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# B850 pigment–protein complex of *Rhodopseudomonas sphaeroides*: Extinction coefficients, circular dichroism, and the reversible binding of bacteriochlorophyll

(photosynthetic bacteria/antenna complexes/pigment–protein interactions)

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ABSTRACT Chromatophores of *Rhodopseudomonas sphaeroides* yield the antenna complex B850 in either of two states, depending on the method of isolation. Methods using dodecyl (= lauryl) dimethylamine oxide yield B850 with an absorption spectrum like that *in vivo*: the bands at 800 and 850 nm, due to the bacteriochlorophyll (Bchl) components Bchl-800 and Bchl-850, are in ratio $A_{800}/A_{850} = 0.65 \pm 0.05$. When B850 is isolated by methods using dodecyl sulfate, the Bchl-800 is attenuated or absent. Bchl assays of these materials and of the isolated antenna complex B875 yielded the following extinction coefficients, ±SD, on the basis of the molarity of Bchl: For B875, $\varepsilon_{875} = 126 \pm 8\ mM^{-1}\ cm^{-1}$. For B850 in the normal (high-Bchl-800) state, $\varepsilon_{850} = 132 \pm 10\ mM^{-1}\ cm^{-1}$. For the individual components of Bchl in B850, $\varepsilon_{850}$ of Bchl-850 = $184 \pm 13\ mM^{-1}\ cm^{-1}$ and $\varepsilon_{800}$ of Bchl-800 = $213 \pm 28\ mM^{-1}\ cm^{-1}$. With these coefficients the molecular ratio of Bchl-850 to Bchl-800 equals 1.8 ± 0.4 for B850 in the high-Bchl-800 state. Starting with B850 depleted of Bchl-800, the addition of dodecyldimethylamine oxide restored the 800-nm absorption band. The 850-nm band became shifted toward the blue, narrowed, and slightly attenuated, and its associated circular dichroism became 20% more intense. Free Bchl added with dodecyldimethylamine oxide accelerated the restoration of Bchl-800 and retarded the attenuation of Bchl-850. We conclude that free Bchl can interact reversibly with a binding site for Bchl-800 in the B850 complex, with dodecyl sulfate favoring dissociation and dodecyldimethylamine oxide promoting association. Thus the reversible dissociation of a native chlorophyll–protein complex has now been demonstrated.

In photosynthetic bacteria the intracytoplasmic membranes contain "antenna" pigment–protein complexes that absorb light and consequently deliver excitation energy to photochemical reaction centers (1). One such antenna complex, the B850 complex of *Rhodopseudomonas sphaeroides* (and a similar complex from *Rhodopseudomonas capsulata*), has been purified and characterized extensively (2–8). The smallest complete unit of B850 probably consists of two polypeptides weighing about 8 and 10 kilodaltons, to which three molecules of bacteriochlorophyll *a* (Bchl) and one carotenoid molecule are bound noncovalently. The optical absorption spectrum in the near infrared shows maxima at 800 and 850 nm. The 850-nm band has been attributed to two Bchl molecules in strong excitonic interaction, and the 800-nm band has been attributed to the third molecule of Bchl, interacting only weakly with the other two. These conclusions are based primarily on assays of Bchl and protein and on spectra of linear and circular dichroism (CD) in optical absorption. The CD spectrum of isolated B850 shows a strong symmetric double (positive and negative) wave centered near the 850-nm absorption maximum, suggestive of dimeric exciton interaction. There is no perceptible CD in the 800-nm region.

Of the two components of Bchl in B850, which we shall designate Bchl-800 and Bchl-850, the former is relatively more labile. It has long been known that during the isolation and further manipulation of intracytoplasmic membrane fragments (chromatophores) the 800-nm absorption band becomes attenuated relative to the 850-nm band. Selective loss of the 800-nm band has also been reported for isolated B850 exposed to light while being frozen and thawed (4). When chromatophores from *R. capsulata* are exposed to Pronase there is a selective loss of Bchl-800, and concurrently one of the polypeptides (8 kilodaltons) is degraded and the carotenoid pigment is released (8). The Bchl-850 and its associated polypeptide (10 kilodaltons) are more resistant to modification by the action of Pronase.

