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IN *CHROMATIUM*, A SINGLE PHOTOCHEMICAL REACTION CENTER OXIDIZES BOTH CYTOCHROME C552 AND CYTOCHROME C555

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SUMMARY

With *Chromatium* cell suspensions, the relative actinic effects of 800- and 890-nm light are the same for the photooxidation of cytochrome C552 as for that of cytochrome C555. The action spectra for the two photooxidations probably are identical.

A single 20-nsec actinic flash causes only about 1/10 as much cytochrome oxidation as does strong continuous light, and the effect of the flash does not vary greatly with conditions. The concentration of a single photochemical reaction center appears to limit the amount of cytochrome oxidation which results from the short flash.

Under some conditions (aerobic whole cells, whole cells under weak continuous illumination, and chromatophores at high redox potentials), a single flash results in C555 oxidation. Under other conditions (anaerobic whole cells in the dark, and chromatophores at low potentials), the flash appears to cause C552 oxidation.

From direct kinetic measurements with chromatophores, $P870^+$ appears to be the oxidant, both for C555 oxidation at high potentials, and for C552 oxidation at low potentials. The rate of the latter reaction is faster than that of the former by a factor of approx. 2.

The same primary and secondary electron acceptors (X and Y) appear to be involved in the oxidation of the two cytochromes. This conclusion rests on measurements of the rate of electron transfer between X and Y under different conditions.

o-Phenanthroline blocks the reaction between X and Y, with no effect on the oxidation of C555 or C552 by a single flash.

INTRODUCTION

Several authors[1–4] have suggested recently that photosynthesis in the purple sulfur bacterium *Chromatium* involves two or more types of photosynthetic systems, which operate at different redox potentials and oxidize different cytochromes. The background for this proposal lies in the studies of OLSON AND CHANCE[5,6], who found that *Chromatium* contains at least three different *c*-type cytochromes. One of these,

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Abbreviations: PMS, *N*-methylphenazonium methosulfate; BChl, bacteriochlorophyll.

cytochrome C552 (also called C553 or C423.5), becomes oxidized under weak illumination of anaerobic suspensions of cells; it is also autoxidizable. Its reduction in the dark after a period of illumination is comparatively slow, requiring a time on the order of 1 min. BARTSCH[8] and BARTSCH AND KAMEN[7] have purified cytochrome C552 from *Chromatium*, and found the soluble cytochrome to have a midpoint potential at pH 7 (denoted as $E_{m,7}$) of +10 mV.

Another cytochrome, which is called C556, C555, or C422, has a slightly different absorption spectrum, and requires stronger actinic light to maintain its oxidation. Cytochrome C555 is not autoxidizable. Its reduction in the dark after illumination is rapid, requiring less than 1 sec. This cytochrome has not been isolated in a form which is clearly recognizable, although CUSANOVICH AND BARTSCH[9] have purified a cytochrome with some of its properties. The $E_{m,7}$ of cytochrome C555 *in situ* is approx. +330 mV (ref. 3).

The mechanism of cytochrome C555 oxidation has been studied with *Chromatium* chromatophores[10–12]. Following a single actinic flash, the primary photochemical reaction is the tranfer of an electron from a special bacteriochlorophyll complex, called P870, to an unidentified electron acceptor, X. Oxidized P870 draws an electron from C555 in a reaction which has a half-time of 2 μsec at 298°K and a quantum yield near 1.0. On a second flash, each P870 is capable of oxidizing a second similar cytochrome[12].

Evidence that a different photochemical reaction center participates in the oxidation of cytochrome C552 comes from the finding of MORITA[1] that the action spectrum for C555 photooxidation differs from that for C552. MORITA[1] found that 890-nm light is most effective for C555 oxidation, and 800-nm light for C552, if one expresses the effectiveness on the basis of incident irradiance. J. BARBER has confirmed the observations of MORITA[1] (personal communication). SYBESMA *et al.*[13–15] have obtained comparable results with a different photosynthetic bacterium, *Rhodospirillum rubrum*, and have presented evidence that the different action spectra do not reflect heterogeneous bacterial cultures. The existence of several kinds of photochemical reaction centers thus could be of fundamental significance in bacterial photosynthesis. CUSANOVICH *et al.*[3], MORITA *et al.*[2], and HIND AND OLSON[4] suggest that *Chromatium* C555 participates in cyclic photophosphorylation, whereas C552 participates in non-cyclic electron transport from substrates such as thiosulfate to pyridine nucleotide.

Remaining in conflict with the action spectra of MORITA[1] are earlier measurements by AMESZ AND VREDENBERG[16]. The earlier experiments indicated that 880-nm light is more effective than is 800-nm light, for exciting both C552 oxidation and BChl fluorescence at wavelengths longer than 900 nm. The action spectra which AMESZ AND VREDENBERG[16] obtained for cytochrome C552 oxidation and for BChl fluorescence appeared to be identical. This implies that in order to cause C552 oxidation, excitons must migrate to B890, the bacteriochlorophyll complex which absorbs maximally at 890 nm, as it is the B890 complex which fluoresces. The results of MORITA[1] suggest that, on the contrary, 800-nm light may be particularly effective in exciting C552 oxidation.

The present paper reports that we were unable to confirm the observation of MORITA[1] of different action spectra for photooxidation of *Chromatium* cytochromes C555 and C552. It then presents several lines of evidence that a single reaction center

involving P870 is responsible for the oxidation of both cytochromes. Finally, it offers an interpretation of the results of MORITA[1], based on this conclusion. SEIBERT AND DEVAULT[17,18] have been investigating this subject simultaneously, and their results can be interpreted similarly.

