

PHOTOCHEMICAL ELECTRON TRANSPORT IN PHOTOSYNTHETIC REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHEROIDES*

II. INTERACTION WITH EXTERNAL ELECTRON

DONORS AND ACCEPTORS AND A

REEVALUATION OF SOME SPECTROSCOPIC DATA

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ABSTRACT Photochemical reaction centers prepared from *Rhodopseudomonas spheroides* were treated with reduced cytochrome c (cyt c), and in some cases with ubiquinone (UQ), and illuminated. The light-induced oxidation of cyt and reduction of UQ were observed, and also the variations in fluorescence of P870. These observations indicated that each reaction center contains a primary photochemical electron acceptor capable of holding just one electron. Depending on the method of preparation, the reaction centers may also contain secondary electron acceptor pools consisting mainly of UQ. The role of native UQ as an electron acceptor could be duplicated by added UQ. The yield of P870 fluorescence increased by a factor of 3–4, at most, during illumination of reaction centers in the presence of an electron donor such as reduced cyt. This suggests that the quantum efficiency for the primary photoact is about 0.7, rather than 0.9–1.0 as concluded in the past from optical absorption measurements. The apparent quantum efficiency for the oxidation of cyt by illuminated reaction centers can be increased by the addition of UQ and is decreased at higher concentrations of the detergent lauryl dimethylamine oxide (LDAO). These treatments do not affect the quantum efficiency of P870 oxidation, measured in the absence of cyt.

INTRODUCTION

A forthcoming article¹ describes the kinetics of the photochemical oxidation and subsequent reduction of P870 in reaction centers prepared from *R. spheroides*. It is shown that UQ, either endogenous or added, can trap electrons so as to delay their return to oxidized P870 (P870⁺ or P⁺).

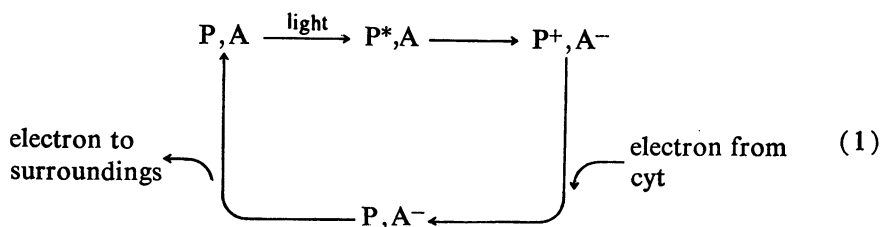
¹ Clayton, R. K., and H. F. Yau. To be published.

Other work (1–4) has shown that in cells and subcellular preparations from photosynthetic bacteria, cyt can deliver electrons rapidly to P870⁺. Electrons are transferred photochemically from cyt, through P870, to primary and secondary electron acceptors including UQ. Ultimately the electrons may cycle back to oxidized cyt, or they may be promoted to the level of reduced-form nicotinamide adenine dinucleotide (NADH). These electron transfers may be coupled to the formation or utilization of adenosine triphosphate (ATP) (see reference 5).

The extent of cyt oxidation can be used to measure the number of electrons delivered photochemically to primary and secondary acceptors. The size of the “electron acceptor pool” in a sample can thus be assayed.

Another way to measure the size of an electron acceptor pool is by analyzing the fluorescence of the sensitizing pigment. This method has been applied by Malkin and Kok (6, 7) to measure the acceptor pools associated with the reaction center of green plant photosystem II. These investigators observed the fluorescence of the light-harvesting chlorophyll that serves this reaction center. The fluorescence rises during illumination because the reaction centers cannot deal with excitation quanta as rapidly as the quanta are being absorbed. Experiments with added oxidizing and reducing agents suggest that electrons accumulate on the reducing side of the photochemical system during illumination (6). The primary electron acceptor then becomes reduced, the reaction center can no longer function, and the fluorescence rises. A suitable analysis of the dynamics of this rise in the fluorescence (see later) shows how many electrons must be transferred through the photochemical system in order to fill all secondary pools and reduce the primary acceptor as well.

Similar behavior is shown by reaction centers from *R. spheroides*, in which the fluorescence is emitted by P870 in direct competition with photochemical utilization (8). In the presence of an electron donor such as reduced cyt c, the fluorescence of P870 rises during illumination as shown by curve *b* in Fig. 1. We assume that the higher level of fluorescence f_{\max} signifies the condition P, A⁻, in which the hypothetical primary acceptor (A) is in its reduced form. The sequence of events is interpreted as follows, where P stands for P870 and P* is P in its singlet excited state:



The system P, A is only weakly fluorescent (f_0 in Fig. 1) because it is photochemically competent. The system P, A⁻ is more strongly fluorescent. As long as a suitable electron donor such as reduced cyt is present, P⁺ is converted to P so rapidly that

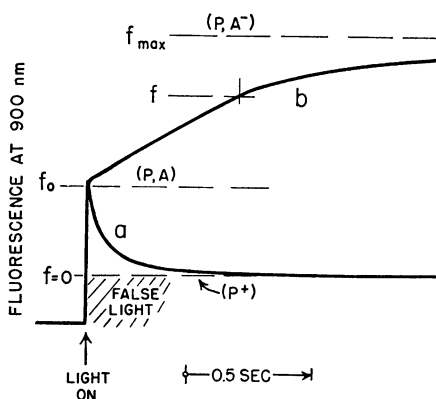
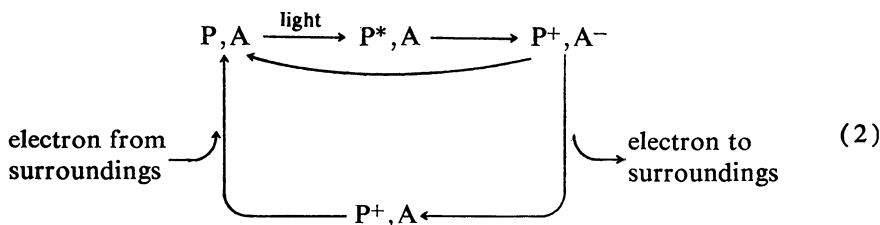


