*PHOTOREDUCTION OF UBIQUINONE AND PHOTOOXIDATION OF PHENAZINE METHOSULFATE BY CHROMATOPHORES OF PHOTOSYNTHETIC BACTERIA AND BACTERIOCHLOROPHYLL**

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A stimulation by phenazine methosulfate (PMS) of photophosphorylation with extracts of *Rhodospirillum rubrum* was first reported by Geller and Gregory,[1] who at the same time presented spectroscopic evidence that PMS was oxidized during the reaction. Jagendorf and Avron[2] subsequently reported that PMS serves as a cofactor for cyclic photophosphorylation in chloroplasts, and considerable interest and effort has since been concentrated on the action of PMS in the electron transfer reactions of photosynthetic systems. The activity of PMS in the photophosphorylation system of *R. rubrum* has been explained in two ways: (*a*) PMS bypasses inhibitor-sensitive and rate-limiting sites of the electron transfer sequence,[3, 4] and (*b*) PMS exerts an oxidation-reduction poising action on the system.[5]

The present communication shows that PMS participates directly in light-induced electron transfer reactions of *R. rubrum*, *Rhodopseudomonas spheroides*, and *Chromatium* chromatophores. These reactions are characterized by a rapid, light-dependent oxidation of reduced PMS and an associated reduction of added ubiquinone (UQ). In a subsequent dark period PMS is reduced by the photoreduced UQ, forming a cyclic system much like that previously described for the coupled photooxidation of ferrocytochrome *c* and photoreduction of UQ by *R. rubrum* chromatophores.[6] Furthermore, PMS can be replaced with the reduced forms of N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) and 2,6-dichlorophenolindophenol (DPIP), and a methanol extract of *R. rubrum* chromatophores (bacteriochlorophyll) also catalyzes the PMS-UQ photoreaction.

Methods and Materials.—*R. rubrum* cells were grown in a medium containing malate, glutamate, acetate, and ammonium chloride as described previously.[7] *Chromatium* was grown as described by Hendley,[8] and the culture medium for *Rps. spheroides* was that reported by Lascelles.[9] Chromatophores were prepared by a 2-min sonic oscillation (Raytheon, 10 kc) of twice-washed whole cells in 10% sucrose buffered at pH 7.8 with 0.1 *M* Tris-HCl. The particles sedimenting between 20,000 and 100,000 × *g* were washed once, suspended in the Tris-sucrose solution, and stored at 0–3°C. Reactions were performed under anaerobic conditions using red light (650 mμ sharp cutoff filter) and followed with a modified Bausch and Lomb Spectronic 505 recording spectrophotometer as outlined by Vernon.[7] UQ_6 was obtained from Mann Research Laboratories, and UQ_2 was a gift of Merck, Sharp and Dohme Research Laboratories. (UQ_6 is the abbreviation used for the ubiquinone containing 6 isoprene units in the side chain. Likewise, UQ_2 contains two such units.) Reduced ubiquinones were prepared by the method of Green and Burkhard.[10] Chlorophyll was determined by the method of van Niel and Arnold.[11]

Results.—The photooxidation of reduced PMS ($PMSH_2$) in the presence of UQ was followed at 388 mμ, a major absorption peak of PMS. Figure 1 shows the

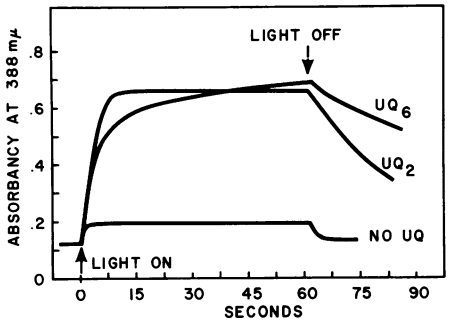

FIG. 1.—Photooxidation of $PMSH_2$ by *R. rubrum* chromatophores in the presence of UQ_2 or UQ_6. The reaction mixtures contained the following (in μmoles): sucrose, 1400; KCl, 28; reduced UQ_2, 0.33, or reduced UQ_6, 0.36 (added in 0.02 ml ethanol) where indicated; PMS, 0.20; Tris (final pH 7.1), 80; and *R. rubrum* chromatophores containing 0.032 mg bacteriochlorophyll in a final volume of 3 ml. The reaction mixtures were made anaerobic by 3 one-min evacuations interspaced by two additions of argon. The reactions were carried out at 25° C under vacuum. PMS was tipped into the reaction mixture from the cap after anaerobic conditions were obtained, and the reduced quinone reduced the PMS chemically prior to illumination.

