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NATURE OF PHOTOCHEMICAL REACTIONS IN CHROMATOPHORES OF *CHROMATIUM* D

I. EFFECTS OF ISOOCTANE EXTRACTION ON THE PHOTOCHEMICAL REACTIONS OF P_{890} AND UBIQUINONE IN CHROMATOPHORES OF *CHROMATIUM* D

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SUMMARY

1. Effects of various experimental conditions on the light-induced reduction of ubiquinone in the chromatophores of *Chromatium* D were investigated.

2. Addition of $Na_2S_2O_3$ or malate and change of aero- to anaerobiosis did not affect the reactions of ubiquinone, thus indicating the lack of non-cyclic electron paths in the isolated chromatophores. 2-*n*-Heptyl-4-hydroxyquinoline-*N*-oxide and antimycin A slowed down the dark reoxidation of photoreduced ubiquinone, yet still suppressed slightly the magnitude of photoreduction of ubiquinone. Evidence was obtained that indicated that the observed absorption change of ubiquinone is caused by a cyclic electron flow in the chromatophores.

3. Extraction of chromatophores with isooctane diminished the light-induced absorption changes in the ultraviolet and near-infrared regions, which were recovered by readdition of ubiquinone. Extraction of less than 80% ubiquinone was without effect, and readdition of only 5% of the endogenous ubiquinone sufficed to cause a noticeable recovery both with respect to the changes of ubiquinone and that of P_{890} in the extracted chromatophores.

4. These facts indicate that a special small portion of endogenous ubiquinone characterized by a strong affinity towards combination with the photochemical reaction site is participating in the cyclic electron transport, which makes the primary photochemical reaction in the chromatophores.

A light-induced decrease occurred at 283 nm that survived extensive extraction with isooctane, probably ascribable to a redox reaction of an unidentified substance contained in the chromatophores.

Abbreviations: Q, ubiquinone; PQ, plastoquinone; HQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide.

INTRODUCTION

It is now widely accepted that a special form of bacteriochlorophyll initiates the photochemical reaction in the chromatophores of photosynthetic bacteria[1–3]. On receiving the excitation energy absorbed by, and transferred through, the bulk of bacteriochlorophyll molecules, this special form of bacteriochlorophyll designated as P_{870} or P_{890} is photooxidized and results in an absorption decrease at the specified wavelength. This reaction is thought to be the most immediate step following the excitation of the pigment system in the photosynthetic apparatus, as indicated by the fact that it occurs at a temperature as low as 1°K (ref. 2). The most probable candidate for the corresponding electron acceptor in this primary reaction of photosynthesis is ubiquinone. It was CLAYTON[4] who has first suggested this idea in 1962, working with *Rhodopseudomonas spheroides*, *Rhodospirillum rubrum* and *Chromatium*.

Recently, VERNON *et al.*[5] suggested that ubiquinone could serve in *R. rubrum* chromatophores as the electron acceptor in a primary photochemical reaction and more recently, Ke *et al.*[6] showed that the photoreduction of ubiquinone in *Chromatium* particles took place at 77°K at a rate comparable to that at room temperature.

We reported previously[7,8] that ubiquinone in *Chromatium* functions as an electron carrier both in non-cyclic electron transport and in cyclic electron transport of photosynthesis. In the non-cyclic flow of electrons, a large portion of the endogenous ubiquinone (20–50% of the total ubiquinone) was found to be involved in the light-induced redox reaction. In contrast, a small fraction (a few percent) of the ubiquinone was involved in the light-induced cyclic electron transport.

In the present study, the reactions of ubiquinone were followed in the isolated chromatophores to discover that they were devoid of one of these two reaction systems present in the whole cells, namely, only the cyclic electron flow, which corresponded to what had been observed in the whole cells as the 'low amplitude' change of ubiquinone, occurred. An attempt was made to investigate the role of ubiquinone in the chromatophores by changing its content by extraction with isooctane and by readding ubiquinone to the extracted chromatophores. Detailed studies of absorption changes in the ultraviolet and near-infrared regions in the presence and absence of added ubiquinone in the extracted chromatophores were also made. The experimental results will be described in the following with special reference to the role of ubiquinone in the photochemical reaction of chromatophores.

MATERIAL AND METHODS

Chromatium strain D was grown anaerobically for 5 days at 30° in an inorganic medium originally described by BOSE[9], containing $NaHCO_3$, Na_2S and $Na_2S_2O_3$. A light intensity of about 8000 lux was provided by tungsten lamps placed around the culturing bottles. Chromatophores were obtained as follows. The cells were crushed in a French pressure cell in 0.06 M phosphate buffer (pH 7.4) containing 0.25 M sucrose and 1% NaCl. The cell debris was removed by centrifugation at 15000 $\times g$ for 15 min, and the supernatant was recentrifuged at 60000 $\times g$ for 1 h. The pellet (chromatophores) thus obtained was dispersed in the same medium and used for spectroscopic measurements.

