Coupled Photooxidation of Bacteriochlorophyll P890 and Photoreduction of Ubiquinone in a Photochemically Active Subchromatophore Particle Derived from *Chromatium**

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ABSTRACT: A photochemically active subchromatophore fragment isolated from *Chromatium* chromatophores after treatment with Triton X-100 shows absorbance decreases at 890 (reaction center bacteriochlorophyll, Bchl) and 275 mμ (ubiquinone) with rise times $\leq 5 \times 10^{-5}$ sec and half-decay times of 0.25 sec. The light minus dark difference spectrum between 220 and 920 mμ consists essentially of changes attributed to the photooxidation of the reaction center Bchl P890 and photoreduction of endogenous ubiquinone. The effects of pH of the suspending medium in the range of 3–14 and that of heating the fragments between 40 and 80° are very similar for both P890 and ubiquinone reactions. Both reactions are independent of temperature from room temperature down to 77 °K. Quantum requirement measurements yielded an average of 6 quanta required for each molecule of P890 oxidized or ubiquinone reduced. Chemical difference spectra of the subchromatophore fragments showed they contain P890 at a ratio of 1:9 to the bulk Bchl. The subchromatophore fragment also contains 3.3 mole % cytochrome relative to the total Bchl. Ubiquinone is present at a high concentration relative to the bulk Bchl, approximately 1:2. The photooxidation of P890 and photoreduction of ubiquinone are both abolished when sufficient ferricyanide or dithionite is present. Presumably when P890 is chemically oxidized or when ubiquinone is reduced, the coupled redox reaction is prevented. When the reduced forms of dyes such as N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD), 2,6-dichlorophenolindophenol (DPIP), or phenazine methosulfate (PMS) are present, the P890 absorbance either becomes smaller (with a faster decay rate) or disappears completely, but the initial absorbance change due to ubiquinone reduction, which is presumed to be coupled to P890 photooxidation, is unaffected. The disappearance of the P890 absorbance change is caused by an efficient transfer of electrons from the reduced dye to the photooxidized P890, which turns over very rapidly so that the transient cannot be seen. In the presence of reduced dyes, a slower secondary ubiquinone reduction can also be observed, which takes place following the initial fast transfer of electrons from P890 to ubiquinone. The P890–ubiquinone reaction has the properties of a primary photochemical reaction in the bacterial system.

Photosynthesis is initiated by a photochemical reaction in which there is an electron transfer between a photoexcited chlorophyll molecule and some closely associated molecule. It is now widely accepted that the major portion of the chlorophyll serves as a primary energy absorber, and only a small fraction of the chlorophyll, presumably present in a specialized environment and thus absorbing at a longer wavelength, traps the absorbed energy and initiates the photochemical reaction. Photooxidation of these special chlorophylls has been observed. In the longer wavelength-absorbing pigment system (photosystem 1) of green plants, a special chlorophyll (P700) undergoes such an oxidation. The corresponding "acceptor" molecule has been assumed to be a highly reducing substance (X) with a redox potential perhaps as low as -600 to -700 mv.

In photosynthetic bacteria, a primary photooxidation has been demonstrated for the long-wavelength-absorbing form of BChl,[1] P890 (or P870 depending on the species). In search for a primary electron acceptor for the bacterial system, Clayton (1962a) reported a light-induced absorption decrease in the ultraviolet region for chromatophores of a blue-green mutant of *Rhodopseudomonas spheroides*, and attributed it to the reduction of endogenous ubiquinone. Also Bales and Vernon (1962) have shown that oxidation of reduced DPIP by *Rhodospirillum rubrum* chromatophores is coupled to the photoreduction of endogenous quinones. Bacterial chromatophores (or solubilized Bchl) also readily catalyze the photoreduction of exogenous ubiquinones by ferrocytochrome c or reduced PMS (Zaugg *et al.*, 1964). In a flash spectrophotometric

[1] Abbreviations used: BChl, bacteriochlorophyll; DPIP, 2,6-dichlorophenolindophenol; PMS, phenazine methosulfate; TMPD, N,N,N',N'-tetramethyl-p-phenylenediamine.
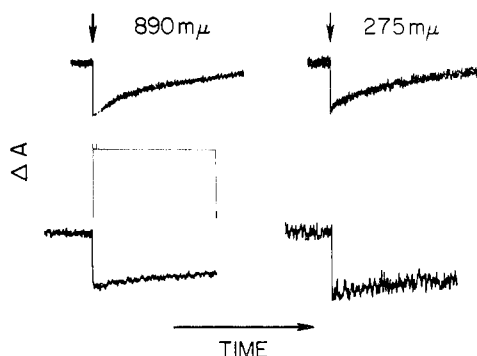
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FIGURE 1: Absorption-change transients at 890 and 275 mμ induced by 20-μsec light flashes (represented by the vertical arrow). The full time spans for the top and bottom tracings are 1 sec and 25 msec, respectively. The signals at the bottom represent approximately the initial 18-msec portion of those at the top. The signals were made at different sensitivities and with an arbitrary number of flashes to yield a size suitable for presentation. The actual ΔA's for the signals are: at 890 mμ,3 $\times$ 10^{-4} (top) and 1.7 $\times$ 10^{-4} (bottom); at 275 mμ, 3 $\times$ 10^{-5} (top) and ~10^{-6} (bottom).

