PHOTOCHEMICAL ELECTRON TRANSPORT IN PHOTOSYNTHETIC REACTION CENTERS

IV. Observations Related to the Reduced Photoproducts

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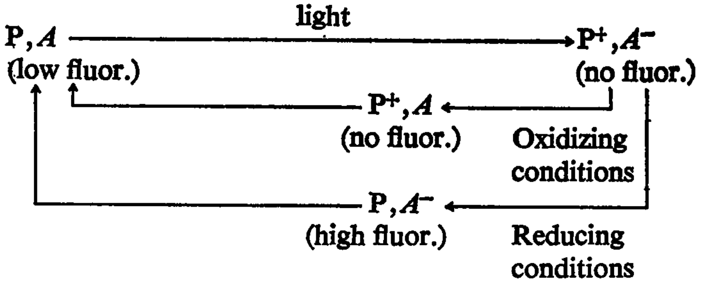
ABSTRACT The formation and dissipation of reduced photoproducts in photochemical reaction centers from *Rhodopseudomonas spheroides* has been studied in three independent ways: by direct chemical reduction, by photochemical reduction (illuminating reaction centers in the presence of weak reductants), and by adding electron acceptors to illuminated reaction centers to reverse the reduction. In every case the reduction is attended by the appearance of an absorption band at 450 nm and the bathochromic shift of a band centered at 305 nm. Both reduction and oxidation of reaction centers, and also photochemical oxidoreduction, cause bathochromic shifts of absorption bands identified with bacteriopheophytin (BPh), and hypsochromic shifts of bands of bacteriochlorophyll (BChl) (P-800 and, in the case of reduction, P-870). Reduction causes relatively large shifts of BPh and small shifts of BChl; the reverse is seen with oxidation and oxidoreduction. Addition of sodium dodecyl sulfate (SDS) to reaction centers suppresses the 450 nm absorption change but not the band shifts associated with BPh and BChl. Under some conditions the 450 nm change and the band shifts show different kinetics, with the kinetics of the band shifts matching those of a transient change in the yield of P-870 fluorescence. New data, on the efficiency of photo-bleaching of P-870 in reaction centers in which part of the P-870 has already been oxidized with ferricyanide, militate against the idea that part of the photochemical bleaching of P-870 is due to reduction of that pigment.

INTRODUCTION

Photochemical activities of reaction centers (RCs) prepared from *R. spheroides* are manifested by the oxidation and reduction of P-870, the specialized BChl that serves as primary electron donor (1):

$$\text{P-870 (absorption band at 865 nm)} \underset{\text{dark}}{\overset{\text{light}}{\rightleftarrows}} \text{P}^+ \text{(oxidized, bleached form).} \qquad (1)$$

The primary electron acceptor A, partner in the photochemical oxidation of P-870, remains hypothetical. Its state of oxidation seems to be indicated (2, 3) by the yield of fluorescence from P-870: the system P,A^-, formed by illuminating reaction centers under reducing conditions, is more strongly fluorescent than P,A, presumably because excitation energy cannot be used for photochemistry unless the electron acceptor is in its oxidized form A. The bleached pigment P^+ is nonfluorescent.



(2)

One should, of course, be cautious in ascribing the altered fluorescence solely to a specific cause such as reduction of the primary acceptor; but if we take this model at face value, then measurements of the fluorescence as a function of the external redox potential (3) have indicated that A has a midpoint potential near -0.05 v, independent of pH and showing a "one-electron" titration curve. Studies of the light-induced change in fluorescence, and of the oxidation of external electron donors such as reduced cytochrome *c* (cyt *c*) or phenazine methosulfate (PMS), have confirmed that the primary acceptor associated with each molecule of P-870 can hold just one electron in its photochemically reduced form (4, 5).

With RCs in the presence of electron donors such as reduced PMS we have seen (6) small light-induced optical absorption changes related to the formation of reduced photoproducts, possibly including the primary acceptor. The principal change that we reported was an absorption increase at 450 nm, but the changes include conspicuous bathochromic (to greater wavelengths) shifts of absorption bands that we ascribe to BPh, and also hypsochromic (to lesser wavelengths) shifts of bands at 800 and 865 nm due to BChl (as P-800 and P-870). Both the BChl and the BPh appear to be normal components of the RC (7).

This paper describes further studies of absorption changes related to the reduced photoproducts in RCs from *R. spheroides*. The measurements have been extended to the ultraviolet, and the changes have been studied in relation to the participation of ferricyanide and ubiquinone (UQ) as secondary electron acceptors. Some effects of SDS are also noted.

The possibility has been raised that the primary acceptor is a second molecule of P-870, with bleaching of the 865 nm band caused by $P \rightarrow P^-$ as well as by $P \rightarrow P^+$.

