Comparative Study of the Circular Dichroism Spectra of Reaction Centers from Several Photosynthetic Bacteria†

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ABSTRACT: The absorption and circular dichroism (CD) spectra of reaction centers from the purple photosynthetic bacteria *Rhodopseudomonas viridis* and *Rhodospirillum rubrum*, and of a preparation from *Chromatium* enriched in reaction centers are compared with those previously reported for reaction centers from *Rhodopseudomonas spheroides*. The reaction centers, with the exception of those from *R. viridis*, appear spectrally very similar. The spectral properties of the bacteriopheophytin present in the reaction centers give no indication that the bacteriopheophytin is strongly interacting with the reaction center bacteriochlorophyll and imply that the bacteriopheophytin has no function in the transfer of electrons. The reaction centers from purple photosynthetic bacteria are compared with those present in green photosynthetic bacteria and higher plants. Significant differences are found among the spectra of photoactive complexes from these three classes of photosynthetic organisms.

The reaction centers of photosynthetic organisms are complexes containing protein, (bacterio)chlorophyll and other primary photoreactants of photosynthesis. These complexes exhibit light-induced, reversible absorption changes when a chlorophyll molecule within the reaction center loses an electron to an unknown primary acceptor. Associated with the reaction centers in purple photosynthetic bacteria there are normally 40–100 antenna bacteriochlorophyll (Bchl)[1] molecules, which function to transfer light excitation energy to the reaction center. Several purified or enriched reaction center preparations have been made from these bacteria (Reed and Clayton, 1968; Reed, 1969; Gingras and Jolchine, 1969; Thornber *et al.*, 1969; Thornber, 1970; Smith *et al.*, 1972). This is in contrast to green photosynthetic bacteria and higher plants from which purified reaction centers have not yet been isolated. Only samples enriched in photoactive pigments have been obtained from these two classes of photosynthetic organisms (Fowler *et al.*, 1971; Olson *et al.*, 1973; Kok, 1961; Vernon *et al.*, 1969; Sane and Park, 1970).

Despite much effort, little is known about the detailed structure or mechanism of action of the photoactive BChl proteins. *In vivo* the reaction centers of purple bacteria are membrane bound within the chromatophore and isolation procedures require the use of a detergent to release and solubilize the reaction center complexes. The presence of detergent in the reaction center preparations is a hindrance to many biochemical analyses such as molecular weight determinations. Even the number of pigment molecules present per protein remains unresolved. Recent measurements (Feher, 1971; Clayton *et al.*, 1972) indicate that reaction centers from *Rhodopseudomonas spheroides* contain two molecules of bacteriopheophytin (BPh) and either three, four, or five molecules of BChl. Whether the BPh plays an important role in the electron-transfer reactions was uncertain. Molecular weight approximations of reaction centers from *R. spheroides* (Feher, 1971), *Rhodopseudomonas viridis* (Thornber and Olson, 1971), and *Rhodospirillum rubrum* (Smith *et al.*, 1972) give estimates ranging from 37,000 to 110,000.

The circular dichroism (CD) of reaction centers from *R. spheroides* in both the oxidized and reduced states has previously been analyzed by Sauer *et al.* (1968). They concluded, on the basis of large changes in the CD spectrum upon oxidation, that the reduced reaction center contains three or more closely coupled BChl molecules which exhibit exciton interaction. In the oxidized state one of these molecules has been converted to a BChl$^+$ radical and the CD evidence for exciton interaction has disappeared. A model for the structure of the reaction center was proposed in which the oxidizable BChl was located between the other BChl molecules. Thus, in the oxidized form, the remaining BChl molecules are separated by a BChl$^+$, the distance between BChl molecules has increased, and resonance interaction is significantly attenuated. More recent research (Philipson and Sauer, 1972; Schultz and Sauer, 1972; Philipson *et al.*, 1972) has confirmed the existence of exciton interaction in photosynthetic materials. On the basis of electron paramagnetic resonance (epr) line-width measurements, Norris *et al.* (1971) and McElroy *et al.* (1972) have proposed that the unpaired electron in oxidized reaction centers is distributed over two BChl molecules.

