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THE LIGHT-INDUCED OXIDATION-REDUCTION REACTIONS OF MENAQUINONE IN INTACT CELLS OF A GREEN PHOTOSYNTHETIC BACTERIUM, *CHLOROPSEUDOMONAS ETHYLICA*

KEN-ICHIRO TAKAMIYA

Department of Biology, Faculty of Science, Kyushu University, Fukuoka (Japan) *

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SUMMARY

1. The light-induced ultraviolet absorption changes of intact cells of a green photosynthetic bacterium, *Chloropseudomonas ethylica*, were investigated spectroscopically under aerobic and anaerobic conditions. Under anaerobic conditions the ultraviolet absorption between 260 and 290 nm increased on illumination and that of wavelengths shorter than 260 nm decreased. The light-*minus*-dark difference spectrum suggested that these absorption changes were caused by menaquinone contained in *C. ethylica*. It was calculated that 60% of the total menaquinone was oxidized on illumination. Under aerobic conditions the photooxidation of menaquinone was abolished but was observed again after making the suspension anaerobic.

2. The time course of photooxidation of menaquinone consisted of a rapid phase and a slow phase. The kinetic behaviour of the menaquinone reaction was generally similar to that of photooxidation of cytochrome(s).

3. The light-induced reaction of menaquinone was compared with those of quinones in other photosynthetic bacteria, *Rhodospirillum rubrum*, *Rhodopseudomonas spheroides*, *Rhodopseudomonas palustris* and *Chromatium* D.

INTRODUCTION

It has recently been demonstrated that photosynthetic bacteria generally contain one or two kinds of quinone as major components[1-4]. The amounts of these quinones are comparable to (or larger than) those of cytochromes, flavins and other components of the photosynthetic electron transport chain in the photosynthetic bacteria. These quinones are reversibly oxidized and reduced in ethanolic solution. Therefore, it is expected that they function as electron carriers in the photosynthetic electron transport chain of the bacteria.

Spectrophotometric methods have usually been employed for investigating the role of endogenous quinones in the photosynthetic apparatus. STIEHL AND WITT[5]

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

* This research was performed in part in the Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Tokyo, Japan.

measured the flash-induced ultraviolet absorption changes of chloroplasts or cells of *Chlorella* by a repetitive flashing technique and suggested that plastoquinone acts in the linear electron transport chain as a link between Photosystems I and II. Plastoquinone served as a redox pool between the two photosystems, and the size of the pool was calculated to be 6 plastoquinone molecules.

From his spectroscopic experiments with *Anacystis nidulans*, AMESZ[6] showed that plastoquinone was oxidized on illumination of light absorbed by Photosystem I and reduced by Photosystem II. This photoreduction of plastoquinone was inhibited by the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea) (DCMU). He also indicated that the amount of plastoquinone that functioned in the oxidation–reduction reaction was relatively small (1 molecule/150 molecules of chlorophyll).

With respect to the role of quinones in photosynthetic bacteria, CLAYTON[7] discovered that the ultraviolet absorption decreased on illumination in the chromatophores of *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides*. He suggested that ubiquinone was reduced on illumination with near infrared irradiation. He also proposed that ubiquinone is the primary electron acceptor in bacterial photosynthesis. However, he and his coworkers[8] recently retracted his proposal, after making a comparison of the oxidation–reduction titrations of P870 fluorescence and the light-induced absorption change at 280 nm.

Beugeling[9] observed the light-induced absorption changes of ferricyanide-treated chromatophore particles in which the bulk of bacteriochlorophyll had been bleached. He found that the kinetics of the absorption changes at 270, 365, 435, 605, 700 and 865 nm were similar. He also found that the amounts of oxidized P890 and of reduced ubiquinone, calculated from the amplitudes of light-induced absorption changes at 890 nm and 270 nm, were approximately equal and that the quantum requirements for the photooxidation of P890 and for the photoreduction of ubiquinone were the same.

KE *et al.*[10] investigated the light-induced absorption changes at 275 and 890 nm of subchromatophore fragments isolated from chromatophores of *Chromatium* after treatment with Triton X-100. The rise times of both absorption changes were found to be $\leqslant 5 \cdot 10^{-5}$ sec. Both absorption changes, attributed to the photooxidation of P890 and photoreduction of endogenous ubiquinone, were independent of temperature from room temperature to 77°K. They suggested that the P890–ubiquinone reaction has the properties of a primary photochemical reaction in the bacterial system. Later, KE[11] found that the rapid change in absorbance had a halftime of 1 μsec or less. He suggested that ubiquinone is involved in or tightly coupled to the initial charge-separation process. He also indicated a possibility that some component other than ubiquinone accepted electrons from photooxidized P890 during the initial charge-separation process.

