Spectral Properties of Reaction Center Preparations from *Rhodopseudomonas spheroides*<sup>*</sup>

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**SUMMARY**

The absorption, circular dichroism, and fluorescence spectra have been measured on oxidized and reduced reaction center preparations from *Rhodopseudomonas spheroides* strain R-26 at 295 and 77° K. The spectra indicate that the 4 bacteriochlorophyll a and 2 bacteriopheophytin a molecules in the reaction center are contained in a single pigment complex. The absorption bands at 893, 804, and 700 in the red and near-infrared each resolve into two separate CD maxima at 77° K. The interaction is very strong between the pigment molecules and their orientations are not coplanar. Oxidation of the reaction center bleaches the 893-nm absorption band of a bacteriochlorophyll dimer and the two corresponding CD maxima disappear. The changes in the low temperature fluorescence and CD spectra suggest that the molecular orientation may change when the reaction center is oxidized.

The reaction center of photosynthetic bacteria contains the specialized components which participate in the primary photochemical reaction. Light energy is utilized by this pigment complex to effect the transfer of an electron from bacteriochlorophyll to the primary electron acceptor, most likely a nonheme iron compound with an EPR signal at g value of 1.82 (3, 4). Studies of reaction centers using absorption and circular dichroism spectrophotometry have shown that the pigment molecules in the reaction center interact very strongly (5) and that reaction centers purified from several different purple photosynthetic bacteria have several similarities (6). The CD spectra of the reaction center preparations from strain R-26 of *Rhodopseudomonas spheroides* were interpreted with the previous bacteriochlorophyll analyses (7) as consistent with a pigment complex containing at least a trimer of bacteriochlorophyll (1, 2) to the primary electron acceptor, most likely a nonheme iron compound with an EPR signal at g value of 1.82 (3, 4). The CD spectra of the reaction center preparations from strain R-26 of *Rhodopseudomonas spheroides* were interpreted with the previous bacteriochlorophyll analyses (7) as consistent with a pigment complex containing at least a trimer of bacteriochlorophyll (5). However, more recent studies have indicated that reaction center preparations from this bacterium contain both bacteriochlorophyll a and bacteriopheophytin a in a molar ratio of 2:1 and most likely contain 2 bacteriopheophytin and 4 bacteriochlorophyll molecules (8, 9). In light of these pigment analyses, further studies of the spectral properties of the reaction center preparations seemed worthwhile.

In the studies of the molecular organization in the reaction center which we report here, the absorption and circular dichroism spectra of oxidized and reduced reaction center preparations from *R. spheroides* have been measured both at 295 and 77° K. Some of these results have been reported previously in preliminary form (10). The fluorescence emission spectra at 77° K have also been measured. These results are consistent with a single pigment complex in the reaction center containing 6 strongly interacting pigment molecules.

**EXPERIMENTAL PROCEDURES**

Materials—Ammonyx LO (lauryldimethyl amine-N-oxide) was a gift from Onyx Chemical Co., Jersey City, N. J. Triton X-100 (octylphenylpolyoxyethanol) was a gift from Rohm & Haas Co., Philadelphia, Pa. Glycerol and potassium ferricyanide of reagent grade were purchased from Matheson.

*R. spheroides* strain R-26 was grown on modified Hutner medium containing succinate as the carbon source, harvested at 4° in 0.01 M Tris-HCl buffer, pH 7.5, containing 0.1% Ammonyx LO.

Analytical Procedures—Absorption spectra were measured in a Cary model 14R recording spectrophotometer and circular dichroism spectra were measured in a sensitive circular dichroism spectrophotometer developed in this laboratory (14). Quartz cuvettes with 10-mm path length were used throughout for room temperature spectra. For 77° K spectra, the reaction center complex was isolated from chromatophores by Triton X-100 fractionation procedure described previously (11) and the reaction center protein subunit was isolated by treating the complex with Ammonyx LO using a procedure similar to those of Feher (12) and Clayton and Wang (13) which will be reported separately. The reaction center preparations were maintained at 4° in 0.01 M Tris-HCl buffer, pH 7.5, containing 0.1% Ammonyx LO.

The absorption and circular dichroism spectra of reaction center preparations measured at 295° K are shown in Fig. 1 and some
FIG. 1. Absorption and circular dichroism spectra of reduced (---) and oxidized (---) reaction center preparations from Rhodopseudomonas spheroides strain R-26 measured at 295°K in 0.01 M Tris-HCl buffer, pH 7.5, containing 0.1 M ammonyx LO. The sample was exposed to only the monochromatic measuring beam and oxidized by the addition of potassium ferricyanide crystals.

