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

I. KINETICS OF THE OXIDATION AND REDUCTION OF P-870 AS AFFECTED BY EXTERNAL FACTORS

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ABSTRACT Photosynthetic reaction centers from *Rhodopseudomonas spheroides* were prepared with the detergent lauryl dimethylamine oxide (LDAO). In contrast to reaction centers made with Triton X-100, these contained no cytochromes and little or no ubiquinone (UQ). The reduction of P-870, after its photochemical oxidation, was studied in these materials with the following results. In reaction centers made with Triton X-100, slow kinetic components (seconds to minutes) could be attributed to secondary electron acceptors or traps. In reaction centers made with LDAO the kinetics were predominantly fast (half-times, 100 msec or less); slower components could be introduced by adding UQ. Added UQ appeared to become bound to reaction centers made with LDAO, but the binding might have meant only that both components were trapped within detergent micelles. Ferricyanide could retard the reduction of oxidized P-870, apparently by capturing electrons from the reducing side of the photochemical system. Under conditions in which the participation of secondary electron acceptors seemed to have been eliminated, the recovery of P-870 was mainly by a first-order process with a half-time of about 60 msec at room temperature and 20–30 msec at about −80°C and below. The transition with decreasing temperature suggested the presence of a mixed population, exhibiting both the 60 and 20 msec components, but variations in the absorption spectra with temperature did not suggest the presence of a mixed population. Absorption difference spectra in the ultraviolet were compatible with the idea that UQ added to reaction centers became reduced in the light.

INTRODUCTION

Photochemical reaction centers have been prepared from chromatophores of *R. spheroides* by a new method (1) using the detergent LDAO, following a suggestion by Dr. R. G. Bartsch.[1] These reaction center particles are smaller (molecular weight

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[1] Essentially the same method was developed independently by G. Feher's group (2) following this suggestion.

less than 10^5) and chemically simpler (nearly devoid of cytochromes and quinones) than the preparations made with Triton X-100 as described earlier (3).

This is the first of a series of articles dealing with the optical properties of these reaction center preparations in relation to photochemical electron transport. Reaction centers show reversible bleaching of an absorption band at 865 nm, reflecting the oxidation of a component of bacteriochlorophyll, P-870, that acts as primary photochemical electron donor. In this article we describe the kinetics of P-870 oxidation as affected by the presence of UQ and other reagents. Also, some effects of low temperature on P-870 oxidation and on the absorption spectra of the preparations are shown and discussed.

MATERIALS AND METHODS

Materials

LDAO was a gift from Onyx Chemical Corporation, Jersey City, N. J., through the courtesy of Mr. R. Sorrentino. UQ (coenzyme Q_6) was purchased from Sigma Chemical Co., St. Louis, Mo.

Reaction centers from blue-green mutant *R. spheroides* were prepared as described in detail elsewhere (1). The method involved preparation of chromatophores, addition of LDAO, centrifugation to remove heavy materials, and fractionation of the supernatant with ammonium sulfate to purify the reaction centers. Absorption spectra of a typical preparation, measured under weak and strong light respectively, are shown in Fig. 1. In the various preparations used in these experiments the ratio of optical density (OD) at 282 nm to that at 802 nm ranged from 1.2 to 1.4.

Alternatively reaction centers were prepared using Triton X-100 instead of LDAO, as described earlier (3). Reaction centers made with Triton contained variable amounts of cytochromes (about one to three molecules per molecule of P-870) and UQ (about 5–20 per P-870) and had an apparent molecular weight approaching 10^6. In contrast the reaction centers made with LDAO were smaller (molecular weight about 10^5) and simpler chemically, containing no detectable cytochromes and less than 1 UQ per 10 P-870. Cytochromes were estimated from oxidized-reduced difference spectra in the alpha band region, using ferricyanide and dithionite as oxidant and reductant. UQ was extracted from freeze-dried reaction centers with 2,2,4-trimethylpentane, transferred to ethanol, and assayed by the OD at 275 nm and the loss of OD at 275 nm on addition of $NaBH_4$. UQ may have been underestimated because of its possible degradation during 2 days dialysis of the material before freeze-drying.

The concentration of reaction centers was based on the assumption that each reaction center contains one P-870 with $\epsilon = 113\ \text{mM}^{-1}\ \text{cm}^{-1}$ and $\Delta\epsilon = 100\ \text{mM}^{-1}\ \text{cm}^{-1}$ at 867 nm (4). In all experiments involving reaction centers in aqueous media, the system contained 0.01 M Tris-Cl, pH 7.5.