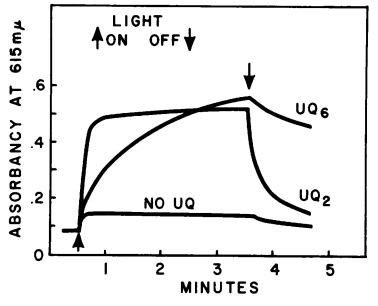

FIG. 2.—Photooxidation of reduced DPIP by *R. rubrum* chromatophores in the presence of UQ_2 or UQ_6. The reaction mixtures contained the following (in μmoles): sucrose, 700; KCl, 14; Tris-HCl (final pH 8.0), 100; UQ_2H_2, 0.16, or UQ_6H_2, 0.20 where indicated; DPIP, 0.10 with UQ_2H_2 and no UQ and 0.20 with UQ_6H_2; and *R. rubrum* chromatophores containing 0.034 mg bacteriochlorophyll in a final volume of 2 ml. Reactions were carried out as described in Fig. 1 except that an atmosphere of argon was used. DPIP was placed in the cap and tipped in after the reaction mixtures were made anaerobic.

absorbancy changes obtained when a system containing *R. rubrum* chromatophores, $PMSH_2$, and either UQ_2 or UQ_6 is illuminated. A few minutes prior incubation in the dark allows reduction of PMS by the added reduced UQ (UQH_2). Although the initial rates of $PMSH_2$ photooxidation (and UQ photoreduction) appear to be nearly the same for both quinones, the reaction with UQ_2 reaches its maximal extent sooner than that with UQ_6. In the dark the reaction is reversed in both cases, and the light-on light-off sequence can be repeated several times without changing the extent or rate of the reactions. In the absence of added ubiquinone an absorbancy change is observed with PMS which had been reduced by anaerobic illumination in white light prior to addition to the reaction mixture. The slight photooxidation of $PMSH_2$ in this case is coupled to endogenous components of the chromatophore (probably quinones) in a reversible system analogous to the reactions observed in the presence of added quinones. The dark reaction in which PMS is reduced by the photoreduced ubiquinone appears to be primarily chemical in nature, since it is not sensitive to 10^{-5} *M* antimycin A. In this respect it differs from the dark reduction of added cytochrome *c* by reduced ubiquinone which is sensitive to antimycin A at 10^{-7} *M*.[6]

Because of relatively slow instrument response it is not possible precisely to determine initial rates of the photoreactions shown in Figure 1. However, in cooperation with Dr. R. K. Clayton of this laboratory, an instrument with more rapid response was used to follow initial reaction rates. From these experiments initial $PMSH_2$ photooxidation rates of 6–8 mmoles/hr/mg Bchl were obtained.

The slight photooxidation of $PMSH_2$ by illuminated chromatophores in the absence of added UQ, shown in Figure 1, is similar to the reactions observed by

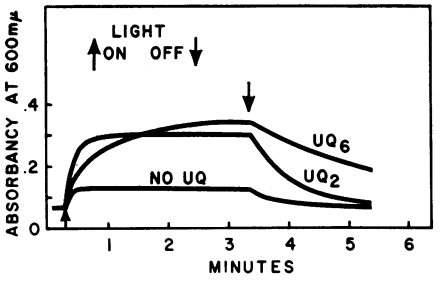

FIG. 3.—Photooxidation of TMPD by *R. rubrum* chromatophores in the presence of UQ_2 or UQ_6. Reaction mixtures contained the following (in μmoles): sucrose, 700; KCl, 14; Tris-HCl (final pH 7.1), 100; UQ_2 or UQ_6 (oxidized where indicated), 0.20; TMPD (in cap), 0.26; and *R. rubrum* chromatophores containing 0.028 mg bacteriochlorophyll in a final volume of 2 ml. Reactions were carried out as described in Fig. 2.