For the extraction of ubiquinone with isooctane, the chromatophores were

washed twice with distilled water by centrifugation at 60 000 × *g* for 1 h, suspended in distilled water and lyophilized. The lyophilized chromatophores were extracted at room temperature with isooctane which had been dried by addition of anhydrous Na_2SO_4. About 500 ml of isooctane were used for extraction of about 1.5-ml amounts of the final pellet of chromatophores. The isooctane solution, orange-yellowish in color, was separated from the chromatophores by centrifugation. The extracted chromatophores were dried *in vacuo* and resuspended in 0.06 M phosphate buffer (pH 7.4) containing 0.25 M sucrose. Sometimes at this stage of preparation, aggregates were formed owing to an incomplete dispersion of the chromatophores. In such cases, the suspension was subjected to sonication for 5 min to obtain a homogeneous dispersion. The aggregates were discarded by centrifugation at 15 000 × *g* for 15 min. The clear supernatant thus obtained was used as the extracted chromatophore fraction for the spectroscopic measurements.

Readdition of the extracts, or pure quinone, to the extracted chromatophores was performed as follows. Small amounts of isooctane extract or solution of pure quinone in isooctane were added to the powder of extracted chromatophores and evacuated to remove the isooctane. The dried chromatophores thus obtained were resuspended in 0.06 M phosphate buffer (pH 7.4) containing 0.25 M sucrose. The aggregates formed were removed as described above.

Ubiquinone-7 and vitamin K_2 contained in the isooctane extracts were isolated and purified by thin-layer chromatography as described previously[10]. The amount of ubiquinone-7 thus purified was determined by measuring the absorption difference at 275 nm in the absolute ethanol solution before and after addition of sodium borohydride. Total contents of ubiquinone-7 in the lyophilized chromatophores were determined in the same way as described above, except that ubiquinone-7 was completely extracted with acetone–methanol (7:2, v/v). With material of low ubiquinone content (*e.g.*, chromatophores 99% extracted with isooctane) a large amount of the samples was extracted exhaustively with acetone–methanol (7:2, v/v) and the amount of ubiquinone-7 thus removed was determined in the same way as described above. Pure plastoquinone-9, ubiquinone-2 and ubiquinone-10 which were kindly supplied by Dr. R. Dilley were used. Vitamin K_3 was purchased from Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan.

Spectroscopic measurements

For the measurements of light-induced absorption changes in the ultraviolet region, a Bausch and Lomb monochromator equipped with an ultraviolet grating furnished the measuring light. A cell compartment containing the chromatophore sample was attached in front of the exit slit of the monochromator. A solar blind type photomultiplier, R166 (Hamamatsu TV Co., Ltd., Hamamatsu, Japan), was used as detector; a liquid filter (*viz.* saturated solution of $NiSO_4$ and $CoSO_4$ in a quartz vessel, 1-cm light path) was placed between the cuvette and the photomultiplier. The actinic light obtained from a tungsten lamp (30 W) through a Wratten 88A filter was illuminated at a right angle to the direction of the measuring beam. The signals from the detector were amplified and recorded.

For the measurements of near-infrared absorption changes, an Aminco-Chance dual wavelength spectrophotometer was used, equipped with a photomultiplier 7102 (Hamamatsu TV Co.). A Wratten 88A filter was placed between the detector

and the cuvette. The actinic light was provided from an iodine lamp (650 W, Ushio Electric Inc., Tokyo, Japan) through a combination of interference filter (maximum transmission, 590 nm; half bandwidth 12 nm) and a Corning No. 5780 blue filter.

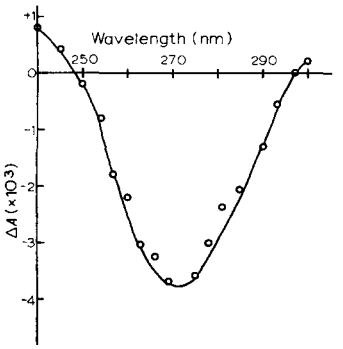


Fig. 1. Light-*minus*-dark difference spectrum of chromatophores of *Chromatium*. Actinic light was provided from a tungsten lamp (30 W) through a Wratten 88A filter. Concentration of chromatophores, 18.4 μM bacteriochlorophyll.

Anaerobiosis was obtained by flushing argon gas three or four times through the chromatophore suspension placed in a Thunberg-type cuvette. All the measurements were carried out at room temperature.

RESULTS

Light-induced absorption changes in ultraviolet region in untreated chromatophores

As reported by previous investigators[4,11,12], the absorbance in the range of wavelengths between 250 nm and 290 nm decreased on illumination of the chromatophores with actinic light mainly absorbed by bacteriochlorophyll. Fig. 1 shows the light-*minus*-dark difference spectrum at the steady state of the change which was obtained within 30 sec of illumination. The difference spectrum, having a trough at about 275 nm and isosbestic points at 248 nm and 297 nm, was similar in shape to the reduced-*minus*-oxidized difference spectrum of pure ubiquinone in ethanol solution except for a rather pronounced sharpness of the slope at 250 nm (*cf.* Fig. 4). This difference spectrum is also similar to those reported previously by CLAYTON and other investigators[4,12,13] working with *Rps. spheroides*, *Chromatium* strain D and *R. rubrum*. As estimated by the magnitude of the light-induced absorption decrease at 275 nm, only 7–10% of the total endogenous ubiquinone was reduced on illumination. The time-courses of absorption changes at various wavelengths tested showed a similar pattern, which was also similar to those reported by CLAYTON and other workers[4,12]. Accurate time-courses, especially the 'on' kinetics, could not be followed because of the limited time response of the amplifier used. The half-time of dark recovery was about 2 sec.