study (Ke et al., 1965) of chlorophyll-catalyzed redox reactions in a system containing chlorophyll, cytochrome, and trimethylquinone, the kinetic evidence also suggests that the initial electron transfer was from the excited chlorophyll to the oxidized quinone. In a recent review, Vernon and Ke (1966) suggested that ubiquinone could serve in R. rubrum chromatophores as the electron acceptor in a primary photochemical reaction, which initiates the cyclic electron-transport sequence. This paper presents evidence for a primary photoreaction between P890 and endogenous quinone in a subchromatophore particle derived from Chromatium. The light minus dark difference spectrum consists essentially of changes attributable to P890 Bchl photooxidation and ubiquinone photoreduction. The similarities of the two reactions toward pH and heat treatment, as well as their temperature independence down to 77°K suggest that the two reactions are coupled, and the primary photochemical reaction in Chromatium produces oxidized P890 and reduced ubiquinone.

Experimental Section

The photochemically active subchromatophore particle from Chromatium was prepared according to procedures described previously (Garcia et al., 1966). To briefly summarize: treatment of Chromatium chromatophores with 3% Triton X-100 produces two fractions which can be separated by sucrose density gradient centrifugation. The lighter fraction, which is photochemically inactive, contains the forms of Bchl which absorb near 800 and 850 mμ. The heavier fraction absorbs near 800 and 890 mμ, shows a light-

induced electron spin resonance signal and absorbance decrease at 890 mμ corresponding to P890 photooxidation, and catalyzes several coupled redox reactions.

The light-induced reactions were carried out with dilute suspensions of the particle in pH 7, 0.01 M phosphate buffer. For measurements of absorbance from 220 mμ to approximately 750 mμ, a 50-mμ wide interference filter with maximum transmission centered at 875 mμ was used to isolate the excitation light. For measuring wavelengths above 750 mμ, a 30-mμ wide interference filter with wavelength centered at 590 mμ was used. When measurements were made in the near-infrared region, corrections were made for the interference in the absorption-change transients due to fluorescence by the use of a CAT computer or a Signal Averager by operating in the "subtract" mode with the measuring beam off.

For measurements in the ultraviolet region, a 200-w continuous-source deuterium arc lamp (Kern and Sprenger, Göttingen, Germany) was used. The lamp was water cooled and powered by a Kepco Model HB250M regulated DC power supply. The reflectance-type ultraviolet interference filter (UV-R-250, -280, and -310, Schott) assembly was mounted in front of the EMI 9558Q photomultiplier for shielding against the 875-mμ excitation light.

For low-temperature reactions, thin films of the chromatophore particles were prepared on quartz plates according to the method of Arnold and Clayton (1960). Details concerning the rapid spectrophotometer and the low-temperature dewar device used in this study were described previously (Ke et al., 1964; Ke and Ngo, 1967). For excitation of longer duration (1–10 sec), an electromechanical shutter constructed of a stepper motor (Cedar Engineering, Minneapolis) was used, which was triggered and timed with a Tektronix Model 162 wave generator and Model 163 pulse generator. The shutter has a rise time of 10 msec. In addition to the Mnemotron Model 400 CAT computer used for most routine experiments, a Fabri-Tek Model 1052 Signal Averager with time resolution of 5 $\times$ 10^{-5} sec was also used for the rise-time studies presented in Figure 1.

Results

Light-Induced Absorption Changes. It was reported earlier that the photochemically active subchromatophore particle derived from Chromatium shows a light-induced absorption decrease corresponding to P890 photooxidation (Garcia et al., 1966). We have now examined in more detail the absorption changes together with the reaction kinetics over the wavelength range of 220–920 mμ. Typical absorption-change transients at 890 and 275 mμ induced by 20-μsec flashes are presented in the top row of Figure 1. The absorption decreases at 890 and 275 mμ, representing photooxidation of P890 and photoreduction of ubiquinone-7, respectively, have half-decay times of approximately 250 msec. The estimated value for the 890-mμ transient
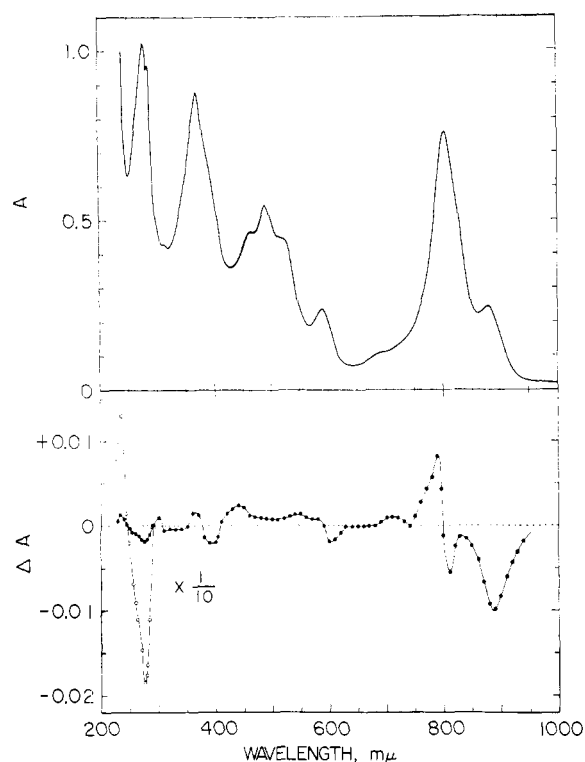
FIGURE 2: Spectral studies. Top: absorption spectrum of the heavy subchromatophore particle derived from *Chromatium*. Bottom: light minus dark difference obtained by 2-sec illumination.