METHODS

Chromatium Strain D was grown photo-autotrophically in the medium of MORITA *et al.*[2]. A radiometer probe (Yellow Springs Instrument Co. Model 65) indicated that the total incident energy was $8 \cdot 10^4$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}, including wavelengths to about 4 μ. The culture temperature was 30°. Cultures were started with inocula of approx. 20 %, and collected after 3 days. They were chilled, filtered twice (through coarse filter paper and then through finer paper), centrifuged, and resuspended with approximately their original volume of cold, fresh, filtered growth medium. For some experiments, the cells were concentrated up to 3-fold. The samples were returned to a temperature of 22° for study.

For a series of experiments similar to those of Fig. 1, cells also were grown at lower and higher light intensities, covering a range of about 10-fold. Varying the light intensity at which the cells grow causes changes in the absorption spectra of the cells[19]. For the same series of experiments, cells also were grown over a temperature range from 25 to 32°, and some cultures were collected after 2 days of growth instead of 3.

For experiments under anaerobic conditions, cell suspensions were bubbled for 5–10 min with nitrogen which had passed over hot Cu (ref. 11). For aerobic conditions, the suspensions were bubbled continuously with air.

The spectrophotometric methods for measuring oxidation and reduction of P870 and cytochrome, after a single laser flash or after a pair of flashes, have been described elsewhere[10,11]. For steady state cytochrome measurements, a strip chart recorder displayed the spectrophotometer output. Faster measurements could be made simultaneously with an oscilloscope. Preparation of chromatophores also followed standard procedures[20].

The actinic light source for the experiments of Fig. 1 was a 300-W tungsten–iodine lamp with a water cell filter, a Schott RG-9 cutoff filter, and 10-nm interference filters (Oriel optics). Similar results were obtained with a 150-W Xe actinic lamp, and a monochromator in place of the interference filters. The incident actinic irradiance was measured with a silicon solar cell (Solar Systems 10-8L) which was calibrated with the radiometer. The portion of the actinic light which the cells absorbed was measured with an integrating sphere with 30 silicon solar cells (Solar Systems 10-4L) on the walls. The wall spaces between the solar cells were painted white. The solar cells were connected in parallel across a small load resistor, and the voltage was measured on a Keithley 140B microvoltmeter. Powdered milk solutions were used as blanks.

Light-induced absorbance changes at different redox potentials were followed in a cuvette specially adapted for anaerobic measurements, in which oxygen-free nitrogen was bubbled through the sample continuously. The redox potential was measured with a Pt foil electrode and a calomel reference electrode (Beckman 39170). A double salt bridge prevented contamination of the sample with Hg from the calomel

electrode. The electrodes were standardized by measuring the redox potential of a saturated solution of quinhydrone at various known pH values, and also by titrations of methylene blue and FMN. A redox buffer mixture containing 100 μM *N*-methylphenazonium methosulfate (PMS, $E_{m,7} = +80$ mV), 100 μM methylene blue ($E_{m,7} = +11$ mV), 100 μM potassium indigotetrasulfonate ($E_{m,7} = -45$ mV), and 112 μM potassium indigodisulfonate ($E_{m,7} = -125$ mV), in 0.1 M potassium phosphate buffer at pH 7.0 was used for the cytochrome oxidation measurements in Figs. 4, 5, and 6A. For the measurements of P870 reduction in Fig. 6B, the redox buffer consisted of 50 μM PMS and potassium indigotetrasulfonate in 0.1 M phosphate buffer at pH 7.0. The redox potential was adjusted by the injection of small portions of 2 mM $Na_2S_2O_4$ in 0.2 mM NaOH. Experiments with 100 μM PMS as the only added redox buffer gave results which were identical to those of Figs. 4, 5 and 6. The definitions of E and $E_{m,7}$, and the $E_{m,7}$ values for the redox buffers are taken from CLARK[21].

RESULTS

The action spectrum of the cytochrome oxidations

Fig. 1 presents an experiment to determine whether the action spectrum for cytochrome C555 photooxidation differs from that for C552. The ordinate indicates the steady state level of cytochrome oxidation which results from illumination with 800-nm or 900-nm light. The abscissa gives the absorbed irradiance of the actinic light. The curve for anaerobic cells (A) is comparable to Fig. 2 in the paper of MORITA[1]. As OLSON AND CHANCE[6] discovered, there are several steps in this curve. The cytochromes which respond to the strongest illumination include C555; that which responds to the weakest is C552. The simpler curve for aerobic cells (B) includes C555 but not C552, as the latter is autoxidized before the illumination.

In Fig. 1 the relative actinic effects of 800- and 900-nm light are the same throughout the curves. Fourteen experiments of this type were conducted, with cells which were grown under a variety of conditions (see METHODS). With every sample of cells, the relative actinic effects of the two wave lengths were independent of the irradiance. These measurements gave no indication that the action spectrum for C552 oxidation (upon weak irradiation of anaerobic cells) differs from that for C555 oxidation (upon strong irradiation of anaerobic or aerobic cells).

The amount of cytochrome oxidation which results from a short flash

If the action spectra for oxidation of C552 and C555 are indistinguishable, the possibility remains that the two cytochromes occur in different photochemical units, but that energy can transfer efficiently between the units. In this case, one would expect that the amount of cytochrome oxidation which results from a single, short flash would be twice as great under conditions which allow both types of units to function, as it is when only one type is functional. Contrary to this expectation, the amount of cytochrome oxidation which results from a short flash does not vary greatly, although a short flash causes the oxidation of only approx. 1/10 of the total cytochrome present in the cells. For example, DEVAULT[22] has found that weak continuous illumination will oxidize essentially all of the C552 in anaerobic cell suspensions with very little effect on the amount of cytochrome which a short flash will oxidize. We confirmed his observations, as Fig. 2 will show.

