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# EVIDENCE FOR MONOMERIC BACTERIOCHLOROPHYLL IN $P_{800}$ OF THE PHOTOREACTION CENTER FROM *RHODOSPIRILLUM RUBRUM*

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SUMMARY

To find out whether weak or strong coupling exists between the bacteriochlorophyll molecules of the photoreaction center, the relative efficiency of energy transfer to $P_{870}$ was measured at 795 nm and at 808 nm, at room temperature and at 77 °K. At room temperature, both relative efficiencies are close to 100 %. However, at 77 °K, 795 nm light has a quantum efficiency of 76 % and 808 nm light has an efficiency of 87 %. These results confirm the fact that $P_{800}$ is formed of at least one short wavelength component and one long wavelength component. Moreover, the short wavelength component is weakly coupled to both $P_{870}$ and to the long wavelength component of $P_{800}$. The conclusion is that the short wavelength component is due to monomeric bacteriochlorophyll. By comparison with other data, all four bacteriochlorophyll molecules of the photoreaction center are inferred to be monomeric.

## INTRODUCTION

Recent experiments have shown that the photoreaction center isolated from wild type *Rhodospirillum rubrum* contains four molecules of bacteriochlorophyll, two molecules of bacteriopheophytin and one molecule of spirilloxanthin [1]. Its porphyrin composition is identical to that of similar preparations obtained from *Rhodopseudomonas sphaeroides* [2, 3]. Interaction between these pigments has been studied by different methods. Strong singlet interaction between three or more bacteriochlorophyll molecules in the reduced state of the primary electron donor has been proposed [4] as an interpretation for the characteristic changes in the circular dichroism (CD) spectra of similar preparations upon oxido-reduction [4–6]. EPR and ENDOR spectroscopy indicate that the free electron of the oxidized primary electron donor is delocalized over two bacteriochlorophyll molecules [7–10]. EPR spectroscopy shows that the excited triplet state is also delocalized over two bacteriochlorophyll molecules [11–13] indicating strong triplet interaction. However, there is also some evidence that the singlet dipoles of the bacteriochlorophyll molecules are not so strongly coupled as to be delocalized. For example the quantum efficiency at 800 nm

for the oxidation of $P_{870}$ is less than unity at room temperature [14–16] and there is evidence that $P_{800}$ emits fluorescence at 77 °K [5]. Moreover, recent photodichroism experiments [17] indicate that $P_{870}$ is formed of two weakly coupled oscillators with irreversible energy transfer.

The present work is an attempt to check whether the coupling is weak or strong between the absorption dipoles of $P_{800}$ and of $P_{870}$. Our test was based on the theoretical work of Förster [18] which predicts that, only in the first case, should energy transfer efficiency depend upon the energy overlap of the emission and absorption bands of the donor and acceptor chromophores. We took advantage of the fact that the energy overlap between $P_{800}$ and $P_{870}$ can be modified by lowering the temperature: between 298 °K and 77 °K, a 25 nm bathochromic shift is observed for $P_{870}$ with only minor changes in the position of $P_{800}$ [5, 19]. One would predict, therefore, that the efficiency of energy transfer would decrease at cryogenic temperatures in only one case: that of weak coupling.

## MATERIALS AND METHODS

Photoreaction center was isolated from wild type *Rhodospirillum rubrum* (ATCC no 11170) by a modification of the method of Noël et al. [20]. The preparations were stored at 4 °C in 25 mM Tris · Cl (pH 8.0) buffer containing 0.03 % dodecyldimethylamine *N*-oxide.

The relative quantum efficiencies of bacteriochlorophyll photooxidation were found by measuring the relative differences in the number of photons absorbed by $P_{800}$ and by $P_{870}$ which cause identical steady state changes in the $P_{870}$ absorbance. Absorbance change was measured with a CARY 14R spectrophotometer modified so as to permit illumination of the sample at 90° to the measuring light beam. Cross illumination was provided by a 650 W tungsten halogen lamp. The intensity of the actinic lamp was controlled by varying the voltage of the power supply (Kepco Model JQE 75-8(M)). The relative energy flux of the light was measured by a Yellow Springs Instrument Co. Radiometer. The actinic wavelength was selected by narrow band Baird Atomic Interference filters placed between the light source and the sample. Another interference filter with maximum transmittance at either 900 nm or 870 nm was placed in front of the phototube in the sample compartment to cut off any scattered light from the exciting beam.