is probably less accurate because of a slight distortion of the initial portion by the fluorescence interference. However, the two decay times are not greatly different.

The first 18-msec portions of the absorption-change transients at the two wavelengths were reexamined with a faster time resolution and are presented in the bottom row of Figure 1. At the time scale used, the rise time for both reactions was estimated to be $\leqq 5 \times 10^{-5}$ sec.

The light minus dark difference spectrum obtained by plotting the absorption changes produced by 2-sec illumination is shown in Figure 2. The absorption spectrum of the subchromatophore particle is also included in the figure for comparison. The difference spectrum consists essentially of changes due to bacteriochlorophyll and ubiquinone present in the particle. The photooxidation of P890 is represented by absorption decreases at 890, 590, and 380 mμ, a broad absorption increase centered at 430 mμ, and a blue shift of the 804-mμ absorption band. The ratio of the 890: 590 mμ change is approximately 5.

The absorption change below 350 mμ is typically that of a ubiquinone, presumably ubiquinone-7 known to be present in relatively high concentration in *Chromatium*. The maximum absorption decrease occurred at 275 mμ, and the maximum absorption increase at 235 mμ. The isosbestic points were at 247 and 290 mμ, in close agreement with those in the difference spectrum
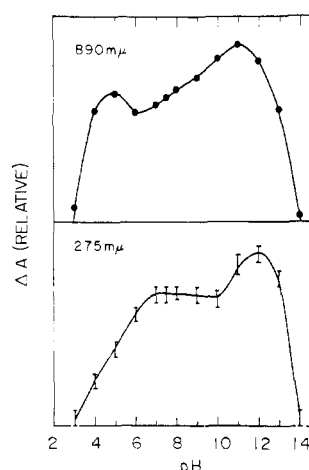

FIGURE 3: Effect of pH of the suspending medium on the relative magnitude of the absorbance changes at 890 and 275 mμ.

of ubiquinone in solution (Crane and Dilley, 1963). As seen from the steady-state difference spectrum, the mole ratio of the extent of ubiquinone photoreduced to P890 photooxidized is approximately 1.7, assuming the millimolar extinction coefficients for ubiquinone and P890 are 12 and 113 mM^{-1} cm^{-1}, respectively.

Effects of pH and Heat Treatment on the 890- and 275-mμ Reactions. Both the photooxidation of P890 and photoreduction of ubiquinone-7 were stable over a wide pH range. The relative magnitude of the reaction at different pH values is presented in Figure 3. For the P890 reaction (Figure 3, top), the magnitude was apparently maximal at pH values of 5 and 11. However, at pH below 4 and above 12, the reaction rapidly deteriorated. In the ubiquinone reaction (Figure 3, bottom), the high pH maximum was also present, but the low pH maximum was absent. The reaction deteriorated steadily below pH 6.5 and very rapidly above pH 12.

Both the P890 and ubiquinone-7 reactions were relatively stable toward heat treatment. When the chromatophore suspension was heated at temperatures up to 40° for 5 min, no change in the magnitude of the reaction resulted. Heating at temperatures above 50° for 5 min decreased the magnitude gradually, and more rapid decay was seen for temperatures above 70°. Very little reaction remained after the particle was heated at 80° for 5 min. As seen from Figure 4, the responses of the two reactions toward heat treatment were nearly the same.

Quantum Requirement for Ubiquinone Photoreduction. Photooxidation of P890 is an efficient reaction, which is consistent with a primary process. The quantum requirements for P890 photooxidation in chromatophores of *Chromatium* and *R. spheroides* were determined previously by Clayton (1962b) to be between 3 and 6. More recently Parson (1967), using the 6943-Å

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TABLE I: Quantum Requirement for Ubiquinone Photoreduction.

