths longer than 700 nm to pass with little attenuation. Bacteriochlorophyll was prepared by the method of Sauer, Lindsay Smith, and Schultz.[10]

Results.—The CD spectra of preparations of pigmented lamellae from a variety of photosynthetic materials show multiple CD bands in the long-wavelength region. In the case of chromatophores from *R. spheroides*, wild type, there are three pronounced components at 793(−), 847(+), and 867.5(−) nm.[8] (The symbols (+) and (−) after the wavelength values indicate the signs of the CD components.) The CD spectrum of chromatophores of the carotenoidless R-26 mutant of this bacterium is shown along with its absorption spectrum in Figure 1. Apart from the missing absorption in the region from 400 to 550 nm because of the absence of carotenoids, there are distinct absorption differences between the mutant and the wild-type chromatophores in the far-red spectral region. The band at 800 nm is weaker in the mutant, and the main absorption peak is at 860 nm. In the wild type, the main peak is at 852 nm, with a pronounced shoulder to longer wavelengths. The CD spectrum of the mutant differs in detail from that of the wild type; the mutant has components at 815(−), 857(+), and 890(−)

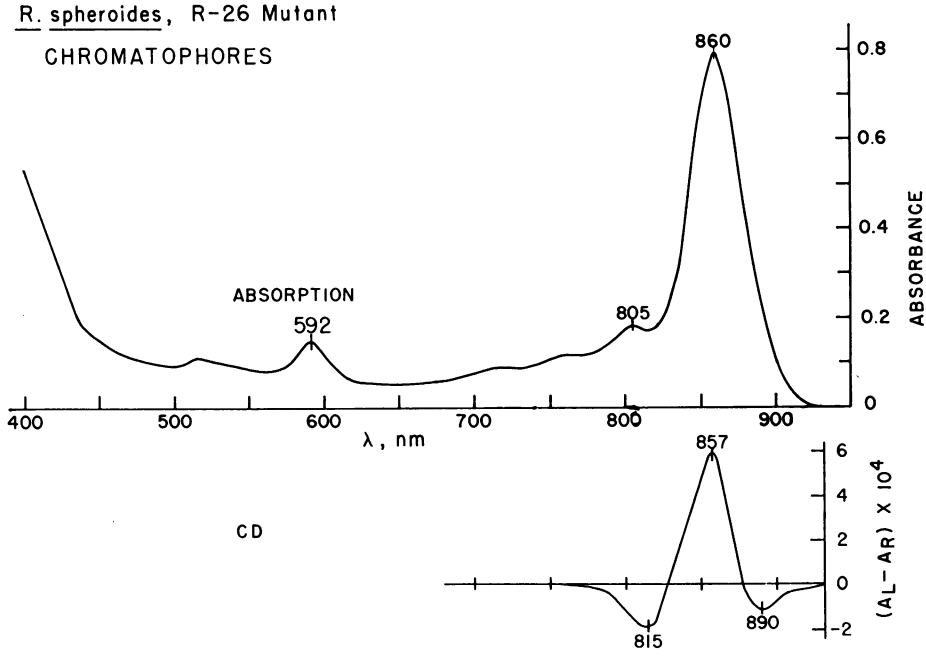


FIG. 1.—The absorption and CD spectra of an aqueous suspension of chromatophores from *R. spheroides*, R-26 mutant; 0.1-cm path.

nm, whereas in the wild type, the corresponding CD components are at 793(−), 847(+), and 867.5(−) nm. As will be seen below, these CD and absorption components are predominantly characteristic of the antenna or light-harvesting pigments.

When the chromatophores of the R-26 mutant are treated with chloroiridate or with Triton X-100, the absorbance in the far-red region decreases approximately tenfold.[2, 3, 6] A small absorption band at 865 nm remains, along with virtually all of the original absorption at 800 nm (Fig. 2). These absorptions are fully photoconvertible and correspond to uncovered P870 and P800 from the reaction centers of the original chromatophores. The integrated intensities are close to a P800:P870 ratio of 2:1. Apparently the antenna pigments, B870, responsible for bulk absorption in the original material have been either removed or converted to species that absorb at shorter wavelengths (the 682- and 757-nm peaks). These are probably oxidized bacteriochlorophyll[11] and bacteriopheophytin, respectively.

