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

III. Effects of Orthophenanthroline and Other Chemicals

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ABSTRACT Reaction centers from *Rhodopseudomonas spheroides* mediate the photochemical oxidation of cytochrome c (cyt c), and show a time-varying fluorescence of P870. Analyses of these effects indicate that the reaction centers contain a primary photochemical electron acceptor capable of holding one electron. Native or added ubiquinone (UQ) can act as a secondary electron acceptor. Orthophenanthroline (*o*-phen) blocks electron transfer from primary to secondary acceptors, and allows the primary acceptor to be exhibited in the foregoing experiments. Other chelators (with the possible exception of 8-hydroxyquinoline) and dichlorophenyldimethylurea (DCMU) are without apparent effect on reaction centers. *o*-Phen also inhibits the primary photochemical act in reaction centers; this effect is prevented by the presence of UQ. 2-*n*-Nonyl-4-hydroxyquinoline-*N*-oxide (NQNO) inhibits the primary photochemistry in reaction centers but does not affect secondary electron transfer.

INTRODUCTION

Two other communications (1)[1] have dealt with the photochemical oxidation of P870 in photosynthetic reaction centers prepared from *R. spheroides*, and with interactions between the primary photoproducts and secondary electron donors and acceptors. UQ was shown to retard the return of electrons to oxidized P870, apparently by acting as a secondary electron acceptor. Denoting the hypothetical primary photochemical electron acceptor by A, and representing electron transfers by arrows, we can entertain the following speculations for the interaction between

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

UQ and reaction centers:

$$\begin{array}{ccc} \text{P870} & \xrightarrow{h\nu} & \text{A} \\ \uparrow & & \downarrow \\ \text{UQ} & \longleftarrow & \end{array} \quad \text{or} \quad \text{P870} \xrightarrow{h\nu} \text{A} \rightleftarrows \text{UQ} \qquad (1)$$

Reduced cyt c, or alternatively reduced phenazine methosulfate (PMS), can donate electrons to oxidized P870, and with repeated photochemical cycling the oxidation of excess cyt can be coupled to the reduction of an equivalent amount of UQ:

$$\text{cyt} \rightarrow \text{P870} \xrightarrow{h\nu} \text{A} \rightarrow \text{UQ}, \qquad (2)$$

where again the arrows represent electron transfers.

We will report here some effects of *o*-phen and a few other substances on the foregoing reactions.

MATERIALS AND METHODS

Reaction centers were prepared from blue-green mutant *R. spheroides*, using either Triton X-100 or lauryl dimethylamine oxide (LDAO), as described elsewhere (2, 3).[1] The reaction centers made with Triton X-100 contained secondary electron acceptor molecules, mainly UQ, that were lacking in the reaction centers made with LDAO. For use in experiments the reaction centers were suspended in water containing 0.01 M Tris-Cl, pH 7.5, and suitable concentrations of either Triton X-100 or LDAO.

Sigma Chemical Co. (St. Louis, Mo.) was the source of all reagents, including 1,10-phenanthroline monohydrate or *o*-phen, except for the following donations: bovine cyt c and NQNO were from Dr. Lucile Smith; Tiron, $(HO)_2(C_6H_4)(SO_3^-)_2$, was from Dr. A. T. Jagendorf; LDAO was from the Research Department of Onyx Chemical Corporation, Jersey City, N. J.

Because of their sparing solubilities in water, certain reagents were dissolved first in ethanol and then diluted (25-fold–100-fold) into the suspension of reaction centers. These reagents included Antimycin A, DCMU, UQ, *o*-phen, NQNO, and 8-hydroxyquinoline. Measurements involving the addition of reagents in ethanol were attended by controls in which the same amount of ethanol was added.

For experiments with bacteriochlorophyll (BChl) in vitro, the pigment was extracted from the light-harvesting membrane fraction of carotenoidless mutant *R. spheroides*. This fraction is a by-product in the preparation of reaction centers. The extraction was made with methanol; the concentrated methanolic solution of BChl was diluted into water containing 0.15% LDAO and 0.01 M Tris-Cl, pH 7.5, for use in photochemical experiments.

Concentrations of reaction centers were based on an extinction coefficient $\epsilon = 113$ mM^{-1} cm^{-1} at 867 nm (1, 4). Quantum efficiencies for the oxidation of P870 were computed from initial slopes of the light-induced bleaching of P870, assuming[2] $\Delta\epsilon = 100$ mM^{-1}cm^{-1} for the ΔOD at 867 nm. Concentrations of cyt c and quantum efficiencies for its oxidation were

[2] As discussed earlier (1), these values of ϵ and $\Delta\epsilon$ might properly be revised to 151 and 133 mM^{-1}cm^{-1} respectively. If so, the computed concentrations of reaction centers and quantum efficiencies for P870 oxidation would be less by a factor of 0.75.