B850 isolated by the method of Clayton and Clayton (2), using fractionating procedures in the presence of the detergents dodecyl (= lauryl) dimethylamine oxide ($DodMe_2NO$) and Triton X-100, shows a "normal" ratio of Bchl-800 to Bchl-850: the ratio of absorbances $A_{800}/A_{850}$ is 0.65 ± 0.05, as in freshly prepared extracts of whole cells. When B850 is isolated by electrophoresis of chromatophores dissociated with dodecyl sulfate ($DodSO_4$), the product is deficient in Bchl-800. The ratio $A_{800}/A_{850}$ is less than 0.4; further dialysis of this material against 0.1% lithium $DodSO_4$ ($LiDodSO_4$) can lead to complete loss of Bchl-800 with relatively little loss of Bchl-850. Thus one can prepare B850 with either a normal or a depleted complement of Bchl-800. By assaying these preparations for Bchl we have estimated separately the molar extinction coefficients of Bchl-800 and Bchl-850. From these data we have obtained molecular ratios of Bchl-800 and Bchl-850 in "normal" B850. Our results are compatible with the popularly accepted ratio of 1:2, but the ratio 2:3 falls within our limits of confidence.

Starting with B850 that is deficient in Bchl-800, we have been able to restore the 800-nm band to its normal (*in vivo*) level by incubating the material with $DodMe_2NO$ plus free Bchl. We assume that the B850 complex has a specific binding site for the Bchl-800; $DodSO_4$ removes Bchl from this site and $DodMe_2NO$ allows the Bchl to become bound again. We can foresee a variety of experiments, involving the binding of Bchl analogs, to test the nature of this binding between Bchl and protein.

## MATERIALS AND METHODS

Cells of *R. sphaeroides* (strain 2.4.1) were grown and chromatophores were prepared as described (2). The B850 complex was isolated by exposing chromatophores (absorbance 50 at 850 nm, in 0.01 M Tris·HCl at pH 7.5) to 1% $DodMe_2NO$ and then using

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Abbreviations: Bchl, bacteriochlorophyll *a*; $DodMe_2NO$, dodecyl (= lauryl) dimethylamine oxide; $DodSO_4$, dodecyl sulfate; CD, circular dichroism.

the fractionating protocol of Clayton and Clayton (2) or else subjecting the material to chromatography through hydroxylapatite (9). In these preparations of B850 we found $A_{800}/A_{850} = 0.65 \pm 0.05$.

Alternatively, the chromatophores were exposed to Tris $DodSO_4$ or $LiDodSO_4$ and B850 was isolated by electrophoresis through polyacrylamide at 4°C as described by Broglie *et al.* (10). This method gave B850 with relatively less Bchl-800; $A_{800}/A_{850} < 0.4$. The 800-nm component could be attenuated further by exposing the isolated B850 to 0.1% $LiDodSO_4$. The method of Broglie *et al.* also yielded the B875 antenna complex in a separate fraction. All fractions were suspended in aqueous 0.01 M Tris·HCl, pH 7.5, with detergents as specified.

Tris $DodSO_4$ was prepared by passing $NaDodSO_4$ through a column of Dowex AG 50W-12X cation-exchange resin, eluting with 0.2 M Tris·HCl, pH 7.5.

The Bchl content of these preparations was assayed by extracting the material with acetone/methanol (7:2, vol/vol) and using $\varepsilon_{770} = 76\ mM^{-1}\ cm^{-1}$ for Bchl in this solvent (11). A correction of 7% was applied for losses, as estimated through "dummy" extractions using Bchl dispersed in aqueous detergent solutions as the source material.

