PIGMENT CONTENT AND MOLAR EXTINCTION
COEFFICIENTS OF PHOTOCHEMICAL REACTION
CENTERS FROM RHODOPSEUDOMONAS SPHEROIDES*

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Running title: Spectroscopic analysis of
reaction centers

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SUMMARY

Reaction center particles isolated from carotenoidless mutant Rhodospseudomonas spheroides were studied with the aim of determining the pigment composition and the molar extinction coefficients.

Two independent sets of measurements using a variety of methods show that a sample with $A_{800} = 1.00$ contains $20.8 \pm 0.8 \mu\text{M}$ tetrapyrrole and that the ratio of bacteriochlorophyll to bacteriopheophytin is 2:1.

Measurements were made of the absorption changes attending the oxidation of cytochrome c coupled to reduction of the photo-oxidized primary electron donor in reaction centers, using laser flash excitation. The ratio of the absorption change at 865 nm (due to the bleaching of P870) to that at 550 nm (oxidation of cytochrome) was found to be 5.77.

These results, combined with other data, yield a pigment composition of 4 bacteriochlorophyll and 2 bacteriopheophytin molecules in a

reaction center. Based on this choice, extinction coefficients are determined for the 802 and 865 nm bands: $\epsilon_{802} = 288 \pm 14 \text{ M}^{-1}\text{cm}^{-1}$ and $\epsilon_{865} = 128 \pm 6 \text{ M}^{-1}\text{cm}^{-1}$. For reversible bleaching of the 865 nm band, $\Delta\epsilon_{865}^{\text{red-ox}} = 112 \pm 6 \text{ M}^{-1}\text{cm}^{-1}$ (referred to the molarity of reaction centers). Earlier reported values of photochemical quantum efficiency are recomputed, and the revised values are shown to be compatible with those obtained from measurements of fluorescence transients.

INTRODUCTION

Photosynthetic reaction center preparations have become a valuable tool in studying the photochemistry and associated electron transport of bacterial photosynthesis.¹⁻¹⁵ When computing stoichiometries and quantum efficiencies in these studies, we have had to rely on earlier values of extinction coefficients determined with relatively crude preparations.¹⁶ Recent refinements in the purification of reaction center particles (rcp's) from Rhodospseudomonas spheroides^{10,17} have allowed an improved evaluation of the molecular composition and optical parameters of these particles. New data on these matters will be reported here.

Absorption spectra of rcp's^{10,12,17} show maxima near 537 and 757 nm identified with bacteriopheophytin (BPh) and maxima near 600, 803, and 865 nm ascribed to bacteriochlorophyll (BChl). Absorption (A) by a sample of rcp's can be correlated with the amounts of BChl and BPh extractable from that sample. If we can decide how many

molecules of these pigments are in one reaction center, we can translate these data into extinction coefficients based on the molarity of reaction centers. For this we must define a reaction center, as either a functional or a morphological entity, not necessarily identical with a rcp. Functionally a reaction center can be defined as that constellation of molecules capable of effecting the photochemical translocation of one electron with the use of one quantum. Morphologically a reaction center is the smallest particle capable of performing the characteristic photochemistry. Recent analyses by Feher, Okamura, Raymond, and Steiner¹⁸ suggest that a morphologically defined reaction center is a distinct smaller part of the rcp as prepared by Clayton and Wang¹⁷ or Feher¹⁰.

MATERIALS AND METHODS

Section I^{*}

Rcp's were isolated from carotenoidless mutant Rhodopseudomonas spheroides strain 2.4.1./R-26 as described by Fehér¹⁰. They were kept cold and in the dark until ready for use. BChl was extracted from Rhodospirillum rubrum by standard methods²⁰. The ratio of the absorption in the red band in ether to that in acetone (1.37) and of the absorption in the blue band to that in the red band in both ether (0.78) and acetone (0.96) agreed very well with the data of Sauer et al.²¹, and their extinction coefficients were used in calculating total moles of pigment. BChl was quantitatively converted to BPh by adding 1/100 volume of 1 M HCl to an acetone solution. The BPh was crystallized from

* D.C.M. contributed the results in Section I, S.C.S. and R.K.C. the results in Section II, and W.W.P. and R.K.C. those in Section III.

acetone-water. Again the spectra agreed quantitatively with published values²¹. The conversion of BChl to BPh in ether required stronger acid: 2% v/v of methanesulfonic acid. All extractions of rcp's and manipulations of the pigments were carried out in a darkroom under dim green light. All solvents were freshly distilled, as was the aqueous HCl. The distilled water was twice re-distilled in quartz, and glassware was carefully cleaned.