Table I summarizes the effects of various experimental conditions on the magnitude of light-induced absorption decrease at 275 nm and the half-time of dark recovery. Aerobiosis and anaerobiosis, or the addition of malate or $Na_2S_2O_3$, did not affect the magnitude of the absorption change. These findings are different from what

TABLE I

LIGHT-INDUCED ABSORPTION DECREASE AT 275 nm IN CHROMATOPHORES OF *Chromatium* D

Reaction conditions	*Additions*	*Concn. (M)*	*Relative magnitude of decrease (%)*	*Half-time of dark recovery (sec)*
Aerobic	None	—	100	2
Anaerobic	None	—	100	2
Aerobic	$Na_2S_2O_3$	$5.9 \cdot 10^{-3}$	100	2
Aerobic	Malate	$2.8 \cdot 10^{-3}$	100	2
Aerobic	HQNO	$3.0 \cdot 10^{-5}$	68	10
Aerobic	Antimycin A	$3.0 \cdot 10^{-5}$	70	10
Aerobic	KCN	$4.3 \cdot 10^{-4}$	96	2

has been observed with intact cells[7,8]; in intact cells of *Chromatium*, we have observed a 'high amplitude' reduction of ubiquinone (20% of the total) on illumination in the presence of $Na_2S_2O_3$ under the aerobic condition and a 'high amplitude' oxidation (50% of the total) on illumination in the presence of malate under the anaerobic condition. On the other hand, the addition of 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO) and antimycin A to the chromatophore suspension decreased the magnitude of the absorption change and elongated the half-time of dark recovery. KCN had no detectable effect. These effects of inhibitors on the light-induced absorption changes of chromatophore are similar to what has previously been reported with respect to the 'low amplitude' photoreduction of ubiquinone in intact cells[8].

Effects of isooctane extraction of chromatophores

Effects of isooctane extraction on light-induced absorption changes in ultraviolet region. To test the possibility that ubiquinone in the chromatophore is acting as an electron carrier in the cyclic electron transport chain or as an electron acceptor closely associated with the reaction of P_{890}, the effects of extraction of ubiquinone were examined. If the absorption change at 275 nm is caused by the change of ubiquinone, it will be abolished by extraction of the ubiquinone from the chromatophores.

It has been reported that quinone, and some carotenoids, are readily extracted from the chromatophores by treatment with isooctane, while chlorophyll resists extraction[14]. These facts were also confirmed in the present study by the measurements of the absorption spectrum (340–900 nm) of the isooctane extract and analysis of the extracted quinones with thin-layer chromatography.

Fig. 2 shows the relationship between the magnitudes of absorption change at 270 nm and the amounts of ubiquinone removed by the isooctane extraction. In this experiment, lyophilized chromatophores were suspended in dry isooctane under stirring with a magnetic stirrer, and aliquots of the suspension were removed at appropriate time intervals for centrifugation. The supernatant isooctane solution was analyzed by thin-layer chromatography. It should be noted that the magnitude of absorption change at 270 nm remained almost unchanged until about 80% of the total ubiquinone had been extracted from the chromatophores. This fact indicates that a large portion of ubiquinone contained in the chromatophore is irrelevant

with the light-induced absorption change at 270 nm. Then the magnitude of light-induced absorption change decreased in step with the increasing degree of extraction until 90% of the total ubiquinone in the chromatophores had been removed. At this stage of extraction, there still remained a significant portion of the capacity for light-induced absorption change (*e.g.*, 30% in the experiment shown in Fig. 2), which was found to survive even extraction of 99% of the ubiquinone (Fig. 3).