Optical absorbance and CD were measured with a homemade single-beam spectrometer using a photoelastic modulator (Hinds International, Portland, OR) to produce alternating (50-kHz) right and left circular polarization in the measuring beam. For absorbance, a voltage proportional to the logarithm of the dc photocurrent was fed to a signal averager (Tracor Northern model TN-1500, Middletown, WI). Wavelength scans of this voltage were stored and appropriate baseline subtractions were made. For CD the 50-kHz component of the photocurrent was isolated with a tuned amplifier (Princeton Applied Research model 124A, Princeton, NJ) and the corresponding voltage, $\Delta V(L - R)$, in which L and R indicate left and right, was divided by a voltage $V$ proportional to the dc photocurrent. With $\Delta V \ll V$, the quantity $\Delta V/V$ is proportional to $\Delta A$ or $A_L - A_R$. This quantity was stored in wavelength scans. The magnitude of the CD signal was calibrated by comparison with published spectra of B850 at known concentrations (4).

## RESULTS

**Extinction Coefficients of the B850 and B875 Complexes.** Fig. 1 shows absorption spectra of purified *R. sphaeroides* chromatophores (solid curve) and of B875 isolated from these chromatophores (long dashes). The second of these spectra was subtracted from the first in such proportion that the contribution of B875 in the chromatophores was deleted. The resulting difference (short dashes) shows the spectrum of B850 in the chromatophores. In other experiments the spectrum of the chromatophores was decomposed by subtracting the spectrum of purified B850 [prepared by the method of Clayton and Clayton (2)], leaving the contribution of B875.

We extracted a known amount of B875 with acetone/methanol to determine its Bchl content and thereby compute its extinction coefficient, which proved to be $\varepsilon_{875} = 126 \pm 8\ mM^{-1}\ cm^{-1}$. We then determined the Bchl content of a known amount of chromatophores. Subtracting the Bchl attributable to B875 (referring to Fig. 1), we could estimate the Bchl content of the B850 component. This information yielded an extinction coefficient for B850. In other experiments the roles of B850 and B875 were reversed in this protocol, so that the extinction coefficient $\varepsilon_{850}$ of B850 was determined directly and the $\varepsilon_{875}$ of B875 was found indirectly. We made 16 such determinations (in both ways), using four different preparations of chromatophores, six of purified B850 and two of B875. The

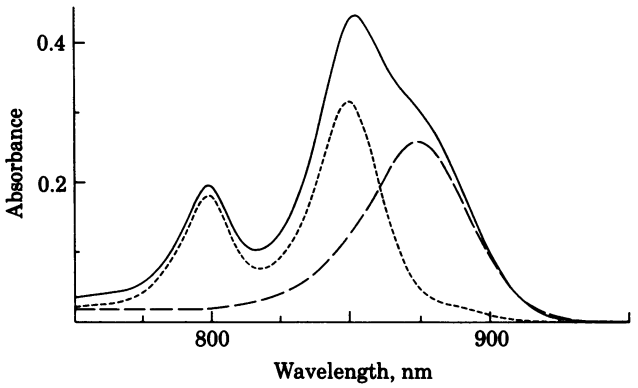


FIG. 1. Solid curve, absorption spectrum of purified chromatophores from *R. sphaeroides*, strain 2.4.1. Long dashes, the B875 antenna pigment–protein complex isolated from these chromatophores by the method of Broglie *et al.* (10). Short dashes, the second of these spectra was subtracted from the first in the right proportion to delete the contribution of B875 in the chromatophores. The result shows the residual spectrum of B850 in the chromatophores. Materials were suspended in 0.01 M Tris·HCl, pH 7.5.

mean (±SD) extinction coefficients are

$$\varepsilon_{875} \text{ of B875} = 126 \pm 8\ mM^{-1}\ cm^{-1} \quad [1]$$

$$\varepsilon_{850} \text{ of B850} = 132 \pm 10\ mM^{-1}\ cm^{-1}. \quad [2]$$

**Removal and Replacement of the 800-nm Component of B850.** Fig. 2 (solid curve) shows the absorption spectrum of B850 prepared by the method of Broglie *et al.* (10) and dialyzed overnight against 0.01 M Tris·HCl, pH 7.5. The small amount of Bchl-800 remaining in this material could be removed entirely (not shown) by dialysis against the same buffer with 0.1% $LiDodSO_4$, overnight at 4°C.