In this paper we examine absorption and CD spectra of reaction centers from *R. viridis* and *R. rubrum* and of a preparation enriched in reaction centers from *Chromatium* (fraction A) (Thornber, 1970). These spectra are compared with one another and with the previous results from *R. spheroides*. The reaction centers from purple bacteria appear spectrally very similar, except for those from *R. viridis* which show some marked dissimilarities, especially in the low-temperature absorption spectrum. No CD evidence of interaction between reaction center BPh and BChl is detected, indicating that the BPh probably does not actively participate in the electron-transfer reactions. A comparison is made between the reaction centers of purple bacteria and those present in green bacteria (Olson *et al.*, 1973) and higher plants (Philipson *et al.*, 1972).

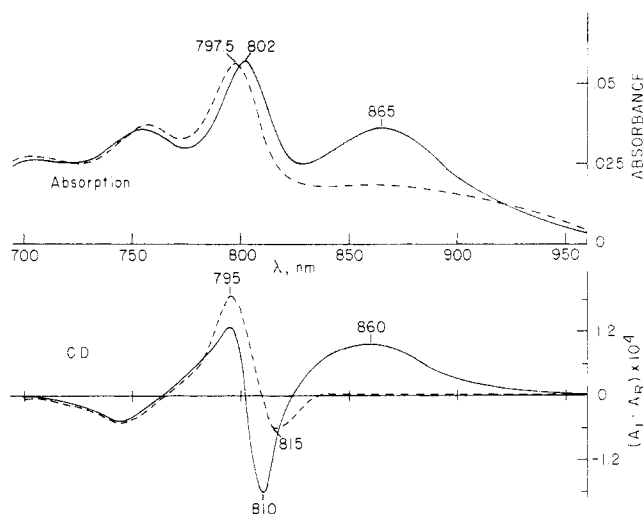[1] Abbreviations used are: BChl, bacteriochlorophyll; BPh, bacteriopheophytin.

FIGURE 1: Absorption and CD spectra of *R. rubrum* reaction centers. Reduced (——) and oxidized (— — —). *T* = 24°, 1-cm path length, 50 mM Tris buffer (pH 8.0). Solid sodium ascorbate was added to reduced sample and potassium ferricyanide crystals were added to oxidized sample.

FIGURE 2: Absorption and CD spectra of *R. rubrum* reaction centers. Reduced (——) and oxidized (— — —). *T* = 24°, 1-cm path length, 50 mM Tris buffer (pH 8.0), A_{802} = 0.52. Sodium ascorbate was added to reduced sample and potassium ferricyanide crystals were added to oxidized sample.

Materials and Methods

The reaction centers from *R. viridis* and *Chromatium* fraction A were prepared in the laboratory of J. M. Olson, using the methods of Thornber (1970, 1971). Key steps in the isolation procedures involve the use of the detergent, sodium dodecyl sulfate, for solubilization and hydroxylapatite chromatography. The *R. rubrum* reaction centers were prepared by W. R. Smith in the laboratory of C. Sybesma (Smith *et al.*, 1972) using sodium dodecyl sulfate and sucrose density gradient centrifugation. All samples were stored in the freeze-dried state and were resuspended in the appropriate buffer just prior to the experiments.

Absorption spectra were recorded using a Cary 14R spectrophotometer and CD spectra were recorded using a Durrum-Jasco J-20 spectropolarimeter with sensitivity extended to 1000 nm. Light-minus-dark difference spectra were obtained in both instruments using side illumination at 530 nm by a projector equipped with a 150-W tungsten lamp. The wavelength of illumination was defined by an interference filter and a Corning 4-94 filter. The incident light intensity was measured to be 1.5×10^5 ergs cm^{-2} sec^{-1}. The photomultipliers were shielded from stray light with a Corning 2-64 filter.