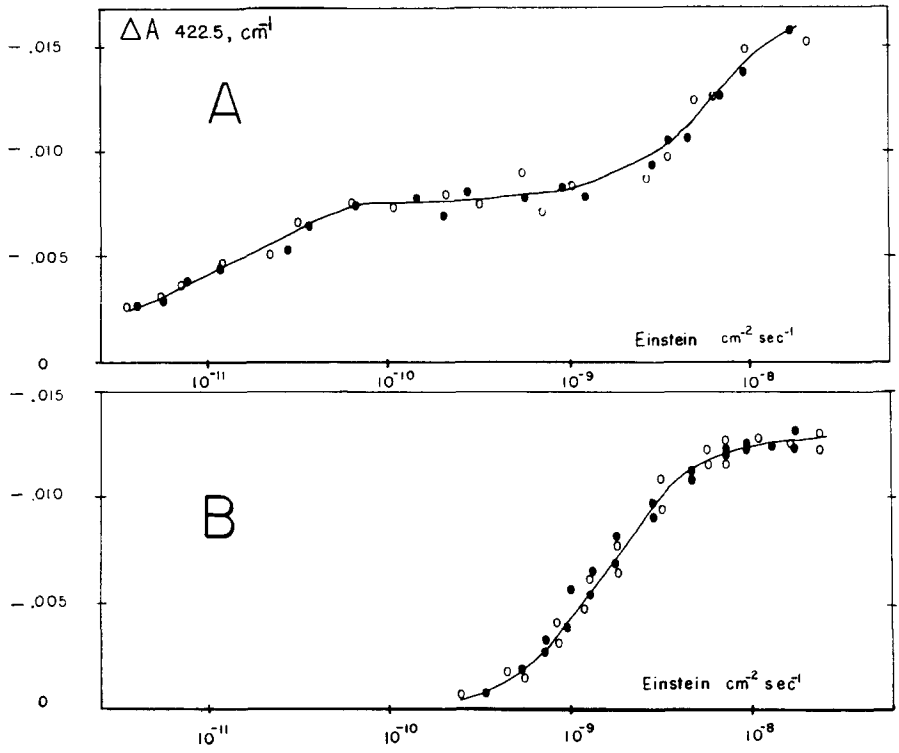

Fig. 1. A. Steady state cytochrome oxidation in an anaerobic *Chromatium* cell suspension, as a function of the absorbed irradiance of the continuous actinic light. The abscissa scale is logarithmic. O—O, 900-nm actinic light; ●—●, 800-nm actinic light. (8-15-69.) B. Same as A, except an aerobic suspension of a different sample of cells (8-11-69.) With a given sample of cells, saturating continuous light generally caused about twice as much cytochrome oxidation under anaerobic conditions as it did under air.

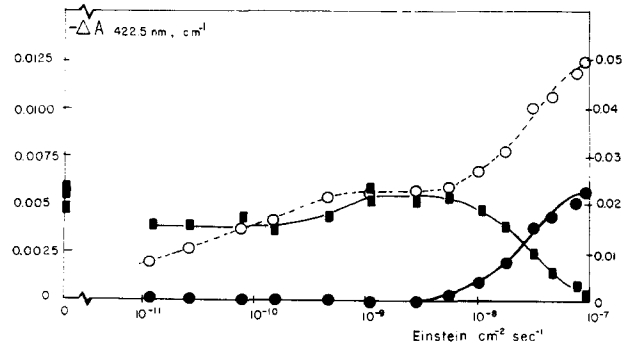

Fig. 2. ■—■, cytochrome oxidation which is caused by a single 20-nsec actinic flash of saturating intensity, superimposed on continuous illumination with the incident irradiance designated on the abscissa. The left-hand ordinate scale applies. Anaerobic cell suspension. The actinic light included a broad band of near infrared radiation from a 75-W tungsten–iodine lamp and Schott RG-N9 filters (4 mm). The expression of the incident irradiance in units of Einstein·cm^{-2}·sec^{-1} was made on the arbitrary assumption that the light was monochromatic, with a wavelength of 800 nm. O---O, steady state cytochrome oxidation caused by the continuous actinic light. Right-hand ordinate scale applies. Note that the maximum steady state oxidation is about 10 times as great as the oxidation which results from a single flash. ●—●, steady state cytochrome oxidation which recovers rapidly (in less than 1 sec) when the actinic light shutter is closed. Right-hand ordinate scale applies. (3-19-69.)

The squares in Fig. 2 indicate the amount of cytochrome oxidation which occurred in response to a single 20-nsec laser flash of saturating irradiance, superimposed on continuous illumination. One can oxidize all of the cytochromes which respond to weak continuous illumination ($1 \cdot 10^{-9}$–$1 \cdot 10^{-8}$ Einstein$\cdot$cm$^{-2}\cdot$sec^{-1}), and the flash still causes approximately the same amount of cytochrome oxidation as it does if the cells are in darkness. The simplest conclusion is that the flash must cause the same primary reaction in both cases, and that the concentration of the primary photochemical reaction center limits the effect of the flash.