Instruments

Absorption spectra were measured with a Cary 14 R Spectrophotometer (Cary Instruments, Monrovia, Calif.). Light-induced changes of OD were measured with a simple split-beam difference spectrometer described earlier (5). In most measurements the measuring wavelength was 863 nm and excitation was at 800 nm. Interference filters prevented scattered

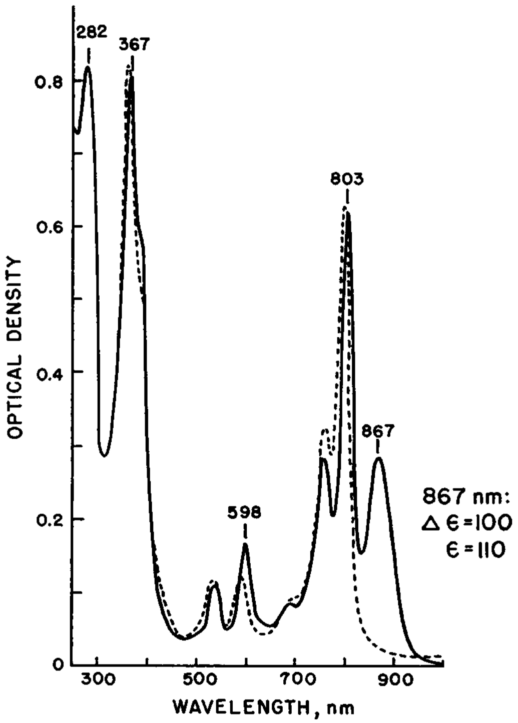


FIGURE 1 Absorption spectra of reaction centers from *R. spheroides*, prepared with LDAO by the method of Clayton and Wang (1). The dashed curve shows the spectrum under strong illumination; note the bleaching of the 867 nm band (P-870).

exciting light from reaching the detectors. In reaction centers the fluorescence was too weak to disturb the measurements of ΔOD. Excitation was delivered by either a 600 w tungsten-iodine lamp or a xenon flash lamp operated at 100 J and 0.1 msec half-time. Signals were recorded with both a strip-chart recorder and a storage oscilloscope. An example of a trace on the oscilloscope is given in Fig. 2. This trace represents one of the more challenging measurements, with a time constant of about 2 msec. The noise corresponded to about 0.002 OD units. Figs. 3–9 show smoothed traces of the data; in these the confidence limits are within 0.002 OD (about 5 arbitrary units in Fig. 7). Measurements at low temperatures were made with an unsilvered Dewar flask designed to support either aqueous samples or dried films, as described earlier (6).

RESULTS AND DISCUSSION

Kinetics Involving Primary and Secondary Electron Acceptors

Fig. 3 shows the bleaching and recovery of P-870 in reaction centers prepared with Triton X-100, signaling the reaction

$$P \underset{\text{dark}}{\overset{\text{light}}{\rightleftarrows}} P^{+}, \qquad (1)$$

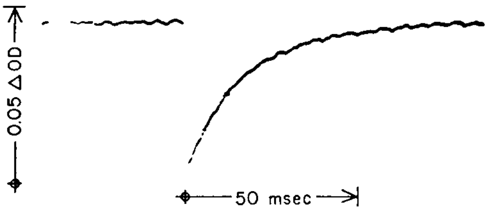


FIGURE 2

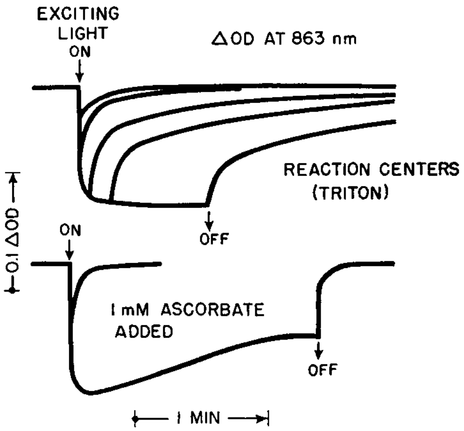
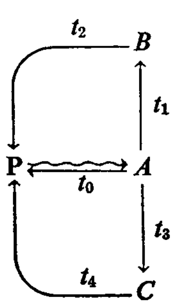


FIGURE 3

FIGURE 2 Light-induced bleaching (oxidation) and recovery of P-870, measured by the ΔOD at 863 nm. Exciting light 800 nm, from a xenon flash lamp. The flash duration was less than 1 msec. The sample was a dried film of reaction centers (made with LDAO) that had been extracted with isooctane. The OD at 865 nm was 0.2.

FIGURE 3 Bleaching and recovery of P-870 in reaction centers made with Triton X-100, measured by the ΔOD at 863 nm. The 800 nm exciting light was 3 mw/cm^2, from a tungsten-iodine lamp. It was turned off at different times in replicate measurements; the traces are superimposed here. Reaction centers at a concentration of 1.5 μM (based on ϵ = 113 mM^{-1} cm^{-1} at 867 nm) were suspended in 0.01 M Tris-Cl, pH 7.5, with 0.05% Triton X-100.

where P stands for P-870. After a relatively short exposure to light, the half-time for recovery was about 3 sec. Prolonged illumination caused a much slower recovery to predominate. The behavior was consistent with the following explanation. The most probable fate of an electron removed from P-870 was to enter a cycle by which it returned to P-870 in a few seconds. After a brief exposure to light, most of the reaction centers recovered in this way, but occasionally an electron found its way into a deep trap or cul-de-sac from which it took minutes (on the average) to return to P-870. After prolonged illumination this less probable event had happened in most of the reaction centers, leaving them in a state from which recovery was very slow. This model is diagrammed below, with the primary electron acceptor denoted A and secondary acceptors B and C. First-order rate constants for electron transfer are indicated by the half-times t_0, $\cdots$, t_4.



$$\begin{aligned} t_0 &= 0.02\text{–}0.1 \text{ sec},\\ t_1 &\ll t_0,\\ t_2 &= 1\text{–}5 \text{ sec},\\ t_3 &\gg t_1,\\ t_4 &= 1 \text{ min or more.} \end{aligned} \tag{2}$$

After an electron has been driven (wavy arrow denoting light reaction) from P to *A*, it may return directly to P or go on to *B* or *C*. The "direct" return to P might actually involve carriers or states not shown in the diagram. Its time constant t_0 seems to be about 50–100 msec at room temperature (see later) and 20–30 msec below about 150°K (7, 8). We do not see this rapid recovery process in reaction centers made with Triton and measured at room temperature, because the electron is transferred very quickly from *A* to *B* ($t_1 \ll t_0$), whence its return to P is slower (t_2 = several seconds). With lesser probability an electron may go from *A* to *C* and then require minutes to return to P.