Results

The room temperature absorption and CD spectra for a dilute suspension of *R. rubrum* reaction centers in both the chemically reduced and chemically oxidized states are shown in Figure 1. The spectra result from the long-wavelength, nondegenerate, Q_y transitions of BChl a and BPh a. Three distinct peaks are seen in the reduced absorption spectrum. Those at 802 and 865 nm are attributed to BChl a transitions and the peak at 755 nm is usually assigned to BPh a (but see Feher, 1971). The 802- and 865-nm components are commonly referred to as P800 and P870, respectively. Upon oxidation there is a bleaching of the 865-nm absorption band and a blue shift of the 802-nm component. These spectral effects, caused by oxidation (whether induced by light or chemicals), are qualitatively similar in all purple bacteria
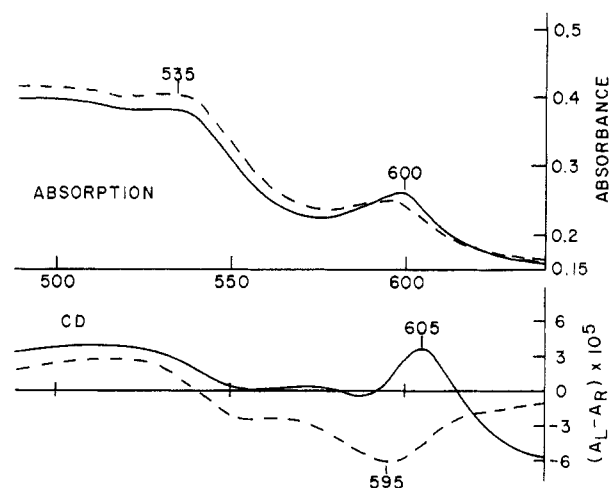
reaction center preparations and produce a characteristic oxidized-minus-reduced absorption difference spectrum (*e.g.*, Figure 5b).

The CD spectrum (Figure 1) for this sample shows components at 860 (+), 810 (−), 795 (+), and 745 (−) nm. (The symbols (+) and (−) after the wavelength values indicate the signs of the CD components.) Oxidation causes the 860-nm peak to disappear, the 810-nm trough largely to disappear, and an increase in magnitude of the 795-nm peak. The CD (in the region of BChl a absorption) has thus changed from a three- to a two-component spectrum. The spectra shown in Figure 1 for *R. rubrum* reaction centers are very similar, apart from some wavelength differences in the oxidized CD spectrum, to those previously described for *R. spheroides* reaction centers (Sauer *et al.*, 1968).

The reduced and oxidized absorption and CD spectra for the 600 and 535 nm (Q_x) transitions of BChl a and BPh a, respectively, are shown in Figure 2 for the *R. rubrum* reaction centers. In these spectra the only significant changes owing to chemical oxidation occur in the region of the BChl a transition. Here, the CD is significantly affected by the redox state of the photoactive complex, going from a positive component at 605 nm to a negative one at 595 nm upon ferricyanide addition. The presence of scattering is responsible for a portion of the absorption in this region, and it is possible that the negatively displaced base line at 650 nm in the CD spectrum of the reduced reaction centers is due to a scattering artifact.

The absorption and CD results (Figure 3) for *R. viridis* reaction centers at room temperature qualitatively resemble those for *R. rubrum*. The major difference is the much longer wavelengths at which the chromophores absorb. Here the photoactive pigments are P830 and P960 by contrast with P800 and P860 in *R. rubrum*. Part of this wavelength shift is explained by the presence in *R. viridis* of BChl b rather than the more common BChl a; in acetone BChl a and BChl b absorb at 771 and 794 nm, respectively (Eimhjellen *et al.*, 1963). The structure of BChl b (Brockmann and Kleber, 1970; Baumgarten, 1971), although not completely known, is closely related to that of BChl a. Also, in this reaction center there is no clearly resolved absorption peak
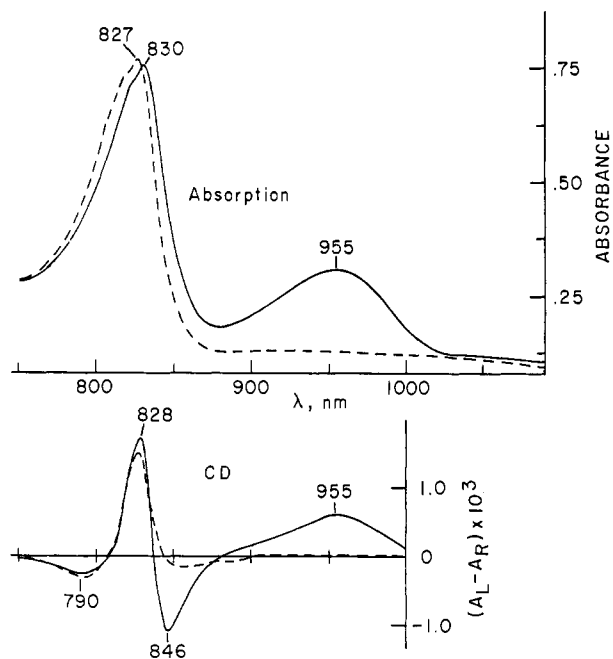
FIGURE 3: Absorption and CD spectra of *R. viridis* reaction centers. Reduced (—) and oxidized (----). *T* = 24°, 1-cm path length, 50 mM Tris buffer (pH 8.0). Samples were reduced with solid sodium ascorbate and oxidized with crystalline potassium ferricyanide.

due to BPh b. The 790 (−) nm component in the CD (Figure 3) implies, however, that this pigment is present; the analogous component, at 745 (−) nm, in *R. rubrum* is clearly associated with the BPh a absorption peak.