Excitation Wavelength (mμ)		Absorbed Quanta (Einsteins/cm³ sec)	Ubiquinone Reduced (moles cm³ sec)	Quantum Requirement (Einsteins/mole)
Peak Transmission	Half-Band Width			
862	858–865	1.59×10^{-10}	2.7×10^{-11}	5.9
		1.38	2.3	6.1
873	868–876	1.76	2.8	6.3
		1.45	2.3	6.3
879	876–883	1.83	3.3	5.5
888	886–894	1.66	2.6	6.4
		1.52	2.4	6.3
900	894–904	1.74	3.0	5.8
		1.32	2.3	5.8

flash from a ruby laser as the excitation source, reported a quantum requirement of 3.6 for P870 photobleaching in *R. rubrum* chromatophores.

We have examined the quantum requirement for ubiquinone photoreduction in the subchromatophore particle by using excitation wavelengths that encompass the far-red-absorbing band. Dilute suspensions with an absorbance of about 0.1 were used for these determinations. Results from two separate series of measurements are summarized in Table I. The peak transmission and the half-band width of the interference filters (Baird-Atomic or Spectrolab) used are also listed in Table I. The results indicate that over a range of excitation wavelengths, an average of 6 quanta was required for one molecule of ubiquinone reduced. A separate determination of the quantum requirement for P890 photooxidation by 590-mμ excitation also yielded a value of about 6.

Temperature Independence of P890 and Ubiquinone Reactions. One criterion for a primary photochemical reaction is its ability to take place at very low temperatures. For instance, P890 photooxidation in chromatophores of *R. spheroides* takes place at temperatures as low as 1°K (Arnold and Clayton, 1960). Earlier, Chance and Nishimura (1960) reported that photo-

oxidation of cytochrome 423 in *Chromatium* cells takes place at 77°K. In the plant photosynthetic apparatus, Vernon *et al.* (1967) recently showed a temperature-independent photobleaching of P700 in the presence of reduced PMS.

Although the P890 reaction is known to occur at very low temperatures, the temperature dependence of the ubiquinone reaction is of great interest in view of its liklihood of being the reaction partner in the primary photochemical reaction. We have examined the temperature dependence of both the 890- and 275-mμ reactions by using dried thin films of the chromatophores mounted in a cold finger frame inside a quartz dewar and excited with flashes of 2-sec duration. Results at several selected temperatures are presented
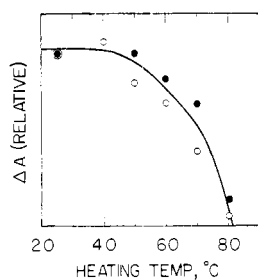

FIGURE 4: Effect of temperature at which the suspension was heated for 5 min on the relative magnitude of the absorbance changes at the 890 (open circles) and 275 mμ (closed circles).
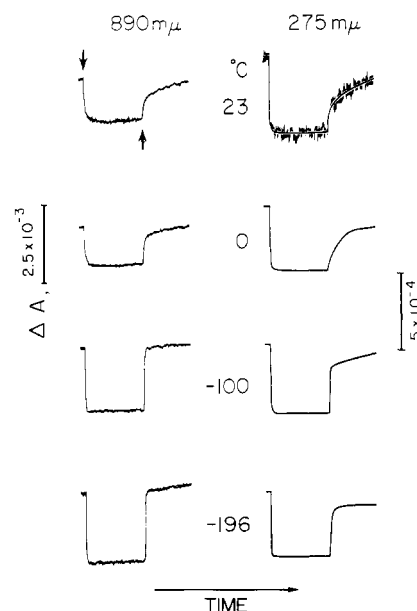

FIGURE 5: Effect of reaction temperature on the absorption-change transients at 890 and 275 mμ.

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in Figure 5. Actual tracings of the P890 reactions are presented, whereas retracings of the 275-mμ reactions are included, with the actual noise level shown for the room temperature reaction only. Over the temperature range from room temperature to liquid nitrogen temperature, the rise time of the P890 reaction appears to be shorter (at lower temperatures). At temperatures below 0°, the magnitude of the P890 reaction increased and the decay became more rapid. The 275-mμ reactions over the same temperature range behaved similarly, except that although 70% of the decay was very rapid, the remaining portion was slower. In fact, the slow portion appeared to be temperature dependent, *i.e.*, slower with decreasing temperatures.

Chemical Composition of the Subchromatophore Particle. Goedheer (1960) showed earlier that the light minus dark difference spectrum of chromatophores in the near-infrared region is very similar to that caused by chemical oxidation and he concluded that the photobleaching represents an oxidation of a bacteriochlorophyll component. Thus, treating the chromatophore particle with ferricyanide would allow an estimation of the P890 content. The untreated minus ferricyanide-treated difference spectrum of a suspension of the subchromatophore particle with an absorbance of 0.48 at 804 mμ is shown in Figure 6. A difference band with ΔA 0.065 appeared at 890 mμ. Taking the millimolar extinction coefficients of the 800- and 890-mμ bands to be 136 and 113 mM^{-1} cm^{-1}, respectively, and assuming that all the 890-mμ absorption change was contributed by P890, the P890 content relative to the bulk chlorophyll is about 1:9. This ratio is substantially higher than 1:50 usually found in bacterial chromatophores, indicating the subchromatophore particle is highly enriched in P890. As can be seen from Figures 2 and 6, the extent of P890 photooxidized in the steady state is only a fraction of that caused by chemical oxidation, approximately 20% in this case. It may be noted from Figure 6 that chemical oxidation also causes an absorption decrease at 590 mμ.