The faster direct recovery process emerges when electron transfer to *B* and *C* is inhibited. This can be brought about in several ways:

(*a*) by lowering the temperature (7, 8; see also later in this article);

(*b*) by removing the secondary electron acceptors, through the use of LDAO instead of Triton or through extraction with solvents (see later);

(*c*) by the action of orthophenanthroline, described in another article in this series;

(*d*) by reducing the secondary substances so that they cannot accept electrons.

The fourth of these methods is illustrated in the lower part of Fig. 3. In the presence of 10^{-3} M sodium ascorbate, the reduction of oxidized P-870 after a short (0.2 sec) period of illumination had a half-time of about 1 sec. This probably reflected a reaction between P^+ and ascorbate; but after a longer exposure to light about half of the recovery was by a much faster process, of half-time 60 msec in this instance. A simple explanation is that during illumination, electrons are transferred from ascorbate through P-870 and *A* to the secondary acceptors *B* and *C*. When these substances are fully reduced, and therefore do not accept electrons from *A*, the recovery of P-870 is by the path with half-time t_0 (see reaction 2). During illumination with ascorbate present, the concentration of P^+ (amplitude of the ΔOD) is seen to pass through a peak and then subside to a lesser value. This is partly because the back reaction is becoming faster, and perhaps also because in some reaction centers the primary acceptor has become reduced and cannot function in the light reaction. In any case the decline in amplitude of the ΔOD affords a visualization of the photochemical filling of electron acceptor pools in the presence of ascorbate.

Effects of Added UQ

In reaction centers made with LDAO, and known to contain little or no UQ, the recovery of P-870 after a flash of light was mainly by a first-order process of half-

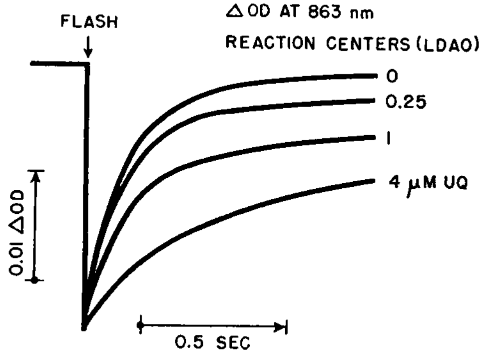


FIGURE 4

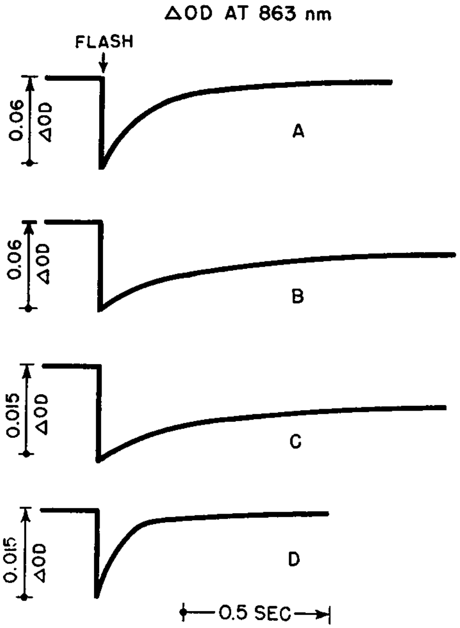


FIGURE 5

FIGURE 4 Flash-induced ΔOD at 863 nm in reaction centers with varying concentrations of added UQ (coenzyme Q_6) as shown. Reaction centers made with LDAO were suspended to 0.9 μM in 0.01 M Tris-Cl, pH 7.5, with 0.05% LDAO.

FIGURE 5 Flash-induced ΔOD at 863 nm in reaction centers made with LDAO. (A) Reaction centers, 2 μM; no added UQ; 0.05% LDAO. (B) The same with 4 μM UQ added. (C) Sample of B diluted four times with 0.01 M Tris-Cl, pH 7.5, containing 0.05% LDAO, measured with four times greater sensitivity (note the vertical scale on the graph). (D) Sample of C with the concentration of LDAO increased to 1.5%.

time about 0.1 sec. The addition of UQ introduced a first-order component of 1.2 sec half-time, competing with the faster one. Details of this effect are shown in Fig. 4 and Table I. Semilogarithmic plots of the decay of ΔOD showed just two major first-order components as reported in the table.

The strong effect of a low concentration of UQ, such as 1 μM in the presence of 1 μM P-870, suggested that the UQ became bound to the reaction centers. This suspicion was confirmed by the experiment shown in Fig. 5. Reaction centers (2 μM) were treated with enough UQ (4 μM) to have a pronounced but not saturating effect on the recovery of P-870. The preparation was then diluted fourfold with water, the spectrometer was made four times more sensitive, and the ΔOD was measured again. The new curve (C in Fig. 5) was very like the old (B), showing that the interaction between UQ and reaction centers was not weakened upon dilution.