LOACH AND SEKURA[12] found that the decay kinetics at 280 nm have the same time dependence as those at other wavelengths (*e.g.* 433, 605, 790 and 890 nm) in chromatophores. They suggested that, if the negative photochange at 275 nm were due to the reduction of a quinone, the decay kinetics reflected a recombination of the primary oxidant and the reductant (reduced quinone) within the chromatophore. They pointed out another possibility that the negative photochange at 275 nm might merely reflect bleaching or shifting of another band of bacteriochlorophyll coincident with its oxidation.

The light-induced ultraviolet absorption changes of intact cells of photosynthetic bacteria have been investigated less actively than those of chromatophores or subchromatophore particles. The authors[13–15] investigated the light-induced ultraviolet absorption changes observed in intact cells of *Chromatium* D and concluded that the absorption changes from 250 to 300 nm are caused by ubiquinone contained in the bacterium. Further, in starved cells of *Chromatium*, a "low amplitude" photoreduction of ubiquinone was observed, which was suggested to be involved in a cyclic electron transport. A "high amplitude" photoreduction or photooxidation of ubiquinone occurred in the presence of substrate. It was suggested that these changes were involved in a non-cyclic electron transport.

PARSON[16] also reported the ultraviolet absorption changes caused by succinate and light in *Rsp. rubrum*. He observed the absorption increase at 275 nm on illumination in whole cells of *Rsp. rubrum* but he threw doubt on the attribution of the light-induced ultraviolet absorption changes to oxidation–reduction reactions of ubiquinone because of the complexity of the absorption changes.

In this article, the light-induced ultraviolet absorption changes in intact cells of *Chloropseudomonas ethylica* will be described. These absorption changes are attributed to the photooxidation of menaquinone contained in this organism. Some characteristics of the photooxidation of menaquinone will be described and compared with reactions of quinones in other photosynthetic bacteria.

MATERIAL AND METHODS

C. ethylica was grown under anaerobic conditions generally according to the original directions of BOSE[17]. An illumination intensity of about 1000 lux was provided by tungsten lamps. The temperature was 28°. After 2 days of culturing, the cell suspension from culture bottles was centrifuged at about 200 × *g* for 5 min and the precipitate was discarded. The supernatant cell suspension was used for spectroscopy.

Spectroscopic measurements were performed in a Hitachi 356 spectrophotometer and an Aminco-Chance dual-wavelength spectrophotometer with a deuterium lamp (Sylvania). The optical path was 1 cm. The measuring radiation for the ultraviolet region was filtered through a combination of a Toshiba UD-25 filter and a liquid filter (mixture, 4:1, v/v, of saturated solutions of $NiSO_4$ and $CoSO_4$ in a quartz vessel, 1-cm light path) placed in front of the photomultiplier. The transmission curve of the combined ultraviolet filter was previously reported[13]. For the measurements of the oxidation of cytochrome, a Corning 9782 glass filter was attached in front of the photomultiplier. The actinic light of 740 nm was supplied at right angles to the measuring light from a Bausch and Lomb high-intensity monochromator. The widths of the entrance and exit slits were 4 mm and 2 mm, respectively. The intensity of the actinic light was $1.3 \cdot 10^3$ ergs·cm^{-2}·sec^{-1} ($0.80 \cdot 10^{-9}$ einstein·cm^{-2}·sec^{-1}), unless otherwise indicated. Anaerobic conditions were obtained by bubbling argon gas into the cuvette for 15 min and placing a layer of liquid paraffin on the suspension. The concentration of chlorobium–chlorophyll in the bacterial cells was measured by the absorbance at 660 nm of the ether extract of the bacterial cells[18]. The measurements were performed at room temperature.