Section II

Cells of Rps. spheroides 2.4.1./R-26 were grown, and chromatophores were prepared, as described in Ref. 17. Whole cells and chromatophores were always used as suspensions in de-ionized water containing 0.01 M Tris·HCl, pH 7.5. Rcp's were isolated according to the method of Clayton and Wang¹⁷ and were used as chilled, dark-adapted suspensions in de-ionized water containing 0.01 M Tris·HCl, pH 7.5 + ± 0.3% (v/v) of the detergent lauryl-dimethyl amine oxide (LDAO)*.

* The concentration of LDAO is not known ac-

LDAO (Onyx Chemical Co., Jersey City, N.J.) was incubated with catalase (≈ 10 μ g per ml of 30% LDAO) for about one hr. at room temperature to remove H_2O_2 ($\approx 0.02\%$). Triton X-100 (Rohm and Haas) was treated with $SnCl_2 \cdot 2H_2O$ to reduce organic peroxides²². Ubiquinone₃₀ (Sigma Chemical Company) was dissolved in ethanol and stored in a freezer.

All absorption spectra and light-induced absorption changes were measured with a Cary 14R spectrophotometer. The monochromator was calibrated against the Q peak (at 550 nm) of reduced equine cytochrome c, and the readings of optical density were calibrated with the use of a standard

curately, since knowledge of its physical properties and of its binding to rcp's is incomplete; there is no simple assay for the concentration of LDAO in a suspension of rcp's. Thus it is not accurately known how much LDAO is carried along, closely associated with the rcp's during manipulations of the concentration of this detergent -- see Results, Section I.

CuSO₄ solution. The solution itself had been calibrated by direct measurements of transmittance. A sample in the Cary spectrophotometer could be cross-illuminated through a port in the sample chamber.

In all extractions of rcp's, the pellets of sedimented, denatured protein were re-extracted once: additional extractions yielded no detectable pigment. BChl was quantitatively converted to BPh by the addition of 1/60 volume of 5 N HCl. For the determination of the extinction coefficient of BPh in a mixture of 7 volumes of acetone and 2 volumes of methanol (acetone:methanol (7:2)), aliquots of acidified extracts of chromatophores, having known spectra, were evaporated to dryness and the BPh redissolved in dry acetone:methanol (7:2), chloroform, and ether. The spectrum of BPh was unchanged by the addition of water to 5% by volume, and the extinction coefficient obtained with dry solvents was applied to the (wet) extracts of rcp's.

Section III

Preparations of rcp's were made both with

LDAO¹⁷ and with Triton X-100¹.

Sigma Type III cytochrome c was reduced with $\text{Na}_2\text{S}_2\text{O}_4$ under N_2 , chromatographed anaerobically on Sephadex G-25, and stored under N_2 at 0° C.

Absorption changes due to photo-oxidation of the primary electron donor and cytochrome were measured after saturating flashes from a Q-switched ruby laser²³. On each measurement, the spectrophotometer signal was digitized and stored in the memory of a Biomation model 802 transient recorder. The results of four measurements then were averaged in a digital computer. The individual measurements were made 20 min. apart, with the sample in darkness between measurements. A shutter introduced the measuring light beam 1-2 msec. before the recording of each measurement began. When necessary, the baseline signal without an actinic flash or flash artifacts that occurred in the absence of the measuring light also were recorded and were subtracted from the full signal in the computer.

RESULTS

Section I

A volume* of exhaustively dialysed rcp's was centrifuged and the pellet extracted with 6 ml, then 1 ml, of acetone. The spectrum of an aliquot of the weighed combined extracts was determined. The mixture of BChl and BPh was quantitatively converted to the latter with HCl, and the spectrum re-determined. Several variations on the basic theme were carried out, with no significant difference in the results: the rcp pellet was lyophilized before extraction, the acetone was buffered with Tris, and the acetone extract was highly diluted into ether to determine the spectra and convert to BPh.