All measurements were performed with the sample in an Air Products Corporation Joule Thomson cryostat. The sample cuvette was 3-mm square in section. The vacuum shroud of the cryostat was fitted with flat windows on three sides so as to permit illumination of the sample with actinic light while measuring its absorbance. For low temperature measurements, the sample was dispersed in 25 mM Tris · Cl (pH 8.0)/0.05 % dodecyldimethyl-amine *N*-oxide containing 50 % glycerol. The sample was kept in the dark for more than 30 minutes before it was cooled, to insure complete reduction of the primary donor. The samples were frozen to a clear glass at 77 °K by nitrogen gas. For room temperature measurements, the samples were dispersed in the same medium but without glycerol. In all cases, the samples were adjusted to $A \leqslant 0.3$ at the wavelength of the actinic beam to prevent too large an error due to non-uniform distribution of actinic intensity through the zone of measurement [16].

RESULTS

Two separate sets of experiments are presented, one at 298 °K and the other one at 77 °K. Several trustworthy data for the energy transfer efficiency from $P_{800}$ to $P_{870}$ at room temperature have been reported previously [14–16] for *Rhodopseudomonas sphaeroides*. However, since our aim was to compare the efficiencies at two temperatures, we felt that both experiments should be performed under identical experimental conditions. Four wavelengths were used for the actinic illumination, namely 795 nm, 808 nm, 870 nm and 900 nm. The latter two were used for comparison purposes. Absorbance changes were followed at 870 nm or/and at 900 nm, according to the case. All measurements were performed under steady state conditions imposed by a long illumination.

*Energy transfer efficiency at 298 °K*

Under steady state conditions, the rate of the photooxidation of $P_{870}$ is equal to the rate of its reduction by the back reaction:

$$\phi EN = R \tag{1}$$

where $\phi$ is the quantum yield of the photochemical reaction, E is the efficiency of energy transfer to $P_{870}$ from the dipole which absorbs the light, $N$ is the photon flux absorbed and R is the rate of reduction of $P^+{}_{870}$. $R$ is a function of the concentration of $P^+{}_{870}$ and, therefore, of $(\Delta A/\Delta A_{max})$ where $\Delta A_{max}$ is the maximal photochemical absorbance change at a given wavelength. If the intensity of illumination at two wavelengths (1 and 2) can be adjusted so as to produce an equal $\Delta A/\Delta A_{max}$, then the rate of the back reaction, $R$, is the same for both wavelengths. The relative efficiency of energy transfer $(E_1/E_2)$ can then be easily calculated from equation 1 which becomes:

$$E_1N_1/E_2N_2 = 1 \tag{2}$$

Experimentally, however, this situation can only be approximated. Fortunately, this is not a serious handicap. If $(\Delta A/\Delta A_{max})_1$ is small and close to $(\Delta A/\Delta A_{max})_2$ the following relationship can be shown to hold, since $R$ is predominantly a first order reaction,

$$\frac{R_1}{R_2} = \frac{(\Delta A/\Delta A_{max})_1}{(\Delta A/\Delta A_{max})_2} \tag{3}$$

and from equation 1,

$$\frac{E_1N_1}{E_2N_2} = \frac{(\Delta A/\Delta A_{max})_1}{(\Delta A/\Delta A_{max})_2} \tag{4}$$

Since we are interested only in relative efficiencies of energy transfer, we do not need to know the absolute photon flux, $N$, absorbed at a given wavelength. We will use instead the quantity $N°$ which is proportional to $N$. $N°$ is calculated from equation 12 in the Appendix, using the absorbance curves for the reduced photoreaction center and the transmittance curves of the optical filters, as shown on Fig. 1.

The results obtained at room temperature are reported in Table I. The relative

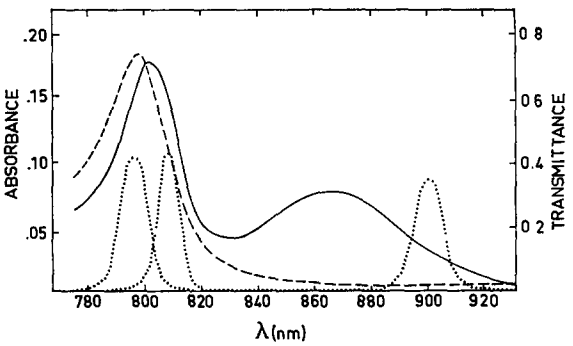


Fig. 1. Absorbance spectra at room temperature of the reduced (—) and of the oxidized (– – –) photoreaction center. The transmittance spectra of the interference filters is indicated by (. . . .).