RESULTS

Light-induced ultraviolet absorption changes of C. ethylica under anaerobic condition

As shown in Fig. 1, the absorbance at 254, 270 and 284 nm (reference wavelength, 290 nm) changed on illumination with light at 740 nm. After the cessation of the illumination these absorption changes returned to the original levels. The absorbance increased at 270 and 284 nm on illumination and decreased at 254 nm.Further, at the former two wavelengths the absorption changes reached a steady state after about 1.5 min but at the latter the absorption change did not reach a steady state

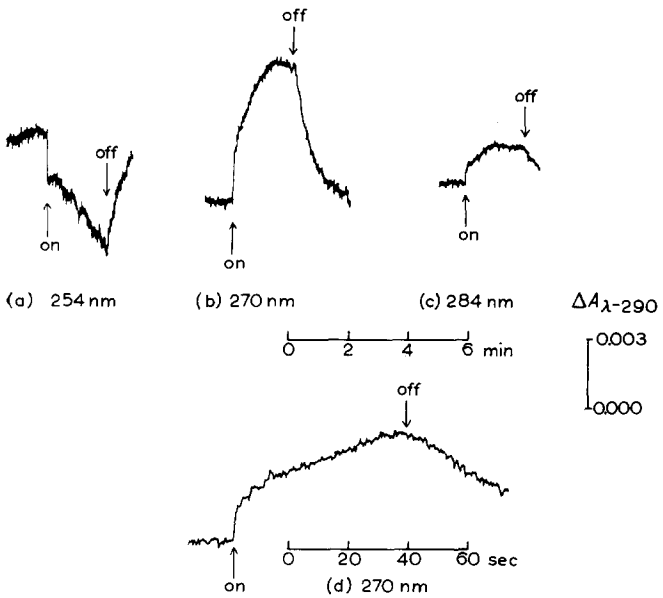


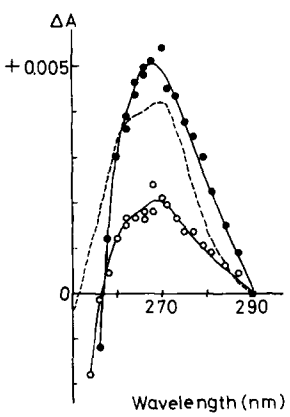
Fig. 1. Time courses of light-induced ultraviolet absorption changes of intact cells of *C. ethylica* Under anaerobic conditions intact cells of *C. ethylica* were illuminated by light at 740 nm. "On" and "off" indicate the times when the actinic light was turned on and off, respectively. The upward deflection corresponds to the increase of absorbance. Measuring wavelengths: (a) 254 nm; (b) 270 nm; (c) 284 nm; (d) 270 nm. Reference wavelength, 290 nm. Light path, 1 cm. Concentration of chlorobium chlorophyll in the suspension of cells, 14.0 μM. For Trace d, the time scale was expanded 6 times.

This observation makes it seem that the nature of the absorption change at around 254 nm is different from that at 270 and 284 nm. At all wavelengths tested the "light-on" kinetics consisted of two phases as shown in Fig. 1 (Trace d). For Trace d, the time scale was expanded 6 times. The absorbance increased rapidly during a few seconds after the start of illumination, increased slowly thereafter, then reached a steady state. (In Trace d, the light was turned off before the absorption change reached a steady state.) On the other hand, no distinct separation of phases was found in the time course of the dark recovery. The recovery curve did not fit the first-order decay kinetics. More detailed analysis of the "light-on" and "light-off" kinetics has not been carried out.

Fig. 2 illustrates the light-*minus*-dark difference spectra of intact cells (solid curves) and the oxidized-*minus*-reduced difference spectrum of pure menaquinone in

ethanolic solution (broken curve). Closed circles illustrate the changes of steady state, and open circles indicate the initial rapid changes (absorption changes at the first 3 sec after illumination started). The two light-*minus*-dark difference spectra had a peak at about 270 nm and an "isosbestic point" at about 257 nm. The shapes of the two difference spectra were similar except that the difference spectrum of the initial rapid change had a shoulder at about 263 nm. The shape of these two light-*minus*-dark difference spectra was similar to that of the oxidized-*minus*-reduced difference spectra of menaquinone, which has been shown to be the principal quinone component in this organism. The "isosbestic point" of the light-*minus*-dark difference spectra was located at a wavelength about 5 nm longer than that of the oxidized-*minus*-reduced difference spectrum of pure menaquinone in ethanolic solution. The shape of these light-*minus*-dark difference spectra was different from that of the oxidized-*minus*-reduced difference spectrum (not shown here) of chlorobium quinone[1], another quinone contained in this bacterium.