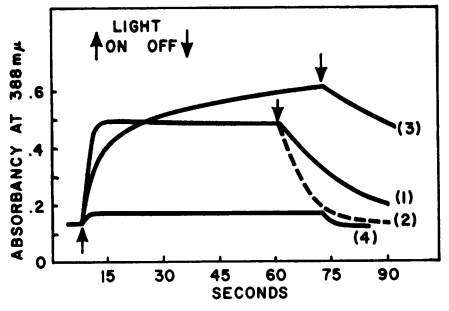

FIG. 4.—Photooxidation of $PMSH_2$ by chromatophores of *Rps. spheroides* and *Chromatium* in the presence of UQ_2 or UQ_6. Reaction mixtures contained (in μmoles): sucrose, 1400; KCl, 28; Tris-HCl, 80 (final pH 7.2); PMS, 0.20; UQ_2H_2, 0.30 (curves 1 and 2) or UQ_6H_2, 0.50 (curves 3 and 4); and chromatophores from *Rps. spheroides* (curves 3 and 4) or Chromatium (curves 1 and 2) containing 0.03 and 0.06 mg bacteriochlorophyll, respectively, in a final volume of 3.0 ml. Reaction mixtures giving curves 2 and 4 contained 0.2% deoxycholate. Reactions were carried out as described in Fig. 2.

Vernon[12] with *R. rubrum* chromatophores and either $DPIPH_2$ or TMPD, in which this photooxidation was apparently coupled to the photoreduction of UQ contained in the chromatophore. These two compounds are also active in a coupled system with added UQ and *R. rubrum* chromatophores, as shown in Figures 2 and 3.

Chromatophores from *Chromatium* and *Rps. spheroides* were shown by Vernon[12] to be active in the photooxidation of $DPIPH_2$ and TMPD in the absence of added UQ. Figure 4 shows that chromatophores from these photosynthetic bacteria will also couple with added UQ. This allows the photosynthetic apparatus in these bacteria to be investigated by means of this reaction, but to date our efforts have been concentrated on *R. rubrum*.

Chromatophores heated for 8 min at 60°C retain their ability to photooxidize $PMSH_2$. This agrees with the observation that photooxidation of added ferrocytochrome *c* in the presence of UQ proceeds rapidly even after heating chromatophores at 60°C for 3 hr,[6] and with previous reports on the heat stability of photooxidation reactions with chromatophores of *R. rubrum*.[13, 14] However, heating in boiling water for 2 min does inactivate the chromatophores. This is shown in Figure 5, where curve 1 represents the activity obtained in the $PMSH_2$–UQ_2 system with boiled chromatophores, and curve 3 shows that no activity is observed in the ferrocytochrome *c*-UQ_2 system. Upon addition of the detergent Triton X-100 a restoration of the $PMSH_2$–UQ_2 activity is obtained (curve 2), but the ferrocytochrome *c*-UQ_2 system remains inoperative (represented also by curve 3). This observation reflects a basic difference in the two reaction systems. $PMSH_2$ appears to react directly with bacteriochlorophyll (see also Fig. 6), while cytochrome *c* appears to react through some endogenous component(s) which is oxidized by the chlorophyll. The structural integrity of the chromatophore which is essential for the cytochrome *c* system is destroyed by heating in boiling water and cannot be

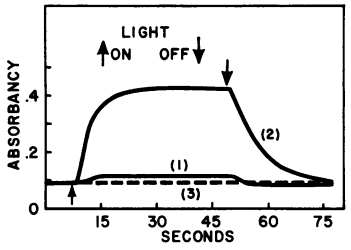

FIG. 5.—Effect of the detergent Triton X-100 on the photooxidation of $PMSH_2$ with heated chromatophores in the presence of UQ_6. Reaction mixtures contained (in μmoles): sucrose, 700; KCl, 14; Tris-HCl, (final pH 7.6) 60; UQ_6H_2, 0.28; PMS, 0.15 (curves 1 and 2, absorbancy at 388 mμ) or cytochrome *c*, 0.08 (curve 3); *R. rubrum* chromatophores, which had been heated in boiling water for 2 min, containing 0.02 mg bacteriochlorophyll; and when used, Triton X-100 at a final concentration of 0.15 per cent (curves 2 and 3). Final volume of the reaction mixture was 2 ml. Curve 3 (absorbancy at 550 mμ) represents the results obtained when the photooxidation of reduced cytochrome *c* was attempted with heated chromatophores both in the presence and absence of Triton X-100 (see ref. 6 for results with unheated chromatophores). Reaction conditions were the same as those outlined in Fig. 2.