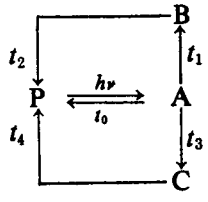
based on $\Delta\epsilon$ (reduced minus oxidized, 550 nm) = 17 $mM^{-1}cm^{-1}$ for equine cyt c, and 19 mM^{-1} cm^{-1} for bovine cyt c (5).[3]

Absorption spectra were measured with a Cary 14R spectrophotometer (Cary Instruments, Monrovia, Calif.). Oxidations of P870 and cyt c were monitored by changes in optical density (OD) at 863 and 550 nm respectively; these light-induced reactions were measured with a split-beam differential spectrophotometer described elsewhere (6).[1] Fluorescence emitted by P870 was measured at 900 nm, using a fluorimeter described earlier (1). Signal-to-noise ratios were as in reference 1 and Clayton and Yau (to be published[1]).

RESULTS AND DISCUSSION

Kinetics of Oxidation and Reduction of P870 As Affected by o-Phen

Fig. 1 (solid curves) shows the light-induced bleaching (oxidation) and recovery of P870 in reaction centers made with Triton X-100, as reported elsewhere.[1] The longer the exciting light was left on, the greater the proportion of slow recovery. This behavior could be explained by the gradual filling of "deep" electron traps during continued photochemical cycling. To account also for a much faster recovery observed under some conditions (for example, at low temperatures), a model was proposed as follows (arrows represent electron transfers):



$$\begin{aligned} t_0 &= \ 0.02-0.1 \text{ sec}, \\ t_1 &\ll t_0, \\ t_2 &= \ 1-5 \text{ sec}, \\ t_3 &\gg t_1, \\ t_4 &= \ 1 \text{ min or more}. \end{aligned} \qquad (3)$$

Electrons are driven photochemically from P870 to the primary acceptor A; they return to P870 either directly or by way of secondary electron acceptors B or C. These secondary acceptors mediate the relatively slow recovery shown by the solid curves in Fig. 1.

Addition of *o*-phen (1 mM) to the reaction centers described in Fig. 1 changed the pattern of bleaching and recovery to the one shown by the dashed curves. After a very short exposure to light the recovery of P870 was very fast, with half-time about 0.06 sec, perhaps corresponding to the direct return of an electron from reduced A to oxidized P870 (t_0 in reaction 3). With longer illumination the quasi-steady-state amplitude of the photo-bleaching increased, as slower components developed in the recovery pattern. Note that the slow recovery paralleled exactly the slower recovery in reaction centers without *o*-phen. This behavior was consistent with the following explanation for the action of *o*-phen. The *o*-phen inhibits electron transfer from the primary acceptor A to secondary acceptors, encouraging a rapid return of electrons from A to oxidized P870. This is similar to the supposed action of DCMU on green

[3] Smith, L. Verbal communication.

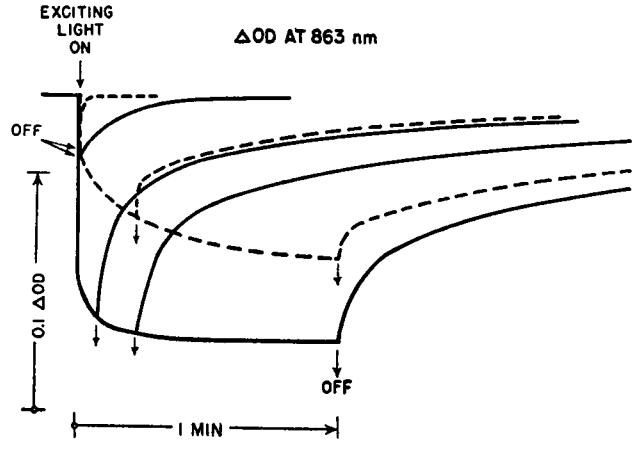


FIGURE 1 Light-induced bleaching (oxidation) and recovery of P870 in reaction centers made from *R. spheroides* using Triton X-100. Curves show the ΔOD at 863 nm. Exciting light (800 nm, 3 mw/cm^2) was turned off at different times in replicate measurements; the traces are superimposed here. Reaction centers, 1.5 μM, in 0.01 M Tris-Cl buffer, pH 7.5, with 0.05% Triton X-100. Solid curves, no other additions (same as in Fig. 3 of Clayton and Yaw[1]); dashed curves, 1 mM *o*-phen added.

plant photosystem II (7), and of *o*-phen on both green plant and bacterial photosystems (8–10). With continued photochemical cycling some electrons get past the "*o*-phen block" and move to the secondary acceptors; the return of such electrons to P870 follows the same time course as if *o*-phen were absent. This last fact supports reaction 3 as an elaboration of the first of the two alternatives shown by reaction 1, rather than any possible elaboration of the second alternative in reaction 1.