Of more interest is the appearance of another band with maximum absorption at 833 mμ. This difference band, which has about the same magnitude as the 890-mμ band, is so strong that the blue shift of the 804-mμ band was barely discernible. The exact nature of the 833-mμ band is not known. It was not present in the chemical difference spectrum reported previously by Goedheer (1960) for *Chromatium* chromatophores. In the light minus dark difference spectrum reported by Duysens *et al.* (1956) for a *Chromatium* extract, there was some indication of a slight bleaching in the 840-mμ region. More recently, Sybesma and Vredenberg (1963) reported a photobleaching at 840 mμ in the green bacterium *Chloropseudomonas ethylicum* which contains *Chlorobium* chlorophyll as the bulk pigment and a small amount of bacteriochlorophyll absorbing at 810 mμ *in vivo*. These authors suggested that the 840-mμ change corresponds to a photobleaching of the reaction center chlorophyll designated as P840. Whether there is any relationship between the 833-mμ band observed here and the reported 840-mμ
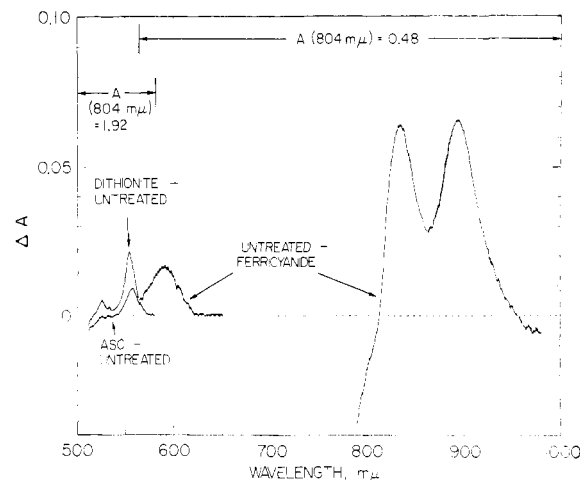

FIGURE 6: Chemical difference spectra of the heavy subchromatophore particle of *Chromatium*. Absorbance of the particle suspension and the chemical treatments are indicated in the figure. Phosphate buffer at 0.01 M and pH 7 was used as the medium.

band remains to be investigated further. The 833-mμ band has not been observed in the chemical difference spectrum of the unfractionated chromatophore of *Chromatium*.

The difference spectra on the left side of Figure 6 were obtained by treating particle suspensions of an absorbance of 1.96 at 804 mμ with two different reducing agents. The smaller difference spectrum (ascorbate treated minus untreated) had its α band at 555 mμ. The larger difference spectrum (dithionite treated minus untreated) had its α band at 553 mμ. The ratio of the two band heights is 1:2, suggesting that cytochrome 553 and cytochrome 555 are present in approximately equal amounts in the subchromatophore particle, and that the two have different redox potentials. Taking the millimolar extinction coefficient of cytochrome 553 as 44.6 (Bartsch and Kamen, 1960), the subchromatophore particle contains about 3.3 mole % of cytochrome relative to the bulk chlorophyll, which is lower than the 5.5 (Newton and Newton, 1957) or 12.6 mole % (Hulcher and Conti, 1960) reported in the literature.

Difference spectra from a ubiquinone assay of an aqueous suspension of a subchromatophore particle by treatment with NaBH₄ showed that the molar ratio of ubiquinone to the bulk chlorophyll is 1:1.8. This represents an enrichment of ubiquinone in the subchromatophore particle by a factor of 2.6 compared with the unfractionated *Chromatium* chromatophores (Fuller *et al.*, 1961).