Figure 1 Time course of the fluorescence at 900 nm emitted by reaction centers made from *R. spheroides* using LDAO. Reaction centers in 0.01 M Tris-Cl, pH 7.5, with 0.1% LDAO and 5 μM UQ. Concentration of reaction centers = 1.0 μM based on $\epsilon = 113 \text{ mM}^{-1} \text{ cm}^{-1}$ at 867 nm. Exciting light, 800 nm, 4.0 mw/cm^2 . Curve a, no electron donor added. The fluorescence declined as the emitter P870 became oxidized (and bleached) to P870⁺. The "false light" signal with all P870 bleached is ascribed to scattered exciting light and emission from chromophores other than P870. Curve b, with 25 μM bovine cyt c (added as the reduced form). The P870 was kept predominantly reduced by the cyt during illumination, and the fluorescence rose (ultimately to f_{max}) as the photochemistry proceeded. The rise is attributed to reduction of the primary electron acceptor A. The maximum level, associated with P, A⁻, could also be obtained by adding Na₂S₂O₄.

no accumulation of P⁺ is observed during illumination.² Light then converts P, A to P, A⁻, and this conversion is extensive when secondary pools become filled so that the flow of electrons out of P, A⁻ to the surroundings is slow. In a steady state the ratio of P, A⁻ to P, A depends on the rate of the light-driven reaction and the rate of P, A⁻ → P, A.

In the dark the reaction centers can be converted almost entirely to P, A⁻, giving a maximal yield of fluorescence, by the addition of Na₂S₂O₄.

If reaction centers are illuminated in the absence of an external electron donor, the reduction of P870⁺ is by electrons from the reducing side of the photochemical system, directly or by way of secondary acceptors (labeled "surroundings"):



Now the state P, A⁻ does not arise. Instead, the P870 is bleached (P → P⁺) and its fluorescence declines in proportion to the fraction bleached (8) (curve a in Fig. 1).

² The transient presence of P870⁺, when reduced cytochrome is present, can be observed if the time resolution is in the range of microseconds and a pulsed laser is used for excitation (1).

We have examined the electron acceptor pools associated with reaction centers from *R. spheroides*, and the role of added UQ as an electron acceptor, using electron-donating systems such as reduced cyt or phenazine methosulfate (PMS). Both the oxidation of the electron donor and the time course of the fluorescence were measured. We present here the results of these studies.

Analysis of the data will necessitate an inquiry into the absolute extinction coefficient and the quantum efficiency for oxidation of P870; see the Appendix.

MATERIALS AND METHODS

Materials

Preparation of reaction centers from *R. spheroides* by two methods has been described elsewhere (9, 10; see also Clayton and Yau, to be published¹). Reaction centers made with Triton X-100 (Sigma Chemical Co., St. Louis, Mo.) (9) contained variable amounts of UQ, 5–20 molecules/P870. Those made with LDAO (10) contained less than 1 UQ/10 P870.¹ Assay of quinone is described elsewhere.¹

For measurements the reaction centers were suspended in 0.01 M Tris-Cl buffer, pH 7.5, containing various concentrations of detergent (Triton X-100 or LDAO), UQ, and electron-donating systems.

PMS and UQ (Coenzyme Q₆) were from Sigma Chemical Co., St. Louis, Mo. Horse heart cyt c was "Fraction VI" from Sigma Chemical Co.; beef heart cyt c was a gift from Dr. Lucile Smith of Dartmouth Medical School. The cyt was reduced with NaBH₄ and the excess BH₄⁻ converted to borate by aeration.

Instruments

Absorption spectra were measured with a Cary 14R spectrophotometer (Cary Instruments, Monrovia, Calif.). Light-induced changes of optical density (OD) were measured with a split-beam differential spectrophotometer described elsewhere (3; Clayton and Yau, to be published¹). Signal-to-noise ratios can be judged from Fig. 2 of Clayton and Yau (to be published¹).

Fluorescence from reaction centers was measured with a simple fluorimeter as shown in Fig. 2, using combinations of filters to separate the exciting light from the measured light

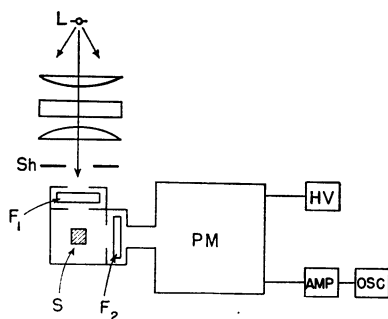


FIGURE 2 Schematic drawing of the fluorimeter. The lamp filament L was focused on the sample S through a water cell (1 inch thick, between the lenses), a shutter Sh, and a filter F₁ (800 nm). Light from the sample passed through filter F₂ (900 nm) to the photomultiplier PM connected to a high voltage supply HV. The photocurrent was delivered to an electrometer amplifier AMP, and the output of AMP was applied to a storage oscilloscope OSC.

The excitation filter F_1 was an 800 nm interference filter; the filter for emitted light, F_2 , was a combination of two Wratten filters, 87B and 87C, (Eastman Kodak Co., Rochester, N. Y.) and a 900 nm interference filter. Light passing through these filters deviated from the optic axis by as much as 15° , causing the band transmitted by each interference filter to become broader by about 10 nm toward lesser wavelengths. The exciting lamp was a 600 w tungsten-iodine (Sylvania "Sun-Gun") lamp (Sylvania Electric Products, Inc., Mountain View, Calif.) The photomultiplier was an RCA Type 70007C (S-1 spectral response, RCA Scientific Instruments, Camden, N. J.) cooled with dry ice. The electrometer amplifier was made from a Philbrick Type 25 C operational amplifier (Philbrick/Nexus Research, Dedham, Mass.) with resistors ranging from 10^6 to 10^9 ohms in the feedback loop. Its output was applied to a storage oscilloscope. Time resolution in the fluorescence measurements was better than 5 msec. Signal-to-noise ratios were greater than 10:1 in all measurements used.