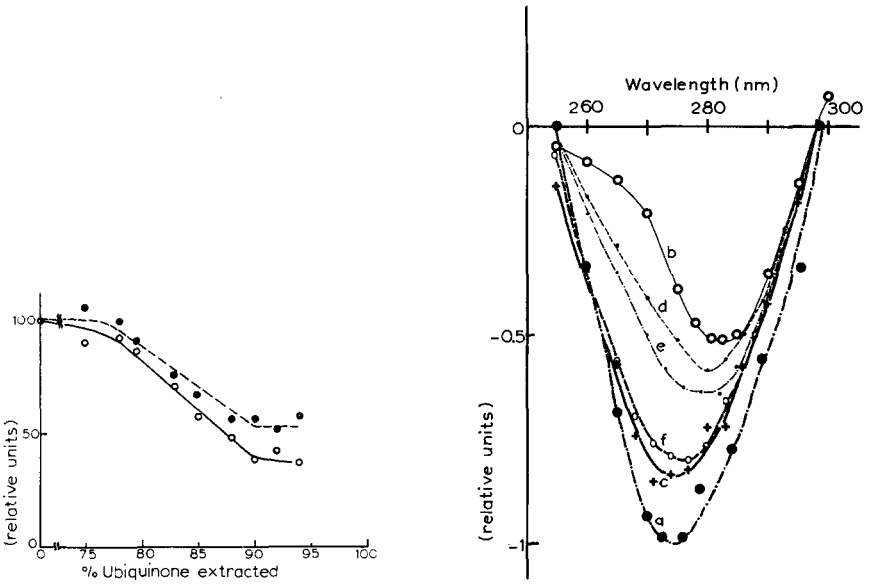


Fig. 2. Relationship between magnitudes of light-induced absorption changes of chromatophores at 270 and 283 nm and the amount of extracted ubiquinone. Absorption change is expressed as percent of the changes in the lyophilized chromatophores. Actinic light was provided from a tungsten lamp (30 W) through a Wratten 88A filter. O — O, 270 nm; ●- - -●, 283 nm.

Fig. 3. Effects of isooctane extraction and readdition of ubiquinone on the light-*minus*-dark difference spectra of chromatophores. a, lyophilized chromatophores; b, extracted chromatophores; c, extracted chromatophores *plus* extract; d, b *plus* Q (5%); e, b *plus* Q (20%); f, b *plus* Q (40%). Amounts of added ubiquinone in percent of total ubiquinone in the lyophilized chromatophores are given in parentheses. The absorption changes were referred to a same concentration of bacteriochlorophyll corresponding to $A_{590\,nm} = 1.0$. One unit in the ordinate corresponds to $5.5 \cdot 10^{-3}$ of absorption change. In all cases, actinic light was provided from a tungsten lamp (30 W) through a Wratten 88A filter.

The surviving portion of the light-induced absorption change was much larger at 283 nm than at 270 nm. These findings seemed to conflict with the assumption that the absorption changes observed at 270–275 nm are caused solely by the changes of ubiquinone. To test this point, the light-*minus*-dark difference spectrum was reinvestigated, using the chromatophores from which more than 90% ubiquinone had been extracted. Curve b in Fig. 3 shows the light-*minus*-dark difference spectrum of the 99%-extracted chromatophores. The trough was located at 283 nm instead of 275 nm in the lyophilized chromatophores, and there was a bump at 270 nm. The alteration caused by the isooctane extraction will be evident from a comparison of Curve b with Curve a, which represents the light-*minus*-dark difference spectrum of the lyophilized chromatophores, which in turn is similar to that of the original

untreated chromatophores (Fig. 1). All the curves in Fig. 3 refer to a same concentration of bacteriochlorophyll in the chromatophores so that the changes in light-responses of the chromatophores may be illustrated by direct comparison of these curves. In these measurements, the half bandwidth of each measuring beam was made 2.5 nm.

The curves shown in Fig. 4 illustrate the spectra for the increments in light-induced absorption change, which were caused by the addition of ubiquinone or readdition of the isooctane extract to the extracted chromatophores. The difference between the lyophilized chromatophores and the extracted chromatophores is also indicated (Curve a−b). It will be seen from these spectra that in all the ubiquinone-fortified samples tested, the light-induced absorption change in the chromatophores consists of two independent portions; namely, the one having a maximum point of decrease at about 283 nm and the other characterized by a maximum at 270 nm. It was the latter part of the absorption change that depended on the contents of ubiquinone in, or its readdition to, the chromatophores measured. These findings will reasonably lead to the inference that the 283-nm component under investigation is the one that survives extensive isooctane treatment. The results shown in Fig. 2 also indicate that the change at 283 nm is more refractile to isooctane treatment.

Moreover, the time-courses of absorption changes were also found to differ in lyophilized and extracted chromatophores. As shown in Fig. 5, the dark recovery of the light-induced absorption changes at wavelengths, 270, 275, and 283 nm were