Even though dilution did not weaken the effect of UQ on reaction centers, the effect was lost (curve D) when the concentration of LDAO was raised from 0.05 to 1.5 %. In fact, the decay became faster than that without added UQ at low LDAO

TABLE I

RECOVERY OF ΔOD AT 863 NM IN REACTION CENTERS*

Concentration of added UQ	Fast decay component		Slow decay component	
	$t_{1/2}$	% of total‡	$t_{1/2}$	% of total
μM	*sec*		*sec*	
0	0.12	94	—	6
0.25	0.10	81	1.5	19
0.5	0.09	70	1.2	30
1.0	0.09	54	1.1	46
2.0	0.12	46	1.2	54
4.0	0.10	33	1.1	67

* Conditions as in Fig. 4 (0.9 μM P-870).

‡ Total ΔOD = 0.025 ±0.001 in each case.

concentration (compare curve D with curve A). The dissociation of UQ from reaction centers at high LDAO concentration might have been expected, since this detergent removes native UQ from the reaction centers. In that case the "binding" of added UQ at low LDAO concentration might mean merely that most of the UQ molecules are trapped in detergent micelles along with one or more reaction centers. When the concentration of LDAO is raised, the binding is lost because a molecule of UQ is usually in a micelle that contains no reaction centers. If so, the binding should be restored by raising the concentration of reaction centers to match the higher concentration of detergent; it is.

Curves A and D in Fig. 5 show that the half-time for $P^+ \rightarrow P$ was reduced from about 0.1 to 0.06 sec when the concentration of LDAO was raised from 0.05 to 1.5% (this was observed whether UQ had been added or not). Therefore the time of 0.1 sec should not necessarily be taken as t_0 in terms of our model (reaction 2). We do not know whether the change from 0.1 to 0.06 sec means that an electron carrier has been removed or that the high concentration of LDAO has wrought a physical change on the reaction centers. A physical change is suggested by the fact that after 24 hr incubation at 1.5% LDAO, the absorption peak of P-870 became shifted from 865 to 852 nm.

The extraction of dried reaction centers with isooctane (2,2,4-trimethylpentane, which should remove UQ among other things) also caused the half-time to become 0.06 sec. This is shown in Fig. 6, which describes the behavior of a dried film of reaction centers. The film was made from an aqueous suspension containing LDAO, so in spite of its rigid appearance it must have contained a high concentration of this detergent as well as a high concentration of reaction centers.

The untreated film showed a half-time of about 1 sec for decay of the ΔOD, an order of magnitude slower than in the aqueous suspension from which it had been made. We do not know the reason for this, but after an overnight soak in isooctane,

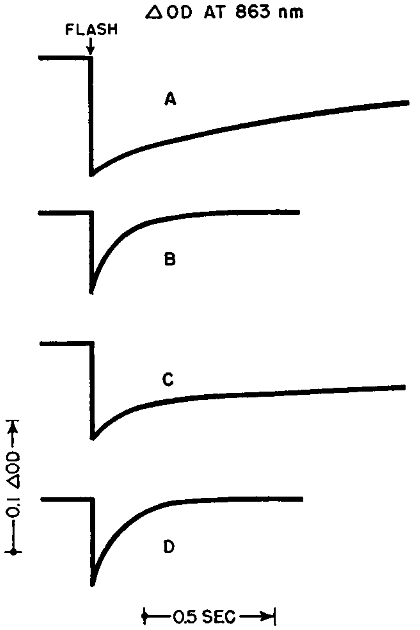


FIGURE 6

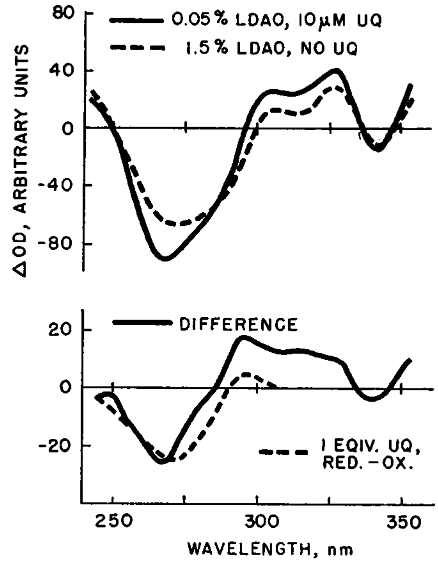


FIGURE 7

FIGURE 6 Flash-induced ΔOD at 863 nm in a dried film of reaction centers made with LDAO. The OD at 865 nm was 0.2. (A) Film prepared from an aqueous suspension containing 0.05% LDAO. (B) The film was soaked in isooctane, dried, and tested again. (C) The same film was then soaked in isooctane containing 2 mM UQ, dried, and reexamined. (D) The same film was extracted once more with isooctane to remove the UQ, and tested.
FIGURE 7 Spectrum of light-induced ΔOD's in reaction centers (1.5 μM) made with LDAO. Exciting light 800 nm, 3 mw/cm^2, 3 sec exposure. The upper curves show the spectra with 10 μM UQ added and 0.05% LDAO (solid curve) and with no UQ and 1.5% LDAO (dashed curve). For both curves the vertical scale was normalized to 100 arbitrary units at 602 nm. The difference between the two curves is plotted below, along with the "reduced minus oxidized" difference spectrum for an equivalent amount of UQ. By equivalent we mean ½ mole of UQ for each mole of P-870 oxidized. P-870 oxidation was based on the ΔOD at 602 nm, which is 21% of that at 865 nm.

followed by drying, the half-time was found to be 0.06 sec. The film was then soaked several minutes in isooctane containing 2 mM UQ, drained, and allowed to dry. This treatment reestablished slower decay components (curve C in Fig. 6) which could be removed by washing again with isooctane.