Analysis of the Data

We wish to compute the concentration of reaction centers and the quantum efficiency of P870 oxidation in our samples. For this we must know (or assume) the values of absolute extinction coefficients of the reaction center pigments, and differential extinction coefficients for their photochemical conversion. These questions are examined in the Appendix. The conclusions are presented in Table I.

There are various reasons for assuming the presence of three, four, or five bacteriochlorophyll (BChl) molecules in each reaction center; see the Appendix. These BChl molecules are generally called P800 and P870, but the absorption bands near 800 and 870 nm are more properly described jointly as features in the absorption spectrum of a closely interacting set of molecules. We shall report our results primarily as computed from the assumption of three BChl molecules per reaction center, but will discuss the consequences of using the alternative assumptions of four or five BChl per reaction center.

Quantities of cyt c oxidized were computed on the basis that the differential ex-

TABLE I
MOLECULAR WEIGHT AND OPTICAL PARAMETERS OF REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHEROIDES*

| Assumed number of BChl molecules per reaction center* | Approximate mol wt of reaction center (18) | Extinction coefficient at 867 nm based on reaction center concn (15) | Differential extinction coefficient for the change at 867 nm (P870 oxidation) | Relative quantum efficiency for P870 oxidation |
|---|--|--|---|--|
| | | $mm^{-1} cm^{-1}$ | | |
| 3 | 80,000 | 113 | 100 | 1.00 |
| 4 | 107,000 | 151 | 133 | 0.75 |
| 5 | 133,000 | 189 | 167 | 0.60 |

* A reaction center is defined as the set of molecules that can effect the photochemical transfer of one electron.

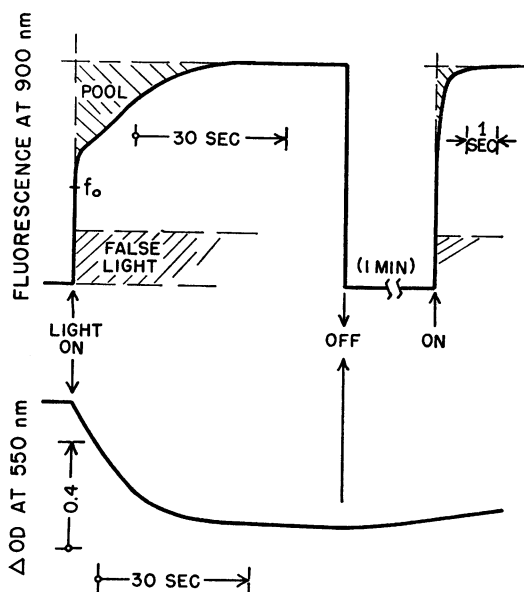


FIGURE 3 Oxidation of added cyt and change in P870 fluorescence induced by illumination of reaction centers made from *R. spheroides* using Triton X-100. The oxidation of cyt c is shown (lower curve) by the loss of OD at 550 nm; $\Delta\epsilon = 17 \text{ mm}^{-1} \text{ cm}^{-1}$. The area above the fluorescence curve (upper) is proportional to the pool of electron acceptor molecules; see text. The sample contained $0.45 \mu\text{M}$ reaction centers (based on $\epsilon = 113 \text{ mm}^{-1} \text{ cm}^{-1}$ at 867 nm), $9 \mu\text{M}$ native UQ, and $50 \mu\text{M}$ equine cyt c added in the reduced form, in 0.01 M Tris-Cl, pH 7.5, with 0.05% Triton X-100. Illumination was at 800 nm, 2.2 mw/cm^2 .

tinction coefficient $\Delta\epsilon$ (reduced — oxidized) at 550 nm equals $17 \text{ mm}^{-1} \text{ cm}^{-1}$ for the equine cyt (11), and $19 \text{ mm}^{-1} \text{ cm}^{-1}$ for the bovine cyt.³

Electron acceptor pools are evaluated from the area “above” a plot of fluorescence vs. time (6, 12), as indicated by the shaded area labeled “pool” in the upper part of Fig. 3. The rationale is as follows. The number of electrons dQ transferred through the photochemical system into the pool in time dt is

$$dQ = I\phi_p dt, \quad (3)$$

where I is the rate of quantum absorption in the sample and ϕ_p is the mean quantum efficiency of the photochemistry. Set ϕ_p equal to $\phi_p(0)$ at the start of illumination. As times passes, some reaction centers enter the state P, A^- and lose their capacity for photochemistry. The quantum efficiency declines in proportion to this conversion. We assume that this is reflected directly by the rise in fluorescence; then if f is the fluorescence at any time t , and ϕ_p is the mean quantum efficiency at that time,

$$\phi_p = \phi_p(0) \left[\frac{f_{\text{max}} - f}{f_{\text{max}} - f_0} \right], \quad (4)$$

³ Smith, L. Verbal communication.

(refer to Fig. 1). When all the reaction centers have been converted to P, A⁻ the fluorescence has reached f_{\max} and $\phi_p = 0$. The total number of electrons Q that must be driven into the pool to achieve this is

$$Q = \int dQ = I \int \phi_p dt, \quad (5)$$

assuming that I remains constant.⁴ Substituting equation 4 in equation 5 we have

$$Q = I\phi_p(0) \int_{t=0}^{\infty} \left[\frac{f_{\max} - f}{f_{\max} - f_0} \right] dt. \quad (6)$$

The element of integration is a strip of height $f_{\max} - f$, normalized to the initial height $f_{\max} - f_0$, and width dt . The integral is thus the area bounded by the fluorescence curve (curve *b* in Fig. 1) and the line above it (f_{\max}), from $t = 0$ onward.

$$Q = I\phi_p(0) \times (\text{Area above fluorescence curve}). \quad (7)$$

In this treatment we have assumed that electrons do not "leak out" of the pool during the measurement; actually this assumption defines the pool as distinguished from its surroundings (oxidized cyt can be taken as part of the surroundings to which electrons flow from the pool). If electrons leave the pool at a nonnegligible rate, the system cannot be "pumped" completely to the level P, A⁻. Then the observed f_{\max} does not represent the greatest possible level of the fluorescence. This can be tested by adding Na₂S₂O₂ to induce the maximum possible level of fluorescence. Also one can measure f_{\max}/f_0 at several exciting light intensities and choose an intensity high enough to assure that f_{\max}/f_0 has reached its greatest possible value.⁵ "Leakiness" of the pool did not pose a problem when we used cyt c as electron donor.