Some spectral parameters of reaction center preparations from Rhodopseudomonas spheroides strain R-26 are listed in Table I. The dark-adapted or reduced reaction center preparation exhibits three absorption bands at 865, 802, and 757 nm in the far-red region and two maxima at 597 and 535 nm. The wavelength maxima and band widths of the positive CD band at 860 nm and the absorption band at 865 nm are very similar. However, the presence of multiple pigment molecules which interact strongly and absorb maximally near 802 nm is evident by the presence of two CD bands having opposite signs and band widths which are much less than the 802-nm absorption band. The negative CD band at 811 nm and the positive CD band at 795 nm are centered at the position of the 802-nm absorption band. Smaller CD bands are also evident from the minimum at 748 nm and the maximum at 603 nm.

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The absorption and circular dichroism spectra of reaction centers measured at 295°K after the addition of excess potassium ferri-cyanide are shown by the dashed lines in Fig. 1 and the spectral changes which occur upon oxidation are shown by the reduced minus oxidized difference spectra in Fig. 2. When the reaction center is oxidized, the 865-nm absorption band bleaches completely and the magnitude of the 597-nm absorption band also decreases. The wavelength maxima of these changes are 862 and 600 nm in the difference spectrum. The corresponding changes in the circular dichroism spectrum are the disappearance of the positive CD bands at 860 and 603 nm. The absorption band at 802 nm shifts to 799 nm with no change in its intensity. The disappearance of the negative CD band at 811 nm and the increase in the remaining 797-nm CD band indicates that the oxidation of the reaction center produces a large change in the interaction of the pigments. The absorption band at 757 nm also shifts slightly toward shorter wave lengths.

The absorption and circular dichroism spectra of reaction centers measured at 77°K are shown in Figs. 3 and 1. At 77°K, the absorption maxima shift toward longer wave lengths and the absorption bands narrow and increase in intensity. These
FIG. 3. Absorption and circular dichroism spectra of reduced reaction center preparations measured at 77°K in pH 7.5 Tris buffer containing 50% glycerol. The absorbance is maximal in the sample exposed only to the monochromatic measuring beam.

FIG. 4. Absorption and circular dichroism spectra of oxidized reaction center preparations measured at 77°K in 50% glycerol. Adding potassium ferricyanide to saturation oxidized approximately 80% of the reaction center preparations as determined by the bleaching at 893 nm.

changes are larger for the bands at longer wave lengths. The 865-nm band shifts 28 nm to 893 nm at 77°K and is resolved into two positive CD bands with maxima at 888 and 877 nm. The double CD band centered near 800 nm remains with the negative band at 809 nm and the positive band at 796 nm. The inflection point near 763 nm at 295°K of a weak positive CD band (see Fig. 1) becomes more pronounced at 77°K, as does the negative CD band at 748 nm. These two bands appear to form a double band centered at 755 nm. At 77°K the absorbance near 535 nm is resolved into two distinct absorption maxima which occur at 533 and 545 nm.

Spectra of oxidized reaction centers measured at 77°K are shown in Fig. 4. Saturation of the glycerol solution with potassium ferricyanide oxidizes approximately 80% of the reaction center preparations as indicated by the absorbance decrease at 893 nm. Both of the positive CD bands at 888 and 877 nm as well as the negative 809-nm band are absent in the spectrum of the oxidized preparation. A change in the pigment interaction in the oxidized reaction center is indicated by the positive 800-nm CD band and the negative 593-nm CD band which are both much stronger than the corresponding bands in the spectra of the reduced preparations (Table I). The inflection at 774 nm of a positive CD band remains after oxidation. The negative CD band at 753 nm and the two positive CD bands at 540 and 528 nm are also much stronger than the corresponding bands in the reduced preparations.

The effect of urea on the pigment complex of the reaction center is shown by the absorption and CD spectra in Fig. 5. The spectra recorded at various times after the addition of this protein-denaturing agent show that the absorption bands shift toward shorter wave lengths coincident with the disappearance of the corresponding CD bands. The rates of the decrease in the 860-nm CD band and the 865-nm absorption band are identical as shown by the time course in the inset of Fig. 5. Disruption of the pigment complex is accompanied by the simultaneous disappearance of both the 803- and 865-nm absorption bands and the formation of a new species which absorbs maximally near 760 nm. An isosbestic point occurs at 783 nm. The loss of the CD bands of the strongly interacting pigments by treating the reaction centers with urea is not accompanied by the formation of any significant new CD bands which can be attributed to the product species.

Fluorescence emission spectra of reduced and oxidized reaction center preparations measured at 77°K are shown in Fig. 6. Fluorescence emission bands occur at 707, 775, 826, and 920 nm and the relative yields of these bands vary greatly depending on whether the preparations are excited at the 597-nm absorption band of the bacteriochlorophyll a molecule or the 535 nm band of the bacteriopheophytin a molecule. The 707-nm band in the oxidized and reduced preparations is most likely emission from a minor impurity of highly fluorescent 2-acetylchlorophyll
The photosynthetic reaction center from *R. spheroides* contains 4 bacteriochlorophyll *a* and 2 bacteriopheophytin *a* molecules (8, 9) within a very specialized environment. The importance of the protein structure to the organization of this pigment complex is evident from the effect of urea on the absorption and circular dichroism spectra of the reaction center (Fig. 5). The occurrence of an isosbestic point accompanying the shift of the absorption bands toward shorter wave lengths and the simultaneous disappearance of the strong circular dichroism band in the presence of the protein-denaturing agent indicate that the pigment molecules are present in a single pigment complex.