Considerations of quantum efficiency have made this possibility unlikely (8). By measuring light-induced bleaching of P-870 against a background of partial chemical oxidation, we have found further evidence against this view.

MATERIALS AND METHODS

Cells of carotenoidless mutant *R. spheroides* strain R-26 were cultivated and made to yield purified RCs as described in detail elsewhere (9). RCs were suspended in H_2O with 0.01 M TrisCl, pH 7.5, and 0.05–1.5% lauryl dimethylamine oxide (LDAO, a gift from Onyx Chemical Corporation, Jersey City, N. J.). The concentration of RCs was based on the assumption that $\epsilon = 113$ mM^{-1} cm^{-1} at 867 nm (4, 9, 10). PMS, orthophenanthroline (*o*-phen), and UQ (coenzyme Q_6) were purchased from Sigma Chemical Co., St. Louis, Mo. In experiments with PMS the formation of pyocyanine was avoided by minimizing the exposure to light of wavelengths below 500 nm.

Absorption spectra and difference spectra were measured with a Cary 14R spectrophotometer (Cary Instruments, Monrovia, Calif.). Light-induced changes of optical density (OD) were measured with a simple split-beam difference spectrometer described elsewhere (8).[1] Exciting light from a 600 w tungsten-iodine lamp was filtered with 2.5 cm of water and a suitable interference filter of bandwidth about 10 nm. The signal to noise ratio in a ΔOD measurement can be judged in Fig. 1 from the record for 338 nm, reproduced carefully from the original trace. These traces were made with a Bausch and Lomb VOM-5 recorder (Bausch and Lomb, Inc., Rochester, N.Y.), of time constant about 0.1 sec.

RESULTS

Experiments with Added Electron Acceptors

We have already shown (6) that when RCs are illuminated in the presence of a suitable electron donor such as reduced PMS, the accumulation of oxidized P-870 is negligible because of the rapid transfer of electrons from the donor:

$$\mathrm{P},A \xrightarrow{\text{light}} (\mathrm{P}^+,A^-) \xrightarrow[\text{Donor} \rightarrow \text{Oxidized donor}]{\text{rapid}} \mathrm{P},A^- \longrightarrow \begin{array}{l}\text{Electron to}\\ \text{secondary}\\ \text{acceptors}\end{array} \qquad (3)$$

Changes of OD may then reflect oxidation of the donor, reduction of A to A^-, and reduction of secondary electron acceptors. The absorption changes attending the formation of reduced photoproducts will collectively be termed $\Delta(R)$ for convenience.

Another way to detect absorption changes associated with the reduced photoproducts is to add an external electron acceptor, and then to abstract the reactions in which reduced photoproducts are reoxidized by the added acceptor, following the photochemical process: (*a*) photochemistry, formation of P^+ and reduced photo-

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[1] The data of Fig. 4 represented by triangles were obtained with a more sophisticated spectrometer employing modulated measuring light (10).

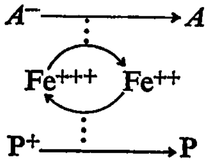
products; (*b*) oxidation of the reduced products, reduction of added acceptor, and further changes in the concentration of P^+. These events could be monitored during illumination, in a system containing potassium ferricyanide as an external electron acceptor. The ferricyanide was accompanied by ferrocyanide in sufficient proportion to minimize any direct chemical oxidation of P-870. Typical concentrations, as used in the measurements shown in Fig. 1, were: RCs, 1.5 μM; ferricyanide, 4 μM; ferrocyanide, 10 μM. Under these conditions the kinetics of P-870 oxidation (also see Fig. 8 of reference 1) suggested the following reactions:

(*a*) A rapid light-induced equilibration between P,A and P^+,A^-, involving an endogenous back-reaction of half-time about 0.1 sec:

$$P,A \underset{\text{rapid}}{\overset{\text{light}}{\rightleftharpoons}} P^+,A^-. \tag{4}$$

Secondary electron acceptors in the RC may also be involved here; the reader can include them under the labels A and A^-.

(*b*) A slower transfer of electrons from endogenous reduced photoproducts to ferricyanide, and also from ferrocyanide to P^+:



(5)

where again the reader can extend the formulation to include secondary acceptors.