Fig. 3 illustrates the light-intensity curve for the light-induced absorption change at 270 nm. The light intensity of the actinic light was decreased by placing neutral density filters between the source of actinic light and the cuvette. The light intensities were measured by a calibrated Kipp thermopile. In the present study, a 14 μM concentration of chlorobium chlorophyll corresponded to about 1.3–1.5 of absorbance at 740 nm of the bacterial suspension. Closed circles show that the total steady-state change was nearly saturated at a light intensity of 1.0 in Fig. 3. The intensity of 1.0 in Fig. 3 corresponded to $1.3 \cdot 10^3$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1} ($0.80 \cdot 10^{-9}$ einstein$\cdot$cm$^{-2}\cdot$sec^{-1}). The open circles in the figure indicate that the initial rapid phase behaved similarly at intensities from 1.0 to 0.1. At intensities below 0.1,



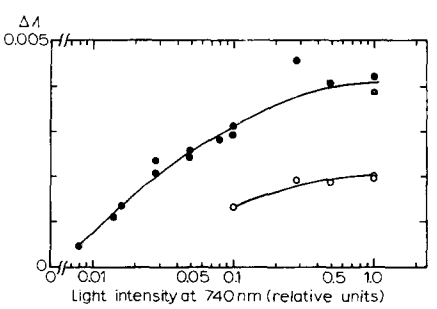


Fig. 2. Light-*minus*-dark difference spectra of intact cells of *C. ethylica* and oxidized-*minus*-reduced difference spectrum of pure menaquinone in ethanolic solution. ●—●, total steady-state change of cells; ○—○, initial rapid change (first 3 sec) of cells. Experimental conditions were the same as for Fig. 1. The broken line represents the oxidized-*minus*-reduced difference spectrum of pure menaquinone in ethanolic solution.

Fig. 3. Relation between magnitude of light-induced absorption change at 270 nm and incident actinic light intensity at 740 nm. ●—●, total steady-state change; ○—○, initial rapid phase (first 3 sec). The actinic light was provided by a Bausch and Lomb high-intensity monochromator. The intensity at 1.0 in the figure corresponds to $1.3 \cdot 10^3$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1} ($0.80 \cdot 10^{-9}$ einstein$\cdot$cm$^{-2}\cdot$sec^{-1}). Concentration of chlorobium chlorophyll, 13.7 μM.

the difference between the rapid and the slow phase was indistinguishable. The light intensity of $1.3 \cdot 10^3$ ergs·cm^{-2}·sec^{-1} at 740 nm ($0.80 \cdot 10^{-9}$ einstein·cm^{-2} sec^{-1}) was about one-tenth or less of the level of light sufficient to saturate the cytochrome oxidation in other purple photosynthetic bacteria such as *Chromatium*[19,20]. This observation seems to reflect the difference of the growth conditions between the two photosynthetic bacteria, *i.e. C. ethylica* can grow under light below 1000 lux which is about one-tenth of that needed by *Chromatium*.

If all the light-induced absorption change at 270 nm be attributed to menaquinone, the light-induced absorption increase at 270 nm corresponds to photo-oxidation of menaquinone. About 60 % of the total menaquinone in the bacterial cells was oxidized on illumination. In this calculation, the difference between 270 and 290 nm of the oxidized-*minus*-reduced difference millimolar extinction coefficient of menaquinone in ethanolic solution, 11.5, was used ($(\varepsilon^{ox.}_{mM} - \varepsilon^{red.}_{mM})_{270\,nm} - (\varepsilon^{ox.}_{mM} - \varepsilon^{red.}_{mM})_{290\,nm} = 11.5$)[21]. The total amount of menaquinone in the cell suspension was calculated from the molar ratio, 1:50, of menaquinone to chlorobium chlorophyll analysed previously in this bacterium[1].

Effect of oxygen on the oxidation–reduction reactions of menaquinone and cytochromes

As with intact cells of *Chromatium*[13], the light-induced ultraviolet absorption changes in the intact cells of *C. ethylica* were found to be affected by oxygen tension in the suspending medium (Fig. 4). As shown in the previous section, under anaerobic conditions the absorption at 270 nm increased on illumination corresponding to photo-oxidation of menaquinone. Under the aerobic conditions produced by bubbling air into the suspension, the increase of the absorption on illumination was abolished and, in many cases, the small absorption decrease on illumination was detected. But this absorption decrease was so small that the light-*minus*-dark difference spectrum could not be obtained under the aerobic condition. As this bacterium took up molecular oxygen (unpublished results), the suspension became anaerobic again within half an hour. As shown in Fig. 4c the light-induced absorption increase at 270 nm was observed again after anaerobiosis. These anaerobic–aerobic cycles could be repeated several