A possible objection to this conclusion is that the laser flash is highly monochromatic. It might, therefore, be absorbed exclusively by one type of photosynthetic unit. To test this proposition, the effect of the laser was compared with that of a Xe flash lamp. The Xe flash had a pulse width of 8 μsec at half amplitude; a Schott RG-N9 filter provided a broad band of near infrared light[11]. With both chromatophores and anaerobic whole cells, saturating flashes from the Xe lamp caused the same amount of cytochrome oxidation as did a saturating laser flash. This excludes theories built on the extreme monochromaticity or brevity of the laser flash.

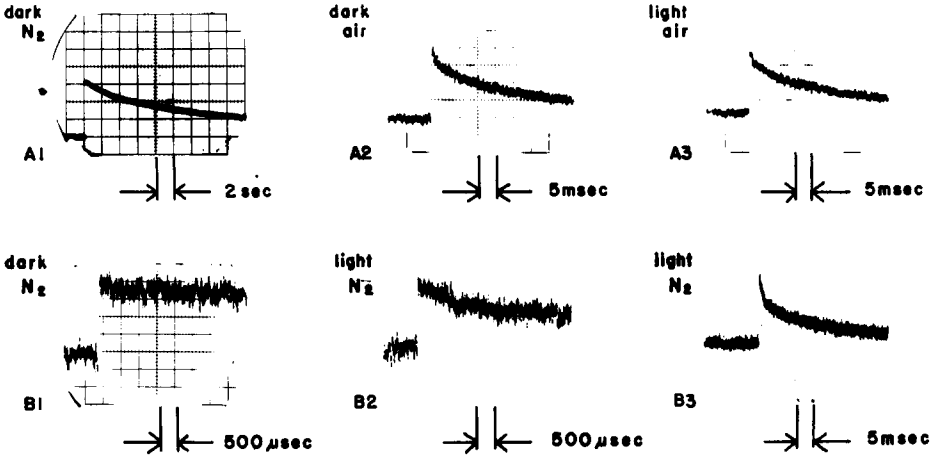

Fig. 3. Cytochrome oxidation and reduction following single flashes of saturating intensity under various conditions. Experiments marked "A" involved one sample of cells. (3-24-69.) Experiments marked "B" involved another sample. (3-15-69.) All of the oscilloscope traces measure the optical transmission at 422.5 nm, with a 1-cm light path. Note that the sweep rates are different in the different measurements. The abrupt upward deflections of the traces indicate the time of the flash. An upward deflection of one major division represents an absorbance decrease of 0.0022 in the A experiments and 0.0011 in the B experiments. For Expts. A2 and A3, the cell suspensions were bubbled with air. Following this treatment, continuous actinic light with an incident irradiance of $1.08 \cdot 10^{-9}$ Einsteins$\cdot$cm$^{-2}\cdot$sec^{-1} (see Fig. 2) caused little or no steady state cytochrome oxidation. For Expt. A3, the suspension was illuminated with continuous actinic light of this intensity. For Expts. B2 and B3, the suspension was illuminated with continuous actinic light with an incident irradiance of $1.44 \cdot 10^{-9}$ Einsteins$\cdot$cm$^{-2}\cdot$sec^{-1} (see Fig. 2). The continuous illumination caused a steady state cytochrome oxidation which was about 39% of the maximum oxidation with strong light. All of the cytochrome reduction was slow after the continuous light shutter closed. Before the measurements of B1, B2, and B3, the sample had been exposed to 31 flashes, superimposed on various levels of continuous light, as part of an experiment like that of Fig. 2. The recovery rates after the earlier flashes were very similar to those shown here, but a greater amount of cytochrome oxidation resulted from a flash superimposed on $1.44 \cdot 10^{-9}$ Einsteins$\cdot$cm$^{-2}\cdot$sec^{-1} continuous incident illumination.

The results of experiments like that of Fig. 2 actually were not quite as simple as this figure implies. The effect of a flash superimposed on weak continuous illumination generally decreased after several trials. On the first few trials, the effect of the flash frequently was slightly greater when the cells were in weak light than it did in darkness. Fig. 3 shows measurements in which weak illumination decreased the effect of the flash. We have no simple explanation for this phenomenon.

Because cytochrome C552 is autoxidizable, a second way of changing the cytochromes which are available for the flash to oxidize is to aerate the cells. Figs. 3A1 and 3A2 show that the flash causes approximately the same amount of cytochrome oxidation, whether the cells are aerobic or anaerobic. The effect of the flash is generally slightly greater with aerobic cells than it is with anaerobic cells. This may be because the primary electron acceptor is partially in the reduced state under anaerobic conditions. Again, the simplest interpretation is that the flash causes the same primary reaction in both cases.

An indication that a single flash causes the oxidation of different cytochromes, depending on whether the cells are aerobic or anaerobic, and whether they are in darkness or weak light, is that the recovery rates vary with these conditions. Figs. 3A1 and 3B1 show that, under conditions which keep C552 in the reduced state (darkness and an inert atmosphere), the recovery after a flash is slow, with a half-time of about 10 sec. The remaining portions of Fig. 3 show that, under other conditions (weak, continuous illumination or an atmosphere of air), the recovery is over 400 times faster. DEVAULT[22], and SEIBERT AND DEVAULT[17,18] have obtained similar results. SEIBERT AND DEVAULT[17,18] also have shown that the difference spectrum of the cytochrome oxidation is that of C552 under the former conditions, and that of C555 under the latter. The following section describes analogous conclusions concerning *Chromatium* chromatophores. The lesser turbidity of chromatophore suspensions permits kinetic measurements of P870 oxidation and reduction which are presently impossible with whole-cell suspensions[10].