We can therefore expect three categories of absorption change: those attending the oxidation and reduction of P, of ferri- and ferrocyanide, and of primary and secondary electron acceptors. In Fig. 1, the changes due to $P \rightarrow P^+$ are shown by the trace measured at 600 nm. At this wavelength the changes $\Delta(R)$ associated with reduced photoproducts are nil (6), and neither ferri- nor ferrocyanide absorbs light. Two features in the 600 nm trace should be noted. There is an initial rapid "light-on" phase and, at the end of illumination, a mixture of fast and slow components of recovery. The rapid phases correspond to the fast interconversion of P,A and P^+,A^-; see equation 4. The slow decay component is identified with a reaction between P^+ and ferrocyanide, in RCs that no longer contained reduced photoproducts to allow a fast back-reaction. These oxidized RCs, P^+,A, had been formed by a reaction with ferricyanide (equation 5). Because the recovery was about 30% "fast" and 70% "slow" in the 600 nm trace of Fig. 1, we estimate that 30% of the RCs were in the state P^+,A^- and 70% in the state P^+,A when the exciting light was turned off. The conversion of P^+,A^- to P^+,A, coupled to the reduction of ferricyanide, took place during illumination and caused a further shift in the balance be-

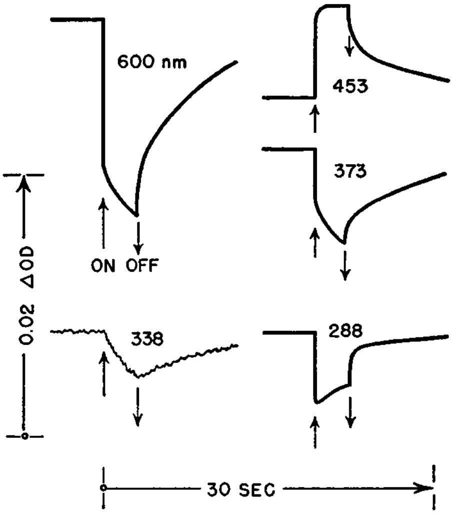


FIGURE 1 Light-induced optical absorption changes in RCs from *R. spheroides*. RCs, 1.5 μM, with 1.5% LDAO, 4 μM $K_3Fe(CN)_6$, and 10 μM $K_4Fe(CN)_6$. Exciting light, 800 nm, 6 mw/cm^2. The record shown for 338 nm is a realistic copy of the actual recorder trace.

tween P and P^+ by slowing the back-reaction. The accretion of more P^+ during illumination is seen as a slow light-on phase in the 600 nm trace.

Although the foregoing explanation of the kinetics of ΔOD at 600 nm is highly speculative, it provides a rational basis for understanding and resolving the kinetics at other wavelengths. We take the trace at 600 nm to represent the kinetics of $P \rightleftharpoons P^+$, unattended by other changes. In contrast, the ΔOD at 338 nm (see Fig. 1) showed no rapid phase and therefore no contribution from $P \rightleftharpoons P^+$. It must be attributed to the reactions of reduced photoproducts and ferri-/ferrocyanide. At all other wavelengths the traces could be decomposed into two components: one having exactly the shape shown at 600 nm and the other the shape shown at 338 nm. The second ("pure slow") component, isolated at 338 nm, could be analyzed further by discounting the contribution due to ferri- $\rightleftharpoons$ ferrocyanide. We estimate (see the last paragraph) that during illumination, 0.7 mole of ferricyanide was reduced for every mole of P oxidized. At the same time, 0.7 equivalents of reduced photoproducts were reoxidized in the reaction with ferricyanide. The amount of P oxidized was computed from $\Delta\epsilon = -20$ mM^{-1} cm^{-1} at 600 nm (4, 9). The ΔODs attributable to reduction of ferricyanide could then be calculated from known spectra of ferri- and ferrocyanide, and subtracted from the observed pure slow component. The residual slow component should reflect the oxidation of reduced photoproducts, $-\Delta(R)$ in our terminology. Its spectrum is shown in Fig. 2, with $-\Delta$OD plotted upward. This was done to facilitate a direct comparison with other graphs, such as Fig. 4, showing $+\Delta(R)$ with positive ΔOD plotted upward. The correction for reduction of ferricyanide is also shown in Fig. 2; note that it is relatively small.