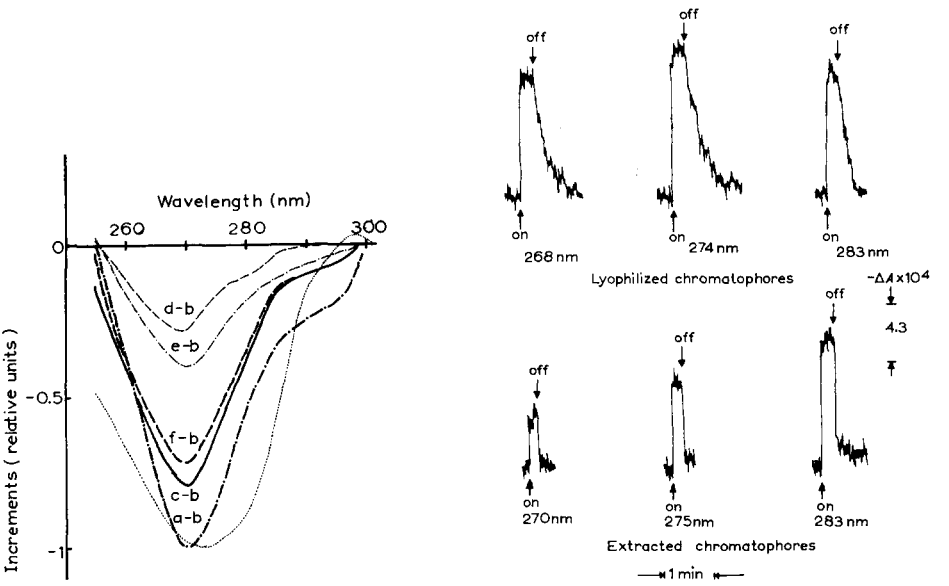


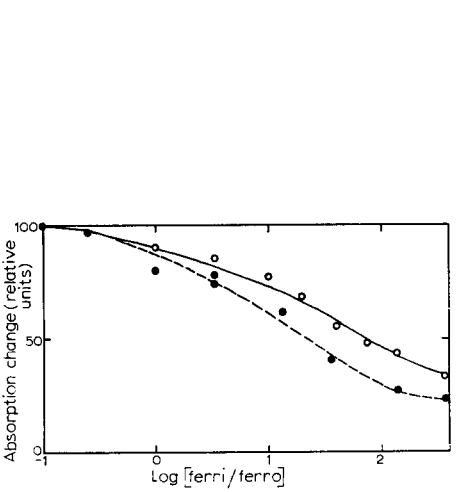
Fig. 4. Difference of difference spectra obtained by subtraction of Curve b from Curves a, c, d and f in Fig. 3. —·—·—, a *minus* b; ———, c *minus* b; – – –, d *minus* b; —·—·—, e *minus* b; ——— f *minus* b. Reduced-*minus*-oxidized difference spectrum (dotted line) of pure ubiquinone in ethanol solution is also indicated.

Fig. 5. Time-courses of light-induced absorption changes in lyophilized (upper) and extracted (lower) chromatophores. Absorbances at 590 nm of lyophilized and extracted chromatophores were 0.22 and 0.35, respectively. Actinic light was provided from a tungsten lamp (30 W) through a Wratten 88A filter.

found to be much faster in the extracted chromatophores than in the lyophilized chromatophores. The similarity in kinetics of the absorption changes at these different wavelengths indicates that the observed absorption changes in the extracted chromatophores were caused by one and the same substance or reaction. However, no detailed study could be made about the 'on' kinetics because of the limitation of the measuring apparatus used.

The readdition of pure ubiquinone (or the isooctane extract) to the extracted chromatophores caused an increase in magnitude of the light-induced changes in the ultraviolet region. However, it was discovered by an experiment not described here that the recovery of absorption in the subsequent dark period showed a slow time-course similar to that observed in the lyophilized chromatophores, there being no initial rapid phase of change which was always the case in the extracted chromatophores.

With the purpose of obtaining information concerning the nature of the light-induced absorption change at 283 nm under investigation, the effect of the environmental redox potential on this change was examined. Fig. 6 illustrated the dependency of this change in the extracted chromatophores towards the redox potential of the reaction medium (actually regulated by varying the ratio of potassium ferri- and ferrocyanide). For comparison, the behavior of the light-induced absorption change at 270 nm in the lyophilized chromatophores was also investigated. A small but definite difference will be noticed between the values of the redox potential of the



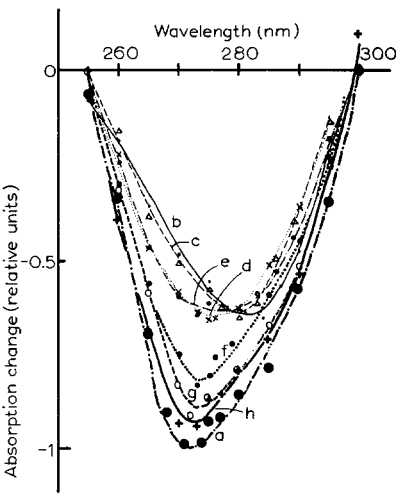


Fig. 6. Relationship between the ratio of ferricyanide to ferrocyanide (logarithmus) in the reaction medium and the magnitudes of absorption changes at 270 nm (lyophilized chromatophores, O—O) and at 280 nm (extracted chromatophores, ●---●). The concentrations of ferricyanide and ferrocyanide were made lower than 0.5 mM to avoid unfavorable effects. Actinic light was provided from a tungsten lamp (30 W) through a Wratten 88A filter.