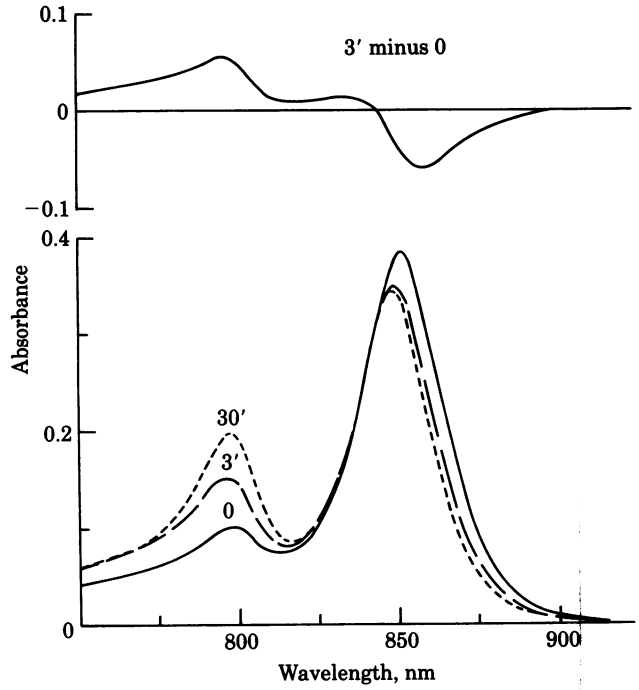


FIG. 2. Solid curve, absorption spectrum of B850 prepared from *R. sphaeroides* by the method of Broglie *et al.* (10) and suspended in 0.01 M Tris·HCl, pH 7.5. Long dashes, the same after 3-min exposure to 0.1% $DodMe_2NO$. Short dashes, after 30-min exposure to 0.1% $DodMe_2NO$. The upper curve shows the difference induced by 3-min exposure to $DodMe_2NO$.

When B850 deficient in Bchl-800 was exposed to 0.1% $DodMe_2NO$ the 800-nm band grew as shown by the dashed curves in Fig. 2. Concomitantly the 850-nm band was shifted toward the blue and also became slightly depressed and narrower. The transitory presence of a little free Bchl (shown by a broad band centered at 770 nm) could sometimes be seen in experiments of this kind.

We speculated that the B850 complex has a specific site for Bchl-800, that dodecyl sulfate removes Bchl from this site, and that free Bchl can be bound to this site when the dodecyl sulfate is replaced by $DodMe_2NO$. The free Bchl is made available because $DodMe_2NO$ dislodges some of the Bchl-850. To test this interpretation we repeated the experiment of Fig. 2, with the same material but with free Bchl (2 $\mu$M) added along with the $DodMe_2NO$. The resulting spectra, with the contribution of free Bchl deleted by subtraction, are shown in Fig. 3. The regrowth of the 800 nm band was accelerated markedly by the presence of added free Bchl (compare Figs. 2 and 3), and the loss of Bchl from the 850-nm band was retarded slightly.

The state of B850 could be cycled back and forth, with Bchl-800 deleted and restored, by exposing the material alternately to $LiDodSO_4$ and $DodMe_2NO$. The results of one such experiment are summarized in Table 1. The B850 had been prepared by the method of Clayton and Clayton (2) and stored in the presence of 0.5% Triton X-100. This material was dialyzed against 0.01 M Tris·HCl, pH 7.5, for 48 hr prior to the start of the experiment. The table shows how Bchl-800 was lost, regained, and lost again during exposures to $LiDodSO_4$, $DodMe_2NO$, and finally $LiDodSO_4$ superimposed on $DodMe_2NO$. The absorbance ratio $(A_{800} - A_{817})/A_{850}$ indicates roughly the relative amount of Bchl-800, because the absorption spectra show a trough at 817 nm between the 800- and 850-nm peaks. The absorption peak near 850 nm declined progressively during the 4 days of this experiment and free Bchl tended to accumulate. Prior to each measurement we bleached the 770-nm absorbance of this free Bchl by exposing the sample to strong white light for 3 min. The association of $DodMe_2NO$ with $LiDodSO_4$ sometimes produced a silky white precipitate that could be removed by centrifugation.