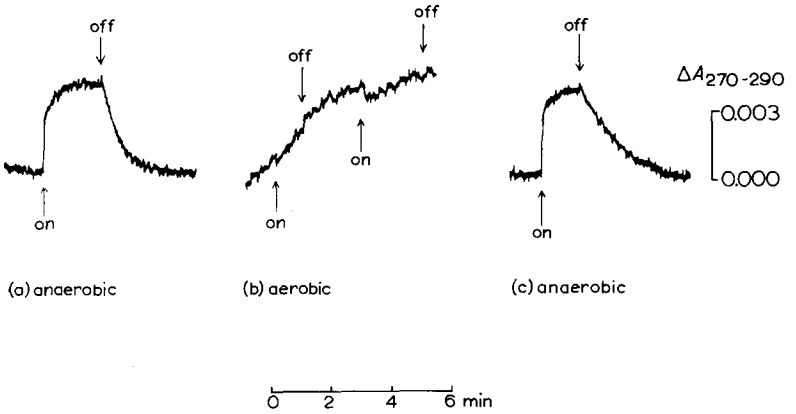


Fig. 4. Effect of oxygen on the light-induced absorption change at 270 nm. (a) Anaerobic condition; (b) aerobic condition obtained by bubbling air into (a); (c) anaerobic condition obtained by making (b) consume oxygen. The experimental conditions were the same as for Fig. 1. Concentration of chlorobium chlorophyll, 14.0 μM.

times. If we assume that the larger portion of the absorption increase at 270 nm was due to the photooxidation of menaquinone, it may be interpreted that the abolition of the photooxidation was probably due to the higher level of oxidation of menaquinone in the dark under the aerobic condition. This observation indicated that menaquinone in intact cells can interact with molecular oxygen enzymically (*i.e.* in an electron transport chain) or non-enzymically.

A similar observation was made in the light-induced oxidation of cytochrome. SYBESMA[22] has reported that the light-induced absorption changes due to cytochromes had biphasic kinetics and each phase seemed to correspond to the oxidation–reduction reaction of each of two cytochromes. In the present study, the light-induced reaction of cytochromes was monitored by the absorption changes at 422 nm (reference wavelength, 440 nm). As shown in Fig. 5, under anaerobic conditions the absorption at 422 nm decreased on illumination corresponding to the oxidation of cytochrome(s).

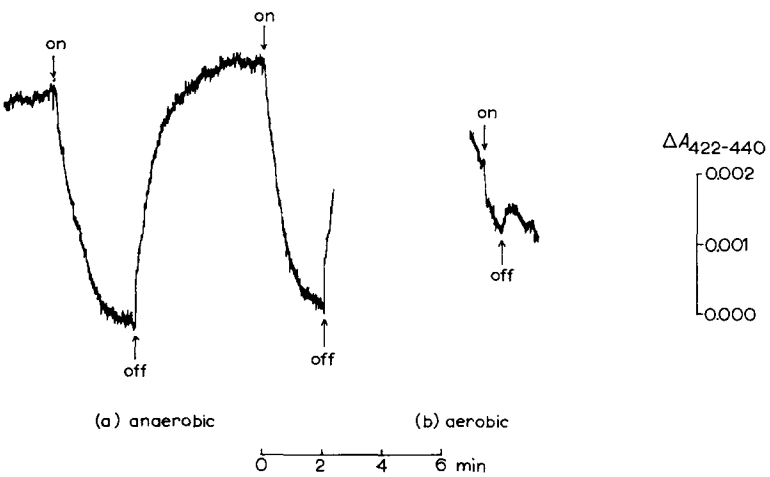


Fig. 5. Light-induced oxidation of cytochromes of intact cells of *C. ethylica*. The absorption change at 422 nm was recorded (reference wavelength, 440 nm). Upward deflection corresponds to increase of absorbance. (a) Anaerobic condition; (b) aerobic condition. Except that the concentration of cells was twice that used for Fig. 1, the experimental conditions were the same.

The absorption change reached the steady state in about 2 min after the actinic light was turned on, and the cessation of illumination caused the recovery of the absorption change. The general profile of the time course was similar to that of the light-induced absorption change in the ultraviolet region described above. This may indicate that these absorption changes are correlated with each other. But a detailed study showed that the kinetics of photooxidation of cytochrome and menaquinone were not identical that a lag of about 5 sec existed in cytochrome oxidation, and that the kinetics of the dark reduction of cytochrome showed a biphasic nature. Judging from the shape of the light-*minus*-dark difference spectrum (not shown here), both C_{422} and C_{419} of SYBESMA'S[22] notation were photooxidized to a similar extent (*cf.* Ref. 23). The biphasic nature of "light-on" kinetics observed by SYBESMA[22] was not clear in the present study. It is probable that the different experimental conditions, *e.g.* light intensity and tension of oxygen, caused the different results between the two investigators. In fact, the light intensity used in SYBESMA'S experiments was about 30

times higher than that used in the present study. Under aerobic conditions the photooxidation of cytochrome(s) diminished. However, a small change, superimposed on a drifting baseline, was observed. The light-*minus*-dark difference spectrum (not shown here) indicated that this absorption change was caused by oxidation of cytochrome, probably C_{422} of SYBESMA's notation. When the cell suspension was made anaerobic again the amplitude of photooxidation of cytochrome(s) recovered to the original amplitude.