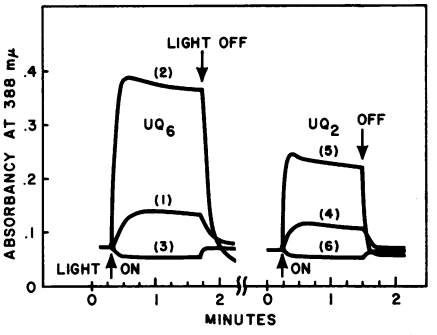

FIG. 6.—Photooxidation of $PMSH_2$ by bacteriochlorophyll in the presence of UQ_2 or UQ_6. Reaction mixtures contained (in μmoles): sucrose, 850; KCl, 17; Tris-HCl, 100 (final pH 7.7); PMS, 0.16 (in cap); UQ_6H_2, 0.24 (curves 1–3), or UQ_2H_2, 0.20 (curves 4–6) added in 0.02 ml ethanol; solubilized asolectin, 0.06% (curves 2 and 5); and a methanol extract of *R. rubrum* chromatophores containing 0.33 mg bacteriochlorophyll (0.05 ml) in a final volume of 2 ml. Curves 3 and 6 represent the results obtained by addition of deoxycholate (final concentration 0.5%) to reaction mixtures giving curves 2 and 5. Curves 1 and 4 were obtained from reactions with no detergent present. Conditions were the same as outlined in Fig. 2.

restored by the addition of detergent. On the other hand, bacteriochlorophyll which has been buried in denatured protein is exposed by detergent action and can react with $PMSH_2$ in the light.

Figure 6 shows that bacteriochlorophyll catalyzes the photooxidation of $PMSH_2$ in the presence of UQ_6 or UQ_2 (curves 1 and 4). The ability of chlorophyll *a*, chlorophyll *b*, chlorophyllin *a*, protoporphyrin IX, and tetraphenylporphyrin to photooxidize $PMSH_2$ in the presence of Triton X-100 and ubiquinones has recently been reported.[15] The reactions observed with bacteriochlorophyll, $PMSH_2$, and ubiquinone are greatly stimulated by the addition of certain detergents. Thus, curves 2 and 5 show the effect of solubilized asolectin (soybean phospholipid) on these reactions. Triton X-100 shows a similar stimulation. Deoxycholate at a concentration of 0.5 per cent inhibits the reaction, even showing some indication of a photoreduction of PMS, which agrees with observations reported recently.[15]

The effect of deoxycholate and Triton X-100 on the $PMSH_2$-UQ reactions in the presence of *R. rubrum* chromatophores was examined. Table 1 shows the inhibitory effect of deoxycholate on the initial rate and extent of the light reaction. The dark reaction, on the other hand, is stimulated. Although stimulation of the dark reaction may contribute to inhibition of the light reaction (since they are competing reactions), it is evident that this alone does not account for all of the inhibition observed. For example, in the presence of 0.07 per cent deoxycholate the rate of the dark reduction of PMS by UQ_6H_2 is the same as the control, but the rate of the light reaction is only 60 per cent of the control, and the extent has been reduced by about 20%. Inhibition of the $PMSH_2$-UQ_6 light reaction occurs at concentrations of deoxycholate which have no effect on the photooxidation of $PMSH_2$ in the

TABLE 1

EFFECT OF DEOXYCHOLATE ON $PMSH_2$-UQ REACTIONS

Final deoxycholate concentration, %	Extent of Light Reaction (Δ A 388 mμ)		Relative Initial Rates			
			Light reaction		Dark reaction	
	UQ_6	UQ_2	UQ_6	UQ_2	UQ_6	UQ_2
0	0.57	0.54	100	100	100	100
0.03	0.57	—	80	—	94	—
0.07	0.46	0.54	60	95	100	165
0.13	0.24	0.52	60	105	140	250
0.20	—	0.45 (0.38)*	—	95 (75)*	—	200 (200)*
1.0	0	—	0	—	—	—