The CD spectrum of the reaction center (RC) preparation resulting from treatment with Triton X-100 is shown in Figure 2. The spectrum is markedly different from that of the untreated chromatophores shown in Figure 1, which demonstrates that the CD bands in the untreated chromatophores arise predominantly from the antenna pigments. In the RC preparation, the absorption band at 865 nm (P870) is associated with a positive CD component, whereas that at 803 nm (P800) is associated with a negative double CD, with a crossing at 802 nm. The double CD band demonstrates that the 803-nm absorption is really an unresolved

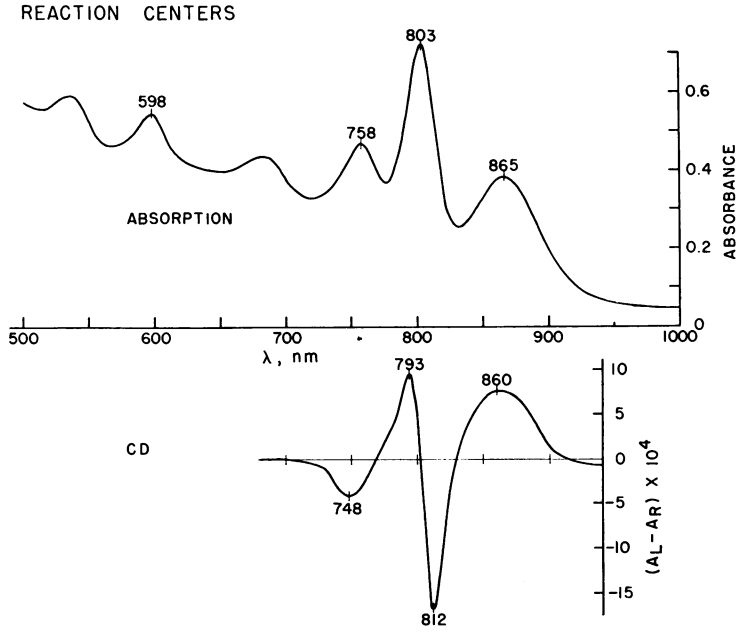


FIG. 2.—Absorption and CD spectra of the reaction centers of *R. spheroides*, R-26 mutant, isolated by the Triton X-100 method of Reed and Clayton; 0.5-cm path.

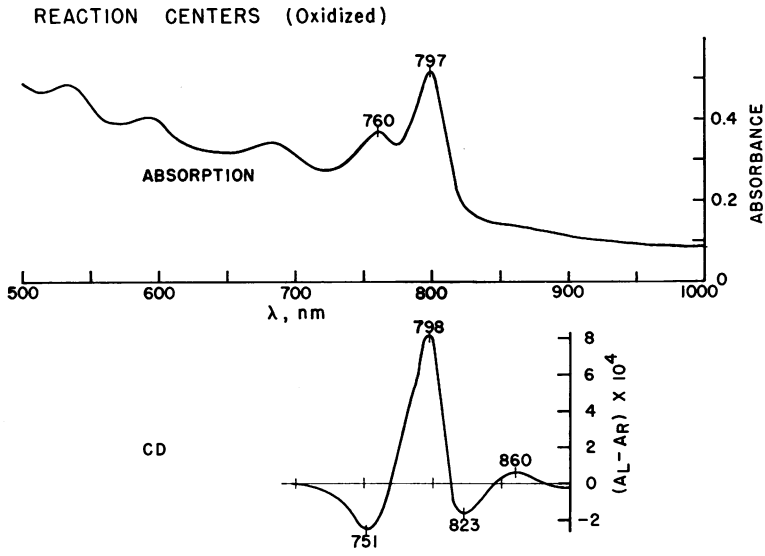


FIG. 3.—Absorption and CD spectra of the reaction centers of *R. spheroides*, R-26 mutant, in the presence of excess ferricyanide; 0.5-cm path.

pair of bands. These are sufficiently close to one another that they are not resolved in the absorption spectrum, even at 77°K.[12] From measurement of the half width (10 nm) of the 803-nm band at 77°K, we estimate the splitting to be less than 80 cm^{-1}.