Table 1. Cycling of B850

| Sequential operations | $\frac{A_{800} - A_{817}}{A_{850}}$ | $A_{850}$, 1-cm path | $\lambda_{max}$ | Bandwidth at half max, $cm^{-1}$ |
|---|---|---|---|---|
| Start | 0.49 | 11.7 | 848.5 | 359 |
| Exposed to 0.1% $LiDodSO_4$ 24 hr, then dialyzed 24 hr against buffer | 0.095 | 8.4 | 850 | 393 |
| Exposed to 0.1% $DodMe_2NO$ for 24 hr | 0.35 | 5.1 | 847.5 | 372 |
| Exposed 24 hr to 0.3% $LiDodSO_4$ superimposed on the 0.1% $DodMe_2NO$ | 0.04 | 3.1 | 851 | 400 |
| Average of 12 preparations with low Bchl-800 | | | 850 | 401 |
| Average of 12 preparations with high Bchl-800 | | | 847.5 | 368 |

B850 isolated from *R. sphaeroides* was exposed alternately to $LiDodSO_4$ and $DodMe_2NO$ in order to delete and restore the absorption band due to Bchl-800. Characteristics taken from absorption spectra are listed. The samples were diluted 1:10 with 0.01 M Tris·HCl, pH 7.5, for absorbance measurements.

Table 1 shows that when Bchl-800 is present the band near 850 nm is relatively narrower and of shorter wavelength, and vice versa when the Bchl-800 is deleted. Average values of the peak wavelength and width of the 850 nm band are shown at the bottom of the Table.

We treated purified chromatophores with $DodMe_2NO$, with the result shown in Fig. 4 for exposure to 0.1% $DodMe_2NO$ for 1 hr followed by 3-min illumination to bleach the 770-nm band of free Bchl. The absorbance of B875 was lost, as expected from previous observations. The 800-nm peak increased, indicating

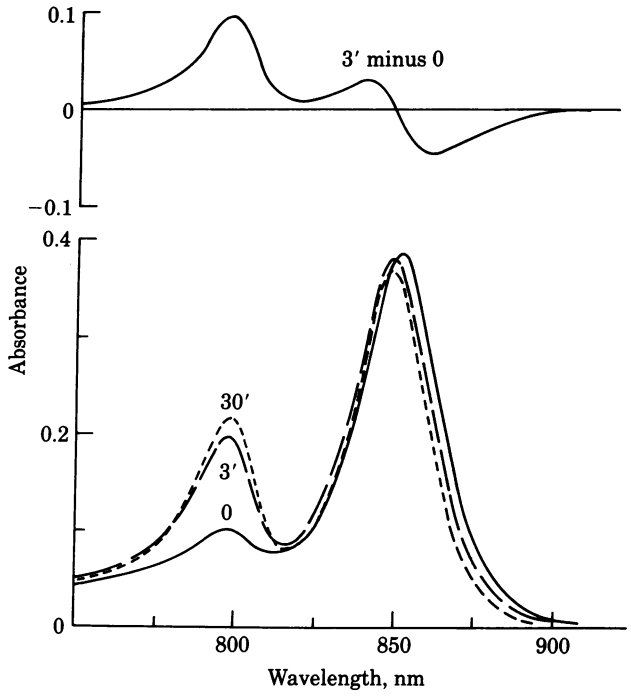


FIG. 3. Conditions as in Fig. 2, except that 2 $\mu$M Bchl was added to the B850 along with the $DodMe_2NO$. The spectral contribution of free Bchl, a broad band near 770 nm, was deleted by subtraction of stored spectra.