* Values in parentheses obtained with the same reaction mixture after 20-min incubation at 25°C. The reaction mixtures contained (in μmoles): sucrose 1400, KCl 28, UQ_2H_2 0.33 or UQ_6H_2 0.36, PMS 0.20, Tris-HCl 80 (final pH 7.1), and *R. rubrum* chromatophores containing 0.032 mg bacteriochlorophyll in a final volume of 3 ml. Reaction conditions were those described in Fig. 1. Deoxycholate was added as indicated. Initial rates were calculated from recorder tracings of absorbancy changes at 388 mμ.

presence of UQ_2. One per cent deoxycholate completely inhibits the photooxidation of $PMSH_2$ with UQ_6 as the electron acceptor.

Triton X-100 differs from deoxycholate in its effect on the light reactions (Table 2). At very low detergent concentrations an inhibition of both reaction rates and extent is seen. As the detergent concentration is increased, the inhibitory effects disappear and, in some cases, stimulations are observed. Here also, as in the experiments with deoxycholate, the effective detergent concentration differs for the two ubiquinones. While Triton X-100 at the higher concentrations suffices to solubilize spinach chloroplasts,[15] it did not remove the bacteriochlorophyll from the chromatophore particle.

Discussion.—The sequence of reactions reported in this communication can be written:

$$(1)\quad UQ + PMSH_2 \xrightarrow{\text{light}} UQH_2 + PMS$$

$$(2)\quad UQH_2 + PMS \xrightarrow{\text{dark}} UQ + PMSH_2.$$

Reaction 1 represents the photochemical reaction catalyzed by the chromatophore (or extracted chlorophyll) and shows the direct involvement of $PMSH_2$ in the electron transfer reaction in the light. $PMSH_2$ can be replaced in the above chromatophore reactions with $DPIPH_2$ or TMPD, as seen in Figures 2 and 3, or with ferrocytochrome *c*.[6]

Clayton has demonstrated that light-induced oxidation of endogenous cytochrome in *Rps. spheroides* and *Chromatium* is coupled to the reduction of quinone contained in the cells.[16] Bales and Vernon[17] have shown that the rapid photooxidation of added $DPIPH_2$ catalyzed by chromatophores of *R. rubrum* produced absorption changes in the particles which are related to quinone reduction. These investigators have considered that the bacteriochlorophyll mediates the photoreaction by directly transferring electrons from the reductant (reduced endogenous cytochrome or reduced dye) to the endogenous quinone. Bose and Gest[18] have offered another explanation for reactions of this type, proposing that a "reverse electron flow" occurs via the enzymatic components of the particle, driven by ATP formed in the light.

The present experiments show that $PMSH_2$, $DPIPH_2$, and TMPD in the presence of illuminated chromatophores can reduce added UQ_2 and UQ_6. These reactions are against the electrochemical potential gradient, are reversed in the dark, and are

TABLE 2

EFFECT OF TRITON X-100 ON $PMSH_2$-UQ REACTIONS

Final Triton X-100 concentration, %	Extent of Light Reaction (ΔA 388 mμ)		Relative Initial Rates			
			Light reaction		Dark reaction	
	UQ_6	UQ_2	UQ_6	UQ_2	UQ_6	UQ_2
0	0.61	0.38	100	100	100	100
5×10^{-4}	0.16	0.13	78	86	33	33
1×10^{-3}	0.03	0.28	30*	100	1*	70
5×10^{-3}	0.05	0.30	40*	100	1*	72
1×10^{-2}	0.03	0.36	30*	120	—	100
5×10^{-2}	0.12	0.42	84	145	130	155
1×10^{-1}	0.28	0.50	100	150	200	140
2×10^{-1}	0.52	—	100	—	210	—