Upon oxidation with a small excess of ferricyanide (Fig. 3) or upon illumination with bright visible light (Fig. 4), the absorption band at 865 nm disappears, whereas the band at 803 nm undergoes a blue shift to 797 nm without change in

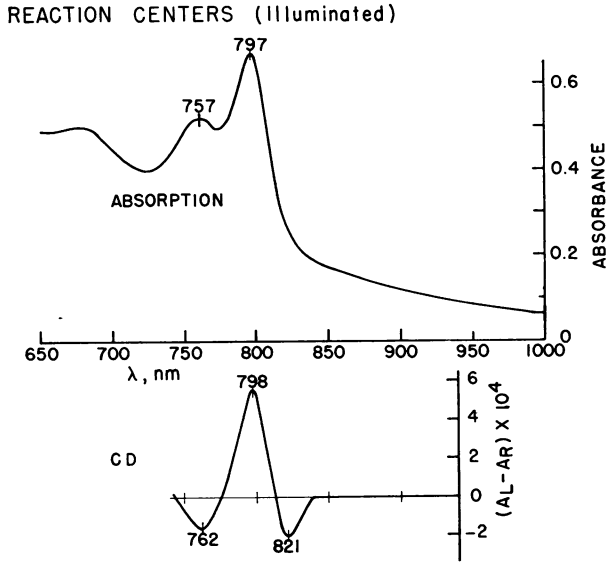


FIG. 4.—Absorption and CD spectra of the reaction centers of *R. spheroides*, R-26 mutant, under side illumination with strong visible light. See text for details of the method; 0.5-cm path.

integrated band area.[5, 6] The photoinduced changes are completely reversible in the dark. The CD spectrum of the chemically oxidized RC preparation (Fig. 3) is similar in shape and magnitude to the CD spectrum of the photoconverted RC fragments (Fig. 4). In the measurement of the photoconverted sample, an instrument feedback loop senses stray actinic light, which leaks through the complementary filter combination and artificially attenuates the CD signal. On the basis of instrument sensitivity tests, this effect has been approximately corrected in Figure 4 by doubling the observed CD signal. The small difference in amplitudes remaining between Figures 3 and 4 may not be significant. The CD spectra both of the chemically oxidized and of the photoconverted reaction centers are distinctly different from the spectrum of the reduced RC preparation (Fig. 2). In Figures 3 and 4, we now see no substantial CD band at 860 nm, and the negative double CD band crossing zero at 802 nm has been changed to a single positive CD band in the photoconverted form. The close agreement of the wavelength of the CD maxima at 798 ± 1 nm and the absorption bands at 797 ± 1 nm demonstrates that there is no significant exciton splitting of this band. The small negative component at 821 nm appears to be real, as it occurs repeatedly. It may result from a component of the absorption of the oxidized bacteriochlorophyll that underlies the tail of the 797-nm band.

In order to aid in interpreting the CD spectral shifts in this long-wavelength region, we have examined the spectrum of pure bacteriochlorophyll in solution. Figure 5 shows the absorbance and CD spectrum of a solution in carbon tetrachloride to which a small amount of pyridine has been added in order to break up aggregates. A similar result is obtained with the addition of methanol. Under these conditions, the bacteriochlorophyll apparently exists as a solvated monomeric species,[10, 12] and the single electronic transition giving rise to the absorption band at 782 nm exhibits a weak, single, positive CD. In a solvent such as acetone or ether, on the other hand, this absorption band of bacteriochlorophyll occurs at 770 nm and the CD is at least five times smaller, lying within the noise level of the current measurements. Why there is this difference from the solvate molecule in carbon tetrachloride is not clear at present.

Discussion.—The preparation by Clayton of photosynthetic bacterial chromatophores in which photoactive reaction center pigments are exposed opens the exciting possibility of learning the molecular arrangement and mode of action of this site of photosynthetic energy conversion. Clayton has observed that the ratio of absorption of P800 to that of P870 is 2 to 1, within experimental uncertainty.[5] Upon excitation with strong light or upon chemical oxidation, the P870 absorption, or one third of the total, bleaches whereas the P800 absorption undergoes a band shift but no bleaching. Evidence from quantitative extraction experiments[5] and the stoichiometry of P870 to cytochrome, which are coupled kinetically,[13] suggest that one molecule in three absorbing beyond 780 nm is involved in the photooxidative bleaching.