The value chosen for $\phi_p(0)$ in this treatment might be based on the efficiency for oxidation of P870 in the absence of electron donor, as determined from the rate of bleaching of the 867 nm band. Alternatively it might be based on the ratio of f_{\max} to f_0 . The latter choice rests on the relationship

$$\phi_p(0) = 1 - f_0/f_{\max}, \quad (8)$$

or more generally,

$$\phi_p = 1 - f/f_{\max}, \quad (9)$$

⁴ The rate of quantum absorption is sensibly constant at a wavelength such as 800 nm, where active (P, A) and inactive (P, A⁻) reaction centers absorb about equally. In any case we could redefine the quantity I as the incident light intensity and compute ϕ_p as the number of electrons transferred per incident quantum.

⁵ If the pool is "leaky" so that the photochemical pumping is not complete in the light steady state, one can correct the measured area by dividing it by y^2 , where y is the fraction of reaction centers converted to P, A⁻ in the steady state. This correction factor can be determined by comparing the observed f_{\max} with the greatest possible f_{\max} , the latter generated by adding Na₂S₂O₄ to the preparation. We have not had to make this correction in our experiments with cyt c as electron donor.

which is valid if fluorescence and photochemical utilization are competing first-order processes by which excitation is quenched (13). This way of choosing ϕ_p is to be preferred, because equations 8 and 9 actually state the premise of our treatment of the fluorescence: that the rise in fluorescence measures the decline in photochemical efficiency that results when P, A is converted to P, A⁻. Note that if equation 6 is combined with equation 8, or equation 5 with equation 9, we have

$$Q = I \int_{t=0}^{\infty} (1 - f/f_{\max}) dt, \quad (10)$$

which employs the value of ϕ_p as dictated by the fluorescence.

RESULTS

Reaction centers made with 1% Triton X-100, and subjected to no purification beyond the initial centrifugation in the presence of the detergent, contained relatively large amounts of UQ, and perhaps other substances that could act as electron acceptors. This is evident in the behavior shown in Fig. 3, which shows a large quantity of cyt oxidized in the light (lower curve) and a large electron acceptor pool as indicated by the fluorescence (upper curve). This sample contained 0.45 μM reaction centers (based on $\epsilon = 113 \text{ mm}^{-1} \text{ cm}^{-1}$ at 867 nm); its content of native UQ was 9 μM . Numerical values (Q) of electron acceptor pools, based on the curves shown in Fig. 3 and on a replicate experiment, are shown in Table II. The smaller pool indicated under "light adapted" was measured after a period of illumination followed by 1 min in the dark, as shown in Fig. 3 (upper right). Conceivably it represents a state in which all secondary pools are filled, but the primary acceptor has unloaded its electron and can accept another. Primary and secondary acceptors can be distinguished more clearly in these measurements by adding *o*-phenanthroline; see the next paper in this series.

TABLE II
ELECTRON ACCEPTOR POOLS (Q) IN REACTION CENTERS
MADE FROM *R. SPHEROIDES* USING TRITON X-100*

| Experiment | Q from Δ (cyt) | Q from fluorescence using equation 10 | |
|------------|-------------------------|---|---------------|
| | | Dark adapted | Light adapted |
| | | $\mu\text{eq/liter}$ | |
| 1 (Fig. 3) | 27 | 21 | 0.42 |
| 2 | 22 | 16 | 0.32 |

* Based on the measurements shown in Fig. 3 and on a replicate experiment. Reaction centers, 0.45 μM .

Reaction centers made with LDAO and suspended in buffer with 0.1% LDAO responded as shown in Table III and in the lowest curve of Fig. 4. The oxidation of cyt showed a rapid phase, possibly reflecting transfer of electrons to the primary acceptor, and a slower phase that would correspond to a secondary acceptor in the reaction centers or in the medium around them.

These preparations fell into two categories. The first kind (top two rows in Table III) had a smaller electron acceptor pool, at least as measured by the oxidation of cyt, and a lower quantum efficiency for cyt oxidation (ϕ_{cyt}). The second kind, with a somewhat larger pool and higher ϕ_{cyt} , is represented by the last four rows in Table III. The two kinds of material also differed in the kinetics of recovery after the light-induced bleaching of P870, in the absence of cyt. The first kind (smaller pool) showed a single first-order component of about 0.1 sec half-time.¹ The second kind showed some proportion of slower recovery; usually 20–50% of the total. These differences suggest that the second kind of preparation was relatively more contaminated with secondary electron acceptors. The differences in the values of ϕ_{cyt} have no obvious explanation, but will be discussed later. In none of these measurements did any oxidized P870 accumulate during illumination as long as reduced cyt was present.

TABLE III
ELECTRON ACCEPTOR POOLS (Q) IN REACTION CENTERS MADE WITH LDAO, AND QUANTUM EFFICIENCIES FOR THE LIGHT-INDUCED OXIDATIONS OF CYT (ϕ_{cyt}) AND P870 (ϕ_p)*

| Preparation No. | Reaction center concn [†] | Type and concn of cyt c | Acceptor per reaction center, Q/P , from cyt oxidation | | Q/P from fluorescence, using equation 10 | $\frac{\phi_{\text{cyt}}}{\phi_p}$ [§] | ϕ_p |
|-----------------|------------------------------------|-------------------------|--|-------|--|---|------------------------|
| | | | Fast phase | Total | | | |
| | μM | μM | | | | | |
| I | 1.0 | Equine, 35 | 0.65 | 1.4 | | 0.55 | |
| II | 1.4 | Bovine, 40 | 0.68 | 1.7 | 0.8 | 0.49 | 0.9 |
| III | 0.7 | Equine, 35 | 1.3 | 1.8 | | 0.68 | |
| IV (Fig. 4) | 1.4 | Bovine, 40 | 1.1 | 2.2 | | 0.75 | |
| V | 1.0 | Bovine, 25 | 1.0 | 1.8 | | | |
| VI | 1.0 | Bovine, 25 | 1.5 | 2.0 | 1.25 | 0.75 | 1.0 |

*Reaction centers were suspended in 0.01 M Tris-Cl, pH 7.5, containing 0.1% LDAO and equine or bovine cyt c.