Significant differences in the absorption properties of *R. viridis* and *R. rubrum* reaction centers are found at 77°K (Figure 4). In *R. viridis* (Figure 4a) the low-temperature absorption spectrum reveals much new structure. In the reduced state five components are resolved. P960 is split into two components absorbing at 929 and 987 nm and P830 shows peaks at 833 and 816 nm and a shoulder (probably due to the BPh b) at 790 nm. Chemical oxidation causes bleaching of both long-wavelength bands and leaves two resolved components at the shorter wavelengths.

The *R. rubrum* reaction centers (Figure 4b) show no new structure at liquid nitrogen temperature. The 865-nm band is shifted to 890 nm, and P800, in both reduced and oxidized states, is narrowed but does not reveal any new components. Feher (1971) has reported that second derivative spectroscopy shows two components in the reduced P800 of *R. spheroides* reaction centers at 77°K. The small peak at 890 nm in the chemically oxidized spectrum is probably due to incomplete oxidation.

Chromatium fraction A is a BChl protein with a molecular weight of about 500,000 and with one photooxidizable (P880) molecule per approximately 45 BChl a molecules (Thornber, 1970). Since the absorption and CD (Figure 5a) spectra in this case, are mostly a function of the bulk BChl molecules rather than of the reaction center itself, more information about the reaction center is obtainable from the light-minus-dark absorption (Figure 5b) and CD (Figure 6d) difference spectra. The absorption difference spectrum (Figure 5b) has the familiar shape of a purple bacterial reaction center with components P800 and P880. The CD difference spectrum (Figure 6d) for this enriched fraction can be compared with difference spectra calculated from the data
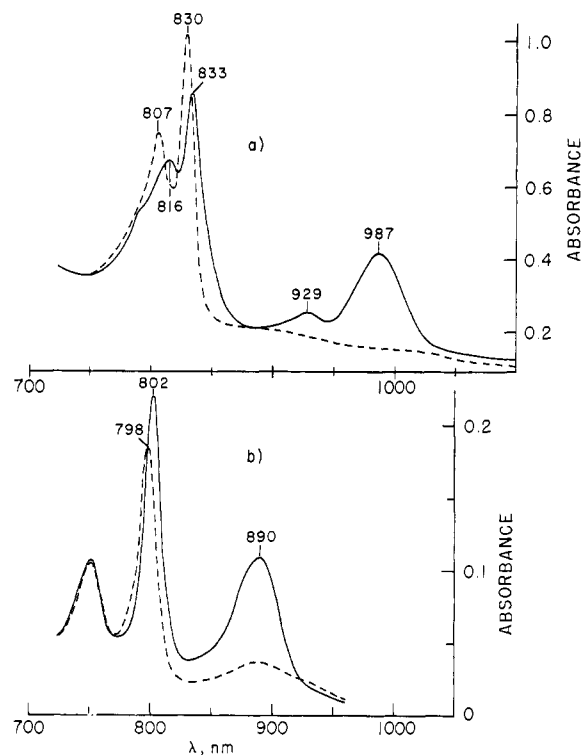