The effect of *o*-phen on the bleaching and recovery of P870 in chromatophores from *Rhodospirillum rubrum* was observed to be similar to that in reaction centers from *R. spheroides*. In both chromatophores and reaction centers, an effect of *o*-phen could be detected at 3×10^{-6} M and a roughly half-maximal effect was produced by 10^{-4} M *o*-phen. In these tests the reaction centers were at 1.0 μM concentration and the chromatophore suspensions were adjusted to contain about 0.2 μM P870.

Using reaction centers made with LDAO we could show that *o*-phen blocks the interaction between reaction centers and added UQ. This is shown in Fig. 2, where we describe the flash-induced bleaching and subsequent recovery of P870. Curve A is for reaction centers (2 μM) in buffer with 0.05% LDAO. The recovery was rapid, with half-time 0.11 sec. Curve B shows the slower recovery that resulted when 20 μM UQ was added; the UQ appeared to act as a secondary electron acceptor. Curve C shows that when 1 mM *o*-phen was added after the UQ, the P870 recovered rapidly once more. The apparent functioning of UQ as a secondary acceptor was abolished by *o*-phen. In fact, the recovery in curve C (half-time 0.07 sec) was faster than in

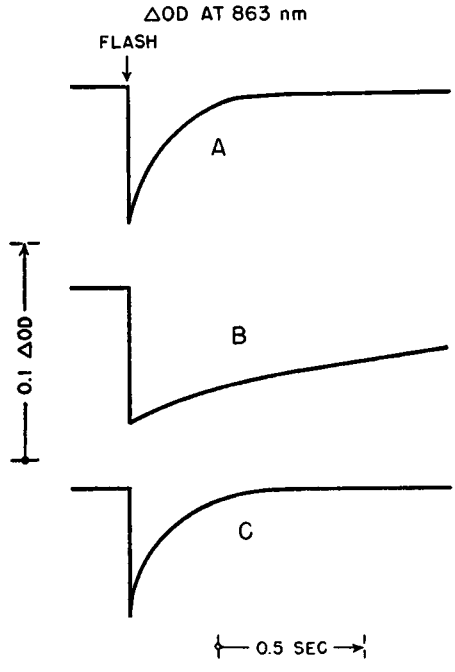


FIGURE 2 Flash-induced bleaching and recovery of P870 in reaction centers made with LDAO. Exciting flash, 800 nm; duration, 1 msec. Reaction centers, 2.0 μM, in buffer with 0.05% LDAO. A, no other additions; B, 20 μM UQ added; C, 1 mM *o*-phen added after the UQ.

curve A, as if the *o*-phen had nullified the small effect of an endogenous secondary acceptor in the reaction centers. It is shown elsewhere[1] that several other treatments, all designed to eliminate the participation of secondary electron acceptors, caused the recovery of P870 to acquire a half-time of 0.06–0.07 sec at room temperature. We take this to be the time constant t_0 in reaction 3.

Even faster recovery of P870 could be induced by adding PMS to the reaction centers. This reagent, added in its oxidized form, seemed to act as a highly effective carrier of electrons from the reducing side of the photosystem to oxidized P870 (compare reference 11). Fig. 3 shows effects of PMS (0.5 mM) and *o*-phen (0.3 mM) on the flash-induced bleaching and recovery of P870 in reaction centers made with LDAO, suspended with 0.25% LDAO and 20 μM UQ. Similar but less striking effects were observed when the UQ was left out. Comparing curve B with curve A we see that PMS caused a relatively fast return of electrons to oxidized P870, especially eliminating all very slow kinetic components. Curve C (compared with curve A) shows the speeding effect of *o*-phen at 0.3 mM concentration. Curve D shows that the recovery was fastest (half-time about 0.04 sec) when both PMS and *o*-phen were present. Evidently the PMS could react effectively with reduced primary acceptor, or with substances proximal to the block imposed by *o*-phen, speeding the return of

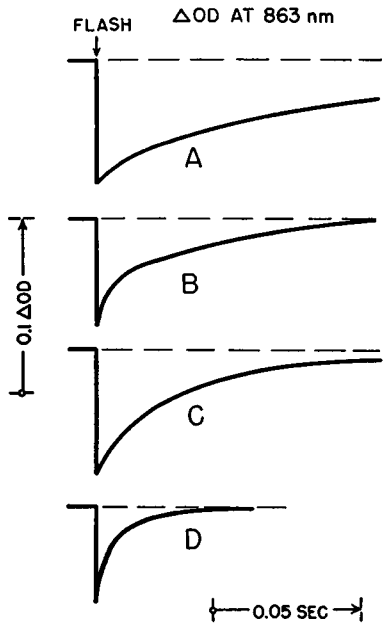


FIGURE 3 Measurements made as in Fig. 2. Reaction centers, 2.0 μM, in buffer with 0.25% LDAO and 20 μM UQ. A, no other additions; B, 0.5 mM PMS (oxidized form) added; C, 0.3 mM *o*-phen (but no PMS) added; D, 0.5 mM PMS and 0.3 mM *o*-phen added.

electrons from these substances to oxidized P870. Electrons in secondary acceptor pools could not be returned as rapidly by PMS (compare curves B and D). Other interpretations of these kinetic patterns are no doubt possible, but the foregoing seems the most simple and natural.