[†] Based on $\epsilon = 113 \text{ mM}^{-1} \text{ cm}^{-1}$ at 867 nm.

[§] From bleaching of the alpha band of cyt, measured at 550 nm.

^{||} From bleaching of P870 in the absence of cyt, using $\Delta\epsilon = 100 \text{ mM}^{-1} \text{ cm}^{-1}$ at 867 nm.

TABLE IV
 SAME AS TABLE III, LAST THREE ROWS BUT IN THE PRESENCE
 OF 1.0% LDAO

| Preparation No. | Q/P from cyt oxidation, fast phase | Q/P from fluorescence (equation 10) | $\frac{\phi_{\text{oyt}}}{\phi_p}$ | ϕ_p |
|-----------------|--|---|------------------------------------|----------|
| IV | 0.67 | | 0.58 | |
| V | 0.68 | | | |
| VI | 0.74 | 0.74 | 0.63 | 0.9 |

Material of the second kind could be made to behave like the first kind by raising the concentration of LDAO to 1% or more; see Table IV. Note that the preparations in this table are the same as the last three in Table III. At the higher concentration of LDAO a slow oxidation of cyt continued as long as the light was kept on, so it was difficult to assess the amount oxidized in relation to an acceptor pool in the reaction centers.

High concentration of LDAO also speeds the recovery of oxidized P870 to its reduced form,¹ and weakens the interaction between reaction centers and added UQ (see Clayton and Yau, to be published,¹ and later, this paper).

UQ, when added to reaction centers made with LDAO, amplified the electron acceptor pool to the extent of two electrons per UQ. This is shown by the three experiments listed in Table V. Preparations IV and VI were the ones listed as such in the previous tables; preparation VII contained 35 μM equine cyt c. The concentration of LDAO in these three experiments was 0.1%. The pools computed from analysis of the fluorescence are seen to be fairly consistent with those based on oxidation of cyt. Note that the addition of UQ appears to have raised ϕ_{oyt} without affecting ϕ_p , the quantum efficiency of P870 oxidation. Preparation VI with 5 μM UQ is the subject of Fig. 1.

Comparing Tables III and IV we saw that a high concentration of LDAO depressed ϕ_{oyt} relative to ϕ_p ; actually ϕ_p was not changed markedly by manipulating the concentration of either LDAO or added UQ. Table VI shows (with preparation IV) that UQ raised ϕ_{oyt}/ϕ_p , at high as well as low LDAO concentration. The depressing effect of high LDAO on ϕ_{oyt} was not as severe when UQ was present.

It was shown earlier that a high concentration of LDAO weakened the interaction between reaction centers and added UQ, judging from the kinetics of recovery (reduction) of oxidized P870. The recovery was slowed by UQ, but not when the LDAO concentration was raised to 1.5%. Fig. 4 (upper curves) shows the effect of a 10-fold increase in LDAO concentration on the photochemical transfer of electrons from cyt c to UQ, again using reaction center preparation IV. The stoichiometry, two cytochromes oxidized for each UQ added, was not altered, but the rate of the reaction was slower at the higher concentration of LDAO. Again the interaction with UQ appears to have been weakened by LDAO.

TABLE V
ELECTRON ACCEPTOR POOLS AND QUANTUM EFFICIENCIES IN
REACTION CENTERS MADE WITH LDAO, SHOWING THE
EFFECTS OF ADDED UQ

| Preparation No. | Reaction center concn | UQ added | Cyt c oxidized by light | Q from fluorescence (equation 10) | $\frac{\phi_{\text{cyt}}}{\phi_p}$ | ϕ_p |
|--------------------|-----------------------------|----------|----------------------------|---|------------------------------------|----------|
| | μM | μM | μM | | | |
| VII | 0.9 | 0 | 0.74 | | | |
| | | 0.25 | 0.97 | | | |
| | | 0.5 | 1.5 | | | |
| | | 1.0 | 2.5 | | | |
| | | 2.0 | 4.2 | | | |
| | | 4.0 | 8.8 | | | |
| IV (Fig. 4) | 1.4 | 0 | 3.1 | | 0.75 | |
| | | 3.0 | 8.3 | | 0.85 | |
| | | 7.0 | 14.5 | | 0.82 | |
| | | 10.0 | 20 | | 0.92 | |
| VI (Fig. 1) | 1.0 | 0 | | 1.25 | | 1.0 |
| | | 5.0 | | 11.3 | | 0.9 |
| | | 10.0 | | 18.6 | | 1.0 |

* Cyt c added, 25–40 μM (see text and earlier tables). LDAO, 0.1%.

TABLE VI
EFFECTS OF UQ AND LDAO ON THE RELATIVE
QUANTUM EFFICIENCIES FOR OXIDATION OF
CYT C AND P870 BY ILLUMINATED
REACTION CENTERS*

| LADO concn | UQ concn | $\frac{\phi_{\text{cyt}}}{\phi_p}$ |
|------------|----------|------------------------------------|
| % | μM | |
| 0.1 | 0 | 0.75 |
| 1.0 | 0 | 0.58 |
| 0.1 | 10 | 0.92 |
| 1.0 | 10 | 0.76 |
| 0.03 | 4 | 0.88 |
| 0.3 | 4 | 0.79 |
| 1.0 | 4 | 0.75 |

* Preparation IV (see earlier tables), reaction centers 1.4 μM . Bovine cyt c, 40 μM .