TABLE I

THE RELATIVE QUANTUM EFFICIENCY, $E$, FOR THE PHOTOOXIDATION OF $P_{870}$, AT ROOM TEMPERATURE, IN THE PHOTOREACTION CENTER FROM *RHODOSPIRILLUM RUBRUM*

| Actinic wavelength $\lambda$ | $\Delta A/\Delta A_{max}$ | $(N^\circ \times 10^{-11})$* | $E$** |
|---|---|---|---|
| 900 nm | 0.204 | 2.14 | 100 % |
| | 0.210 | 2.18 | |
| | 0.226 | 2.31 | |
| | 0.199 | 2.11 | |
| 808 nm | 0.199 | 2.215 | 99.5±6.0 % |
| | 0.196 | 1.94 | |
| 795 nm | 0.219 | 2.300 | 96.8±5.4 % |
| | 0.211 | 2.456 | |
| | 0.225 | 2.305 | |

* $N^\circ$ is expressed in photons $cm^{-2} \cdot s^{-1}$, see equation 12.

** Calculated as $E = \dfrac{\overline{(\Delta A/\Delta A_{max} \cdot 1/N^\circ)_\lambda}}{\overline{(\Delta A/\Delta A_{max} \cdot 1/N^\circ)_{900}}}$

efficiencies of energy transfer from 808 nm and 795 nm to $P_{870}$ were calculated from equation 4. The values of 99.5±6.0 % and 96.8±5.4 % are to be compared with an efficiency of 93 % for the transfer from 800 nm which has been reported for another preparation [14, 16].

*Energy transfer efficiency at 77 °K*

Unlike the complex kinetics found at room temperature, the back reaction at 77 °K is a simple first order reaction [10] such that, under steady state conditions,

$$R = k_b[P^+{}_{870}] \tag{5}$$

were $k_b$ is the rate constant of the back reaction. From equations 1 and 5, one obtains

$$\Delta A/\Delta A_{max} = \phi EN/k_b\ [P_{870}]_Y \tag{6}$$

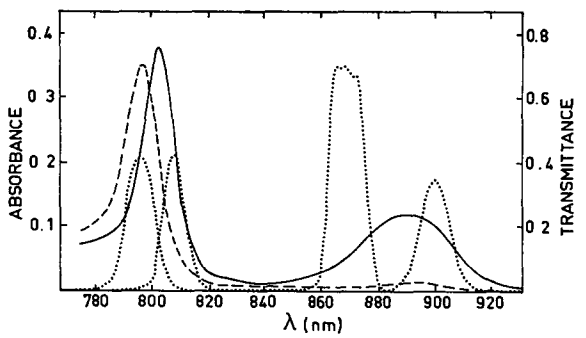


Fig. 2. Absorbance spectra at 77 °K of the reduced (—) and of the oxidized (– – –) photoreaction center. The transmittance spectra of the interference filters is indicated by (. . . .).