FIGURE 4: Low-temperature absorption spectra of *R. viridis* (a) and *R. rubrum* (b) reaction centers. Reduced (——) and oxidized (----). *T* = 77°K, 3-mm path length, glycerol–50 mM Tris (pH 8.0) 1:1, v/v). Samples were reduced with sodium ascorbate and oxidized with potassium ferricyanide.
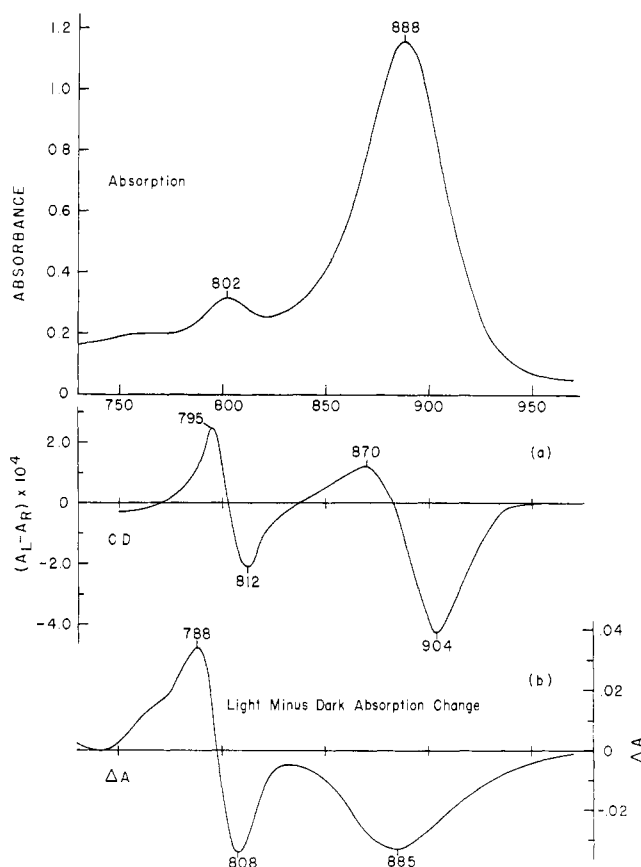

FIGURE 5: Spectra of *Chromatium* strain D, fraction A. (a) Absorption and CD. (b) Light-minus-dark difference absorption. *T* = 24°, 1-cm path length, 50 mM Tris (pH 8.0). 3.3 μM phenazine methosulfate. Activation wavelength, 530 nm.
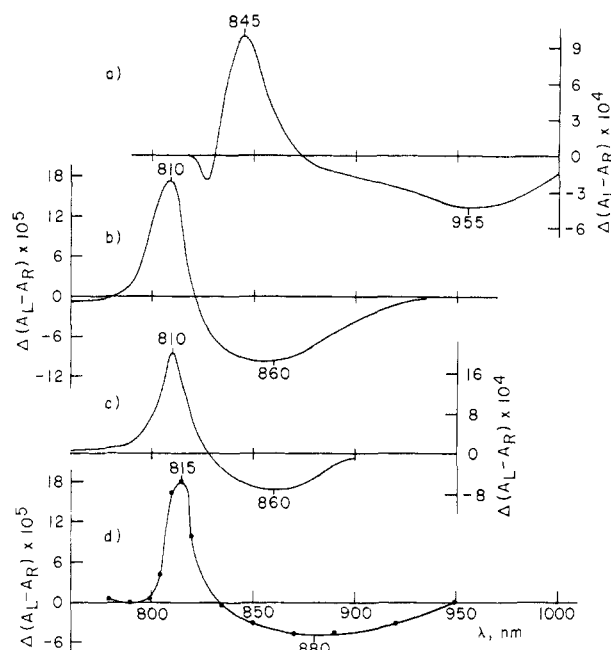
FIGURE 6: Oxidized-minus-reduced difference CD spectra. (a) *R. viridis* reaction centers. Calculated from data in Figure 3. (b) *R. rubrum* reaction centers. Calculated from data in Figure 1. (c) *R. spheroides* reaction centers. Calculated from data in Sauer *et al.* (1968), Figures 2 and 3, normalized to equivalent concentrations. (d) *Chromatium* fraction A. Light-minus-dark CD spectrum. Activation wavelength, 530 nm.

for the purified reaction centers of *R. viridis*, *R. rubrum*, and *R. spheroides* (Sauer *et al.*, 1968) (Figure 6a–c). Again the *Chromatium* reaction center appears to be quite similar to other purple bacteria reaction centers.

Discussion

The CD spectrum of a BChl protein is a sensitive measure of BChl–BChl interaction within the protein. A perturbation of the protein which affects the spatial arrangement of BChl molecules with respect to one another can result in large changes in the CD. Furthermore, if the interaction is strong enough, *i.e.*, an exciton interaction (Kasha *et al.*, 1965), as it appears to be in many photosynthetic materials (Philipson and Sauer, 1972; Schultz and Sauer, 1972; Philipson *et al.*, 1972), theory predicts a characteristic CD spectrum with multiple components of both positive and negative sign (Tinoco, 1963). For nondegenerate transitions the number of resolved components will always be less than or equal to the number of interacting molecules. Pure BChl in solution exhibits a small CD with a single positive component in the long-wavelength region.