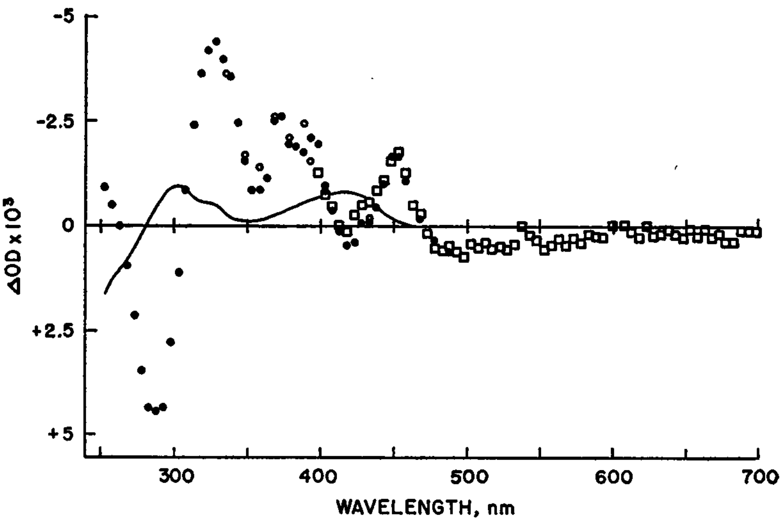


FIGURE 2 The ΔODs of Fig. 1, and similar ones at other wavelengths, were processed to abstract the pure slow component as displayed at 338 nm in Fig. 1 (see text for details). The estimated ΔOD attributable to reduction of ferricyanide, shown by the solid line in this figure, was discounted. The residual "slow component" is plotted here. The three sets of symbols refer to three separate experiments, in which the conditions stated under Fig. 1 prevailed. Negative ΔOD has been plotted upward for reasons given in the text.

Fig. 2 confirms our earlier finding (6) that the spectrum of Δ(*R*) shows a peak at 450 nm. It also shows a symmetrical wave suggesting the shift of a band centered at 305 nm, and a peak near 380 nm. It will be shown later that these changes are accompanied by shifts of the absorption bands near 535 and 760 nm, associated with BPh. Since the Soret maximum of BPh is near 365 nm, both the 380 nm peak and the trough at 355 nm in Fig. 2 could reflect a shift of that band. The data in Fig. 2 suggest a small band shift centered near 535 nm (BPh); this is clearer in other experiments (see Figs. 4 and 6). All of these shifts are bathochromic for reduction and hypsochromic for reoxidation of electron acceptors in the RCs.

In another experiment we used UQ as an external electron acceptor, with *o*-phen added in order to modify the kinetics of the reaction. We had found earlier (5) that *o*-phen inhibits the transfer of electrons from primary photoproducts to UQ. The *o*-phen, by erecting a barrier between components of the RC and added UQ, seemed to allow a kinetic distinction between the rapid accumulation of endogenous photoproducts and the slower discharge of electrons to the added UQ. In any event a mixture of RCs, *o*-phen, and UQ (see the legend of Fig. 3) gave light-induced absorption changes similar to those shown in Fig. 1. These data, processed exactly as before, gave the spectrum plotted in Fig. 3. This spectrum has the same features as that in Fig. 2. The wave around 305 nm is not symmetrical in Fig. 3; the half-wave from 260 to 305 nm is smaller than that from 305 to 350 nm. This could reflect the reduction of added UQ, which would impose a negative ΔOD on the spectrum between about 250 and 300 nm.

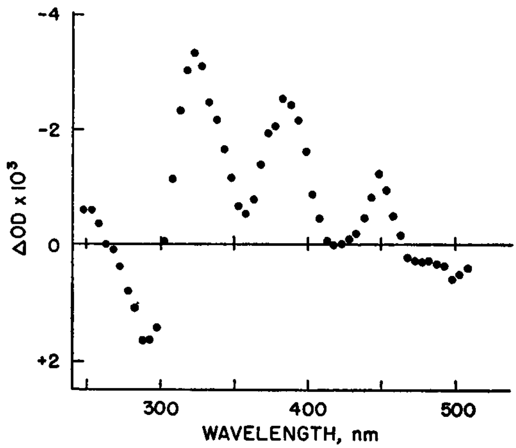


FIGURE 3

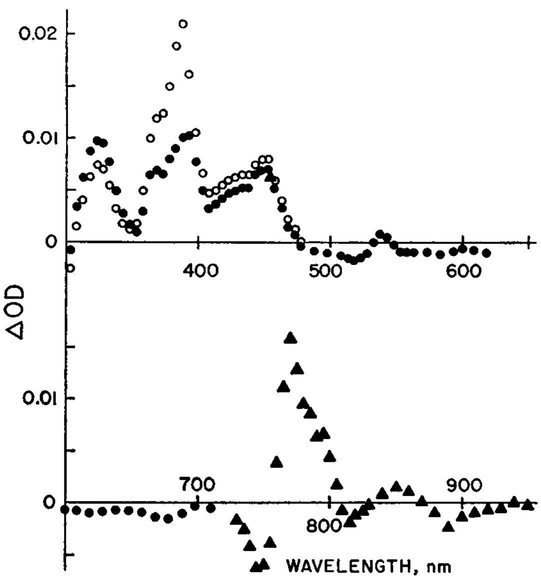


FIGURE 4

FIGURE 3 RCs, 30 μM, were mixed with 80 μM UQ and 500 μM o-phen. Excitation at 800 nm, 6 mw/cm^2, caused ΔODs similar to those shown in Fig. 1 (the measuring beam path in the sample was 0.5 mm). The pure slow component (see text) is plotted here, with negative ΔOD upward.