The extraction was repeated with smaller

* The volume was chosen so as to give a value of 10 - 20 for the product of the volume (ml) times the absorption by the rcp's at the peak near 803 nm (A_{800}). This allowed work with a constant number of rcp's.

samples, using 17 different solvents. Many of these solvents caused difficulties, either failing to extract the pigments completely or producing complex BChl spectra (probably arising from mixtures of different solvation isomers of BChl, as in dimethylsulfoxide). Two useful solvents were dichloromethane and pyridine; these gave the same ratio of BChl to BPh (2:1) as was found with acetone.

The results of these solvent extractions of rcp's suggest that the pigments are buried in a strongly hydrophobic core of the protein, surrounded by a more polar shell containing the detergent LDAO. The relatively low permeability by "poor" solvents (such as hexane, methyl-cyclohexane, benzene, *o*-xylene, diethyl ether, diisopropyl ether, and formamide) and the retention of some photo-activity by the rcp's after extraction with these solvents are evidence suggesting the presence of a polar shell in rcp's. This shell apparently is disrupted by "good" solvents (such as acetone, dichloromethane, and pyridine), and the protein is denatured. "Powerful" solvents (such as dimethylsulfoxide, dimethylformamide,

and N-methylformamide) so scramble the protein structure that it is insoluble even in sodium dodecyl sulfate.

A complication in the spectral analyses was caused by the detergent LDAO, strongly bound to the rcp but extractable with "good" organic solvents. The LDAO forms tight complexes with BChl in most solvents. In acetone the resulting spectral changes were minor in the near ultraviolet and far red bands of BChl (peak $\Delta\epsilon$ +1.5% and -4.2%, respectively), but can cause larger errors in estimating the ratio of BChl to BPh. The sharpening of the 576 nm band in acetone on complexing is large: the full bandwidth at half-maximum decreases from 38 nm to 33 nm; the increase in peak absorption is 11.5%. Titration of BChl with LDAO showed that it is approximately saturated with LDAO on extraction from rcp's. Only a lower limit of several LDAO molecules per BChl, i.e. >10 per rcp, could be set. These results were confirmed upon chromatography of the pigment on sugar. The pigments ran faster on the column in the presence than in the absence

of LDAO, and the separation of BChl and BPh was hindered. These effects could be duplicated by adding LDAO to BChl. Repeated chromatography did separate the complex, but differential losses were too great for this procedure to be of use for analysis. We therefore based our analyses on the spectral properties of BChl and BPh in the presence of the saturating amounts of LDAO bound to the rcp.

The spectral analysis yielded two pieces of information: the concentration of total pigment in a sample of rcp's having $\Lambda_{800} = 1.00$ and the ratio of BChl to BPh. The ratio was obtained from the following equation:

$$\frac{\text{BChl}}{\text{BPh}} = \frac{A_2 \epsilon_1^P - A_1 \epsilon_2^P}{A_1 \epsilon_2^C - A_2 \epsilon_1^C}$$

where A_1 and A_2 are the absorption at two wavelengths, ϵ_1^P is the extinction coefficient for BPh at wavelength 1, ϵ_1^C the same for BChl, etc. The wavelengths 1 = 522 nm and 2 = 576 nm were chosen to maximize the differential extinction coefficients and thus minimize the error. For these wavelengths, the normalized extinction

coefficients in the presence of LDAO are: $\epsilon_1^P = 1$, $\epsilon_2^P = 0.078$, $\epsilon_2^C = 0.855$, and $\epsilon_1^C = 0.128$. Both the far red and visible bands gave the same ratio, but the near ultraviolet band did not, indicating that some ultraviolet-absorbing material other than BChl and BPh was also extracted. It is known¹¹ that ubiquinone is extracted from the rcp's.

The same ratio of BChl to BPh was also obtained by data at a single wavelength before and after conversion of BChl to BPh. The following equation was used:

$$\frac{\text{BChl}}{\text{BPh}} = \frac{A' - A}{A - \frac{\epsilon^C}{\epsilon^P} A'}$$

where A is the absorption of the mixture of BChl and BPh at a given wavelength and A' is the absorption after the addition of HCl. For

768 nm, $\frac{\epsilon^C}{\epsilon^P} = 4.17$ and for 522 nm, $\frac{\epsilon^C}{\epsilon^P} = 0.128$.

The total pigment was most easily obtained following total conversion to BPh. It was also obtained from the spectra of the original extract, with the use of the ratio of BChl to BPh.

Table 1 shows a representative series of data. The weighted results of 5 separate analyses (>10 ratio determinations) were: BChl:BPh = 1.97 ± 0.10 ; $20.8 \pm 0.8 \mu\text{M}$ pigment in a sample having $A_{800} = 1.00$.