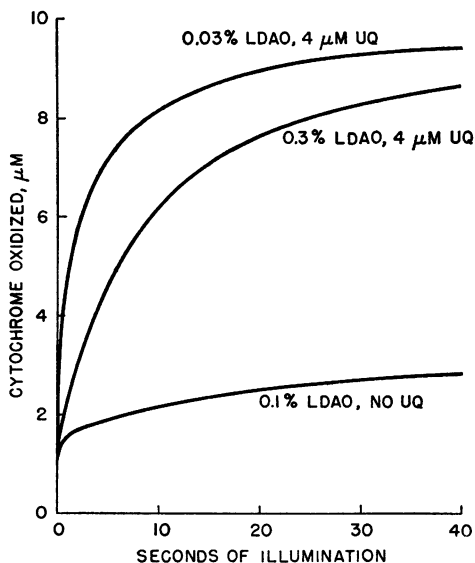


FIGURE 4 Light-induced oxidation of cyt by reaction centers made with LDAO, showing effects of added UQ and of different concentrations of LDAO. Reaction centers (preparation IV in Tables III–VI), $1.4 \mu\text{M}$, with $40 \mu\text{M}$ bovine cyt c in 0.01 M Tris-Cl, pH 7.5. Concentrations of UQ and LDAO are shown.

The transfer of electrons from cyt to UQ is attended by absorbancy changes in the ultraviolet as well as at 550 nm . Fig. 5 (upper curve) shows the change in absorption spectrum caused by illuminating a sample containing $10 \mu\text{M}$ bovine cyt c and $5 \mu\text{M}$ UQ, with reaction centers at $0.7 \mu\text{M}$ and 0.1% LDAO. Any changes due to components of the reaction centers would be small compared with the ones shown, which reflect mainly the oxidation of cyt and the reduction of UQ. The change in OD at 550 nm showed that $9.4 \mu\text{M}$ cyt was oxidized. The changes shown in the upper curve are fairly consistent with the expected changes for the oxidation of $9.4 \mu\text{M}$ cyt (lower solid curve) and the reduction of an equivalent concentration, $4.7 \mu\text{M}$, of UQ (lower dashed curve).

Returning to a consideration of Figs. 1 and 3, we note that after illumination, the higher yield of fluorescence induced by light subsided and eventually returned to the lower level f_0 . In preparations with reduced cyt as electron donor, the recovery of low fluorescence matched the recovering capacity for light-induced oxidation of more cyt (fast phase) and also for light-induced ΔOD 's related to the reducing side of the photochemical system (14). The correspondence between fluorescence and capacity for cyt oxidation was verified for reaction centers made with either Triton X-100 or LDAO; only the latter were used to examine the relationship with the other ΔOD 's (14).

A mixture of PMS (about $20\text{--}500 \mu\text{M}$) and sodium ascorbate (1 mM) could be used

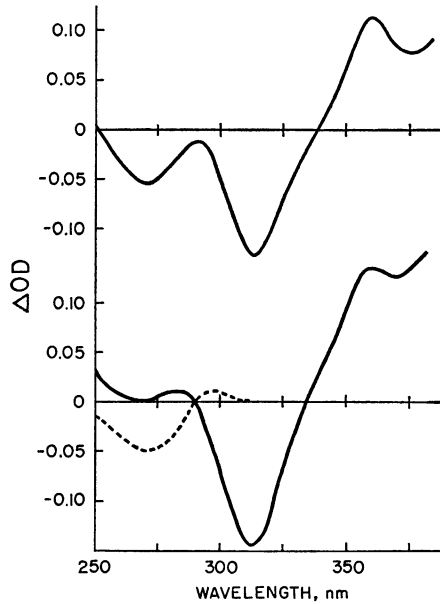


FIGURE 5 Upper curve, spectrum of light-induced changes in a sample containing $0.7 \mu\text{M}$ reaction centers (made with LDAO), 0.1% LDAO, $10 \mu\text{M}$ bovine cyt c, and $5 \mu\text{M}$ UQ. Spectra were measured in a Cary 14R spectrophotometer (1 cm path), before and during illumination of the sample with 800 nm light at 2.2 mw/cm^2 . The difference between the two spectra is shown here. The change at 550 nm showed a light-induced oxidation of $9.4 \mu\text{M}$ cyt c. Lower curves, difference spectra for the oxidation of $9.4 \mu\text{M}$ bovine cyt c (solid curve) and reduction of $4.7 \mu\text{M}$ UQ (dashed curve).

in place of reduced cyt as an electron-donating system with reaction centers, but PMS appeared to have an anomalous quenching effect on the fluorescence of P870. At $50 \mu\text{M}$ concentration it caused a reduction of 20–50% in both f_0 and f_{max} .

DISCUSSION

To a first approximation the foregoing results indicate that each reaction center contains a primary photochemical electron acceptor, able to hold just one electron, distinct from secondary acceptors. Native or added UQ can contribute to the secondary pool, with the expected stoichiometry of two electrons per UQ. This picture is supported by light-induced variations in the yield of P870 fluorescence as well as by the time course of cyt oxidation.

The exact conclusions depend on the choice of optical parameters used in the computations. For example, if we had chosen four BChl per reaction center rather than three, with $\Delta\epsilon (867 \text{ nm}) = 133$ rather than $100 \text{ mM}^{-1} \text{ cm}^{-1}$ (see Table I), we would have computed ϕ_p to be about 0.7 rather than 0.9–1.0. A decided advantage of this revised choice is that the observed changes in fluorescence would become compatible with equation 8, since f_0/f_{max} was generally found to be 0.3. Under this

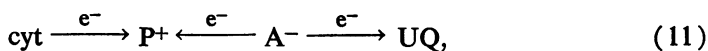
assumption the computed values of Q/P and ϕ_{cyt}/ϕ_p , as in Tables III and IV, would be 33% greater. In the first two rows of Table III, Q/P (from the fast phase of cyt oxidation) would become 0.9 while ϕ_{cyt}/ϕ_p would become 0.7. Similarly in Table IV the revised values of Q/P would be 0.9–1.0, and ϕ_{cyt}/ϕ_p would be 0.8.