In contrast to oxidized PMS, the addition of ferricyanide acted only to retard the recovery of oxidized P870 to its reduced form, and ferrocyanide did not speed this recovery.[1] Apparently the ferricyanide can take electrons from the reducing side of the photosystem, but ferrocyanide is a poor donor to oxidized P870 whereas reduced PMS is an excellent donor.

Electron Acceptor Pools As Revealed in the Presence of o-Phen

We showed earlier (1) that when reduced cyt c was added as a donor of electrons to oxidized P870, as in reaction 2, the magnitudes of electron acceptor pools associated with each reaction center could be assayed, both from the extent of the light-induced cyt oxidation and from the transient changes in the fluorescence of P870. We have found that when these measurements are repeated with *o*-phen present, a distinction can apparently be drawn between primary and secondary electron acceptor pools. This technique automatically defines "primary" and "secondary" as proximal and distal, respectively, to the site of the *o*-phen block. We will see that the primary ac-

ceptor pool, as defined in this way, amounts to just one electron per P870 (or per reaction center).

Let us consider first the photochemical oxidation of cyt c by reaction centers made with Triton X-100, as shown in Fig. 4 (the same preparation was described by Fig. 3 in reference 1). The amount of cyt oxidized (lower curves) was about 60 molecules per reaction center. The addition of 1 mM *o*-phen (dashed curve) slowed the reaction, presumably by limiting the rate at which electrons could leave the primary acceptor and go on to secondary acceptors. With *o*-phen present, the onset of light-induced cyt oxidation was biphasic; there was an initial "rush" followed by a slower reaction. The initial fast phase amounted to 0.78 electrons per reaction center in this experiment. We assume that this fast phase reveals the transfer of an electron from cyt c, through P870, to the primary acceptor A (see reaction 2). The slower but much larger phase reflects the continued photochemical driving of electrons into secondary acceptor pools; the rate of this process gives a quantitative measure of the effectiveness of the *o*-phen block.

A similar but less striking result was obtained with reaction centers made with LDAO (Fig. 5; here the oxidation of cyt is plotted in terms of μM concentration rather than ΔOD at 550 nm). The more rapid phase revealed in the presence of *o*-

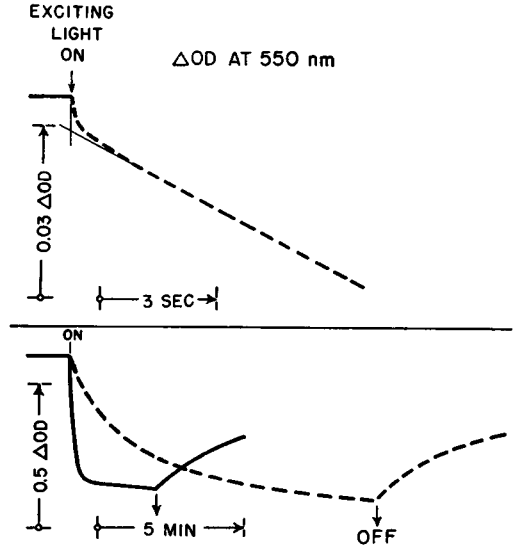


FIGURE 4 Light-induced oxidation of cyt c added to reaction centers made with Triton X-100. Reaction centers, 0.45 μM, in buffer with 0.05% Triton X-100 and 50 μM equine cyt c. Endogenous UQ content 9 μM. Cyt oxidation was measured by the ΔOD at 550 nm. Exciting light 800 nm, 2.2 mw/cm^2. Solid curve, no other additions (same as in Fig. 3, reference 1); dashed curves, with 1 mM *o*-phen. In the upper curve the scale is expanded to show the earliest part of the reaction.

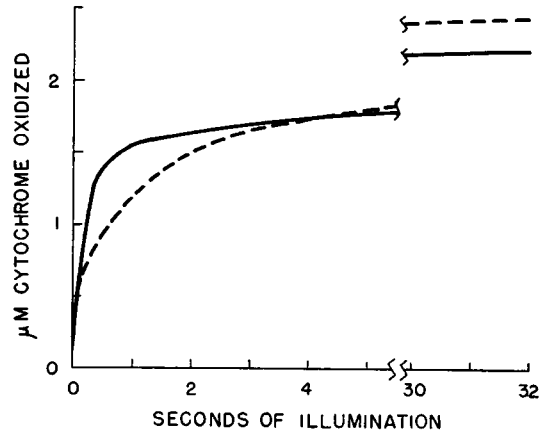


FIGURE 5 Similar to Fig. 4, but with reaction centers made with LDAO and suspended at 1.0 μM concentration in buffer with 0.05% LDAO and 25 μM equine cyt c. The ordinate shows μM cyt oxidized as computed from the ΔOD at 550 nm. Exciting light 810 nm, 5 mw/cm². Solid curve, no additions; dashed curve, with 1 mM *o*-phen.

phen amounted to about 0.7 cyt's oxidized per reaction center. Without *o*-phen about 1.4 cyt's per reaction center were oxidized rapidly and an additional 0.9 much more slowly.