Section II

The number of moles of pigment (BChl plus BPh) in a preparation of rcp's having a known spectrum was determined by measuring the amounts of BPh after extraction of the reaction center pigments with acetone:methanol (7:2), followed by acidification to convert BChl to BPh. The measurements were based on the absorption at 525 nm.

ϵ_{525} for BPh in acetone:methanol (7:2) was found to be $2.34 \pm 0.03 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ by comparing the absorption at this peak for BPh in acetone:methanol (7:2) to those due to equal amounts of BPh in solvents for which the extinction coefficients have been reported in the literature²⁴⁻²⁶.

Two control experiments were carried out

to check for effects of LDAO on the spectra of reaction center pigments in solution. The first was a concentration series with LDAO in which chromatophores from Rps. spheroides 2.4.1./R-26 were extracted according to the procedure used for the measurement of the total pigment in rcp's. The second was a similar concentration series with LDAO using suspensions of rcp's containing 0.05% Triton X-100 (and probably also a small, unknown, amount of LDAO). LDAO at a concentration as high as 0.6% in the suspension of chromatophores prior to extraction had no apparent effect on the spectrum of BPh in the extracts of chromatophores, and there was no apparent effect of LDAO at any of the concentrations (up to 1% in the suspension before extraction) on the spectrum of BPh in extracts of rcp's. Any effects of LDAO on the visible band of BChl (see Section I) did not affect this analysis based on the absorption peak of BPh.

Nine separate extractions were performed, using three preparations of rcp's. Representative data are shown in Table 2. The result agrees with that of Section I: 21 ± 1 nmoles of BPh are

extracted from a one-ml sample of rcp's having $A_{600} = 1.0$. If the ratio of BChl to BPh is 2:1 (see Section I; also see below), then two thirds of these 21 nmoles, or 14 nmoles, is due to BPh made from BChl originally present in the sample.

The ratio of BChl to BPh in the reaction center was determined by measuring the absorption due to BPh at its peak in the visible region in an extract of rcp's before and after acidification to convert BChl to BPh. The solvent used for the extractions was methanol:ether (6:4). Before acidification, some of the absorption at 527 nm (the location of the peak due to BPh in methanol:ether (6:4)) was due to the tail of the BChl band (peaking at about 606 nm). This contribution was estimated by fitting the experimentally measured spectrum (by means of a "least squares" procedure) with spectra of BChl and BPh. The latter spectra were obtained from a methanolic extract of cells of Rps. spheroides 2.4.1./R-26 which was diluted into methanol:ether (6:4). A small correction was applied to

the spectrum of BChl to discount the spectral contribution of BPh extracted along with BChl from the cells.

Two types of controls were done to check for any effect of LDAO in this experiment. The first was to apply the same procedure used with rcp's to a suspension of chromatophores from Rps. spheroides 2.4.1./R-26 which contained 0.3% LDAO (or the appropriate volume of H₂O). There was no significant effect from the 0.3% LDAO on the peak height of the visible band of BPh in the suspension of chromatophores. However, in extracts containing LDAO, the peaks of the visible bands of BChl and BPh were blue-shifted by one nm relative to their positions in extracts without LDAO. The second type of control was a concentration series with LDAO using rcp's in the presence of 0.05% Triton X-100 (and probably also a small basal amount of LDAO). When the concentration of LDAO was 0.3%, small spectral changes were evident, and the calculated ratio of BChl to BPh decreased by $\pm 6\%$ (relative to the values obtained with 0 and 0.1% LDAO). Therefore this

control and the one with chromatophores indicate that the presence of $\geq 0.3\%$ LDAO in the suspensions of rcp's probably introduced error in the determinations of the ratio of BChl to BPh but that the error was not serious at 0.3% LDAO.

Four determinations of the ratio of BChl to BPh yielded the value 2.0. Two additional trials were carried out, in which the sample was acidified immediately after the solvent was added (instead of several minutes later, after a spectrum had been recorded of the nonacidified extract). This would minimize the exposure of BChl to LDAO, and it did result in a somewhat higher value of the ratio (2.1 for both trials). The simplest conclusion from these results is that the ratio of BChl to BPh in the reaction center is 2BChl:1BPh, a result which agrees with that of Section I.

