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LIGHT-INDUCED ELECTRON TRANSPORT IN *CHROMATIUM* STRAIN D

II. LIGHT-INDUCED ABSORBANCE CHANGES IN *CHROMATIUM* CHROMATOPHORES

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SUMMARY

The light-particle preparations, described previously as derived from chromatophore fractions of *Chromatium*, exhibit absorbance changes on steady-state illumination with actinic light. Conditions for optimal absorbance changes were established and used to study the effects of variation in redox potential effective at the light-activated electron transport system. The results indicate that at least six components participate in photo-induced changes in oxidation states; two of these can be correlated with the known cytochrome components—cytochrome *c-552* and cytochrome *c-555*. The natures of the remaining components remain to be established. A model is proposed for the electron transport mechanisms activated by light, the salient feature of which is the existence of two functionally different pathways of photo-activated electron movement. One of these involves a pigment–heme protein complex consisting of the 890 mμ active center bacteriochlorophyll coupled to cytochrome *c-555*, cytochrome *cc'* and a third cytochrome (component No. 3); the other consists of a 905 mμ active center bacteriochlorophyll coupled to cytochrome *c-552* and a low potential component (P_{-135}). A rationalization of all observations on photometabolism of *Chromatium* on the basis of this model is presented.

INTRODUCTION

The photosynthetic bacteria, because of their unicellular nature and position in the evolutionary scale[1], offer unique opportunities for the study of light-induced electron transport. Researches to the present have been focused on light-induced absorbance changes associated with bacteriochlorophyll (BChl)[2–4] and variations in oxidation states of cytochromes[5,6]. *Chromatium* Strain D is a particularly useful organism for such studies because of minimal spectroscopic interference by accessory photoactive pigments, such as carotenoids, and the large magnitude of the absorbance changes due to cytochromes. Further, the anaerobic character of this organism obviates complications which may arise from occurrence of reactions for the utilization of oxygen.

Abbreviations: BChl, bacteriochlorophyll; PS buffer, 0.1 M potassium phosphate–10% sucrose (pH 7.5).

Upon illumination of *Chromatium* suspensions, two major absorbance changes have been associated with BChl. One centered at 883 mμ has been attributed to the photo-oxidation of a BChl molecule, designated P890 (ref. 7); and the other, centered at 808 mμ, has been designated P800 (ref. 7), and is considered to arise from a blue shift of a pigment absorbing at 803 mμ. The present interpretation[7] is that P800 and P890 are separate molecules, P890 being the so-called 'active center' BChl which initiates light-induced electron transport and P800 a 'light harvesting' BChl in close association with P890.

Four pigments, assumed to be cytochromes, have been noted in intact cells by OLSON AND CHANCE[5]. They labeled these cytochromes, according to their Soret absorption maxima, as C-423.5, C-426, C-422 and C-430, and found that C-423.5 and C-426 were oxidized by molecular oxygen and that C-426 formed a complex with CO. Several similar studies with both intact cells and chromatophores have been reported[6,8].

Various suggestions as to the mechanism of light-induced electron transport in *Chromatium* have been presented[5,6]. Interpretations have been complicated by a lack of chemical characterization of the samples studied, a lack of correlation between properties of the changes observed and those of chemically characterized components, and difficulty in determining the relation of the changes observed to light-induced electron transport. As described in Paper I of this series[9], we have found it possible to isolate subcellular particles ('light particles') which provide a better system than obtained heretofore for studies on light-induced electron transport. We report here the characterization of the light-induced absorbance changes of these light particles (a variety of chromatophores) isolated from *Chromatium*, particularly as related to oxidation–reduction (redox) potential.

METHODS

Chromatium light particles were prepared as described previously[9] and suspended in 0.1 M potassium phosphate–10 % sucrose (pH 7.5) (PS buffer).

All spectra were obtained using a Cary 14R recording spectrophotometer. For illumination of the sample compartment, a beam of actinic light was directed through a hole in the side of the sample compartment. The light source was a Sylvania D16 W 21.5-V lamp, attached to a variable voltage d.c. power supply.

The actinic light was passed through a heat filter consisting of 2 cm of water, then through a condensing lens focused on the sample cuvette, and finally through a Schott–Jena interference filter (approx. 10 mμ half band width). The intensity of the actinic light was measured with a YSI-Kettering Model 65 radiometer.

Special cuvettes were constructed to monitor oxidation–reduction potential and pH during the measurement of light-induced absorbance changes. These cuvettes were patterned after those of LOACH[10] and HARBURY[11] and designed to fit the sample compartment.

The cuvettes were constructed from precision-bore rectangular Pyrex cuvette blanks (1 cm × 1 cm internal diameter) (obtained from Fisher and Porter Co.), as shown in Fig. 1A. 2-mm capillary tubing was attached to the bottom of each cuvette outside the path of the measuring and reference beams.

The sample cuvette had a 24/25 outer standard taper joint sealed to its top.