Fig. 7. Effects of addition of various quinones on the light-*minus*-dark ultraviolet difference spectrum of extracted chromatophores. Molar ratio of the added quinones to bacteriochlorophyll was 1:10 in each case. a, lyophilized chromatophores; b, extracted chromatophores; c, b *plus* vitamin K_2; d, b *plus* vitamin K_3; e, b *plus* PQ-9; f, b *plus* Q-7; g, b *plus* Q-2; h, b *plus* extract. The absorption changes were referred to a same concentration of bacteriochlorophyll corresponding to $A_{590\,nm} = 1.0$. One unit in the ordinate corresponds to $7.7 \cdot 10^{-3}$ of absorption change. Actinic light was provided from a tungsten lamp (30 W) through a Wratten 88A filter.

medium, 500 mV and 470 mV, corresponding to 50% maximum changes at 270 nm and 280 nm, respectively.

Fig. 7 illustrates the effects of addition of various quinones on the light-*minus*-dark difference spectrum of the extracted chromatophores. In this experiment, each quinone was added at a concentration (mole/mole) one-tenth that of bacteriochlorophyll present in the extracted chromatophores. The curves were drawn to correspond to equal concentration of bacteriochlorophyll. Vitamin K_2, which is one of the natural constituents of the chromatophore of *Chromatium* had almost no effect; vitamin K_3 and plastoquinone-9 also had only insignificant effects on the magnitude of absorption change but the peak of the difference spectrum was shifted slightly to the shorter wavelength side. It will be noticed that the photochemical activity of the quinones in the chromatophores largely depends on the nature of the nucleus and the substitutions of the molecule. On the other hand, both ubiquinone-2 and ubiquinone-7 restored the original magnitude, as well as shape, of the difference spectrum. Thus, the difference in number of isoprenoid residues in the molecule does not seem to influence the capacity for recovery.

Effects of isooctane extraction on light-induced absorption changes in near-infrared region. Light-induced absorption changes in the near-infrared region in *Chromatium* chromatophores have been extensively investigated by many investigators[1,2,11]. In most cases, an absorption increase at 785 nm and absorption decreases at 805 nm and 880–890 nm were observed on illumination of the chromatophores or bacterial cells. Curve a in Fig. 8 illustrates the occurrence of such typical absorption changes in the lyophilized chromatophores. The chromatophores used in this experiment were the same as those used in the experiment shown in Fig. 3. 99% removal of the ubiquinone by isooctane extraction (Curve b) greatly diminished the light-induced absorption changes of the lyophilized chromatophores. Readdition of the extracted material (Curve c), or pure ubiquinone (Curves d–f) as well, to the extracted chromatophores restored the initial pattern of light-induced absorption changes. An addition of only a small fraction of ubiquinone contained in the original chromatophores (40% of the total ubiquinone) was required for full recovery of the absorption change. A 50% recovery in magnitude of the original steady-state level change was obtained by the addition of 5% of the total ubiquinone-7.

It will be worth mentioning here that the effects of extraction and readdition of ubiquinone were almost similar with respect to the light-induced absorption changes at 270 nm and at the near-infrared region (*i.e.*, 785 nm, 805 nm and 880 nm). Table II shows the percent recovery of the capacity for the light-induced absorption changes at these wavelengths caused by the addition of varied amounts of ubiquinone to the extracted chromatophores (data taken from Figs. 4 and 8). The magnitude of the difference in light-induced absorption changes in the lyophilized and the 99%-extracted chromatophores for each wavelength was taken as 100%. The results of addition of isooctane extract are also indicated in the table. The parallelism in behaviors of the absorption changes compared will be evidently seen from the figures in the table.

Similarity in behaviors of the ultraviolet and near-infrared absorption changes were also discovered on progressive removal of ubiquinone of chromatophores with isooctane. Fig. 9 shows the relationship between the magnitudes of the light-induced steady-state level changes of absorptions at 785, 805 and 880 nm and the

amounts of ubiquinone removed from the lyophilized chromatophores. It will be noticed that the decreases in absorption changes at these wavelengths first emerge after about 75 % of ubiquinone had been removed by extraction. There follows an approximately linear decrease from 75 % to 90 % removal of the ubiquinone. Also with respect to the infrared absorption change, there was no complete abolition of the light-induced absorption change by the isooctane treatment. However, the proportion of survival of the light-induced absorption changes in the near infrared