For the determination of $\Delta\epsilon_{865}^{\text{red-ox}}$, a measurement was made of the fraction of the 865 nm absorption that is lost when rcp's are subjected to illumination intense enough to cause maximal bleaching. The rcp's, $A_{800} = 0.72$, were in the presence of 30 μM ubiquinone₃₀ to retard the

dark re-reduction of the photo-oxidized primary donor¹². The sample was illuminated with saturating white light, causing 88% of the absorption at 865 nm to be reversibly bleached. Controls showed that the trace of ethanol added with the ubiquinone₃₀ in the experimental sample had no apparent effect. Tests with added reductant showed that a negligible fraction of the rcp's initially contained oxidized primary electron donor.

It is now possible to list optical parameters for reaction centers, using a range of assumptions for the number of BChl molecules in a reaction center. Because of the strong electronic interactions between the pigment molecules, it is not meaningful to assign specific molecules to each band in the complex reaction center spectrum. However, one can still assign molar extinction coefficients to the reaction center. This has been done in Table 3.* The relative height of the 865 nm band in rcp's apparently varies slightly in different preparations; a representative value of 2.25 has been used in Table 3 for the computation of ϵ_{865} from ϵ_{802} .

* The values of extinction coefficients quoted in Ref. 19 are too large by 10% because of an arithmetical error. They should read $\epsilon_{800} = 2.9 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and $\epsilon_{865} = 1.2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ for 6 pigment molecules per reaction center.

Section III

A differential extinction coefficient for the absorption change reflecting photochemistry in rcp's can be determined by coupling the photochemistry to a well-characterized chemical process, the oxidation of mammalian cytochrome c. A molar differential extinction coefficient at 865 nm, presumably for the single-electron transfer in rcp's, can be computed from the known coefficient for oxidation of the cytochrome. This determines the molar extinction coefficient of the 865 nm band, for reaction centers defined functionally in terms of single-electron transfer. In turn this specifies the number of BChl molecules per reaction center, when coupled with data on the extraction of pigments from rcp's.

Figure 1A shows the oxidation of horse heart cytochrome c by rcp's, following a short actinic flash. Immediately after the flash, the absorption at 550 nm increased, reflecting the photo-oxidation of the primary electron donor, P^{27} . If no cytochrome had been added, the re-

turn of the signal to the baseline required several seconds (not shown). In the presence of cytochrome, cytochrome oxidation followed, giving a larger absorption decrease with a half-time of about 2 msec. In many experiments, a small, variable part of the cytochrome oxidation occurred with a half-time of about 20 μ sec., in agreement with the results of Ke et al.⁷, but most of the reaction was much slower. The kinetics did not appear to vary with pH between 7.5 and 8.5, nor did increasing the LDAO concentration to 1.5% or replacing the LDAO by 0.05% Triton X-100 affect them markedly.

Figure 1C affords a measurement of the absorption change due to the initial photo-oxidation of P, and Figure 1B shows that, within experimental error, all of the P^+ was reduced again as the cytochrome became oxidized. In the absence of cytochrome, P^+ reduction was negligible on this time scale. The rate of P^+ reduction in Figure 1B is slightly slower than the rate of cytochrome oxidation in Figure 1A. This probably resulted from the sequence of the meas-

urements. In this experiment, the cytochrome measurements were made first, and the rate of the reaction decreased somewhat during the experiment. Experiments in which P was measured first gave results that were consistent with this interpretation.

The initial absorption decrease due to oxidation of P in Figure 1C is 0.079; the net absorption decrease due to cytochrome oxidation in Figure 1A is 0.0138. The ratio is 5.72. Six similar experiments, four of which employed rcp's prepared with LDAO, and two of which used rcp's prepared with Triton X-100, gave an average of 5.77 ± 0.10 for this ratio, in complete agreement with the ratio of 5.8 that Ke et al.⁷ reported for reaction centers prepared with Triton X-100.

Recent measurements of $\Delta\epsilon_{550}^{\text{red-ox}}$ for equine cytochrome c range from 19.6 to 21.1 $\text{mM}^{-1}\text{cm}^{-1}$ (Refs. 28-31). With a value of $20.4 \pm 0.7 \text{ mM}^{-1}\text{cm}^{-1}$, the ratio 5.77 gives $\Delta\epsilon_{865}^{\text{red-ox}} = 118 \pm 4 \text{ mM}^{-1}\text{cm}^{-1}$, within experimental error of the $\Delta\epsilon_{865}^{\text{red-ox}} = 112 \pm 6 \text{ mM}^{-1}\text{cm}^{-1}$ that is predicted on the assumption of 4 BChl per reaction center (see Table 3).