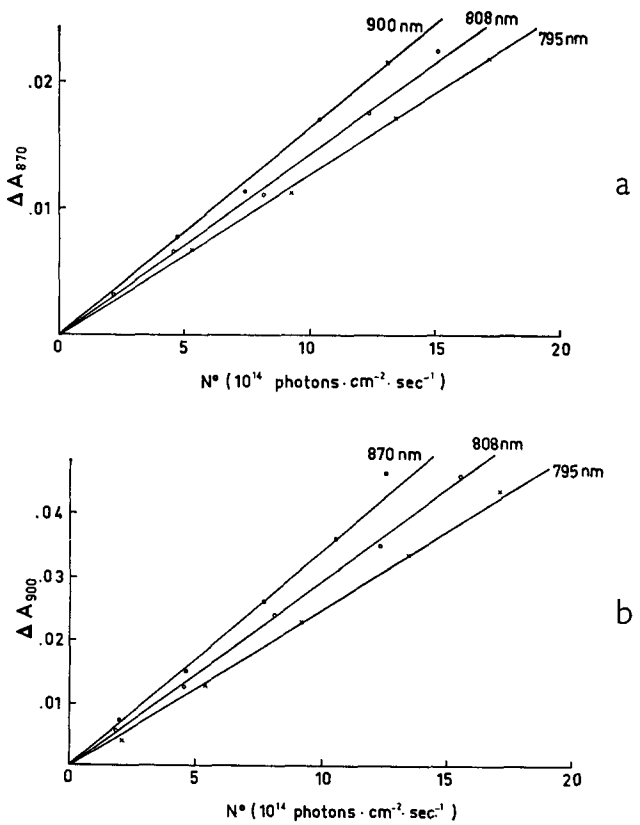


Fig. 3. (a) The fractional change in absorbance at 870 nm ($\Delta A/\Delta A_{max}$) of photoreaction center at 77° K as a function of the relative absorbed photon flux, $N°$. As indicated on the figure, excitation wavelengths were 795 nm, 808 nm and 900 nm. (b) The fractional change in absorbance at 900 nm ($\Delta A/\Delta A_{max}$) of the photoreaction center at 77 °K as a function of the relative absorbed photon flux, $N°$. As indicated on the figure, excitation wavelengths were 795 nm, 808 nm and 870 nm.

where $[P_{870}]_T$ is the total concentration of the photoreaction center in the sample. Again $N^\circ$ was used instead of $N$ in the calculations. The convolution of $N^\circ$ was obtained from the absorption and transmittance curves shown on Fig. 2 according to equations 10 and 12 (see Appendix).

Experimentally, plotting the absorbance change against increasing values of absorbed light intensity yields a straight line as predicted by equation 6. Fig. 3a shows the curves obtained by plotting $\Delta A_{870}$ against $N^\circ$ of various wavelengths, 795 nm, 808 nm and 900 nm. Taking the slope of the latter curve as unity, the relative efficiencies of energy transfer are obtained. Within the same experiment and, therefore, with the same preparation, $\Delta A_{900}$ was measured instead of $\Delta A_{870}$ (Fig. 3b). As expected, the results are the same within experimental error. The average of three experiments is $76.5 \pm 3.8\,\%$ for 795 nm actinic light and $87.1 \pm 1.4\,\%$ for 808 nm actinic light.

## DISCUSSION

Our results amount to the following two complementary observations. (1) The energy transfer efficiency of $P_{800}$ to $P_{870}$ decreases when the temperature is lowered from 298 °K to 77 °K; this is especially clear for excitation with 795 nm light. (2) At 77 °K, the relative efficiency of energy transfer is lower with 795 nm than with 808 nm light. The second observation is a confirmation that $P_{800}$ is composed of at least two dipoles. This is already known from other spectroscopic techniques, namely absorption [19, 21], CD [4–6] and linear dichroism [21, 22] spectroscopy. It shows moreover, that these dipoles are not strongly coupled or, in other words, that $P_{800}$ is not a dimer band. As to the first observation, it clearly indicates that no strong coupling exists between the short wavelength component of $P_{800}$ and $P_{870}$.

Since the short wavelength component of $P_{800}$ is not strongly coupled either with $P_{870}$ or with the long wavelength component of $P_{800}$, it may be safely attributed to monomeric bacteriochlorophyll. It is generally agreed that the single CD band at 798 nm in the oxidized state of the primary electron donor ($P_{ox}$) is a monomer band without any chromophore-chromophore interactions [4, 5, 21]. Our own data indicate that this is also true for the reduced state of the primary electron donor.

Although our results show that the short wavelength component is a monomer band even in the $P_{red}$ state, it is uncertain whether or not the long wavelength component is also a monomer band. Because we cannot uniquely resolve the $P_{800}$ band into its two components, we cannot obtain a unique value for the efficiency of energy transfer from each component. Our results do indicate that the efficiency of energy transfer from the long wavelength component is greater than 87 % at 77 °K. We cannot exclude the possibility that it might be as high as 100 % which would be consistent with the hypothesis of Vermeglio and Clayton [21] that $P_{870}$ and the long wavelength component of $P_{800}$ are dimer bands. This hypothesis could be modified so as to take into account our finding that $P_{870}$ is composed of at least two absorption dipoles [17]. It would then state that a bacteriochlorophyll dimer gives rise to the long wavelength component of $P_{800}$ and to one component of $P_{870}$. Even when modified in this manner, the model encounters several difficulties. It fails to explain why, at cryogenic temperatures, a large bathochromic shift is undergone by only one band ($P_{870}$) of the proposed dimer and not by the other ($P_{812}$). Another difficulty is that,