These values for the rapid phase of cyt oxidation, 0.78 and 0.7 per reaction center, were based on reaction center concentrations computed from "ϵ = 113 $mM^{-1}cm^{-1}$ at 867 nm" (see Materials and Methods). A recent reevaluation (1) suggests that ϵ might instead be taken as 151 $mM^{-1}cm^{-1}$. This would change our estimates of reaction center concentration, making the fast phase of cyt oxidation amount to 1.0 and 0.9 per reaction center in the cases shown in Figs. 4 and 5 respectively. These experiments therefore suggest that each reaction center contains a primary photochemical electron acceptor capable of holding one electron. The block imposed by *o*-phen is between the primary and all secondary acceptors, but *o*-phen does not inhibit the direct return of electrons from A to oxidized P870.

The foregoing results were verified in numerous experiments, some of which are listed in Table I. In some cases the rapid phase of cyt oxidation corresponded to only about 0.4 per reaction center (reaction center concentration based on ϵ = 113 $mM^{-1}cm^{-1}$ at 867 nm), but in these cases the *o*-phen had inactivated some of the reaction centers (see later). This inactivation is indicated in Table I by a lesser quantum efficiency of P870 oxidation (column headed ϕ_p). When the inactivation is taken into account, all of the data support the existence of a primary acceptor capable of holding one electron.

When reaction centers are treated with an electron donor such as reduced cyt c, the fluorescence of P870 rises during illumination (1, 12). As with green plant photosystem II, the rise apparently reflects the conversion of reaction centers to the

TABLE I

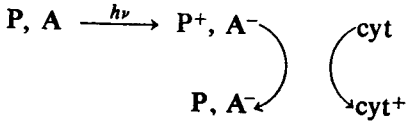
EXTENT OF THE LIGHT-INDUCED OXIDATION OF CYT C ADDED TO REACTION CENTERS FROM *R. SPHEROIDES*, AND QUANTUM EFFICIENCIES ϕ_p AND ϕ_{cyt} FOR THE OXIDATIONS OF P870 AND CYT C*

Prepara-tion	Reac-tion center concn	Cyt c, type and concn	Detergent‡ type and concn	*o*-phen	UQ	Cyt oxidized, molecules per reaction center		ϕ_p	$\frac{\phi_c}{\phi}$
						Fast phase	Total		
	μM	μM	%	mM	μM				
As in Fig. 4	0.45	Equine, 50	Triton X-100, 0.05	0	(native)		60	0.92	
				1.0		0.78	60	0.95	
As in Fig. 5	1.0	Equine, 25	LDAO, 0.05	0	0	1.4	2.3	1.00	0
				1.0	0	0.7	2.5	0.85	0
I (reference 1)	1.0	Equine, 35	LDAO, 0.1	0	0	0.65	1.4	0.92	0
				1.0	0	0.40	1.7	0.60	0
IV (reference 1)	1.4	Bovine, 40	LDAO, 0.1	0	0	1.1	2.2	0.94	0
			" "	0.3	0	1.0	1.7	0.92	0.
			" "	0	10			1.00	0.
			" "	0.3	10			1.00	0.
			LDAO, 1.0	0	0	0.67		0.82	0.
			" "	2.0	0	0.42		0.58	0.
			" "	0	10	0.93		0.83	0
			" "	2.0	10	0.64		0.82	0.

* The value of ϕ_p was determined before cyt was added. Effects of *o*-phen, UQ, and varying detergent cc centration are shown.

‡ The same detergent had been used in preparing the reaction centers.

photochemically inactive form in which the primary acceptor is reduced:



(4)

This is demonstrated in Fig. 6, for the same reaction center preparation that was the subject of Fig. 4. Curve *a* shows the rise in fluorescence without *o*-phen; this is the same as in Fig. 3 of reference 1, but on a more expanded time scale. Curve *b* shows the more rapid rise that occurred when 1 mM *o*-phen was present. It can be shown (1, 13) that the area "above" the fluorescence curve, as indicated by the shaded area above curve *b*, is proportional to the size of the electron acceptor pool. In the present case the area above curve *b* represents 0.82 electrons per reaction center, whereas the