Other assumptions for the number of BChl per reaction center would require $\Delta\epsilon$ values significantly different from the experimental value (e.g.: 84.4 ± 4.2 and $141 \pm 7 \text{ mM}^{-1}\text{cm}^{-1}$ for the assumptions of 3 or 5 BChl per reaction center, both ruled out, in addition, by the value of 2 for the ratio of BChl to BPh).

DISCUSSION

Two lines of evidence favor a choice of 4 BChl and 2 BPh as the pigment composition of the reaction center. This choice in turn will determine which values for the optical parameters for reaction centers (listed in Table 3) should be adopted.

We have presented two independent series of determinations of the ratio of BChl to BPh in rcp's. Both series of measurements agree that the ratio is 2 BChl:1 BPh.* This ratio, along with some spectral observations, allows a choice to be made of the minimum composition of pigments in reaction centers. At room temperature, the absorption spectrum of rcp's contains a band in the visible region, associated with BPh, which is markedly broad and flat (see Ref. 10 or 17, Fig. 1). At low temperatures, this band is resolved into two bands of similar magnitudes¹³. This could represent a splitting of a degeneracy in the energy levels of BPh, resulting in the appearance of two bands in its spectrum in the visible region. However, BChl (or BPh) does not have a high de-

* After completion of this work, the paper by Reed and Peters³² appeared, giving results in agreement with ours.

gree of symmetry^{33,34}; it is more likely that the two BPh bands resolved at low temperature are due to the presence of a dimer of BPh in the reaction center. The foregoing evidence argues in favor of an even number of molecules of BPh per reaction center; the measured ratio of 2 BChl:1 BPh therefore favors the choice of 4 BChl:2 BPh as the minimum pigment composition of the reaction center.

Strong support for the choice of 4 BChl and 2 BPh as the composition of pigment in the reaction center comes from studies of the oxidation of cytochrome c by the photo-oxidized primary electron donor^{5,35-37}. The comparison of the ΔA_{550} due to the oxidation of cytochrome c with the ΔA_{865} which recovers in the coupled reaction yields a value of $\Delta \epsilon_{865}^{\text{red-ox}}$ which agrees well with the value that is predicted on the assumption of 4 BChl per reaction center.

Thus these considerations support the choice of 4 BChl and 2 BPh as the pigment complement of the reaction center. The resulting revision of $\Delta \epsilon_{865}^{\text{red-ox}}$ from $100 \text{ mM}^{-1} \text{ cm}^{-1}$ (Ref. 13) to $112 \text{ mM}^{-1} \text{ cm}^{-1}$ removes an apparent discrepancy between the values of the quantum efficiency of the primary photochemistry measured by the bleaching at 865 nm (e.g.: Refs. 5,13,35,37) and by the rise in fluorescence from the 865 nm

band in rcps or from bulk BChl in chromatophores^{2,13,23}. The representative values for the quantum efficiency of $1.02 \pm .12$ (Ref. 5), 0.9 ± 0.14 (Ref. 13), and 1.0 ± 0.15 (Ref. 13) become 0.81 ± 0.10 , 0.79 ± 0.12 , and 0.88 ± 0.13 when recomputed with the use of $\Delta\epsilon_{865}^{\text{red-ox}}$ of $112 \text{ mM}^{-1}\text{cm}^{-1}$ instead of 93 or $100 \text{ mM}^{-1}\text{cm}^{-1}$ (Refs. 5 and 13, respectively). A representative value for the quantum efficiency measured from the rise in fluorescence is 0.7 (Ref. 13); the values for the quantum efficiency measured in the two ways now agree within the limits of error in the measurements. These values are also compatible with values for the quantum efficiency, determined by measurements of electron spin resonance^{5,38}.

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Table 1. A representative set of data on pigment extraction of rep's from Rhodospseudomonas sphaeroides. See the text for an explanation of the calculations.