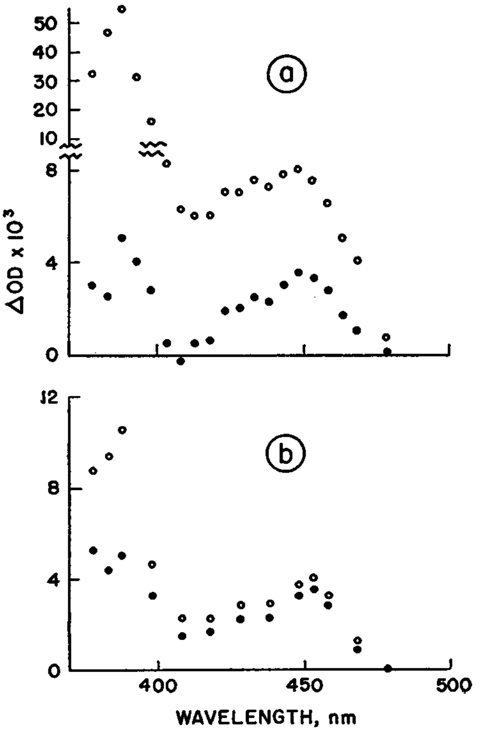
FIGURE 4 Circles: absorption changes induced in RCs, 2 μM, with 1.5% LDAO, 25 μM PMS, and 1 mM sodium ascorbate added. Exciting light, 800 nm, 6 mw/cm^2. The open circles show the total ΔOD at the end of 3 sec of illumination. The filled circles show the endogenous component, Δ(*R*), abstracted as stated in the text. The difference between open and filled circles is the ΔOD attributed to oxidation of PMS. Triangles: Δ(*R*) for 2.5 μM RCs, 0.1% LDAO, PMS, and ascorbate as before; values scaled to 2 ΔM RCs by multiplying the ordinate by 0.8. Excitation, 30 sec at 600 nm, about 1 mw/cm^2.

Experiments with PMS Added As an Electron Donor

Having described two experimental constructions that purportedly display −Δ(*R*), let us turn to a repetition of the kind of experiment described earlier (6) and symbolized by equation 3, with PMS as electron donor. This experiment was aimed at generating Δ(*R*) by forming reduced photoproducts while avoiding any accumulation of P^+. RCs were illuminated in the presence of PMS (25 μM), with 10^{-3} M ascorbate present in order to generate reduced PMS. The light-induced ΔODs are shown in Fig. 4. The open circles, and all of the symbols at wavelengths above 480 nm, show the total ΔOD at the end of a chosen period of illumination. Circles refer to an experiment with 2 μM RCs; exciting light 800 nm, 6 mw/cm^2, 3 sec. The triangles represent an experiment using 600 nm exciting light, about 1 mw/cm^2, 30 sec. The total ΔOD in these experiments is the sum of two components: the endogenous change Δ(*R*) and the change Δ(PMS) due to oxidation of PMS. The two components could be separated on the basis of independent knowledge of their spectra. The spectrum of Δ(PMS) was measured by adding $NaBH_4$ to oxidized PMS. We assume that the spectrum of Δ(*R*) is given in Fig. 2. By analyzing the ΔOD at any two wavelengths (we used 388 and 453 nm for this), requiring ΔOD (total) = Δ(*R*) + Δ(PMS), we could deduce the relative contributions of the two components at all other wavelengths and thus abstract Δ(*R*). The spectrum of Δ(*R*) abstracted in this way is shown by the filled circles and triangles in Fig. 4. It agrees well with the spectra shown in Figs. 2 and 3, and extends the spectrum through the near infrared. The similarities among Figs. 2, 3, and 4 encourage us to believe that we were measuring the same process, manifested by Δ(*R*), in each kind of experiment.

Fig. 4 shows clearly the waves describable as bathochromic shifts of the absorption bands of BPh at 535 and 760 nm. In addition there are small hypsochromic shifts of the 800 and 865 nm bands of BChl (P-800 and P-870).