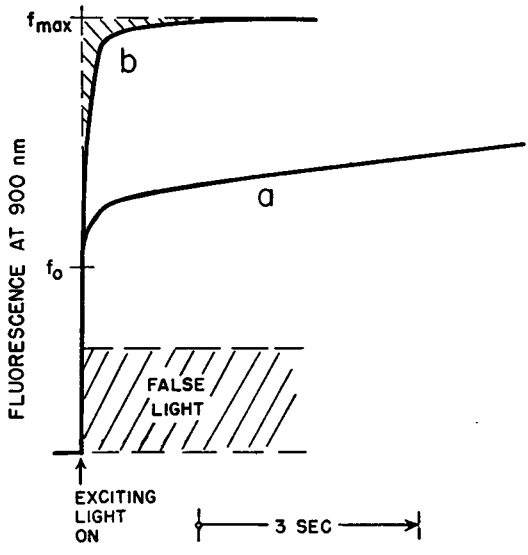


FIGURE 6 Changes in P870 fluorescence induced by illumination of reaction centers made with Triton X-100. Sample with added cyt as in Fig. 4; see also Fig. 3 of reference 1. The "false light" is the residual signal that can be observed with the P870 entirely bleached, during illumination without added cyt. Exciting light 800 nm, 2.2 mw/cm^2. Curve *a*, sample with cyt as stated; curve *b*, 1 mM *o*-phen added. Further discussion in the text.

area above curve *a*, not shown in its entirety, corresponds to 47 electrons per reaction center (see reference 1 for analysis). These data can be compared with the first two rows of Table I. Again, *o*-phen appeared to define an acceptor pool equivalent to about one electron per reaction center.

Similar results were obtained using reaction centers made with LDAO. In one experiment the pool as defined by the fluorescence was 1.5 electrons per reaction center without *o*-phen and 0.91 electrons per reaction center with 1 mM *o*-phen.

Inhibition of Photochemistry by o-Phen

Table I shows that in some cases *o*-phen inhibited the quantum efficiency ϕ_p of the photochemical oxidation of P870. This efficiency was computed from the initial slope of the light-induced ΔOD at 863 nm, in reaction centers without added cyt. There appeared to be no inhibition as long as either native or added UQ was present. The inhibition was more severe at higher concentrations of LDAO (see Table II); this might be related to the fact that LDAO weakens the interaction between reaction centers and UQ.

If the oxidation of cyt c is coupled closely to that of P870, a decline in ϕ_p caused by *o*-phen should be paralleled by a lower quantum efficiency for cyt oxidation, ϕ_{cyt}. This was the case, but in addition there may have been a slight reduction of ϕ_{cyt} relative to ϕ_p, as indicated by the figures in the last column of Table I. See reference 1 for a fuller discussion of the ratio of ϕ_{cyt} to ϕ_p.

TABLE II

o-PHEN INHIBITION OF THE QUANTUM EFFICIENCY ϕ_p OF P870 OXIDATION IN REACTION CENTERS MADE WITH LDAO AND LACKING UQ*

ϕ_p	LDAO concn, per cent		
	0.02	0.1	1.5
Before *o*-phen was added	0.93	0.97	0.93
7 min after addition of 1 mM *o*-phen	0.72	0.76	0.55
70 min after the addition of 1 mM *o*-phen	0.63	0.52	0.30

* The inhibition was more pronounced at higher concentrations of LDAO. Reaction centers, 0.75 μM, in 0.01 M Tris-Cl, pH 7.5, with LDAO as shown. Quantum efficiencies were computed from the initial slope of the light-induced bleaching at 863 nm.

We undertook to determine whether the inhibition of ϕ_p by *o*-phen meant that all the reaction centers were affected somewhat, or some of them were inactivated entirely. This question was tested by measuring the maximum possible light-induced bleaching at 863 nm in reaction centers with different concentrations of *o*-phen. In one experiment the reaction centers were adjusted to an OD of 0.15 at 863 nm (near the peak of P870). The expected ΔOD for the complete oxidation of P870 in such a preparation would be −0.13 at 863 nm; this could be confirmed by chemical oxidation using ferricyanide. The maximum possible light-induced bleaching was determined by measuring the ΔOD at a succession of exciting light intensities and extrapolating to infinite intensity. The extrapolation was facilitated by plotting the data in reciprocal form, as shown in Fig. 7. The values of maximum | ΔOD | obtained from these plots are listed in Table III for reaction centers to which different concentrations of *o*-phen had been added. All samples had been incubated with the *o*-phen for 3 hr before measurement. It can be seen that at increasing concentrations of *o*-phen there was a progressively higher residue of reaction centers that could not be bleached by light. The remaining (bleachable) fraction of reaction centers was denoted x and equated to maximum | ΔOD |/0.13. This active fraction is also listed in Table III.