* Because of small absorbancy change the rate is only approximate.
Reaction mixtures contained (in μmoles): sucrose 900, KCl 18, Tris-HCl (final pH 7.5) 100, PMS 0.16, UQ_6 or UQ_2 0.15, and *R. rubrum* chromatophores containing 0.02 mg bacteriochlorophyll in a final volume of 2.2 ml. Reaction conditions were the same as described in Fig. 2. Triton X-100 was added as indicated. Initial rates were calculated from recorder tracings of absorbancy changes at 388 mμ.

best explained in terms of a direct transfer of electrons from the electron donor molecule to UQ via the bacteriochlorophyll upon illumination. In the case of $PMSH_2$ and UQ, the reaction proceeds in the presence of extracted bacteriochlorophyll or with boiled chromatophores treated with detergent, and in these cases the ATP-forming system is not operative.

Although the photooxidation of $PMSH_2$ by chromatophores appears to be a direct electron transfer reaction mediated by bacteriochlorophyll, it is not possible at this time to say if $PMSH_2$ and UQ react directly with chlorophyll or with chromatophore components which are intimately associated with the bacteriochlorophyll at the reaction center. It appears that the anaerobic photooxidation of ferrocytochrome *c* requires structurally intact chromatophores and some endogenous chromatophore component(s), since this reaction is not catalyzed by boiled chromatophores in the presence of Triton X-100, or by extracted bacteriochlorophyll.

The effect of detergents on $PMSH_2$ photooxidation by chromatophores is complex. The nature of the detergent and the length of the ubiquinone isoprenoid side chain appear to influence the response of the photoreaction to detergent action. The effect of deoxycholate, as seen in Table 1 and Figure 6, is essentially the same as observed previously for the photooxidation of $PMSH_2$ in the presence of chloroplasts (or chlorophyll *a*) and ubiquinone.[15] In those studies it was shown that deoxycholate caused a reversal of the reactions (e.g., a photoreduction of PMS and a dark oxidation of $PMSH_2$). An explanation of this phenomenon is not possible at this time. However, in view of the rapid photoreduction of PMS when trimethyl-*p*-benzohydroquinone ($E_0' = +0.102$ V[19]) is used in place of ubiquinone in the absence of deoxycholate,[15] either a change in equilibrium between the oxidized and reduced forms of PMS and UQ or an alteration of the redox potential of one of the reactants might be suspected.

An explanation of the inhibition of Triton X-100 at low concentrations which disappears at higher concentrations would also be premature at this point. Since the detergent is capable of restoring activity to heated chromatophores (Fig. 5), it is likely that stimulations seen at higher concentrations result from the exposure to PMS and UQ of bacteriochlorophyll which, in the absence of detergent, could not catalyze the reaction.

Summary.—Chromatophores of photosynthetic bacteria catalyze a rapid, light-dependent oxidation of reduced phenazine methosulfate ($PMSH_2$), 2,6-dichloro-

phenolindophenol and N,N,N′,N′-tetramethyl-*p*-phenylenediamine. These photooxidations are coupled to reduction of ubiqinone (UQ_2 or UQ_6). A back reaction occurs in the dark, allowing for cyclic electron flow. Deoxycholate inhibits the light reaction whereas Triton X-100 inhibits at low concentrations and stimulates the reaction at higher concentrations. Chromatophores are inactivated by heating in boiling water, but subsequent addition of Triton X-100 restores the $PMSH_2$ photooxidation activity. The $PMSH_2$-UQ reaction is also catalyzed by extracted bacteriochlorophyll. These data indicate that the chromatophore catalyzes the photoreaction via bacteriochlorophyll which catalyzes a direct transfer of electrons from the donor molecule to UQ under the influence of light.

The authors gratefully acknowledge the technical assistance of Miss Georgia Helmer during the course of this investigation, and are indebted to Dr. R. K. Clayton for the use of his equipment and for his collaboration on the experiments with the kinetics of the reaction. Appreciation is also expressed by Dr. B. Burnham for furnishing *Rps. spheroides* cells.

* Contribution no. 118 from the Charles F. Kettering Research Laboratory. The term "chromatophore" designates the particulate fraction prepared by sonic oscillation and sedimenting between 20,000 and 100,000 × *g* during centrifugation.

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