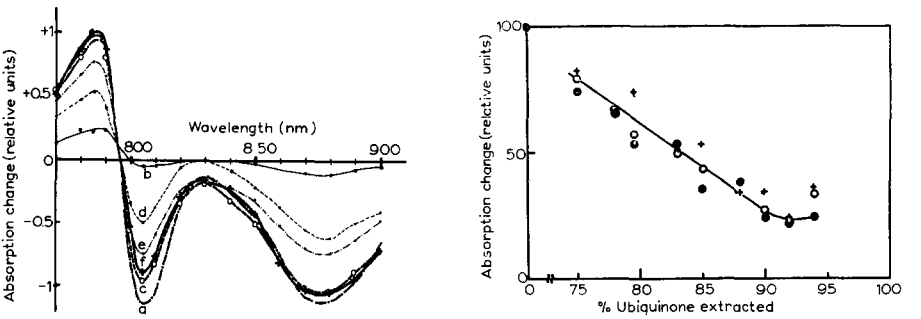


Fig. 8. Effects of isooctane extraction and readdition of ubiquinone on the light-*minus*-dark infrared difference spectra of chromatophores. a, lyophilized chromatophores; b, extracted chromatophores; c, b *plus* extract; d, b *plus* Q (5 %); e, b *plus* Q (20 %); f, b *plus* Q (40 %). Amounts of added ubiquinone in percent of total ubiquinone in the lyophilized chromatophores are given in parentheses. The chromatophore samples were the same as those used in the experiment shown in Fig. 3. The absorption changes were referred to a same concentration of bacteriochlorophyll corresponding to $A_{590\,nm} = 1.0$. One unit in the ordinate corresponds to $2.2 \cdot 10^{-2}$ of absorption change. The actinic light was provided from an iodine lamp (650 W) through a combination of an interference filter (maximum transmission, 590 nm) and a Corning No. 5780 filter.

Fig. 9. Relationship between the magnitudes of light-induced absorption changes of chromatophores at 785 nm (●), 805 nm (○) and 880 nm (+) and the amount of extracted ubiquinone. Absorption changes were expressed in percent of the changes observed in the lyophilized chromatophores at each wavelength. The chromatophore samples used were the same as those used in the experiment shown in Fig. 2. The actinic light was provided from an iodine lamp (650 W) through a combination of an interference filter (maximum transmission, 590 nm) and a Corning No. 5780 filter.

TABLE II

RECOVERIES OF LIGHT-INDUCED ABSORPTION CHANGES (AT 270, 785, 805 AND 880 nm) OF EXTRACTED CHROMATOPHORES

Data were taken from Figs. 4 and 8.

Conditions of chromatophores	*Additions*	*Relative recoveries of magnitude of light-induced absorption changes* (%)			
		270 nm	*785 nm*	*805 nm*	*880 nm*
Lyophilized	None	100	100	100	100
Extracted	5 % Q	28	33	39	50
Extracted	20 % Q	40	74	63	62
Extracted	40 % Q	72	106	83	92
Extracted	Extract	79	111	78	94

was usually by far lower than that in the ultraviolet. For instance, in the chromatophores from which 99% of the original ubiquinone had been removed by extraction (*cf.* Figs. 3 and 8), about two-thirds of the absorption change at 283 nm were retained, while only one-tenth each of the original absorption changes was detected in the near-infrared region (*i.e.*, 785 nm, 805 nm and 880 nm). This fact indicated that the changes observed at the 283-nm component of the light-induced absorption change (see above) does not represent any change of bacteriochlorophyll which makes the cause of the light-induced changes observed at these wavelengths.

In the same series of experiments, the specificity of quinones was also investigated. It was discovered that the addition of vitamin K_2, vitamin K_3 or plastoquinone-9 could not recover the light-induced absorption changes at the range of wavelengths 770–900 nm, while complete recovery was obtained by the addition of ubiquinone-2, ubiquinone-7 and ubiquinone-10 to the extracted chromatophores. These findings concerning the recovery by quinones of the light-induced absorption changes in the near-infrared region are similar to those observed above with respect to the changes in the ultraviolet region.

Any change in environmental redox potential caused by the isooctane treatment of the chromatophores cannot be the cause of the observed decreases in magnitudes of the light-induced absorption changes in the ultraviolet and the near infrared, since the occurrence of such decreases on extraction with isooctane and the recoveries on readdition of ubiquinone were also confirmed by the measurements performed in a medium, in which the oxidation–reduction potential was adjusted to a given fixed level by the use of an oxidation–reduction buffer, *i.e.*, a mixture of potassium ferri- and ferrocyanide (1:10).

DISCUSSION

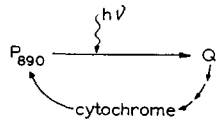
In the previous paper, we have reported the occurrence of a 'low amplitude' photoreduction of ubiquinone in the starved intact cells of *Chromatium* in the absence of substrate such as thiosulfate under aerobic condition. This photoreduction of ubiquinone was inhibited by the addition of some inhibitors, such as HQNO and antimycin A. From these findings, we concluded then that the 'low amplitude' photoreduction of ubiquinone observed in the intact cells of *Chromatium* was caused by the cyclic electron transport of photosynthesis[8].

The following similarity in behaviors will be noticed between the 'low amplitude' photoreduction of ubiquinone observed in intact cells and photoreduction of ubiquinone in the chromatophores of the same organism described in the present study. In both cases, the magnitude of photoreduction of ubiquinone amounted to 5–10% of the total. Both changes were observed irrespective of the presence or absence of substrates, such as thiosulfate or malate. The time-courses of the two changes were similar; both were inhibited by HQNO but unaffected by KCN. We concluded from these facts that the photoreduction of ubiquinone in the chromatophores corresponds to the 'low amplitude' photoreduction in the intact cells, and therefore is caused by the cyclic electron transport of photosynthesis.