even at 35 °K [21], the 893 nm absorption band is not resolved into its components, presumably a monomer and a dimer band. Another piece of negative evidence comes from the difference spectrum between state $P^r$ and state $P_{red}$ [23]. This difference spectrum is similar to the $P_{ox}$ minus $P_{red}$ difference spectrum, except in the 800 nm region: $P^r$ appears to be spectrally similar to $P_{ox}$ as far as $P_{870}$ is concerned, but it is more akin to $P_{red}$ in the 800 nm region. State $P^r$ is thought to be triplet in character [23]. One would expect dimer bands to disappear together, whether the bleaching is due to oxidation or to the transition to a triplet state.

For these reasons, we favor the hypothesis that $P_{800}$ is due to two bacteriochlorophyll molecules which are in their monomeric state. On the other hand, on the basis of photodichroic experiments [17], $P_{870}$ can be attributed to two bacteriochlorophyll molecules with irreversible singlet energy transfer between them. We feel, therefore, that there is no strong singlet exciton coupling between the four bacteriochlorophyll molecules in the photoreaction center. This implies, in turn, that the CD spectra would best be interpreted as indicating interactions between the bacteriochlorophyll molecules and their environment [24] rather than strong interactions between like chromophores [4, 5, 21]. It must be noted that the proposed absence of singlet exciton dimeric states is not inconsistent with the existence of electronic or triplet 'dimer' states which have been found by EPR spectroscopy [7—13].

## APPENDIX

The fraction of the photon flux, $M_\lambda$, absorbed at a given wavelength by the photoreaction center preparation is related to its absorbance at that wavelength, $A_\lambda$ by equation 7,

$$M_\lambda = 1-10^{-A_\lambda} \tag{7}$$

On illumination with actinic light, a fraction, $\Delta A/\Delta A_{max}$, of the absorbance disappears. Hence, the absorbance of the sample under illumination is described by

$$A_\lambda = A_\lambda^{red}\,(1-\Delta A/\Delta A_{max})+A_\lambda^{ox}\,(\Delta A/\Delta A_{max}) \tag{8}$$

In equation 8, $A_\lambda^{red}$ is the absorbance at a given wavelength of the preparation in the $P_{red}$ state and $A_\lambda^{ox}$ is the absorbance of the preparation in the $P_{ox}$ state.

$\overline{M}_\lambda^{red}$, the fraction of the total photon flux at wavelength $\lambda$ that is absorbed by the reduced species is given by

$$\overline{M}_\lambda^{red} = M_\lambda\,(M_\lambda^{red}/M_\lambda^{red}+M_\lambda^{ox}) \tag{9}$$

or, from equations 7, 8 and 9,

$$\overline{M}_\lambda^{red} = [1-10^{-[A_\lambda^{red}(1-\Delta A/\Delta A_{max})+A_\lambda^{ox}(\Delta A/\Delta A_{max})]}] \cdot \frac{[1-10^{-A_\lambda^{red}(1-\Delta A/\Delta A_{max})}]}{[1-10^{-A_\lambda^{red}(1-\Delta A/\Delta A_{max})}]+[1-10^{-A_\lambda^{ox}(\Delta A/\Delta A_{max})}]} \tag{10}$$

The total photon flux, $N$, absorbed by the photoreaction center in the $P_{red}$ state, through the interference filter obeys the relationship

$$N = g\,N^\circ \tag{11}$$

where $g$ is a proportionality factor due to the geometry of the sample.

Since the band width of the interference filters used in this experiment is small (10 nm), one can assume the energy to be constant within this bandwidth. With this assumption and making use of Planck's law, the following equation can be derived:

$$N^0 = \frac{E_m \lambda_0}{hc \Sigma_\lambda T_\lambda} \Sigma_\lambda \bar{M}_\lambda^{red} \frac{T_\lambda \lambda}{\lambda_0} \tag{12}$$

In equation 12, $E_m$ is the energy flux measured by the thermopile, $h$ is Planck's constant, $c$ is the speed of light, $\lambda_0$ is the wavelength of maximal transmittance of the filter and $T_\lambda$ is transmittance at wavelength $\lambda$.

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