We repeated the experiment with PMS, adding or withholding UQ as indicated in the legend of Fig. 5. The added UQ, serving as a terminal electron acceptor, supported the oxidation of a greater amount of PMS; however, the magnitude of Δ(*R*)



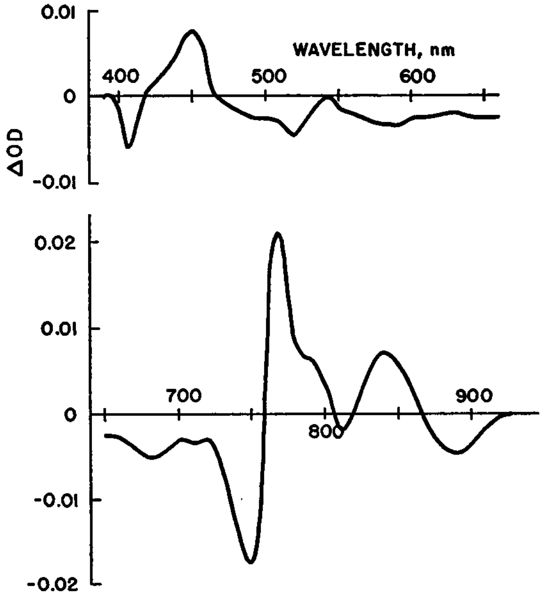


FIGURE 5

FIGURE 6

FIGURE 5 Similar to Fig. 4. Exciting light, 800 nm, 0.7 mw/cm², 3 sec. RCs, 2 μM; PMS, 25 μM; sodium ascorbate, 1 mM. (*a*) 0.02% LDAO, 20 μM UQ. (*b*) 2% LDAO, no UQ.
FIGURE 6 Difference spectrum induced by adding a few milligrams of $Na_2S_2O_4$ to 3 ml of RCs, 2.9 μM, in 0.01 M TrisCl, pH 7.5, with 0.1% LDAO.

generated during illumination was the same whether UQ had been added or not. These results are shown in Fig. 5. Evidently a saturating level of reduced photoproducts, responsible for $\Delta(R)$, could be sustained during illumination, even with added UQ present.

In all the foregoing experiments we could quantify the changes $\Delta(R)$ in terms of equivalents of reduced photoproducts. For example, in the experiment of Fig. 4 we found that $\Delta(R)$ at 450 nm was 0.007 in a sample containing 2 μM RCs (1 cm path). Independent experiments (4, 5) had shown that the electron-accepting capacity amounted to just one electron per RC. We therefore found $\Delta(R)$ (450 nm) = 0.007 for 2 μeq, or $\Delta\epsilon = 3.5\ \text{mM}^{-1}\ \text{cm}^{-1}$. Similar values were found in the other experiments.

Direct Chemical Reduction and Oxidation of RCs

Reduction of RCs with sodium dithionite, $Na_2S_2O_4$, caused absorption changes similar to those shown in Fig. 4 for photochemical reduction; see Fig. 6. Note the peak at 450 nm, the bathochromic shifts around 535 and 760 nm, and the hypsochromic shifts of both P-800 and P-870.

Illumination of RCs to which no electron donor had been added caused the characteristic ΔODs that attend oxidation of P-870; see Fig. 1 in either reference 1 or 9. These changes included bleaching of the band at 865 nm (P-870), a partial bleaching of the 600 nm band (only part of this band is associated with P-870, the rest with

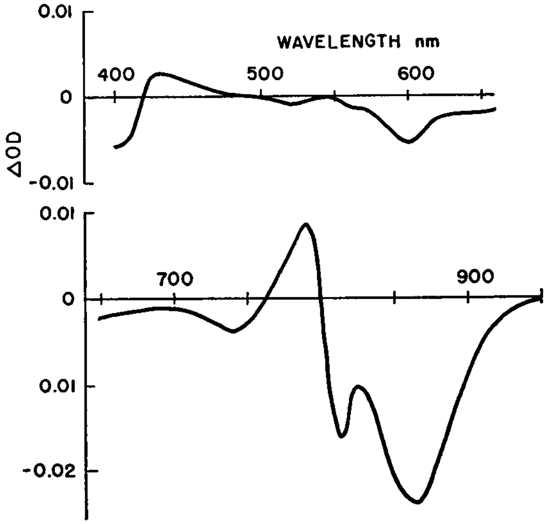


FIGURE 7 Difference spectrum induced by adding H_2O_2 (about 10^{-3} M) to RCs, 2.7 μM, with 0.1 % LDAO.

P-800), and an increase in OD centered near 430 nm. These effects have their counterparts in the difference spectrum for oxidation of BChl in vitro (11). The photochemical oxidation of P-870 is attended also by a small bathochromic shift around 760 nm (BPh), obscured by the much larger blue shift of P-800. All of these features are seen in a difference spectrum for the chemical oxidation of RCs, elicited with H_2O_2 and shown in Fig. 7 (compare Fig. 1 in reference 12). The shift around 760 nm as well as the one around 800 nm can be discerned in earlier examples of light-induced and oxidation-induced difference spectra, for cells and chromatophores of purple bacteria as well as RCs (12–14). These band shifts are thus induced by chemical oxidation and photochemical oxidoreduction of RCs as well as by reduction, but the relative magnitudes are strikingly different in the case of reduction.[2]

Is P-870 an Electron Acceptor As Well As a Donor?