We have now recorded three distinct treatments: illumination in the presence of ascorbate, extraction with isooctane, and addition of 1.5% LDAO, that cause oxidized P-870 to become reduced with a half-time of 0.06 sec. The same half-time is induced by adding 10^{-4} M orthophenanthroline to reaction centers, regardless of the way in which they were prepared and treated with LDAO or UQ (see a later article in this series). We take 0.06 sec provisionally to represent the room temperature value of t_0 in our model.

The slowing of $P^+ \rightarrow P$ by UQ could mean that the UQ is actually accepting electrons from A^- and giving them to P^+, passing reversibly through its reduced form UQH_2. Alternatively the UQ, because of its electron affinity, could retard the movement of electrons from A^- to P^+ without participating in the cycle in the usual sense:

$$\begin{tikzcd} P \cdot A \cdots UQ \arrow[d, shift right, "h\nu"'] & \\ P^+ \cdot A^- \cdots UQ \arrow[u, shift right] \arrow[r, shift left] & P^+ \cdot A \cdots {}^-UQ \arrow[l, shift left] \end{tikzcd} \tag{3}$$

This would be an example of a physical effect of UQ on the reaction kinetics.

We do not have decisive information on these alternatives. It is clear that when an electron donor such as reduced cytochrome is added, the charge on P^+ is neutralized rapidly by an electron from the donor and the electron on the reducing side of the photoact moves freely to UQ, so that reaction centers can cause photochemical oxidation of excess cytochrome coupled with the reduction of an equivalent amount of UQ (published in the second article in this series); but without an added electron donor to reduce P^+, it is not clear that UQ can become reduced. Efforts to see an electron spin resonance signal corresponding to the semiquinone, using reaction centers made with LDAO, mixed with UQ, and exposed to brief flashes of light, have not succeeded. Spectra of light-induced ΔOD's in the ultraviolet,[2] for reaction centers with and without added UQ, are shown in Fig. 7. The major features of both spectra, including most of the bleaching centered near 275 nm, can be ascribed to the oxidation of P-870, as Loach has shown by comparisons with spectra of oxidized bacteriochlorophyll in vitro (9). The difference between the two curves could accommodate the difference spectrum (reduced-oxidized) of one equivalent (one-half mole) of UQ per mole of P-870 oxidized, but the correspondence is not particularly striking.

If UQ is reduced in the usual sense, two H^+ are incorporated with every two electrons, and the reaction should be favored at lower pH values. Conversely the oxidation of reduced UQ should be retarded at lower pH. This point was tested in an experiment like the one shown in Fig. 4, using the following conditions: reaction centers, 1 μM in 0.05 M Tris-Cl with 0.1% LDAO. [UQ] = 0 and 4 μM respectively, at pH 7, 8, and 9. Without UQ the kinetics of ΔOD (863 nm) were the same at all three pH values. With UQ the slow component made up 75% of the total ΔOD at each pH, but the half-time of this component varied with pH as follows:

pH = 7, $t_{1/2}$ = 1.45 sec
" 8, " 1.1 "
" 9, " 0.64 "

These results support, at least qualitatively, the idea that the slow component of $P^+ \rightarrow P$ is coupled to the oxidation of reduced UQ.

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[2] For these measurements the tungsten measuring beam lamp in the spectrometer was replaced with a deuterium arc lamp.