Any downward revision of ϕ_p , however, as by the choice of four BChl per reaction center, would reflect in an embarrassing way on earlier reports (1–3) of the efficiency of cyt oxidation by illuminated chromatophores and reaction centers. In those reports ϕ_{cyt} was given to be 0.9–1.0, and equal to ϕ_p if the latter was computed from $\Delta\epsilon = 90\text{--}100 \text{ mM}^{-1} \text{ cm}^{-1}$ at the wavelength of maximum bleaching of P870. It appeared that every cyt was oxidized through a reaction with P870⁺. If we now accept $\Delta\epsilon (867 \text{ nm}) = 133 \text{ mM}^{-1} \text{ cm}^{-1}$ (Table I) we are led to conclude that in some cases the oxidation of cyt was more efficient than the oxidation of P870.

A comprehensive reevaluation of these questions should await a new and more accurate determination of the extinction coefficients of reaction centers. At present these coefficients are based on measurements made with relatively crude materials (15; see also Appendix, this paper).

Irrespective of the choice of optical parameters, we must deal with the fact that in reaction centers made with LDAO and exposed to reduced cyt c, the addition of UQ raises the apparent quantum efficiency of cyt oxidation, while a high concentration of LDAO lowers ϕ_{cyt} . Measurements of the same preparations without cyt showed that ϕ_p is unaffected by UQ or LDAO concentration. The addition of reduced cyt probably does not change ϕ_p , because the initial level of P870 fluorescence, f_0 in Fig. 1, is the same whether cyt is present or not. Finally let us note that no accumulation of P870⁺ was observed during illumination as long as reduced cyt was present.

One could argue that a rapid internal reaction (a cycle or a back reaction) between P870⁺ and electrons in the reaction centers competes with a reaction between P870⁺ and cyt,



and the balance of this competition is shifted by UQ or LDAO. In particular, UQ retards the flow of "internal" electrons to P⁺ and allows the cyt to compete more favorably as electron donor. This explanation encounters one difficulty. If an internal reaction is to compete significantly with cyt oxidation, it must be rapid enough to prevent the observation of P870⁺ under our experimental conditions. Now, in preparations without added cyt we can observe the kinetics of "internal" electron transfer to P⁺; this reaction has a half-time of 20 msec or more.¹ It is not so fast that it prevents the observation of P⁺. If there were a faster internal reaction, undetected with our spectrophotometer, it should have some effects on the observed kinetics and the apparent quantum efficiency of P870 oxidation. These effects should vary with UQ and LDAO concentration, and with the intensity of the exciting light. No such effects were observed.

We conclude that there is no "hidden, very fast" internal reaction between P870⁺ and electrons in reaction centers without added cyt. Whether such a reaction could be introduced by the addition of reduced cyt, and modulated further by UQ or LDAO, remains open to speculation. One could argue that a component in the reaction centers becomes reduced when the reduced cyt is added, and this component then becomes an effective electron donor to P870⁺. Alternatively the presence of cyt could alter the physical state of the reaction centers so as to speed an internal electron transfer. Some such explanation seems to be required to explain the variations in $\phi_{\text{cyt}}/\phi_{\text{P}}$.

It was noted that in reaction centers with added reduced cyt, the recovery of low fluorescence after illumination matched the restoration of the capacity for light-induced cyt oxidation (fast phase) and for light-induced ΔOD 's related to the reducing side of the photosystem (14). All of these phenomena appeared to signal the recovery of the primary electron acceptor to its oxidized (functional) form. In reaction centers made with Triton X-100, the secondary pool remained in a reduced state long after the primary acceptor appeared to have recovered; see the two fluorescence transients at the top of Fig. 3.

We conclude that each reaction center from *R. spheroides* contains a primary photochemical electron acceptor capable of holding one electron, distinct from a secondary electron acceptor pool that consists largely of UQ. The secondary pool can be simulated by adding UQ to reaction centers from which the native UQ had first been removed.

The changes of P870 fluorescence during illumination are consistent with this view, and indicate that the quantum efficiency for the primary photochemistry might be about 0.7 rather than 0.9–1.0 as concluded from optical absorption measurements.

We are indebted to Dr. D. C. Mauzerall for communicating the results of some recent analyses of the pigments in reaction centers.

Some of the experiments described here have been performed by students of the Physiology Course at the Marine Biological Laboratory, Woods Hole, Mass., during the summers of 1969–1971.

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APPENDIX

Spectroscopic Analysis of Reaction Centers

Absorption spectra of reaction centers from *R. spheroides* show bands at 757, 802, and 867 nm, with areas in roughly 1:2:1 proportions. In the past the 802 and 867 nm bands have been attributed (15) to two molecules of P800 and one of P870 respectively, but it is probably not realistic to identify these bands with distinct molecules. More properly the pair of bands⁶

⁶ There may be more than two bands due to BChl; Feher (18) has reported a shoulder on the long

should be regarded as the joint property of a set of closely interacting BChl molecules in the reaction center. It remains to be settled whether the number of such molecules is three, as we have assumed in the past, or some other small integer (see later).

The 757 nm band can be ascribed separately to bacteriopheophytin (BPh). In earlier spectroscopic investigations (15) one of us (R. K. C.) found that the extraction of reaction center pigments with acetone and methanol yielded only substances with the absorption spectra of BPh and BChl, plus a degradation product (probably oxidized BChl) with an absorption peak at 680 nm. The amount of BPh obtained in the extract was commensurate with the height of the 757 nm band in the reaction centers. This BPh was considered at the time to be a contaminant, but it appears now to be an intrinsic part of the reaction center. The ratio of BPh to BChl has approached a constant value ($OD\ 757/OD\ 867 = 1.1$) in ever more refined reaction centers, and this ratio in reaction centers from *Rhodospirillum rubrum* is the same as in those from *R. spheroides*.⁷

Let us now define a reaction center as that constellation of molecules that can effect the photochemical transfer of a single electron, possibly (but not necessarily) to a distinct acceptor molecule. The choice of a "one-electron" photochemistry is based on the following facts.