The possibility has been considered (8) that as one specialized molecule of BChl serves as photochemical electron donor, a second one might serve as acceptor. Conversion of BChl to a reduced radical should change its absorption spectrum in much the same way as conversion to an oxidized radical. Therefore one can ask if the observed bleaching of P-870 is due partly to $P \rightarrow P^-$ and partly to $P \rightarrow P^+$,

$$P, P \xrightarrow{\text{light}} P^+, P^-. \tag{6}$$

In this view one quantum can, in principle, cause the bleaching of two Ps. The observed quantum efficiency of 0.7–1.0 Ps per quantum (4, 8) militates against this model. Further evidence was sought by exposing RCs to various oxidation potentials (using mixtures of ferri- and ferrocyanide) to bleach part of the P-870 by oxidation, $P \rightarrow P^+$, and then studying the light-induced bleaching of the remaining part. If there are two P-870 per RC, one functioning normally as electron donor and the other as electron acceptor, they nevertheless can both be bleached by chemical oxidation using ferricyanide, since the 865 nm band can be bleached completely (and reversibly) in this way. A redox titration for this chemical bleaching obeys a Nernst equation for a single chemical entity of midpoint potential of about 0.4–0.5 v, with the oxidized and reduced forms differing by one electron (15, 16, and unpublished measurements in our laboratory using RCs). Under partial bleaching we can compute, from a binomial distribution, the fraction of RCs in the states P,P; P,P^+; and P^+,P^+. When half of the total P-870 is bleached, for example, the distribution should be 25%:50%:25% for these three states respectively. Now, in the model of equation 6, the state P,P^+ should be unresponsive to light. P^+ cannot be expected to act as an electron donor. It might serve as an acceptor, taking an

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[2] It was R. T. Wang who first noticed and stressed, in our laboratory, the qualitative similarity of the band shifts caused by oxidation and by reduction in RCs.

electron from P in a photochemical reaction, but this rearrangement, $P, P^+ \xrightarrow{\text{light}} P^+, P$, would not be attended by a net change in the amounts of P and P^+. Therefore, under partial chemical oxidation, a fraction of the P-870 (in reaction centers in the state P, P^+) should resist photobleaching. In fact, we observed that with every degree of partial chemical bleaching of P-870, using ferricyanide, the residual 865 nm band could be bleached completely by light. Moreover, the quantum efficiency (using light absorbed by P-870 itself) for photobleaching of the residual P-870 was not lowered by partial chemical bleaching. In short, we found no indication of a component of P-870 that resisted photobleaching. We take this as strong evidence against the model of equation 6.

DISCUSSION

A working hypothesis, seemingly shared by all investigators of the photochemistry of photosynthesis, is that there exists an essential primary electron acceptor (A, X, Q, etc., in various terminologies) partner to the photochemical oxidation of the donor, P-870 in the case of RCs from *R. spheroides*. We can imagine that, after the primary photoact, the electron can interact with other entities in the RC before going to P^+ or to an external acceptor. This interaction might be sequential, in the sense that the state of reduction (the electron) is passed *in toto* along a chain of carriers A, B, C, $\cdots$. One could imagine a more complicated network of electron transport, or a pool of several substances competing for capture of the electron. Finally we can suppose that the electron interacts with a variety of substances, perhaps altering their optical properties, without losing its principal involvement with a primary acceptor A.

These possibilities are illustrated by the nature of the electron spin resonance (ESR) signals observed in illuminated RCs and other preparations (17–20; also G. Feher, M. Y. Okamura, and J. D. McElroy, personal communication). The well-known signal attributed to P^+ (8, 17) was found to be accompanied by an extremely broad signal, of bandwidth more than 10^3 gauss, which was attributed to A^- (17, 18). Subsequently Loach and Hall (19), studying subchromatophore preparations from *Rhodospirillum rubrum*, found still another signal, with $g = 2.0050$ and 7.0 gauss bandwidth, and ascribed *it* to the reduced primary acceptor. Feher et al. (reference 20 and personal communication) then observed a signal like that of Loach and Hall in RCs from *R. spheroides*, but only if the RCs had been exposed to 0.1% SDS along with 0.02% LDAO. The SDS released iron that had been bound to the RCs. The present consensus is that the signal of Loach and Hall is a property of A^-, that iron is not essential (the preparations of Loach and Hall contained less than one Fe per three P-870), but that iron in the RC can interact with A^-, broadening the ESR signal enormously. The data do not afford a choice between the varieties of interaction mentioned in the last paragraph.