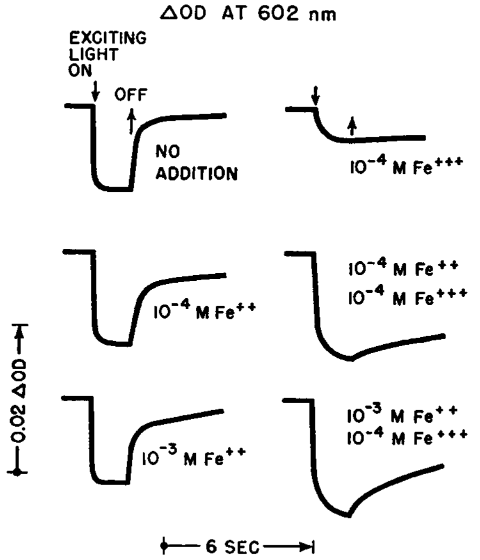

FIGURE 8

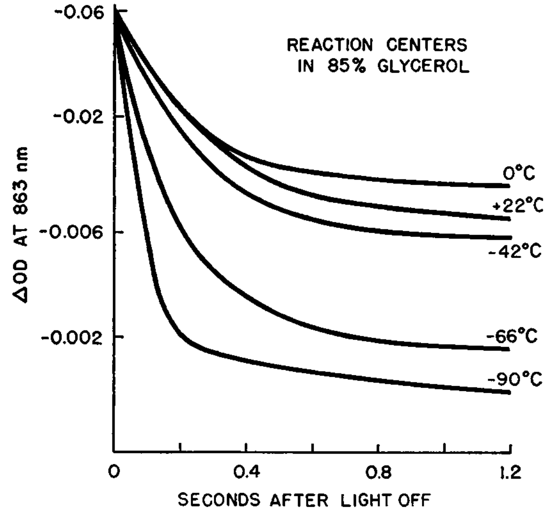

FIGURE 9

FIGURE 8 Light-induced oxidation of P-870, measured by the ΔOD at 602 nm, in reaction centers prepared with LDAO. Reaction centers, 1.0 μM in 0.05 M Tris-Cl, pH 7.5, with 0.1% LDAO. The effects of different concentrations of potassium ferricyanide and ferrocyanide, denoted Fe^{+++} and Fe^{++} respectively, are shown. Exciting light was 870 nm, 5.7 mw/cm², 1.5 sec exposure.

FIGURE 9 Semilogarithmic plots of the ΔOD at 863 nm after flash-induced bleaching, in reaction centers made with LDAO and measured at various temperatures as shown. Reaction centers were suspended in 85% glycerol + 15% H_2O containing 0.01 M Tris-Cl, pH 7.5. The final concentration of LDAO was 0.05%. The OD at 865 nm was 0.2.

Interactions with Ferri- and Ferrocyanide

It is well known (10, 11) that P-870 interacts with ferri-/ferrocyanide, the redox couple P^+-P having a midpoint potential between +0.4 and +0.5 v. Ferricyanide also interacts with the reducing side, apparently removing electrons from primary and/or secondary acceptors. This is suggested by the effects of ferri- and ferrocyanide on the kinetics of ΔOD (863 nm) in reaction centers made with LDAO, as shown in Fig. 8. First of all, if the potential is set too high (upper right trace), some P-870 is oxidized chemically and cannot participate in the light reaction; but if this is avoided, the principal effect of ferricyanide is to retard markedly the reduction of oxidized P-870. This is shown in all the right-hand traces, with 10^{-4} M ferricyanide. The rate of $P^+ \rightarrow P$ is then governed by the concentration of ferrocyanide, suggesting that ferricyanide traps electrons from the reducing side of the photoact, and P^+ must be reduced by ferrocyanide:

$$A^- \rightarrow A,\ Fe^{+++} \rightarrow Fe^{++} \quad \text{and} \quad Fe^{++} \rightarrow Fe^{+++},\ P^+ \rightarrow P \qquad (4)$$

The slight retardation induced by adding ferrocyanide alone (left-hand traces) might have been caused by the inevitable accompanying trace of ferricyanide. In well purified reaction centers the decay of the ΔOD is affected strongly even by 4 μM ferricyanide, with [P-870] = 1 μM.

These observations seem to contradict earlier findings (12) that electron carriers such as methylene blue or cytochrome *c*, when added in their oxidized forms, can *accelerate* the reduction of oxidized P-870; however, in the earlier studies the reaction centers had been made with Triton X-100. In these preparations the rate of $P^+ \rightarrow P$ without added reagents was slow; $t_{1/2}$ = several seconds (see Fig. 3), associated with the presence of native UQ. The added electron carriers then facilitated the cycling of electrons back to oxidized P-870. Moreover, in contrast to leucomethylene blue or reduced cytochrome, ferrocyanide is a relatively ineffective donor of electrons to oxidized P-870.

Absorption changes were discovered recently (13) which reflect redox changes in reaction centers on the reducing side of the photoact. It is shown in another article in this series that these changes are affected by ferricyanide but not by UQ, in a manner suggesting that the former reagent but not the latter can take electrons entirely away from the reaction centers after illumination.

Kinetics and Spectra at Low Temperatures

The decay of ΔOD (863 nm) after an 800 nm exciting flash was measured at temperatures down to −90°C in reaction centers mixed with glycerol, and to −130°C in dried films of reaction centers. The decay curves were plotted semilogarithmically (see Fig. 9) and analyzed into first-order components by progressive subtraction, beginning with the slowest component. The results of this analysis were unequivocal as long as only one or two major components were present, but if three or more components emerged, the manner of their resolution became somewhat subjective and arbitrary.

Reaction centers made with LDAO and suspended in 85% glycerol (15% H_2O containing 0.01 M TrisCl, pH 7.5) with 0.05% LDAO behaved as shown in Fig. 10. In this figure the ordinate is temperature and the abscissa gives the half-time for each decay component. The amplitude (ΔOD) of each component is given by the height of the bar that represents it in the graph. Thus in Fig. 10 we show at 23°C a major component of ΔOD = 0.05, with a half-time of 0.09 sec, and two small components ($\Delta OD < 0.005$) at 0.5 sec and > 10 sec. We see that for the major component the half-time was 0.09 sec down to about −40°C, and then fell to 0.025 sec as the temperature was lowered to −80°C. At still lower temperatures, down to 1°K, the half-time remains between 0.02 and 0.03 sec (7, 8).