Direct evidence that P870$^+$ oxidizes both C555 and C552

CUSANOVICH *et al.*[3] have discovered that one can study C552 photooxidation in chromatophores, simply by lowering the redox potential. As Figs. 4 and 5 show, we confirmed their finding that C552 undergoes photooxidation at redox potentials below 0 mV, and C555 at potentials above +200 mV. Fig. 5 gives the α-band spectra of the cytochromes which undergo photooxidation after a single flash at +240 mV and at −25 mV. Fig. 4 shows that the changeover from C555 to C552 occurs as one lowers the redox potential through the region +100 to −20 mV.

It is now possible to ask two direct questions. Do P870 oxidation and reduction occur in response to a single flash at low redox potentials, and, if so, does the reduction rate match the rate of C552 oxidation? The answer to both questions appears to be yes. Fig. 6A shows the rate of cytochrome photooxidation at redox potentials of +220 and −25 mV. The half-times of the cytochrome oxidations are approx. 2.0 and 0.8 μsec, respectively, Fig. 6B shows the rate of P870$^+$ reduction after a saturating flash at redox potentials of +240 and −60 mV. The rates correspond to half-times of approx. 2.0 and 0.8 μsec, respectively.

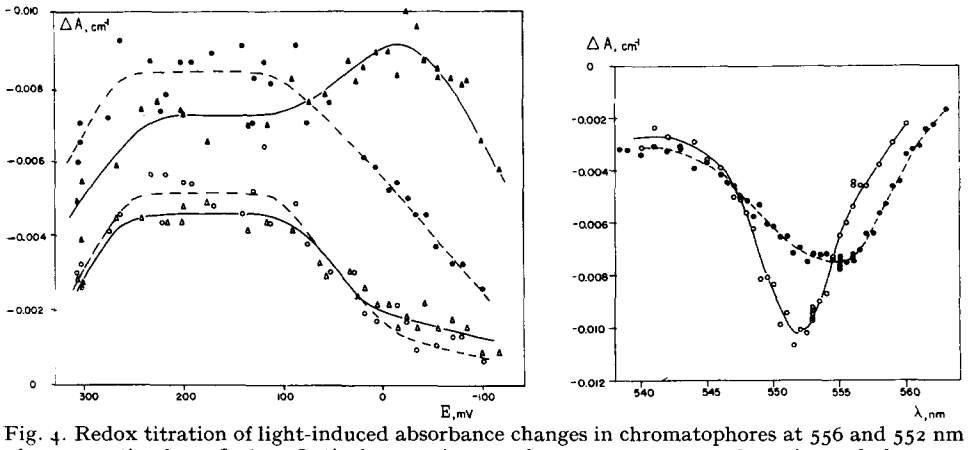

Fig. 4. Redox titration of light-induced absorbance changes in chromatophores at 556 and 552 nm after saturating laser flashes. Optical transmittance changes were measured continuously between 1 and 7 msec after the flash, with an oscilloscope sweeping at 1 msec/cm. In some potential regions appreciable reduction of the cytochrome occurred during this interval. The data are corrected for this recovery by extrapolation close to the time of the flash. Measurements with higher sweep rates indicated that the initial transmittance changes were complete within about 10 μsec after the flash, both at 552 and at 556 nm. Bacteriochlorophyll concentration, 72 μM; 1-cm light path; monochromator band pass, 3.6 nm. ●---●, absorbance changes at 556 nm due to a first flash; ○---○, absorbance changes at 556 nm due to a second flash, 1.0 msec later; ▲—▲, absorbance changes at 552 nm due to a first flash; △—△, absorbance changes at 552 nm due to a second flash, 1.0 msec later. (9-19-69.)

Fig. 5. Spectra of light-induced absorbance changes in chromatophores at different redox potentials. Conditions as in Fig. 4; data are extrapolated toward time of flash, as described in the legend to Fig. 4. ●---●, absorbance changes following a single flash in the redox potential region of +200 to +240 mV; ○—○, absorbance changes following a flash in the redox potential region of −30 to −10 mV. (9-19-69.)

Evidence that the same primary and secondary electron acceptors participate in the photooxidation of C555 and C552

Recent work[11,12] has provided another reaction which is linked to the primary photochemical reaction, and which should vary with the conditions if *Chromatium* contains two types of photochemical reaction systems. This is the transfer of an electron from the primary electron acceptor, X, to a secondary acceptor, Y. One can follow this reaction by measuring the rate at which the photochemical apparatus regains its ability to function, after a saturating actinic flash which reduces X completely. The rationale is that the primary photochemical reaction between P870 and X cannot occur so long as X remains in the reduced state.

Fig. 7 shows the ability of a second flash to cause cytochrome oxidation as a function of time after a saturating flash. In anaerobic cell suspensions, a time on the order of 1 msec must elapse before X returns halfway to its equilibrium level. This is considerably longer than the 80 μsec which *Chromatium* chromatophores require for the same process[11], probably because a lower redox potential prevails in anaerobic cells and Y exists largely in a reduced state. According to a recent report by CRAMER[23], anaerobic cell suspensions can maintain a redox potential as low as −200 mV. Fig. 7 shows that aerating the cells decreases the half-time to about 200 μsec, in accordance with this interpretation.