Our CD observations are entirely consistent with the interpretation that the reaction center consists of a trimer of coupled bacteriochlorophyll molecules associated with donors and acceptors of electrons. The presence of three components in the CD spectrum at 860, 812, and 793 nm cannot be accounted for by

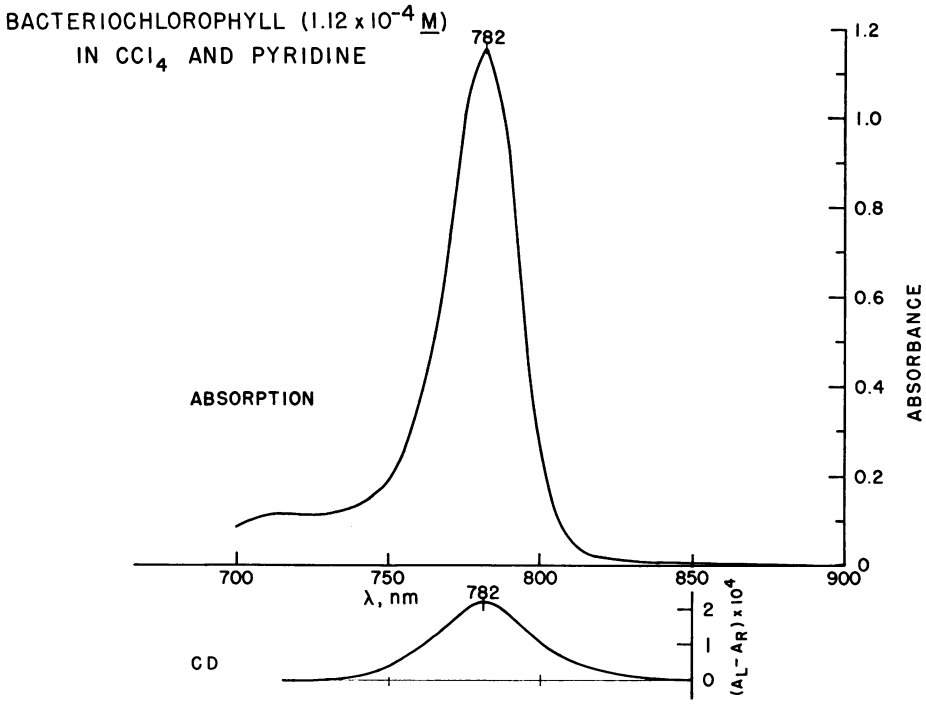
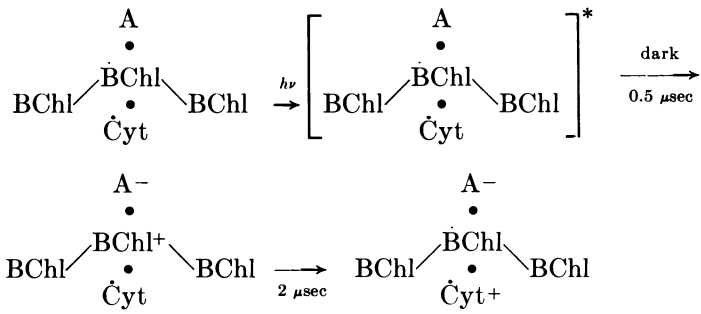


FIG. 5.—Absorption and CD spectra of purified bacteriochlorophyll complexes with pyridine in carbon tetrachloride; conc 1.12×10^{-4} M and path length 0.1 cm.

fewer than three molecules. The postulate that the three molecules are coupled together is confirmed by observations that the P870 and P800 behave identically under a variety of conditions with respect to kinetics, electrochemical potential, and phenotypic variation.[2, 4, 14] The change in the CD spectrum near 800 nm that accompanies the bleaching of P870 also supports this postulate.

Clayton has proposed a model for the reaction center, recently given support by the studies of pulsed laser-induced absorbance changes by Parson,[13] in which a bacteriochlorophyll molecule (BChl) is closely coupled to an electron acceptor (A), possibly ubiquinone, and a donor, cytochrome (Cyt). Upon illumination by 30-nsec flashes, the BChl in *Chromatium* transfers an electron to the acceptor in less than 0.5 μsec, and in the following dark period, it becomes reduced by the cytochrome in 2 μsec. On the basis of the absorption and CD evidence described in this paper, we propose an expanded model in which two other bacteriochlorophyll molecules also participate in the reaction center. In the dark, the three molecules form a trimer with large exciton interaction. The close correspondence in behavior of the P870 and P800 features supports the model where the three molecules are all interacting with one another. An energy level diagram that illustrates this interaction is shown in Figure 6. It must be emphasized that in this model, the P800 and P870 absorption and CD bands are characteristic of the aggregate and not of the individual molecules.[15] Upon illumination, one molecule, which is near an electron acceptor, is oxidized and there is little evi-

dence of any remaining electronic coupling between the two unbleached BChl molecules. A schematic picture of the events described above, based on Clayton's original ideas,[1] can be represented by