We may suppose that in this material the decay was direct (see reaction 2), with t_0 changing from 0.09 to 0.025 sec between −40 and −80°C. Alternatively we could claim that at room temperature the reaction involved a secondary carrier *B*, with

t_2 = 0.09 sec. Then at lower temperatures the electron transfer from *A* to *B* became slower (t_1 increased), so that the pathway involving *B* was "frozen out." At −80°C and below, only the direct pathway (t_0) operated.

Now let us examine the behavior of a dried film of reaction centers extracted with isooctane, as shown in Fig. 6 (curve B). The kinetics of $P^+ \rightarrow P$, shown in Fig. 11, presented a pattern distinct from that of reaction centers in glycerol. The half-time was about 0.06–0.07 sec at room temperature and a little under 0.02 sec at −100°C and below. At intermediate temperatures the decay did not show the gradual progression of a single component, as in Fig. 10. Instead, both components (0.06 and 0.02 sec) were represented in varying proportions. This pattern suggested a mixed population of reaction centers, in either of two states corresponding to the two decay times. This could come about after excitation if electrons found their way into either of two different traps (secondary acceptors). The changing ratio of the two components with temperature could result if electron transfer into each kind of trap was dependent on the temperature, but the two temperature dependences were dissimilar. The half-times for electron transfer from these traps to oxidized P-870 would be 0.06 and 0.02 sec, independent of the temperature. Heretofore we have identified the fastest decay (especially at 1°K) with a direct reversal of the primary photoact, re-

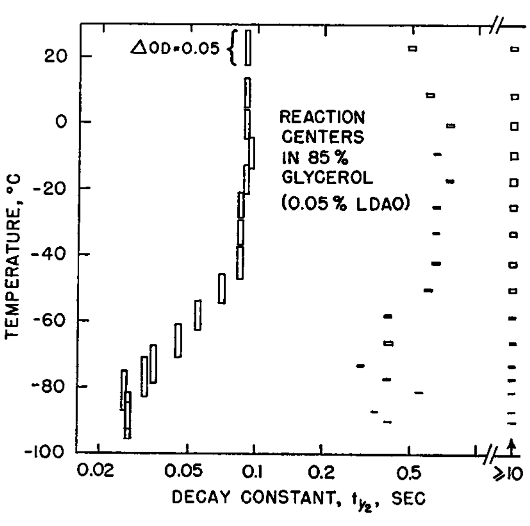

FIGURE 10

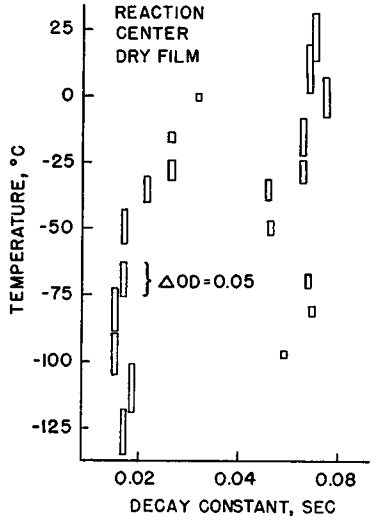

FIGURE 11

FIGURE 10 Decay curves like the ones in Fig. 9 were analyzed into first-order components by successive subtraction; the results are shown here (reaction centers in glycerol as in Fig. 9). At a given temperature, each component is represented by a bar. The amplitude of the component is indicated by the height of the bar. The horizontal position of the bar shows the half-time and the vertical position (center of bar) shows the temperature. Some of the data were obtained during cooling of the sample and some during warming; the results were the same at any one temperature.

FIGURE 11 Same as Fig. 10, but for a dried film of reaction centers extracted with isooctane, as described in Fig. 2 and in curve B of Fig. 6.

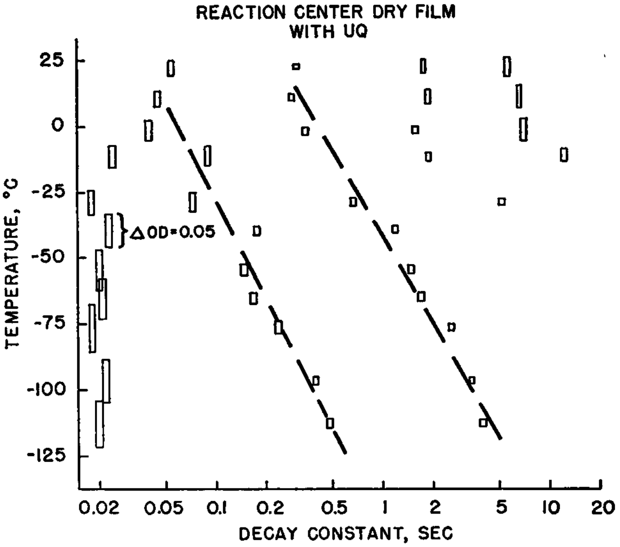

FIGURE 12 Same as Fig. 11, but for a dried film of reaction centers with added UQ as in curve C of Fig. 6.

serving the notion of secondary acceptors to explain the slower processes; however, we can entertain the possibility of secondary electron acceptors or states even along the so-called direct pathway.

As a variation of the foregoing picture, the reaction centers could be involved in a temperature-dependent structural change analogous to a phase transition,[3] going between two physical states characterized by different values of t_0 (0.06 and 0.02 sec). We shall return later to the question of other evidence for a physical transition at low temperatures.