The most germane conclusion from Fig. 7 is that the X^- oxidation rate does not differ markedly if one compares anaerobic cells in darkness with anaerobic cells in

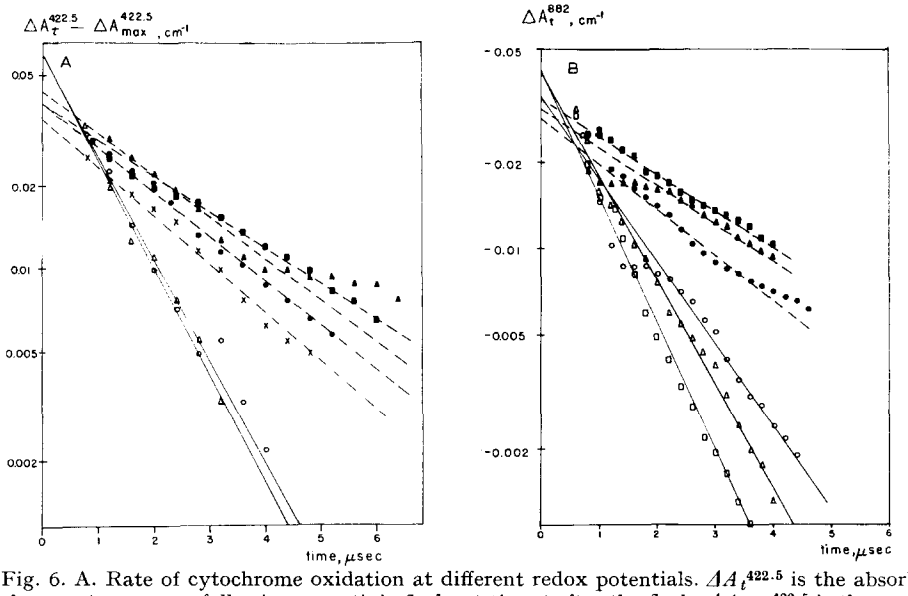

Fig. 6. A. Rate of cytochrome oxidation at different redox potentials. $\Delta A_t^{422.5}$ is the absorbance change at 422.5 nm following an actinic flash, at time t after the flash. $\Delta A_{max}^{422.5}$ is the maximal absorbance change following the flash, and is measured at approx. 8 μsec after the flash. Bacteriochlorophyll concentration, 92 μM. ●, ▲, ■, ×, absorbance changes due to single flashes in the redox potential region of +218 to +222 mV. ○, △, absorbance changes due to single flashes in the redox potential region of −25 to −24 mV. Other measurements indicated that the rate of cytochrome oxidation is the same at +240 as at +220 mV, and the same at −60 as at −25 mV. Ordinate scale is logarithmic. (9-11-69.) B. Rate of P870$^+$ reduction at different redox potentials. ΔA_t^{882} is the absorbance change at 881.9 nm following an actinic flash at time t after the flash. These data are corrected for an artifact which is due to bacteriochlorophyll fluorescence, and which is significant for the first 2 μsec after the flash. The correction was obtained by measuring the fluorescence alone, according to the first method of PARSON[10]. Bacteriochlorophyll concentration, 73 μM. ●, ▲, ■, absorbance changes due to flashes at a redox potential of +240 mV. ○, △, □, absorbance changes due to flashes in the redox potential region of −66 to −53 mV. Ordinate scale is logarithmic. (10-9-69.)

weak light. Thus, the rate appears not to depend directly on whether the flash causes the oxidation of C552 or that of C555. The simplest conclusion is that the same primary and secondary electron acceptors mediate the photooxidation of the two cytochromes.

Fig. 4 shows the dependence of X^- oxidation on the redox potential. The open symbols in the figure indicate the amount of cytochrome oxidation which results upon illumination of chromatophores with a second flash, 1 msec after the first. At high potentials, X^- reoxidation is complete in less than this time[11]. At potentials low enough to reduce Y, the second flash has a smaller effect. (Separate measurements gave a half-time of 5 msec for X^- oxidation in chromatophores between −10 and −70 mV.) The measurements at 556 and 552 nm in Fig. 4 both indicate that Y reduction occurs over the potential range +100 to 0 mV. A comparison of Figs. 4 and 7 suggests that the endogenous redox potential does not rise through this range, upon weak, continuous illumination of anaerobic cells. The discussion below will return to this point.

The effect of o-phenanthroline

MORITA *et al.*[2] have proposed that a third cytochrome, called C426, can undergo photooxidation independently of C555 or C552. Their evidence for this suggestion was

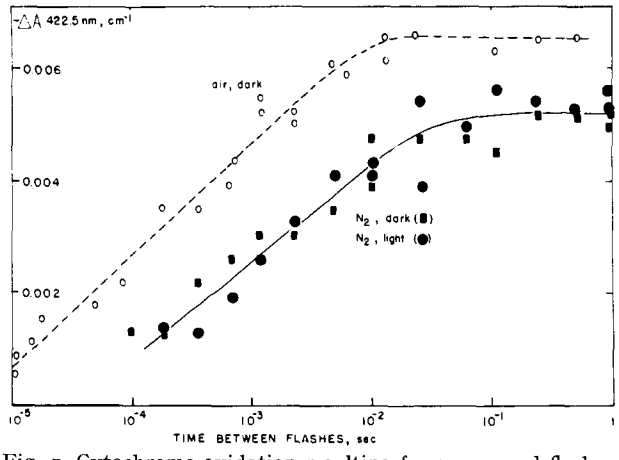

Fig. 7. Cytochrome oxidation resulting from a second flash, as a function of time after the first flash. The abscissa scale is logarithmic. See PARSON[11,12] for technical details on this type of experiment. O---O, aerobic cell suspension (3-28-69); ■—■, anaerobic cell suspension (a different sample); ●—●, anaerobic suspension (same sample as was used for the closed squares), with $1.08 \cdot 10^{-9}$ Einsteins$\cdot cm^{-2} \cdot sec^{-1}$ continuous incident illumination (see Fig. 2). (3-21-69.) Under anaerobic conditions in the dark, the cytochrome recovery after the second flash was slow, with a half-time on the order of 10 sec, suggesting that the second flash induced oxidation of a second C552 heme.