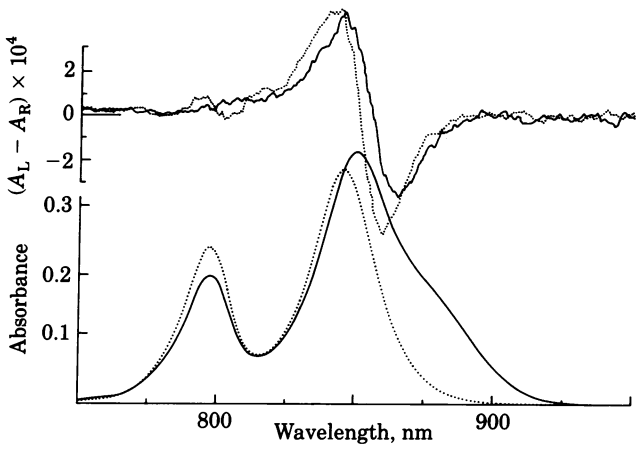


FIG. 4. Absorption and CD spectra of purified chromatophores from *R. sphaeroides*. Solid curves, chromatophores in 0.01 M Tris·HCl, pH 7.5. Dotted curves, the same after 1-hr exposure to 0.1% $DodMe_2NO$ plus 3-min strong white illumination just before measurement.

that abour 20% of the binding sites for Bchl-800 had been vacant and could be occupied by Bchl in the presence of $DodMe_2NO$. Intact cells, suspended in 30% albumin for optical clarity, showed a 5% increase of the 800-nm band when treated for an hour with 0.3% $DodMe_2NO$.

**Optical Extinction Coefficients of Bchl-800 and Bchl-850.** Having access to B850 with high or low Bchl-800 content, we undertook to find the individual molar extinction coefficients of Bchl-800 and Bchl-850 in the complex, as distinguished from the coefficient $\varepsilon_{850}$ given earlier (Eq. 2) for the complex as a whole. Starting with a concentrated suspension of B850 in 0.01 M Tris·HCl, pH 7.5, we diluted one aliquot 1:10 with Tris buffer and extracted another aliquot by a 1:10 dilution into acetone/methanol. Comparing absorption spectra,

$$A_{770}/\varepsilon_{770} = A_{800}/\varepsilon_{800} + A_{850}/\varepsilon_{850}, \quad [3]$$

in which the left side refers to the acetone/methanol extract, with $\varepsilon_{770} = 76\ mM^{-1}\ cm^{-1}$ (11) and the right side refers to the comparable concentration of undenatured B850. The extinction coefficients on the right are for the individual components Bchl-800 and Bchl-850. The absorbance $A_{800}$ in Eq. 3 has been corrected for the "tail" of the 850-nm band, estimated to be 4% of the peak. This procedure can be applied to two samples of B850, one with high and one with low Bchl-800, and the two examples of Eq. 3 can then be solved simultaneously to yield $\varepsilon_{800}$ of Bchl-800 and $\varepsilon_{850}$ of Bchl-850. To do this correctly, however, we must modify Eq. 3 to take account of the width of the 850-nm band, which depends on the state of the complex as shown in Table 1. We assume that the concentration of Bchl-850 is proportional to the area of the 850-nm band, or approximately to the product of peak absorbance and bandwidth at half-maximum. An equivalent statement is that the extinction coefficient $\varepsilon_{850}$ has one value in material with high Bchl-800 and a 9% lesser value (in proportion to the 9% broader band; see Table 1) in material with little or no Bchl-800. Reserving the symbol $\varepsilon_{850}$ for the case of high Bchl-800, we can write $\varepsilon_{850}/1.09$ in place of $\varepsilon_{850}$ when applying Eq. 3 to material with low Bchl-800.

We applied the foregoing procedure to two samples of B850 with low Bchl-800 and five with high Bchl-800, solving 10 pairs of equations and finding

$$\varepsilon_{800} \text{ of Bchl-800} = 207 \pm 24\ mM^{-1}\ cm^{-1} \quad [4]$$

$$\varepsilon_{850} \text{ of Bchl-850} = 185 \pm 12\ mM^{-1}\ cm^{-1}. \quad [5]$$

Failure to take account of the changing bandwidth of Bchl-850 led to very high and erratic values of $\varepsilon_{800}$, ranging from 250 to nearly 500 $mM^{-1}\ cm^{-1}$.