Several measurements of the 275-mμ absorbance decrease were also made with freeze-dried subchromatophore particles which were subsequently extracted with hexane for 2–5 hr. However, the magnitude of the absorbance change was practically unaltered by extraction, which removed 10–50% ubiquinone
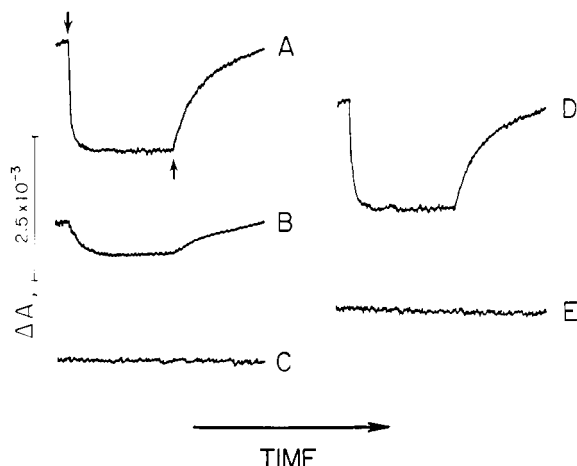
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FIGURE 7: Effect of chemical oxidant and reductant on the 890-mμ absorbance change. (A) Particle suspension alone; (B) particle partly oxidized by ferricyanide; (C) particle completely oxidized by ferricyanide; (D) particle plus ascorbate; (E) particle plus dithionite. The full time span for all signals was 4 sec. Arrows indicate lights on and off.
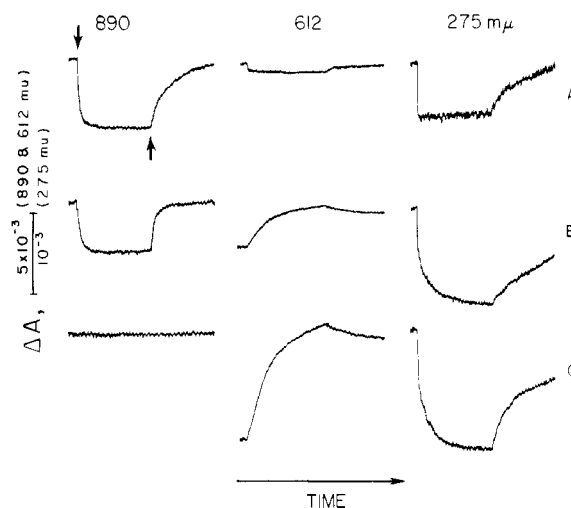

FIGURE 8: Effect of TMPD on the absorbance changes at 890, 612, and 275 mμ. Row A: particle suspension alone; row B: plus 10^{-6} M TMPD; row C: plus 10^{-5} M TMPD. The full time span for all signals was 4 sec. Arrows indicate lights on and off.

and variable amounts of carotenoids. These experiments indicate that only the bulk ubiquinone was extracted, and that the ubiquinone giving rise to the 275-mμ absorbance decrease was tightly bound to the reaction center BChl.

Effect of the Redox Environment on the Primary Reactions. Since the photobleaching of P890 represents an oxidation reaction, it would be expected that the presence of ferricyanide, by chemically oxidizing the P890, would eliminate the light-induced 890-mμ absorption decrease. Simultaneous measurements of the absorption-change transients and the chemical difference spectra showed that when the P890 was only partially oxidized by ferricyanide, the remaining P890 apparently could still undergo photochemical oxidation. However, the rise time of the reactions appeared to be somewhat slower. When the difference spectrum showed maximum chemical oxidation, photobleaching ceased to occur. Simultaneously, the 275-mμ absorption decrease also disappeared. Some typical absorbance changes are shown in Figure 7.

The presence of ascorbate had little effect on the rise time, the magnitude, or the decay time of the 890-mμ absorption-change transient. However, the presence of dithionite eliminated the P890 as well as the ubiquinone reaction completely (see right side of Figure 7). The stronger reducing power of dithionite presumably completely reduced the ubiquinone, thus preventing the coupled photochemical redox reaction, in which ubiquinone serves as an electron acceptor.

Effects of the presence of reduced dyes on the absorption-change transients at 890 and 275 mμ were also examined. When TMPD was present at 10^{-6} M, as shown by the transients in row B of Figure 8, the onset of the 890-mμ transient became slower and

the decay faster. The small absorption decrease at 612 mμ associated with BChl photooxidation was now replaced by a slower absorption increase representing TMPD oxidation. In addition, a continued slower reduction of ubiquinone also took place. When TMPD concentration was increased to 10^{-5} M, the 890-mμ transient disappeared, but the 612- and 275-mμ transients were enhanced. These experiments indicate an efficient transfer of electrons from the reductant TMPD to the photooxidized P890. At 10^{-5} M TMPD, the decay rate at 890 mμ must be faster than that of the onset, thus the transient could not be seen, even though the P890 was turning over. The feeding of electrons into the system by TMPD is represented by its oxidation, as shown by the absorbance increase at 612 mμ. Under these conditions, the photoreduction of ubiquinone, which was coupled to the P890 oxidation should not be affected, as shown by the unchanged rapid portion of the onset reaction at 275 mμ. The continued slower reduction of ubiquinone would then represent a secondary reaction of endogenous quinone which is not directly associated with P890 at the reaction center.

The effects of reduced PMS on the 890- and 275-mμ reactions are similar to those of TMPD, as shown in Figure 9. The presence of oxidized PMS alone has little effect (Figure 9, row B). Small amounts of reduced PMS ($\sim 10^{-7}$ M) appeared to retard the decay at 890 mμ (Figure 9, row C). At higher reduced PMS concentrations, the 890-mμ decay became more rapid, and eventually the transient disappeared (Figure 9, rows D and E). At the same time, at 275 mμ, a slower phase reduction occurred, and its rate was proportional to the concentration of reduced PMS. One feature that is different from the TMPD case is that,

with reduced PMS present (PMS was reduced by ascorbate), a slower reduction continued even when the light was turned off. This probably represents a chemical reduction of ubiquinone by reduced PMS. The effect of DPIP is similar to that of PMS.