When UQ was added to a dried film of reaction centers, as exemplified by curve C in Fig. 6, the kinetic pattern for recovery of P-870 became quite complicated. Fig. 12 shows that at room temperature there were several decay components slower than 0.1 sec; these were attenuated below about −10°C. There was some indication that these components became slower as the temperature was lowered; see the dashed lines sketched in the figure. At sufficiently low temperatures the component of 0.02 sec half-time predominated, as in other preparations.

To explore the possibility of a physical transition in reaction centers at low temperatures we measured absorption spectra and noted several features as functions of the temperature. These features included the wavelengths and OD's at the P-800 and P-870 peaks, and the half-bandwidth of P-870. Results are shown in Fig. 13, for reaction centers in H_2O-glycerol (0.05% LDAO) and in dried films.

The peak of P-800 remained at 802 nm as the temperature was lowered, but the peak of P-870 moved toward greater wavelengths. If there were a transition between

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[3] Dr. David Mauzerall suggested this possibility to us.

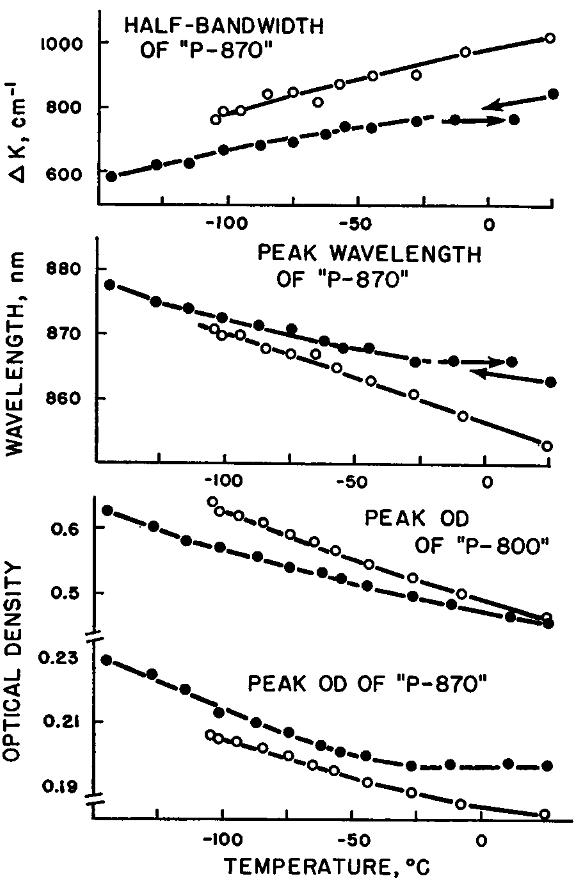

FIGURE 13 Parameters in the absorption spectra of reaction centers measured at various temperatures. Filled circles: dried film as in curve A of Fig. 6. Open circles: reaction centers in glycerol-H_2O as in Fig. 9. The half-bandwidth of P-870 was measured as follows: the wavelength was recorded at the absorption maximum and at the position of half-maximum OD on the long wave side of the peak. These wavelengths were converted to wave number (reciprocal of wavelength, centimeters^{-1}) and their difference was doubled to yield Δk.

two distinct states, one "low temperature, long wave" and the other "high temperature, shorter wave," with a mixed population at intermediate temperatures, then the absorption band should be broadest at those intermediate temperatures where the population is heterogeneous, becoming sharper at the extremes of temperature. Instead, the half-bandwidth was found to diminish monotonically with decreasing temperature.

The only clear indication of a sharp transition was seen in the peak OD of P-870, and then only in the dried film: the OD was constant down to −30°C and then abruptly began to rise with further decrease in the temperature. This was also the temperature at which the 0.06 sec decay gave way to the 0.02 sec component (see Fig. 11).

CONCLUSIONS

We have seen that the dynamics of electron transport in bacterial photosynthetic reaction centers, after the photochemical oxidation of P-870, can be described reasonably in terms of primary and secondary electron acceptors. While the model of reaction 2 can lay no claim to uniqueness or completeness, it has provided a sensible basis for discussing our observations. Alternative models can invoke mechanisms that we usually describe as physical rather than chemical; a so-called physical effect of UQ (for example) is likely to depend on its affinity for electrons (compare reactions 2 and 3). In the presence of reagents such as ferricyanide and UQ the fate of an electron, after its photochemical removal from P-870, should depend on the relative electron affinities of P^+, UQ, and $Fe(CN)_6$, as well as on details of structural organization. The relative solubilities of these components in the phases of the system should also be influential; for example ferricyanide, but not UQ, can easily diffuse in the aqueous phase, away from the detergent micelles that hold reaction centers.

Reaction centers made with Triton X-100 differ from those made with LDAO in that the former contain native UQ and display slow components in the kinetics of rereduction of oxidized P-870. These properties are conferred on the reaction centers made with LDAO when UQ is added. Both the native and the added UQ can be reduced through a photochemical reaction coupled to the oxidation of an external donor such as cytochrome. Thus the apparent function of UQ in vivo, to form an electron acceptor pool, can be reconstituted by adding UQ to the "stripped" reaction centers.

We are indebted to Dr. David Mauzerall for illuminating discussions.
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