Another way to estimate these extinction coefficients is to assume that the intrinsic intensity of absorption is the same for each component. This quantity is proportional to $\int(\varepsilon/k)dk$ (12), approximated by $\varepsilon_m \Delta k/k$, in which $\varepsilon_m$ is the extinction coefficient at the maximum of the band, $k$ is the wave number ($cm^{-1}$) at the maximum, and $\Delta k$ is the bandwidth at half maximum. Then

$$\varepsilon_{850}\Delta k/k \text{ (Bchl-850)} = \varepsilon_{800}\Delta k/k \text{ (Bchl-800)} = \varepsilon_{875}\Delta k/k \text{ (Bchl of B875)} \quad [6]$$

Taking $\varepsilon_{875} = 126 \pm 8\ mM^{-1}\ cm^{-1}$ for B875 (Eq. 1), and measuring the widths of the various absorption bands, application of Eq. 6 gave

$$\varepsilon_{800} \text{ of Bchl-800} = 219 \pm 15\ mM^{-1}\ cm^{-1} \quad [7]$$

$$\varepsilon_{850} \text{ of Bchl-850} = 183 \pm 12\ mM^{-1}\ cm^{-1}, \quad [8]$$

in good agreement with the values expressed in Eqs. 4 and 5. If we assign equal weight to the values found by each method we obtain average values

$$\varepsilon_{800} \text{ of Bchl-800} = 213 \pm 28\ mM^{-1}\ cm^{-1} \quad [9]$$

$$\varepsilon_{850} \text{ of Bchl-850} = 184 \pm 13\ mM^{-1}\ cm^{-1}. \quad [10]$$

The ratio of absorbances $A_{850}/A_{800}$ ranged between 1.4 and 1.7 for different samples of B850 in the "high Bchl-800" state and in the spectra of cells and chromatophores from which the contribution of B875 had been subtracted. The molecular ratio of bchl-850 to bchl-800 in these preparations is given by

$$[\text{Bchl-850}]/[\text{Bchl-800}] = (A_{850}/A_{800})(\varepsilon_{800}/\varepsilon_{850}) \quad [11]$$

and with $\varepsilon_{800}$ and $\varepsilon_{850}$ taken from Eqs. 9 and 10 we find

$$[\text{Bchl-850}]/[\text{Bchl-800}] = 1.8 \pm 0.4. \quad [12]$$

This is consistent with the accepted value of 2 and is not incompatible with a ratio of 3/2.

Consider a sample of B850 with Bchl-850 at 1 mM concentration, so that from Eq. 10 its absorbance for a 1-cm path is 184 at the 850-nm peak. If there is one molecule of Bchl-800 for every two of Bchl-850 in this sample, the total Bchl concentration is 1.5 mM. Then the extinction coefficient at 850 nm for the complex as a whole is

$$\varepsilon_{850} \text{ of B850} = (184 \pm 13)/1.5 = 123 \pm 12\ mM^{-1}\ cm^{-1} \quad [13]$$

on the basis of the molarity of Bchl. This can be compared with the value 132 ± 10 reported in Eq. 2.

**CD.** In the foregoing experiments we routinely measured CD spectra as well as absorption spectra. Fig. 4 (upper curves) shows the conspicuous positive and negative branches, symmetric about a zero crossing near 850 nm, associated with the Bchl-850 of B850. This CD has been described in several publications from K. Sauer's laboratory, especially by Sauer and Austin (4). A persistent feature of these CD spectra is that the zero crossing is located about 3 nm to the long-wave side of the 850 nm absorption peak. Note that $DodMe_2NO$ causes a blue shift of the CD bands along with the 850-nm absorption band.

CD spectra similar to those in Fig. 4 were obtained with purified B850. Starting with B850 depleted of Bchl-800, the addition of $DodMe_2NO$ to restore Bchl-800 caused the main absorption peak to shift from about 851 to 847 nm, and concomitantly the zero crossing in the CD spectrum shifted from about 854 to 850 nm. At the same time the CD of Bchl-850 became about 20% more intense.