Discussion

Although the available time resolution of our instrumentation (10^{-5} sec) does not rule out the possibility of another substance as the primary electron acceptor, the rapid onset times of both the 890- and 275-mμ reactions suggest that photooxidation of the reaction center P890 coupled to the photoreduction of endogenous ubiquinone is the primary photochemical event in *Chromatium* chromatophores. If some other unknown compound functions between the P890 and ubiquinone it would have to transfer electrons very efficiently to ubiquinone, since the P890–ubiquinone reaction proceeds rapidly at $-196°$ and also accounts for all aspects of the light minus dark difference spectrum of the chromatophores. Although there is some uncertainty in the decay rates for the two reactions, the general shapes of the tracings for both reactions are similar. Furthermore, treatment of the particle by heating causes a disappearance of the two signals in a similar manner, indicating that the two are functionally linked. Examination over a wide range of pH values again shows a similar response to the two signals. The response of the P890 signal is displaced somewhat to higher pH values, but these differences could reflect a change in the absorbance properties of the two components at the different pH values due to ionizations and configurational changes.

Loach and Sekura (1967) have recently suggested that the 280-mμ change could reflect a bleaching or shifting of another band of bacteriochlorophyll. However, the differential effects of reduced dyes on the 890- and 275-mμ absorbance changes shown in Figures 8 and 9 are not consistent with such an alternative explanation.

The difference spectrum induced by light shows changes only in Bchl and ubiquinone; there is not any apparent change in the cytochrome region of the spectrum. This indicates that the reaction between the Bchl in P890 and the ubiquinone is not only the fastest, but is essentially the only one observed in the particles. Further evidence for the primary nature of the reaction is found in the fact that lowering the temperature to $-196°$ does not prevent the reaction from taking place, indeed it proceeds at a faster rate at the lower temperature, as do the subsequent reactions in the dark. Apparently the transferred electron is trapped in the vicinity of the P890, allowing for a fast back reaction.

Although the information obtained shows a coupled photoreaction between P890 and ubiquinone, there are some indications that the photochemical reaction may not be restricted to this simple electron-transfer reaction. The stoichiometry of the reaction, as determined from the initial rates of the absorbance changes for P890
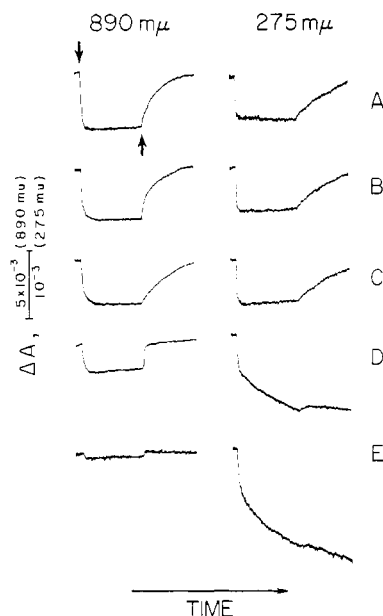

FIGURE 9: Effect of reduced PMS on the absorbance changes at 890 and 275 mμ. Row A: particle suspension alone; B: plus 10^{-6} M PMS; C–E: sample in B plus additional ascorbate at 10^{-7}, 5×10^{-7}, and 10^{-6} M, respectively. The full time span for all signals was 4 sec. Arrows indicate lights on and off.

and ubiquinone (cf. Table I) and using the absorption coefficients given earlier, varies from the expected ratio of 2 moles of P890 oxidized/mole of ubiquinone reduced. The evidence presented by Loach and Sekura (1967) indicates that the photooxidation of the reaction center Bchl involves a one-electron change, while the photoreduction of the accompanying acceptor (ubiquinone in this case) involves a two-electron change. Therefore the observed mole ratio of 1 does not agree with the expected value. Furthermore, the decay kinetics of the two signals are not exactly parallel. For instance, in the data shown in Figure 6, the P890 is restored to its original state more quickly than is the ubiquinone, and more completely at lower temperatures. In the data shown in Figure 8, the unchanged dark recovery rates at 275 mμ suggest that ubiquinone reoxidation must be coupled to some oxidant other than P890 when a reduced dye is present. The quantum yield data also show that the photoreduction of ubiquinone is more efficient than is the photooxidation of P890. All these data taken together indicate that the P890 may be interacting with some other component in the particle, such that the oxidized P890 is reduced back to its original state at least in part by interaction with this other compound. A likely candidate for such a compound is the cytochrome contained on the particle. Experiments performed to date, however, have been unable to show any significant photooxidation of the cytochrome. The question of a possible other reductant for oxidized P890 in the subchromatophore particle remains to be determined.