The oxidized species, $BChl^+$, has an absorption spectrum entirely different from that of the reduced BChl.[6] As a consequence, the illuminated reaction centers no longer contain three bacteriochlorophyll molecules, but only two. The evidence from the CD spectra (Figs. 3 and 4) suggests that the two BChl molecules that remain are not strongly interacting in the chemically oxidized or photoactivated state. Figure 6 illustrates the absence of exciton splitting in the oxidized state. This may result from the fact that the BChl molecules are separated by a greater distance (i.e., by the $BChl^+$) as suggested in the model above, or from the fact that the long-wavelength absorption oscillators have an unfavorable relative orientation.

The rotational strength giving rise to CD for any two interacting molecules, at least in the approximation involving point electric transition dipole moments, depends upon a triple product involving the two transition dipoles and a vector joining the centers of the two molecules.[16, 8] For several simple orientations, including completely parallel or completely perpendicular transition moments, the triple product is zero. Although the rotational strength, $R_{\pm}$, increases linearly with increasing distance, the interaction energy V_{12} varies with the inverse third power of distance. As a consequence, the exciton-band splitting decreases rapidly toward zero with increasing intermolecular distances, and the positive and negative CD components exactly cancel one another in the limit of infinite separation.

We favor the model in which the centers of three bacteriochlorophyll molecules in the reduced reaction center are in a linear or somewhat bent arrangement. Upon excitation, the central molecule is removed by virtue of photooxidation, and the distance between adjacent interacting molecules now is nearly doubled, without any necessary molecular motion. The observed concurrent blue shift of the center of gravity of the P800-P870 absorption is consistent with this picture, as blue shifts are always observed for the long-wavelength transitions of chlorophyll molecules upon decreasing aggregation.[8, 10] Furthermore, the positive CD observed at 798 nm in the oxidized RC preparation is similar in sign and shape to that for monomeric bacteriochlorophyll in solution (Fig. 5). This absorption at 797 nm is still 15-nm to longer wavelength than is observed in simple solution

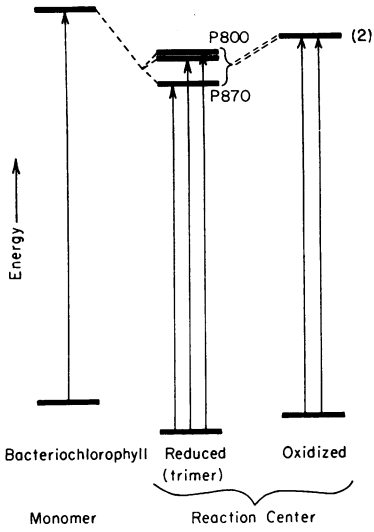


FIG. 6.—Electronic energy level diagram for bacteriochlorophyll as monomers and in reaction centers.

spectra; however, this may result from a strongly polarizable intermediate environment of the P800 molecules in their lipoprotein matrix. It should be noted that asymmetric molecules, including $BChl^+$, in the immediate environment may contribute to the rotational strengths of the bacteriochlorophyll chromophores.

Summary.—Studies of the circular dichroism and absorption spectra of the bacteriochlorophyll molecules in chromatophore fragments of *R. spheroides* (R-26 mutant), with antenna pigments removed, suggest that there are large exciton interactions among the reaction center molecules. The evidence from the CD spectra supports an interpretation in which the reaction center contains a trimer of bacteriochlorophylls. Upon activation by visible light or upon chemical oxidation, one of the molecules is converted to its oxidized form, and the remaining two molecules no longer exhibit evidence of any strong electronic interaction. A model is proposed in which the reaction center consists of two bacteriochlorophyll molecules separated by a third, which in turn is in close proximity to an electron acceptor and an electron donor (cytochrome).

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