that *o*-phenanthroline appeared to block photooxidation of C552 and C555, but not that of C426. However, MORITA *et al.*[2] did not provide absorption spectra to support their identification of the cytochrome which is resistant to *o*-phenanthroline, and their basis for the identification appears to be insecure. CUSANOVICH *et al.*[3] and HIND AND OLSON[4] subsequently interpreted C426 in terms of other cytochromes. In spite of the uncertainty over the identity of C426, it is important to consider whether *o*-phenanthroline reveals a photochemical system other than the one which oxidizes C552 and C555.

Fig. 8 shows the effect of *o*-phenanthroline in *Chromatium* chromatophores. It is clear that *o*-phenanthroline has essentially no effect on the first actinic flash, but that it does inhibit the action of the second flash. In collaboration with Mr. WILLIAM PENDERGRASS, we made similar measurements with aerobic and anaerobic cell suspensions and with chromatophores at −25 mV. The results of all of these measurements were similar to those of Fig. 8, except that higher concentrations of *o*-phenanthroline were necessary in the whole-cell experiments, presumably because Fe^{2+} in the suspension medium competed for the inhibitor. Contrary to the report of MORITA *et al.*[2], we found that *o*-phenanthroline did not prevent steady state photooxidation of cytochromes in aerobic cells under continuous illumination, although it did increase the intensity of the illumination which was required to bring about the oxidation.

The simplest interpretation of our results is that *o*-phenanthroline has no direct effect on P870 oxidation, nor on the oxidation of C556 or C552 by P870, but that it does block the reduction of Y by X^-. R. K. CLAYTON AND D. FLEISCHMAN (personal communication) have reached a similar conclusion in studies of two other species of photosynthetic bacteria, *Rhodopseudomonas viridis* and *Rhodopseudomonas spheroides*. If one accepts this view, there remains no evidence that the photooxidation of cytochrome C426 occurs in an independent photosynthetic system.

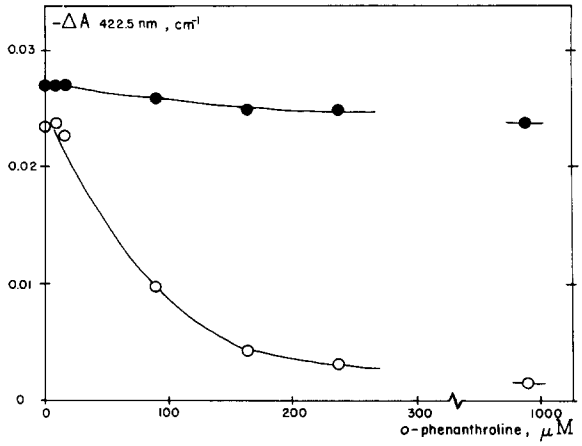

Fig. 8. Cytochrome oxidation resulting from the first or second flash, as a function of the concentration of *o*-phenanthroline. Anaerobic chromatophores; 42 μM bacteriochlorophyll; 3.3 μM PMS; 0.1 M glycylglycine (pH 7.4). The redox potential was not measured in this experiment; in other experiments, chromatophores under similar conditions established a redox potential of +250 to +300 mV. *o*-Phenanthroline was added in 5-μl portions of ethanol; ethanol alone had no effect. ●—●, cytochrome oxidation on the first flash; ○—○, cytochrome oxidation on a second flash, 1.0 msec later. (9-13-69.)

DISCUSSION

J. P. THORNBER (personal communication) recently has isolated from *Chromatium* a BChl–protein complex which contains P870, along with approx. 40 molecules of light-harvesting BChl per particle. Significantly, the complex also contains both cytochrome C552 and cytochrome C555, and both cytochromes are capable of undergoing oxidation upon illumination. The light-harvesting BChl in his preparation absorbs predominantly at 890 nm. From all indications, this BChl represents the B890 which AMESZ AND VREDENBERG[16] implicated in the immediate transfer of energy to the reaction center. Most of the light-harvesting BChl in intact cells or chromatophores absorbs at 800 and 850 nm, and exists in other protein complexes which J. P. THORNBER (personal communication) has isolated as well. GARCIA *et al.*[24] have isolated a reaction center complex similar to that of J. P. THORNBER, although the cytochromes in their preparation have not proved susceptible to photooxidation.

The efficiency of migration of energy from the outlying light-harvesting complexes to the complex which contains B890 and P870 ordinarily is quite efficient. In anaerobic cell suspensions, the quantum yield for C552 photooxidation under weak light is near 1.0 (refs. 18, 25). Under aerobic conditions, the quantum yield of C555 oxidation can be near 1.0 (refs. 10, 26). If the two reactions occurred in different photochemical systems, the high quantum yield of both of these cytochrome oxidations would suggest that only one system can work at a time, and that an energy switch directs excitons efficiently to whichever type of reaction center is functional. But this conclusion would conflict with the view that the two cytochrome oxidations have different action spectra. The action spectra could differ only if energy transfer between the two systems were limited.