A side-arm was attached and tooled to hold a gas-chromatography type silicone rubber septum 3 mm × 1 mm for introduction of reagents by syringe needle. Both the sample and reference cuvettes were fitted with inner syringe-type fittings on the capillary side-arms. Kel-F syringe needle hubs could be attached to these fittings. The two cuvettes were connected, as shown in Fig. 1A, by 0.056-inch internal diameter Teflon tubing with a Kel-F syringe hub (Hamilton Co. No. 86509) at either end. The reference cuvette was also fitted with a syringe-type fitting at the top for connection to the gas train. A No. 4 silicone rubber stopper (Arthur Thomas Co.) was fitted with a Radiometer platinum electrode (No. P101), an Arthur Thomas Co. combination electrode (No. 4858-L15), a 5-inch 24-gauge stainless steel needle for gas inlet and a

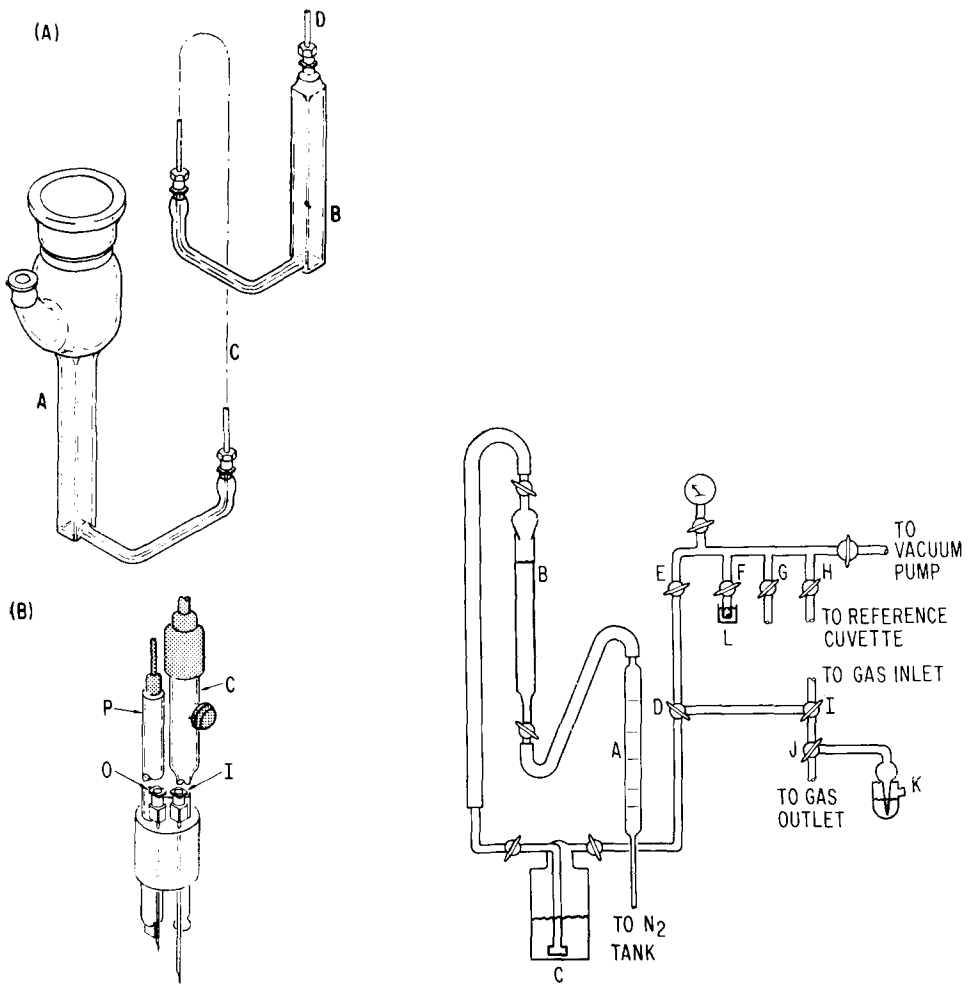

Fig. 1. (A) Anaerobic cuvette assembly. A, sample cuvette; B, reference cuvette; C, Teflon tubing connecting A and B; D, Teflon tubing leading to gas train. (B) Electrode assembly; P, platinum electrode; I, gas inlet; O, gas outlet; C, combination electrode.

Fig. 2. Gas train. A, flowmeter; B, quartz column containing MnO; C, hydration vessel; D, I and J, 2-mm T stopcocks; E, F, G and H, 2-mm stopcocks; K and L, water traps.

3-inch stainless steel 20-gauge needle for gas outlet, as shown in Fig. 1B. The stopper could then be placed in the ground glass joint on the sample cuvette and the sample cuvette used as a closed reaction vessel with gas stirring and electrodes for measuring pH and redox potential.