Against this background let us consider the optical correlatives of photochemical reduction in RCs. We did not take steps to remove bound iron, and we have no decisive knowledge about the presence or absence of secondary acceptors in our RCs. Earlier experiments with the same material (4) showed that the total electron-accepting capacity, in RCs illuminated in the presence of reduced cytochrome *c* as electron donor, was just one electron per P-870. This would seem to rule out the participation of secondary electron acceptors, unless the reduction of a secondary acceptor leaves an RC still incapable of reacting photochemically, even though the primary acceptor has been restored from A^- to A.

We must interpret the absorption changes $\Delta(R)$ exemplified in Figs. 2–4, and also the rise in fluorescence of P-870 associated with reduction (3, 4). The spectrum of $\Delta(R)$ can be resolved into two categories: first, the bathochromic shifts of bands associated with BPh at 365, 535, and 760 nm, plus hypsochromic shifts of the BChl bands at 800 and 865 nm; second, the appearance of a band at 450 nm with a shoulder at 430 nm, together with a wave suggesting the bathochromic shift of a band centered at 305 nm. The second category bears some resemblance to the difference spectrum for the formation of the anionic ubisemiquinone radical from fully oxidized UQ, as reported by Land et al. (21). The latter spectrum shows a peak at 445 nm with a shoulder at 425 nm, and the shift of a band from 275 to 320 nm, giving the appearance of a wave centered at 295 nm. Loach's new ESR signal has also been characterized by Feher et al. (20, and personal communication) as resembling that of a semiquinone.

After the discovery of the new ESR signal (19), we reexamined the absorption changes with SDS added to RCs, as described by Feher et al. (20, and personal communication). The kinetics of light-induced changes were so altered by this treatment that we have not been able to isolate ΔODs associated with reduction, but we did obtain a difference spectrum by reduction with dithionite, differing from the one shown in Fig. 6. The band shifts associated with BPh and BChl remained intact, but there was no trace of the 450 nm peak. This preliminary result suggests that the 450 nm component requires interaction with a substance (iron? ubiquinone?) that can be removed by SDS.[3] Our SDS-treated RCs showed reversible photobleaching of P-870, but no sign of the new ESR signal of Loach and Hall.

The band shifts of BPh and BChl remain as possible correlatives of the primary photochemistry, in RCs stripped of secondary interactants. These shifts are seen, in different proportions, in response to chemical reduction (Fig. 6), chemical oxidation (Fig. 7), and photochemical oxidoreduction (Fig. 1 of reference 1 or 9). They may result from electric fields due to the oxidized and reduced entities in the RC, and as such may eventually prove useful as indicators of charge distribution.

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[3] We also saw an effect of SDS on the absorption spectrum of BPh in RCs. Without SDS, the band near 535 is a doublet, resolved at liquid nitrogen temperature into two equal peaks at 530 and 543 nm, suggesting dimer interaction of two BPh molecules. When 0.1% SDS was added, the band was sharpened to a single one at 537 nm.

We have further preliminary evidence that the 450 and 770 nm components of $\Delta(R)$ reflect separable events, the latter (band shifts) being correlated with the changes in P-870 fluorescence. In an earlier communication (6) we showed that in RCs exposed to 0.05% LDAO and reduced cytochrome *c*, the light-induced ΔOD at 430 nm (representing the 450 nm band) followed the same kinetics as the transient rise in fluorescence (Δf). We have now found, using reduced PMS as electron donor and 1% LDAO, that the ΔOD at 455 nm and Δf show different kinetics, with ΔOD (455 nm) relaxing more rapidly than Δf after illumination. The kinetics of ΔOD at 770 nm matched those of Δf under these conditions. Specifically, the ΔOD (455 nm) showed 50% recovery 17 sec after the end of illumination, whereas ΔOD (770 nm) and Δf required 35 sec for 50% recovery. These unexpected results deserve further study. They suggest that band shifts and high P-870 fluorescence can be associated with secondary events, events that recover more slowly than the capacity for photochemistry. These observations do not rule out the possibility that the band shifts and the high fluorescence are associated *also* with the primary photochemistry.

In conclusion, we have observed some new optical absorption changes associated with photochemical reduction in RCs, but none of these changes serves to identify a primary electron acceptor. Perhaps the acceptor is not a distinctive molecule, but rather a special configuration of the RC protein: a region of high density of positive charge, able to trap electrons from excited P-870 and then pass them on to secondary acceptors such as UQ.

Note Added in Proof. Feher et al. (22) have identified the ESR signal of Loach and Hall (19) as due to anionic ubisemiquinone, UQ^-.

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