The high quantum yields for the two cytochrome oxidations, and the finding of J. P. THORNBER (personal communication) that the P870–B890 particle contains

both cytochromes support the thesis that P870 mediates the oxidation of both cytochromes. Accepting this conclusion, however, leaves the problem of explaining the different action spectra which MORITA[1] and J. BARBER (personal communication) have observed.

In considering this problem, one should note that the abscissa in Fig. 2 of MORITA[1] gives the incident actinic irradiance, whereas our Fig. 1 gives the absorbed light. Recalculating his data on the basis of absorbed light, using the absorption spectrum in Fig. 1 of his paper, reveals that 803- and 904-nm light were essentially equally effective at low irradiances. This agrees with our results. The disagreement arises, therefore, only at high irradiances, at which MORITA[1] found a marked decline in the effectiveness of 803-nm light, relative to 904 nm. Rather than attribute this decline to the emergence of a second type of photochemical unit, we prefer to speculate that high intensity illumination can cause a drop in the efficiency of energy transfer to B890 from the other light-harvesting bacteriochlorophyll complexes. There are several recent reports which support this speculation circumstantially, although our own measurements give no indication of such a drop.

First, MURATA[27] and MURATA AND SUGAHARA[28] have found that actinic illumination of chloroplasts or algae causes reversible changes in the chlorophyll *a* fluorescence yield, in addition to the fluorescence yield changes which are due to reactions of the System II trap. MURATA AND SUGAHARA[28] suggest that their observations could result from a decrease in the efficiency of energy transfer to the chlorophyll complex which fluoresces most strongly. Uncouplers block the fluorescence yield changes, suggesting that the decrease in energy transfer accompanies the development of a high energy state. A similar explanation may apply to the experiments of MORITA[1] with *Chromatium*. Our experiments may have failed to show this effect because of a looser energy coupling in our cell suspensions.

Second, for C555 oxidation in *Chromatium* chromatophores, SUZUKI *et al.*[29] have obtained an action spectrum which is identical with that which MORITA[1] obtained for C552 oxidation in whole cells. In both cases, 800- and 900-nm light was essentially equally effective, if one expresses the data on the basis of absorbed light. The report of SUZUKI *et al.*[29] confirms that an inefficient utilization of 800-nm light is not necessarily a feature of C555 oxidation, and supports the view that a special disruption of energy transfer occurred in some of the experiments of MORITA[1]. SUZUKI *et al.*[29] also found that the addition of detergents to *Chromatium* chromatophores causes reversible alterations in the action spectrum for C555 oxidation. A disruption of energy transfer to B890 will explain this effect.

In conclusion, the appearance of different action spectra for the oxidation of cytochromes C552 and C555 could result from the necessity of measuring the two reactions under different conditions. In strong actinic light, there may occur a decrease in the efficiency of energy transfer to B890. MORITA[1] and FORK AND AMESZ[30] have pointed out that other special conditions which elicit C555 photooxidation may cause such changes in energy transfer.

The significance of the difference between the rates of oxidation of cytochromes C552 and C555 (Fig. 6) is not completely clear. SEIBERT AND DEVAULT[18] have found similarly that the rate of C552 oxidation in anaerobic *Chromatium* cells is about twice as great as that of C555. The rates of the cytochrome oxidation reactions in whole cells appear to be identical with those which occur in chromatophores.

DEVAULT *et al.*[31,32] have found that cytochrome oxidation in anaerobic cell suspensions occurs with a half-time of 2 msec after a flash, if the temperature is below 120°K. Below this point, the rate is independent of the temperature, at least down to 4.4°K. As the low temperature cytochrome oxidation does not occur in aerobic cells or in aerobic chromatophores[10,26], it may involve C552. If so, C552 must be able to react directly with $P870^+$, without involving C555. The finding that at room temperature C552 oxidation is twice as fast as C555 oxidation would agree with this analysis. We therefore favor the working hypothesis that $P870^+$ can react directly with either C552 or C555. The alternative is that P870, C555, and C552 form a linear electron transport chain, in which only C555 reacts directly with $P870^+$, and ferricytochrome C555 oxidizes ferrocytochrome C552. This alternative is more cumbersome, because it requires that the rate of C555 oxidation by $P870^+$ must vary somehow with the redox potential. It also requires the postulate that the low temperature cytochrome oxidation involves C555, but that the low temperature reaction is somehow defective at high redox potentials.

The observation that weak illumination of anaerobic cells does not cause a major increase in the X^- reoxidation rate (Fig. 7) suggests that the endogenous redox potential does not rise substantially during the illumination. Because an actinic flash causes C555 photooxidation in illuminated, anaerobic cells (Fig. 3 and ref. 18), it appears that a moderately low redox potential *per se* does not block cytochrome C555 photooxidation. The preference for C552 photooxidation at low redox potentials (Figs. 4 and 5) evidently hinges on the availability of reduced C552, rather than on any obstruction to C555 oxidation.

All of the present evidence is consistent with the view that the same photochemical reaction center, involving P870 and the same primary and secondary electron acceptors, is responsible for the oxidation of two quite different cytochromes. The physiological significance of this complexity remains open to speculation[2-4]. The problem also remains of exposing the relationships between the C552 and C555 hemes which undergo oxidation after single flashes and the additional hemes which respond to continuous light.

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Since submitting this report, we have found that, if one omits PMS, the cytochrome oxidation which results from a second flash (Fig. 4, open symbols) does not vary with E in the region + 100 to −50 mV. The $E_{m,7}$ of Y thus may be lower than Fig. 4 suggests.

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