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Since the photooxidation of P890 is a one-electron reaction, it would be expected that a corresponding one-electron reduction of the ubiquinone to the semi-quinone form would take place. The light-induced (2-sec illumination periods) difference spectrum of the heavy *Chromation* particle, however, shows a difference spectrum in the ultraviolet region, whose shape and isosbestic points correspond to the difference spectrum between oxidized and completely reduced quinone, which is a two-electron change. The speed of the light-induced absorbancy change at 275 mμ does not allow any information to be gained concerning a possible sequential reaction leading from the quinone through the semireduced to the completely reduced form. The stability of the 275-mμ absorbance, once the light-induced level has been reached, shows that there is not detectable conversion of the semireduced form to the completely reduced ubiquinone after the initial reaction has taken place. We therefore conclude there is a rapid conversion of the initially produced semiquinone form of ubiquinone into completely reduced form by some mechanism which is not yet known.

As isolated after treatment with Triton X-100, the heavy particle is enriched in the photochemical apparatus of the chromatophore. The particles contain both a higher ratio of P890 to total Bchl, as well as a higher proportion of endogenous ubiquinone. By comparison of the absorption spectrum of the two fractions prepared by Triton X-100 (Garcia *et al.*, 1966), it is apparent that there is more of the 880 form of Bchl in the heavy particle, while the lighter particle contains more of the 800 and 850 forms of BChl. The latter two forms of BChl serve as light-harvesting pigment, and do not themselves enter into the photochemical reactions (Clayton, 1962b). Experiments performed on other purple photosynthetic bacteria show that Triton splits the chromatophore into two particles, one of which is small and one which appears to be derived from the membrane. One can imagine, therefore, that the photochemical unit is a separately formed entity which contains the reaction center P890, ubiquinone, and related electron-transfer components, and that this photochemical unit then associates with the membrane system of the chromatophore so that the shorter wavelength forms of BChl contained therein can serve as light-harvesting molecules for the photochemical unit. Since the Triton treatment destroys all activity for photophosphorylation, it is not possible to advance any information concerning the completeness of the cyclic electron-transfer system in the large particle. Judging from the kinetic data shown in Figure 5, however, it would appear that the usual cyclic electron flow which occurs

subsequent to the primary photochemical act in chromatophores is not very significant in the particles. Therefore, the particles are a particularly good system for a study of the primary electron-transfer reaction in the bacterial chromatophore.

References

Arnold, W., and Clayton, R. K. (1960), *Proc. Natl. Acad. Sci. U. S. 46*, 769.

Bales, H., and Vernon, L. P. (1962), *in* Bacterial Photosynthesis, Gest, H., San Pietro, A., and Vernon, L. P., Ed., Yellow Springs, Ohio, Antioch, p 269.

Bartsch, R. C., and Kamen, M. D. (1960), *J. Biol. Chem. 235*, 825.

Chance, B., and Nishimura, M. (1960), *Proc. Natl. Acad. Sci. U. S. 46*, 19.

Clayton, R. K. (1962a), *Biochem. Biophys. Res. Commun. 9*, 49.

Clayton, R. K. (1962b), *Photochem. Photobiol. 1*, 305.

Crane, F. L., and Dilley, R. A. (1963), *Methods Biochem. Anal. 11*, 279.

Duysens, L. N. M., Huiskamp, W. J., Vos, J. J., and vanderHart, J. M. (1956), *Biochim. Biophys. Acta 19*, 188.

Fuller, R. C., Smillie, R. M., Rigopoulos, N., and Yount, V. (1961), *Arch. Biochem. Biophys. 95*, 197.

Garcia, A., Vernon, L. P., and Mollenhauer, H. (1966), *Biochemistry 5*, 2399.

Goedheer, J. C. (1960), *Biochim. Biophys. Acta 38*, 389.

Hulcher, F. H., and Conti, S. F. (1960), *Biochem. Biophys. Res. Commun. 3*, 497.

Ke, B., and Ngo, E. (1967), *Biochim. Biophys. Acta 143*, 319.

Ke, B., Treharne, R. W., and McKibben, C. (1964), *Rev. Sci. Instruments 35*, 296.

Ke, B., Vernon, L. P., and Shaw, E. R. (1965), *Biochemistry 4*, 137.

Loach, P. A., and Sekura, D. L. (1967), *Photochem. Photobiol. 6*, 381.

Newton, J. W., and Newton, G. A. (1957), *Arch. Biochem. Biophys. 71*, 250.

Parson, W. W. (1967), *Biochim. Biophys. Acta 131*, 154.

Sybesma, C., and Vredenberg, W. J. (1963), *Biochim. Biophys. Acta 75*, 439.

Vernon, L. P., and Ke, B. (1966), *in* The Chlorophylls, Vernon, L. P., and Seely, G. R., Ed., New York, N. Y., Academic, p 596.

Vernon, L. P., Ke, B., and Shaw, E. (1967), *Biochemistry 6*, 2210.

Zaugg, W. S., Vernon, L. P., and Tirpak, A. (1964), *Proc. Natl. Acad. Sci. U. S. 51*, 232.

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