The light-induced change of the absorption spectrum, mainly a bleaching of the 867 nm band, is mimicked by chemical oxidation as with ferricyanide (16). A redox titration curve for the absorbancy change has the one-electron shape; the reduced and oxidized states differ by one electron (midpoint potential about 0.45 v) (16, 17). If a partial conversion (partial bleaching of the 867 nm band) is effected by oxidation with ferricyanide, the remainder shows a seemingly normal light reaction (unpublished experiment in our laboratory). Thus the chemical removal of a single electron from the set of chromophores comprising a reaction center has the same effect on these chromophores as the photochemical reaction.⁸

We may now ask how many molecules of BChl and of BPh are in each reaction center.

Initially we assigned three BChl to each reaction center because the 802 nm and 867 nm bands have areas in about 2:1 ratio. Also the extraction of oxidized reaction centers, in which the 867 nm band (but not the 802 nm band) was bleached, yielded just two-thirds as much BChl as the extraction of "normal" (unbleached) reaction centers (15). In these experiments ferricyanide had been used to bleach the 867 nm band in the reaction centers. Now S. C. Straley⁹ has achieved the same result in our laboratory, using light rather than ferricyanide to effect the bleaching. Straley confirmed the finding that beached reaction centers yielded two-thirds as much BChl into a methanolic extract as did unbleached reaction centers. She also showed that if ascorbate was added to the extract of bleached reaction centers, the long wave absorption band of BChl (near 770 nm) increased and equaled that in the extract of unbleached reaction centers. This indicated that the extract of photo-bleached reaction centers contained oxidized BChl, which could be reduced by adding ascorbate.

Recently Feher (18) obtained a ratio of one Fe to five Mg atoms in extensively dialyzed reaction centers, suggesting five BChl rather than three if the Fe is assigned a specific function.

wave side of the 802 nm band as well as the usual 867 nm band. We have found that this shoulder is evident in some preparations but not in others; where it seems missing it may be hidden beneath the main 802 nm band.

⁷ Wang, R. T. Unpublished experiments in our laboratory.

⁸ There are lesser effects, especially a bathchromic shift of the 757 nm band (unpublished experiments by S. C. Straley), that appear to be associated with the reduced photoproduct, or with electric fields from both the positively and negatively charged products. Because of these effects, the difference spectra induced by light may differ very slightly from those induced by chemical oxidation, especially between 700 and 800 nm.

⁹ Straley, S. C. Unpublished experiments.

Finally, an estimate of the number of chromophores per reaction center can be based on the ratio of BPh to BChl. Absorption spectra of reaction centers show a band at about 535 nm, attributable to BPh. This rather broad band is resolved at liquid nitrogen temperature into two peaks of equal height, at 530 and 543 nm, suggesting that each reaction center contains two molecules of BPh in slightly different physical states. Mauzerall,¹⁰ using contemporary reaction center preparations, has confirmed that the principal chromophores are indeed BChl and BPh, and has found a ratio of one BPh to two BChl. Straley⁹ has confirmed this finding also. On this basis each reaction center contains two BPh and four BChl.

From the foregoing observations and arguments we should consider the possibility of three, four, or five BChl per reaction center. Let us now compute extinction coefficients corresponding to these values. A reaction center sample in which the concentration of BChl (based on extraction) is 1.0 μM shows, for 1 cm path, an OD of 0.088 at the 802 nm peak ("P800") and 0.038 at the 867 nm peak ("P870") (15). If there are three BChl per reaction center, the concentration of reaction centers in this sample is $\frac{1}{3}$ μM . Then at 1.0 μM concentration of reaction centers, the OD would be 3×0.038 or 0.113 at 867 nm. Thus the extinction coefficient (ϵ) of reaction centers at 867 nm is $113 \text{ mm}^{-1}\text{cm}^{-1}$ if each reaction center contains three BChl molecules. If each reaction center contained four or five BChl, the sample of 1.0 μM BChl concentration would be judged to contain $\frac{1}{4}$ or $\frac{1}{5}$ μM reaction centers, and ϵ at 867 nm would be computed to be 151 or $189 \text{ mm}^{-1}\text{cm}^{-1}$, based on the reaction center concentration.

For convenience we will continue to call the 867 nm band P870, and because this band suffers the most radical change when an electron is removed from the pigment complex, we will speak of the oxidation and bleaching of P870. We should remember, however, that it is only a primitive abstraction to identify this band exclusively with one BChl molecule, independent of the others in the reaction center.

In determining the quantum efficiency of P870 oxidation we measure the rate at which the 867 nm band is bleached. We then apply the computation

$$\Delta C = \Delta\text{OD}/\Delta\epsilon, \quad (12)$$

where ΔC is the concentration of reaction centers converted (millimoles per liter), ΔOD is the change in optical density measured with 1 cm path, and $\Delta\epsilon$ is the change in extinction coefficient [(millimoles per liter)⁻¹ \times centimeters⁻¹], at the wavelength of measurement, that attends the reaction. The change in absorption spectrum shows an 88% loss of OD at the 867 nm peak, so the differential extinction coefficient $\Delta\epsilon$ is 88% of the absolute coefficient ϵ at that wavelength. The value of $\Delta\epsilon$ at 867 nm is therefore 100, 133, or $167 \text{ mm}^{-1}\text{cm}^{-1}$ assuming three, four, or five BChl per reaction center. If a quantum efficiency were computed to be 1.00 on the basis of three BChl per reaction center, the computed efficiency would be 0.75 or 0.60 on the basis of four or five BChl per reaction center.

The molecular weight of a reaction center, based on the dry weight per mole of BChl, has been estimated (18) to be about 80,000 assuming three BChl per reaction center. The assumptions of four or five BChl per reaction center would then lead to molecular weights of about 107,000 or 133,000 respectively. These values of molecular weight are all more or less consistent with a particle diameter of about 80 Å as seen in the electron microscope (18). Thus the reaction center as defined functionally seems to coincide with the morphological entity.

The foregoing parameters are summarized in Table I in the Materials and Methods section.

¹⁰ Mauzerall, D. C. Verbal communication.

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