The addition of $DodMe_2NO$ to chromatophores or to B850 also gave rise to a small feature around 800 nm in the CD spectrum, discernible in Fig. 4: a double wave similar to the much larger one around 850 nm. The magnitude of this effect seemed to be correlated with the amount of Bchl-850 already present and not with the increase of this component induced by $DodMe_2NO$.

## DISCUSSION

The B850 antenna complex of *R. sphaeroides* in the living cell has a "normal" complement of Bchl-800: probably one molecule for every two of Bchl-850. When isolated by a method using $DodSO_4$ the B850 has little or no Bchl-800. Exposure to $DodMe_2NO$ then brings about several changes: The 800-nm absorption band of Bchl-800 is restored. The 850-nm band becomes narrower, somewhat attenuated, and blue-shifted to 847 nm, while the associated CD becomes about 20% more intense. Addition of free Bchl facilitates the restoration of Bchl-800 and inhibits the loss of Bchl-850 during this conversion. It is therefore unlikely that a component of Bchl-850 is converted *in situ*

to Bchl-800. Rather, the loss and recovery of the 800-nm band reflects the interaction of free Bchl with a binding site for Bchl-800.

The changes of Bchl-850 are more complex, and at least two interpretations can be entertained. One possibility is that the 850-nm band has two components: a relatively stable major one and a more labile longer-wave one. The latter is lost when $DodMe_2NO$ is present and can be restored by the binding of free Bchl when $DodMe_2NO$ is replaced by $DodSO_4$. This model fits nicely with the spectra shown in Figs. 2 and 3, but it is difficult to reconcile with the accepted stoichiometry of just two molecules of Bchl-850 (a dimer) per minimal unit of B850.

Alternatively, we can assert that all of the observed changes in the 850-nm band result from the action of $DodMe_2NO$ on just one kind of dimeric Bchl-850. These changes include a narrowing and blue shift of the absorption band and an intensification of the CD, perhaps due to a slight change in configuration of the dimer. We assume that independent of these effects there is some nonselective conversion of Bchl-850 to free Bchl. This attrition of Bchl-850 is ameliorated by externally added Bchl. The difference spectra of Figs. 2 and 3 are at least superficially compatible with this model.

We are not yet able to choose between these interpretations, although the latter fits more readily into our present view of the composition of B850. A choice should become possible through a more careful analysis of the difference spectra induced by $DodMe_2NO$, under conditions that allow more striking separation of the major effects: attrition of Bchl-850 and shifting and narrowing of the surviving 850-nm band.

The 850-nm absorption band of B850, and the corresponding symmetric CD spectrum, have been interpreted in terms of a Bchl dimer for which the exciton components are nearly degenerate and hence merge into one unresolved absorption band. If the two exciton components are of equal strength the absorption band should be symmetrical and the zero crossing in the CD spectrum should coincide with the peak of absorption. In fact, the zero crossing of CD is about 3 nm to the long-wave side of the absorption maximum; this can be seen in published spectra of B850 and of other antenna complexes such as the B860 of carotenoidless mutant *R. sphaeroides*. This probably means that the exciton components are unequal in absorption, as analyzed by Bolt and Sauer (6, 7). However, it is conceivable that the greater part of the CD comes from a minor component with absorption centered 3 nm to the long-wave side of the major 850-nm peak. Perhaps the major features in the CD spectra of photosynthetic tissues do not always correspond to their seemingly obvious counterparts in absorption spectra.

We have demonstrated the reversible binding of a chlorophyll to a site (Bchl-800) in a photosynthetic chlorophyll-protein complex. Also we have tenuous evidence, in the protection against attrition afforded by free Bchl, for the reversible binding of Bchl-850. These results suggest a variety of experiments to explore the nature of the binding: competitive bindings of analogs such as bacteriochlorophyll *b*, chlorophylls *a* and *b*, pheophytins, chlorophyllides, and heme. This could be manifested by the ability of such analogs to block the binding of Bchl *a*.

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