$A_{800} \times \text{Volume}$ (ml) of Sample	Acetone Extract	Absorption			
		745 nm	768 nm	522 nm	576 nm
15.2	(1) 5.15 ml rep's				
	(2) 250 μ l (1) + 500 μ l acetone	--	0.925	0.198	0.283
	(3) (2) + 10 μ l 1 M HCl	0.892	0.295	0.480	0.036
	$\frac{\text{BChl}}{\text{BPh}}$ via 2 wavelengths (522 and 576 nm):	2.00			
	$\frac{\text{BChl}}{\text{BPh}}$ via acid:	2.06 (using A_{768})			
		2.04 (using A_{522})			
Total nanomoles of pigment = 300; therefore 19.8 μ M pigment in a sample having $A_{800} = 1.00$.					
Absolute extinction coefficients ($\text{M}^{-1}\text{cm}^{-1}$):					
for BPh, $\epsilon_{745} = 4.75 \times 10^4$ $\epsilon_{522} = 2.50 \times 10^4$					
for BChl, $\epsilon_{768} = 6.90 \times 10^4$ $\epsilon_{576} = 1.76 \times 10^4$					

Table 2. A representative set of data for the measurement of reaction center pigments as the amount of BPh in acidified acetone:methanol (7:2). The ϵ_{525} used for BPh in acetone:methanol (7:2) was $2.34 \pm 0.03 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ (see text). Prior to extraction, rcp's were suspended in 0.01 M Tris-HCl, pH 7.5 and containing 0.3% LDAO.

A ₈₀₀ of Reaction Center Particles	Raw Data:*	Average	nmoles BPh in 1.0 ml of Sample Having A ₈₀₀ = 1.0
	A ₅₂₅ in Acidified Extract (In- cludes Re- extraction)		
16.3	0.381	0.38 ± 0.01	20 ± 1
	0.383		
	0.372		
	0.390		

* This is the A₅₂₅ of 4.07 ml containing the pigment from 0.20 ml of the suspension of rcp's.

Table 3. Optical parameters for reaction centers of Rhodospseudomonas spheroides.

Assumed Number of BChl per Reaction Center*	A ₈₀₀ per ml of Sample Containing One nmole of Reaction Centers [†]	ε ₈₀₂ , mM ⁻¹ cm ⁻¹	ε ₈₆₅ [‡] , mM ⁻¹ cm ⁻¹	Δε ₈₆₅ ^{red-ox} [#] , mM ⁻¹ cm ⁻¹
2	0.144 ± 0.007	144 ± 7	63.9 ± 3.2	56.3 ± 2.8
4	0.288 ± 0.014	288 ± 14	128 ± 6	112 ± 6
6	0.432 ± 0.022	432 ± 22	192 ± 10	169 ± 8

* Assumptions of odd numbers of BChl per reaction center are ruled out by the finding of a value of 2 for the ratio of BChl to DPh (see Sections I and II).

† Computed from the step: a one-ml sample of rcps having A₈₀₀ = 1.00 contains $\frac{13.9}{x}$ nmoles reaction centers, where x is the number of BChl per reaction center, and 13.9 is the number of nmoles of BChl contained in a one-ml sample having A₈₀₀ = 1.00. This latter number (13.9) is 2/3 of the 20.8 nmoles of tetrapyrrole contained in a one-ml sample having A₈₀₀ = 1.00 (see Sections I and II).

‡ ε₈₆₅ is computed by the relation: $\epsilon_{865} = \frac{\epsilon_{802}}{2.25}$. The use of the value 2.25 for the ratio $\frac{\epsilon_{802}}{\epsilon_{865}}$ is explained in the text.

Computed with the assumption that 88% of the absorption at 865 nm is reversibly bleached in the primary photoact (see text).

FIGURE LEGEND

Figure 1. Absorption changes on photo-oxidation of the primary electron donor P and cytochrome c. The sample contained rcp's (prepared with LDAO) giving an Λ_{800} of 0.25, 0.5% LDAO, 11 μM cytochrome c, and 1.7 μM ubiquinone₃₀ in 3.1 ml of 0.01 M Tris-HCl, pH 7.5. The cuvette had a 1-cm light path and was rendered anaerobic by bubbling with N₂ through a rubber septum. In each trace, a downward deflection represents an absorption decrease. The same sample was used for all of the measurements in the Figure.

A. Cytochrome oxidation, measured at 550 nm, with a monochromator bandpass of 1.65 nm.

B. P oxidation and reduction, measured at 865 nm, with a monochromator bandpass of 2.3 nm. A downward drift that occurred in the baseline without a flash has been subtracted from the full signal.

C. P oxidation, as in B but on a shorter time scale. Artifacts that occurred at the time of the flash have been subtracted from the full signal.

C

865 nm

0.1 msec

$\Delta A = 0.0203$