If the effect of *o*-phen were all-or-none, so that the "active" reaction centers suffered no loss of photochemical efficiency, then the measured value of ϕ_p should be proportional to the active fraction x. We measured ϕ_p and found that this was indeed the case, within the probable accuracy of the measurements (again see Table III).

Thus *o*-phen is able to prevent the primary photochemical act; this effect is distinct from the blocking of electron transfer from primary to secondary acceptors as described earlier. Both of these effects might involve a reaction between *o*-phen and the primary acceptor, but the former (prevention of photochemistry) does not occur when UQ is present. The two effects therefore involve different kinds of interaction.

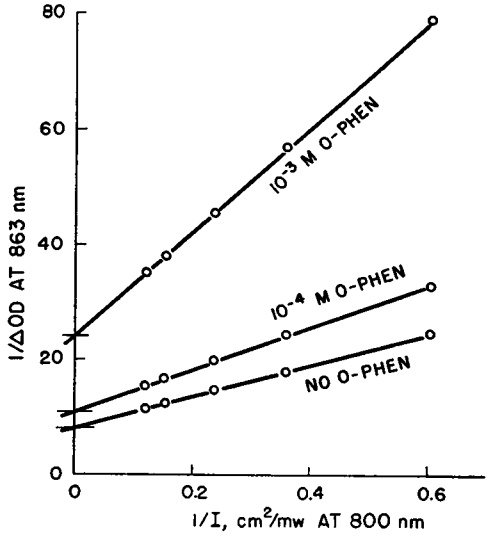


FIGURE 7 Bleaching of P870 in reaction centers made with LDAO. The steady-state ΔOD is plotted against exciting light intensity in double reciprocal form to facilitate extrapolation to infinite light intensity ($1/I = 0$; intercept on the vertical axis). Reaction centers, 1.5 μM, in buffer with 0.1% LDAO and *o*-phen as shown on the graph.

TABLE III

SEVERAL ASPECTS OF THE INACTIVATION OF REACTION CENTERS BY *o*-PHEN*

Concn of *o*-phen	Maximum |ΔOD| at 863 nm‡	*x*, fraction of active reaction centers, corresponding to maximum |ΔOD|	Relative ϕ_p	Relative amount of cyt oxidized in fast phase	*x*, computed from the increase in P870 fluorescence, assuming a linear relationship
M					
0	0.13	1.00	1.00	1.00	1.00
2×10^{-5}	0.115	0.90	0.93	1.15	0.97
10^{-4}	0.095	0.75	0.83	0.74	0.88
10^{-3}	0.042	0.33	0.41	0.42	0.62
10^{-2}	0.018	0.14	0.15	0.20	0.12

* Reaction centers as in Fig. 7.

‡ Maximum | ΔOD | was obtained from Fig. 7, extrapolating to $1/I = 0$. This was taken to indicate the fraction *x* of active reaction centers, equal to 1.00 with no *o*-phen added. For comparison we record relative values of the quantum efficiency of P870 oxidation and of the extent of cyt oxidation (fast kinetic phase). Finally we list the fraction of active reaction centers as computed from the increase of P870 fluorescence, assuming a linear relationship (see the text).

In measurements with cyt c as electron donor we found that the acceptor pool, assayed from the fast phase of cyt oxidation, diminished at higher concentrations of *o*-phen. This diminution was roughly in proportion to the fraction of reaction centers that had been rendered inactive; again see Table III. Thus the inactive reaction centers did not contribute to the electron acceptor pool. This explains the low values for cyt oxidation (fast phase) that appear among the data in Table I.

The inactivation of reaction centers by *o*-phen was attended by an increased fluorescence from P870, measured at the onset of illumination. At high concentrations of *o*-phen the value of f_0 was raised almost to the level f_{max} shown in Fig. 6. A simple assumption would be that inactive reaction centers show the same high fluorescence as reaction centers in which the primary acceptor has become reduced. In that case the *o*-phen–induced rise in fluorescence should be a linear function of the fraction of reaction centers rendered inactive, becoming f_{max} at 100% inactivation. We tested this by computing a set of values of x as a linear function of the rise in fluorescence. The agreement between this predicted x and the value of x computed from ΔOD was poor; see Table III. We can only say that there was a qualitative correspondence between decreased photochemical utilization of quanta and increased fluorescence, in reaction centers inhibited with *o*-phen.

The ability of *o*-phen to inhibit photochemistry involving BChl was tested further with an aqueous dispersion of BChl solubilized with 0.15% LDAO. The BChl had been extracted from the light-harvesting apparatus of *R. spheroides* and was used without further purification; see Materials and Methods. The reaction mixture contained BChl with PMS and sodium ascorbate as an electron-donating system, and UQ as an electron acceptor. Light-induced oxidation of PMS was measured by the ΔOD at 390 nm, with the results shown in Table IV. The presence of 1 mM *o*-phen inhibited the rate of the reaction nearly 50%; at 0.1 mM *o*-phen the inhibition was almost as great.