Since CD is more sensitive than absorption spectroscopy to protein conformation and intermolecular interaction, a comparison of the CD spectra of reaction centers from different organisms can reveal similarities or differences between the BChl–protein complexes that may be undetectable in absorption experiments. We find that *R. spheroides* and *R. rubrum* reaction centers are spectrally extremely similar and may even have identical configurations.

The techniques of absorption and CD difference spectroscopy show that the reaction center in *Chromatium* fraction A also has much in common with the *R. spheroides* reaction center, the main difference being the shift of the long-wave-

length transition from 865 to 885 nm. The source of this is probably a slightly altered arrangement of reaction center BChl a molecules, possibly due to the presence of the antenna BChl a in this preparation.

R. viridis reaction centers exhibit unique spectral properties, especially at liquid nitrogen temperature where five resolved components are found in the reduced absorption spectrum. The five-component spectrum implies at least five reaction center pigment molecules (both BChl b and BPh b). From the shape of its absorption and CD spectra (Figure 3) and from its spectral behavior upon oxidation (Figures 3 and 6a) it is obvious that the reaction centers from the BChl b containing *R. viridis* have much in common with those from other purple bacteria. It is thus possible that more components are present, but unresolved, in the reaction centers from the other purple bacteria. The fact that two components at 929 and 987 nm (Figure 4a) are bleached in the oxidized states raises the possibility that *R. viridis* contains two different reaction centers. If this is correct, it remains to be determined whether both reaction centers reside simultaneously on the same protein or whether they are separate proteins which can be isolated from one another. Experiments must also determine whether both photoactive components, P930 and P990, play identical roles in the electron-transport pathways. Anomalous titration curves (Thornber and Olson, 1971) for *R. viridis* reaction centers have already pointed to the presence of reaction centers with different midpoint potentials.

BPh a, originally thought to be a contaminant (Clayton, 1966) in reaction center preparations, has been found in stoichiometric amounts in more recent preparations (Clayton *et al.*, 1972) and is now considered to be an intrinsic part of reaction centers. Yau (1971) has shown that BPh a in purified reaction centers of *R. spheroides* (R-26 mutant) is capable of sensitizing the photooxidation of reaction center BChl a. The 745 (−) (Figure 1) and 790 (−) nm (Figure 3) CD components of the *R. rubrum* and *R. viridis* reaction centers, attributable to BPh, undergo no significant changes upon oxidation, implying that the BPh molecules are not interacting strongly with the oxidizable BChl molecules. This conclusion is further supported by the spectra of the *R. rubrum* reaction centers in the Q_x region (Figure 2). The 535- and 600-nm absorption peaks are well separated and can be assigned with confidence to BPh and BChl, respectively. In the CD spectrum the component due to BChl a changes dramatically with oxidation, while the BPh a spectrum remains essentially unchanged. The sensitization of the reaction center photooxidation by radiation absorbed by BPh a (Yau, 1971) may result from excitation transfer similar to that originating in absorption by the antenna pigment BChl of unfractionated chromatophores. Such light-harvesting pigments do not appear to play a direct and necessary role in the reaction center photochemistry.

Although reaction centers from green photosynthetic bacteria (P840) and higher plants (P700) have not yet been isolated, the absorption and CD difference spectra of samples enriched in P840 and P700 have recently been studied (Olson *et al.*, 1972; Philipson *et al.*, 1972) in this laboratory. The photosystem I reaction center from spinach chloroplasts (Philipson *et al.*, 1972) appears to have at least two Chl a molecules coupled by exciton interaction. The light-minus-dark difference CD spectrum for the enriched P700 preparation has two components of opposite sign, similar to what is found for purple bacterial reaction centers (Figure 6). The signs of the long- and short-wavelength components for the chloroplast preparations are reversed relative to those from

bacteria, however. Although the spinach and purple bacterial reaction centers may resemble one another in having strongly interacting chromophores, the detailed arrangement of pigments within these different reaction centers is significantly different.

The reaction centers from green bacteria (Olson *et al.*, 1973) appear to be fundamentally different from those in purple bacteria or higher plants. Although the difference absorption spectrum for the green bacteria has two negative components, the difference CD spectrum shows only a single negative component. Thus, the CD studies of reaction centers from three major classes of photosynthetic organisms reveal significant differences among the different classes of reaction centers on this sensitive level of examination.

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