The red and near-infrared absorption bands of the reaction center can be assigned to either the bacteriochlorophyll *a* or the bacteriopheophytin *a* molecules from the low temperature fluorescence of the pigment complex. Since the molecules interact very strongly with each other as evidenced by the large dichroism (Table I), some of the properties of the reaction center are necessarily those of the individual molecules and some are the properties of the entire complex; and although the absorption bands of bacteriochlorophyll and bacteriopheophytin can be greatly affected by the molecular environment (16, 17), the close similarity between the yellow absorption bands in the reaction center preparations and in purified pigment preparations allows considerable confidence in assigning the 555-nm absorption band to the bacteriopheophytin *a* molecules in the reaction center (measured at 295°K) and the 597-nm absorption band to the bacteriochlorophyll *a* molecules (9). The 760-nm absorption band must be the QY band of the bacteriochlorophyll *a* molecules since it emits fluorescence having a high yield at 774 nm in reduced or 772 nm in oxidized preparations at 77°K (Fig. 6) and this fluorescence is excited more effectively at the 535-nm QX absorption band of the bacteriopheophytin molecules. These assignments as QY and QX bands of bacteriopheophytin are also consistent with the opposite signs of the negative CD bands at 753 nm and the positive bands at 540 and 528 nm in the oxidized reaction center preparations at 77°K (Fig. 4). The absorption bands at 504 and 583 nm must be the QY bands of the bacteriochlorophyll molecules in the reaction center because absorption at the 504-nm QX bands of bacteriochlorophyll *a* more effectively excites the 823-nm fluorescence in oxidized reaction center preparations and both the 826 and 920 fluorescence in the reduced preparations.

In the low temperature spectra the absorbance and CD maxima of the six pigments in the reaction center are resolved. The reduced reaction center preparations exhibit three red and near-infrared absorption bands both at 295 and 77°K but each of these three absorption bands resolve into two distinct maxima in the low temperature circular dichroism spectra. Two positive CD maxima at 888 and 877 nm occur near the 803-nm bacteriochlorophyll absorption band. Double CD bands having opposite signs are centered at the wave lengths of the 504-nm absorption band of the reaction center bacteriochlorophyll and at the 760-nm band of bacteriopheophytin. The presence of at least two components giving rise to the absorption band near 800 nm has been indicated by the second derivative of low temperature optical absorption spectra (12). The presence of 2 bacteriopheophytin molecules in slightly different environments is also evident from the presence of two absorption maxima at 533 and 545 nm in the absorption spectra measured at 77°K (Figs. 4 and 5).

The spectral properties of the reaction center preparations indicate that the molecules are not coplanar. The long wave length band in absorption spectra of bacteriochlorophyll and bacteriopheophytin arises from the QY transition dipole which is polarized within the plane of the tetrahydrroporphyrin ring (18, 19) and increased interaction between the pigment molecules shifts the wave length maximum toward longer wave lengths (20, 21). Coupling between the molecules of the pigment com-

**FIG. 6.** Spectra of fluorescence emission from reduced (---) and oxidized (---) reaction center preparations measured at 77°K in 0.01 M Tris buffer, pH 7.5. The excitation wave lengths were 595 nm at the yellow absorption band of the bacteriochlorophyll molecules in the *upper spectra* and 533 nm at the absorption band of the bacteriopheophytin molecules in the *lower spectra.*
plex produces the rotational strength of the reaction center and the strong coupling occurs between transition dipoles which do not have coplanar orientations. Strong coupling within the reaction center is particularly evident from the double circular dichroism bands having opposite signs which are centered near 802 nm (Figs. 1 and 3). The absorption band at longest wavelength and the two positive CD maxima are most likely those of 2 bacteriochlorophyll molecules in the reaction center which are located centrally within the pigment complex to interact with the largest number of molecules. The intermediate position of the absorption band near 802 nm and the pair of CD bands having opposite signs are consistent with the properties expected from a pair of bacteriochlorophyll molecules which are more peripherally located in the complex and which are more perpendicularly oriented. The CD bands near the 757- or 760-nm absorption band of the bacteriopheophytin molecules in the reaction center are smaller (Table I) consistent with a weaker coupling of these pigment molecules. However, when the reaction center is oxidized, the two long wavelength CD bands of bacteriochlorophyll are absent and the CD bands of the remaining unbleached molecules are larger (Table I). The changes in the absorption, fluorescence, and CD spectra which occur when the reaction center is oxidized indicate that the interaction between the molecules is considerably different in the oxidized reaction center and suggest that a change in the orientation of the pigment molecules may occur. Further details of the structure of this pigment complex in the photosynthetic reaction center must await further polarization measurements.

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