TABLE IV

PHOTOCHEMICAL ELECTRON TRANSFER FROM REDUCED PMS TO UQ SENSITIZED BY BChl AS AN AQUEOUS DISPERSION IN 0.01 M TRIS-Cl, pH 7.5, CONTAINING 0.15% LDAO*

Concn of *o*-phen	−ΔOD at 390 nm during 1st 6 sec of illumination
mM	
0	0.015
0.1	0.010
1.0	0.008

* The reaction was monitored by the ΔOD at 390 nm; we list the change during the first 6 sec of illumination, as affected by *o*-phen. BChl, 10 μM; UQ, 20 μM; PMS, 25 μM; Na ascorbate, 1 mM. Exciting light 770 nm, 5 mw/cm².

Effects of Chemicals Other Than o-Phen

The evidence that *o*-phen reacts with the primary electron acceptor in reaction centers was intriguing in view of the known affinity of *o*-phen for various metal ions including Fe^{++} and Fe^{+++}. We were thus prompted to test the effects of other metal chelators, and of some other substances known to affect electron transport in photosynthetic systems. We looked for effects on the light-induced bleaching and recovery of P870, and on the fluorescence of P870 during illumination, with and without added UQ. The following compounds had no detectable effect at the concentrations indicated, at pH 7.5:

Ethylenediaminetetraacetic acid	0.01 M
Ethylene glycol-*bis*-(β-aminoethyl ether)N,N,N',N'-tetraacetic acid	0.01 M
Tiron	0.05 M
DCMU	0.01 M
Antimycin A	0.001 M
α,α'-Dipyridyl	0.01 M

At 0.001 M, 8-hydroxyquinoline had a slight effect, similar to that of 10^{-5} M *o*-phen. The relative ineffectiveness of the former might have been because of its limited solubility in water.

NQNO proved to have a marked effect on the photochemical oxidation of P870 in reaction centers made with LDAO, but only after prolonged exposure. The effect was an inhibition of ϕ_p together with an increase in fluorescence, but in contrast to *o*-phen, NQNO had no marked effect on the kinetics of electron transport beyond the primary photoact.

TABLE V
NQNO INHIBITION OF THE QUANTUM EFFICIENCY OF P870 OXIDATION AND RAISING OF THE FLUORESCENCE OF P870 MEASURED AT THE ONSET OF ILLUMINATION*

Concn of NQNO	Relative ϕ_p	Relative f_0
M		
0	1.00	7
2×10^{-7}	0.85	7.5
2×10^{-6}	0.56	9
2×10^{-5}	0.24	12
2×10^{-4}	0.12	20

* Reaction centers, 0.8 μM, made with LDAO and suspended in 0.01 M Tris-Cl, pH 7.5, with 0.05% LDAO. NQNO was added, and the measurements were made after periods of incubation at room temperature. The effects of NQNO were negligible at 10 min but were nearly maximal at 3 hr. We show the data after 18 hr. Exciting light 800 nm, 3 mw/cm^2.

The inhibition of ϕ_p by NQNO, and the rise in fluorescence, was negligible during the first 10 min of incubation, but was nearly maximal after 3 hr at room temperature. Table V shows the effects measured after 18 hr. The rise in fluorescence was not a linear function of the decrease in ϕ_p ; we could not make a simple quantitative correlation between these two effects.

CONCLUSIONS

These experiments support a conclusion drawn in the preceding communication of this series: that reaction centers contain a primary acceptor capable of holding one electron, and secondary acceptors including UQ. PMS can interact with both primary and secondary acceptors, cycling electrons back to oxidized P870. *o*-Phen inhibits the transfer of electrons from the primary acceptor to secondary acceptors including added UQ. This is sufficient to account for the action of *o*-phen on cells and chromatophores on photosynthetic bacteria. The ability of *o*-phen to block electron transfer from primary to secondary acceptors is seen also in green plant photosystem II, but DCMU, which is especially active in this way in green plants, had no effect on reaction centers.

o-Phen also inhibits the photochemical oxidation of P870 in reaction centers, provided that no UQ is present. This effect probably does not occur in cells or chromatophores, which contain UQ. Reactions of BChl in vitro were also found to be inhibited by *o*-phen, even with UQ present.

The two effects, inhibition of primary photochemistry and blocking of secondary electron transport, may be two manifestations of an affinity of *o*-phen for the primary electron acceptor; however, the selective suppression of the former effect by UQ suggests that two different kinds of interactions are involved. A variety of agents including potent chelators of metal ions did not seem to have any effect on photochemistry or electron transport in reaction centers. NQNO did inhibit the photochemical act, whereas LDAO, another "$-N^+ -O^-$" compound, did not. Evidently the reaction centers, being simple derivatives of the complete photosynthetic apparatus, provide only a limited basis for understanding the actions of various inhibitors of photosynthesis.

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