The 'high amplitude' photoreduction of ubiquinone, which has been observed in intact cells of *Chromatium* under aerobic condition and in the presence of thiosulfate, was not observed in the chromatophores[8]. This fact will indicate that the isolated

chromatophores lack the enzyme(s) catalyzing the electron transport between thiosulfate and ubiquinone or may it be that some of these catalysts had been inactivated during the preparation of the chromatophores. Readdition of the supernatant of the 60000 × *g* 1-h centrifugation to the chromatophore suspension did not recover the lost capacity for the 'high amplitude' photoreduction of ubiquinone in the presence of thiosulfate. A similar circumstance was encountered with respect to the 'high amplitude' photooxidation of ubiquinone lost from the chromatophores.

The addition of HQNO and antimycin A slowed down the dark reoxidation of the photoreduced ubiquinone in the chromatophores. This fact indicates that HQNO and antimycin A inhibited the cyclic electron flow at a site localized at the oxidizing side of the ubiquinone in the cycle. This assumption is in agreement with the conclusion that HQNO inhibits the reduction of the photooxidized cytochrome in the dark described by previous workers[15,16]. However, it has also to be noted that the addition of these inhibitors also caused a decrease in magnitude of the absorption change in the steady-state level if we assume the following sequence of electron flow in the cyclic reaction:



In general, a suppression of the rate of dark recovery would cause an increase in magnitude of the change of steady-state level, provided that the intensity used was sufficiently high to saturate the light-induced change in the steady-state level. Therefore, the fact that the magnitude of the change in steady-state level of absorption was decreased by the addition of HQNO and antimycin A suggests that these inhibitors are also inhibiting to some extent the light reaction of ubiquinone.

We have conclusively shown in the above experiments that it is a special portion of the endogenous ubiquinone which is responsible for the photochemical reduction of the ubiquinone in the chromatophores. The removal of the bulk (*e.g.*, 80%) of the ubiquinone was found to cause no decrease in capacity for the photoreduction. Moreover, it was shown that this special portion of ubiquinone is characterized by its strong affinity towards the site of the photochemical reaction of the chromatophore. Readdition of small amounts (about one-half) of ubiquinone contained in the original chromatophores sufficed for recovery of a significant portion of the lost capacity for photoreduction. These facts are also in conformity with the statements made by previous investigators concerning the behaviors of plastoquinone in the chloroplasts in higher plants[17]. KE *et al.*[6] have also discovered no alteration in light-induced absorption change at 275 nm when they had extracted 10–50% of the endogenous ubiquinone contained in the chromatophores of *Chromatium* D.

The extraction of the bulk of ubiquinone had little effect on the light-induced changes at 785, 805 and 880 nm. Readdition of small amounts of ubiquinone to the extensively extracted chromatophores fully recovered the light-induced reactions of P-substance. This marked parallelism in responses of photochemical reduction of ubiquinone and photochemical oxidation of P-substance in the chromatophores sup-

ports the view that ubiquinone strongly bound to the chromatophores plays the role of electron acceptor in the primary reaction of the chromatophore. In fact the reaction of P-substance required the presence of ubiquinone in the chromatophores. It has also to be noted in this connection that the reaction underlying the 283-nm component of absorption change is indifferent of the reaction of P-substance.

The finding of the present study that the light-induced absorbance change of the chromatophores in the ultraviolet region consists of two components will also deserve mentioning. The one at 270 nm is evidently due to the change (photoreduction) of ubiquinone, endogenous, or readded to the extracted chromatophores. The pattern and the position of the spectrum of this component are in general agreement with those of the reduced-*minus*-oxidized difference spectrum of ubiquinone (dotted line in Fig. 4) although there are some differences in precise shape between the two spectra, which might probably be due to the difference in states of ubiquinone: in alcoholic solution or in the architecture of the chromatophores. On the other hand, the change having its maximum at 283 nm is independent of the presence of ubiquinone, taking place in the lyophilized as well as in the isooctane-extracted chromatophores. In the lyophilized (or partially extracted) chromatophores the change of 283-nm component seems to occur superimposed to the absorption change of ubiquinone in the light.

No definite evidence has been obtained concerning the nature of the substance responsible to the 283-nm component of absorption change. The mode of dependence of the absorption change on the redox potential of the medium (Fig. 6) suggests that the 283-nm component is a reflection of a light-induced redox reaction of a substance contained in the chromatophores. The possibility that it represents a special state of ubiquinone bound to the structure of the chromatophores is excluded by the following way of reasoning. The magnitude of the absorption change at 283 nm retained in the 99%-extracted chromatophores is still so high to amount to several fold (actually, 4- to 5-fold in the case of Curve b in Fig. 3) of what can actually be expected if all the ubiquinone remaining in the extracted chromatophores were to be photoreduced. It will be unreasonable to assume such an extraordinary high value of absorption coefficent of ubiquinone as required to account for the observed high level of absorption change at 283 nm. At the present step of study, it will be tentatively concluded that a substance other than ubiquinone is responsible to the absorption change at 283 nm. An additional support in favour of this view was furnished by the finding that the light-induced absorption changes at 283 nm and 270 nm showed some difference with respect to their dependence on the oxidation–reduction potential of the reaction medium (Fig. 6).

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