DISCUSSION

The following observations in the present study suggest that the light-induced absorption changes in the ultraviolet region are intimately related to the photooxidation of cytochromes and to other photosynthetic reactions in *C. ethylica*. The most probable candidate for the component responsible for the ultraviolet absorption changes is the menaquinone contained in this bacterium. First, the ultraviolet absorption at around 270 nm changed reversibly on illumination with light absorbed by chlorobium chlorophyll and the light-*minus*-dark difference spectrum obtained was similar to the oxidized-*minus*-reduced difference spectrum of pure menaquinone in ethanolic solution. Second, the calculated amount of menaquinone oxidized by illumination was comparable to the total amount of menaquinone present in the cells (about 60 % was oxidized by illumination). Third, the kinetic profiles of cytochrome oxidation and the ultraviolet absorption changes are generally similar to each other.

In spite of these features, it remains possible that the ultraviolet absorption band of P840 partially contributes to the observed light-induced absorption changes. In order to clarify this point, another type of experiment such as the chemical extraction method[24] will be required.

C. ethylica does not have the ubiquinone that is contained in many other photosynthetic bacteria, but contains menaquinone and chlorobium quinone as the major quinones. Let us suppose a general role of quinones as electron carriers in the photosynthetic electron transport in all the photosynthetic bacteria, either menaquinone or chlorobium quinone (or both) may have a similar function in *C. ethylica*. As described above (in RESULTS), the spectroscopic observations indicate that menaquinone, but not chlorobium quinone, functions in the photosynthetic electron transfer of this organism. This is the first known example of the functioning of a quinone other than ubiquinone in photosynthetic bacteria. The photosynthetic bacteria other than *C. ethylica* have a common quinone, ubiquinone. In the chromatophores of *Rsp. rubrum*, *Rps. spheroides*, *Chromatium* photoreduction of ubiquinone has been investigated[7–12]. Oxidation–reduction reactions of rhodoquinone, which is contained in chromatophores of *Rsp. rubrum*, and of menaquinone, which is contained in chromatophores of *Chromatium*, have not been reported. Photoreduction of ubiquinone was also investigated in the chromatophores of *Rps. palustris* (unpublished data). In intact cells of photosynthetic bacteria other than *C. ethylica*, oxidation–reduction reactions of ubiquinone have also been investigated by the author in a series of measurements of light-induced ultraviolet absorption changes (published in part in refs. 13–15).

The direction of the reaction (*i.e.* oxidation or reduction of quinone on illumination) differed under various experimental conditions (*e.g.* anaerobic or aerobic condition, *etc.*). Table I summarizes these results. The quinone(s) found spectroscopically

TABLE I

OXIDATION–REDUCTION REACTION OF QUINONE ON ILLUMINATION OF SOME PHOTOSYNTHETIC BACTERIA

UQ, ubiquinone; RQ, rhodoquinone; MQ, menaquinone; CQ, chlorobium quinone.

Species	*Quinones present*	*Reactive quinones*	*Intact cell*		*Chromatophore*
			Aerobic	*Anaerobic*	
Rsp. rubrum	UQ RQ	UQ RQ?	red.	ox.	red.
Rps. spheroides	UQ	UQ	red.	ox.?	red.
Rps. palustris	UQ	UQ	red.	ox.	red.
Chromatium D	UQ MQ	UQ	red.	ox. red.	red.
C. ethylica	MQ CQ	MQ	red.?	ox.	?

to be involved in the light-induced oxidation–reduction reaction is indicated in the column of "reactive quinones". The direction of the reaction of quinone under various conditions is also shown.

The chromatophores of *C. ethylica* that were obtained by sonic treatment did not show the light-induced ultraviolet absorption changes in the present study. As the reaction of cytochrome was not detected in the chromatophores, it may be that the photochemical reaction is inactivated during the preparation of chromatophores.

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