The electrodes were connected to an Instrumentation Laboratories Deltamatic pH/mV Electrometer Model 145. pH was measured with the combination electrode. Redox potential was measured, using the Pt electrode and the reference side of the combination electrode (Ag–AgCl). The Pt–Ag–AgCl couple was calibrated by measuring the potential at a known pH of a saturated solution of quinhydrone[12] (2 times crystallized from hot water).

We denote E_h as the measured potential, E_m, the midpoint of a particular couple measured under given conditions, and E_0', the midpoint potential of an electromotive couple as reported in the literature at pH 7.0. (The terms E_h and E_m are used as suggested by CLARK[12].)

The sample cuvette holder had three open sides. The reference cuvette was placed in an unmodified 1-cm cuvette holder.

Samples were deaerated and stirred by bubbling with pre-purified N_2 purchased from Airco. This gas contained approx. 10 ppm oxygen. Because this oxygen concentration was troublesome when trying to maintain low redox potentials, a gas train was constructed to produce oxygen-free N_2 and to facilitate movement of samples from one cuvette to another (Fig. 2).

The N_2 was scrubbed free of oxygen by passing it through a quartz tube filled with MnO (ref. 13). MnO reacts rapidly with oxygen to form MnO_2 and enables production of N_2 containing less than 1 ppm oxygen.

The components of the gas train, up to the hydration vessel, were interconnected with butyl rubber tubing. All components after the hydration vessel were interconnected by 12/2 Pyrex ball and socket joints greased with Apiezon vacuum grease. The gas inlet and outlet were connected to the gas train by double-hubbed 0.056-inch internal diameter Teflon tubes through a Kel-F adapter (Hamilton Co. No. 86506). The reference cuvette was connected similarly to the gas train.

For the determination of redox potentials, several redox buffers were used to poise the potential and couple efficiently with the electrodes. The chromatophores were suspended in one of the following reaction mixtures and then the redox potential was adjusted to the desired level, either by addition of 1 % sodium dithionite dissolved in 10 mM NaOH, or by permitting the endogenous reducing system of the particles to act.

1. Buffer A (for the redox range 520 to 350 mV): 100 μM potassium ferricyanide ($E_0'=430$ mV)[12] in PS buffer.

2. Buffer B (for the redox range 400 to 150 mV): 20 μM potassium ferricyanide and 20 μM 2,6-dibromobenzenoneindol-3'-carboxyphenol (purchased from Matheson Scientific, Los Angeles, Calif.) ($E_0'=250$ mV)[12] in PS buffer.

3. Buffer C (for the redox range 180 to 0 mV): 300 μM $FeCl_3$–10 mM disodium EDTA ($E_0'=117$ mV)[12] in PS buffer.

4. Buffer D (for the redox range 100 to −200 mV): 300 μM $FeCl_3$–10 mM $K_2C_2O_4$ ($E_0'=2$ mV)[12], and 100 μM indigotetrasulfonic acid (purchased from Wilshire Chemical Co., Gardena, Calif.) ($E_0'=-46$ mV)[12] and 100 μM indigodisulfonic acid ($E_0'=-125$ mV)[12] in PS buffer.

RESULTS

General properties

Deaeration by bubbling chromatophore suspensions with purified N_2 in the cuvette system described was found to be highly effective. Although there was no reason to expect the particles to couple efficiently with the redox electrodes in the absence of redox buffer, the removal of oxygen by bubbling with N_2 caused a downward shift of the redox potential. When a stable potential was obtained, the suspension was assumed to be completely deaerated. It was found that exhaustive deaeration of the light particles suspended in PS buffer, resulted in establishment of a potential in the range 250 to 200 mV.

Fig. 3. gives the light *minus* dark steady-state difference spectra of a suspension of light particles at two different actinic intensities. These spectra were similar to those previously reported for *Chromatium*[5,6].

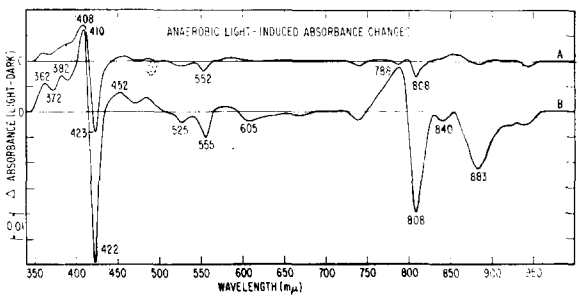

Fig. 3. Light-induced absorbance changes, light particles. BChl concentration 35 μM, anaerobic conditions at pH 7.5 (PS buffer). A. $I = 0.2 \cdot 10^4$ erg·cm^{-2}·sec^{-1}. B. $I = 2.2 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}.

The 883 mμ and 808 mμ difference peaks have been mentioned. The difference peak in the positive direction at 788 mμ is taken as the trough[10] of the 808 mμ difference peak. The 605 mμ difference peak probably arises from the light-sensitive component of BChl with an absorption peak at 590 mμ. All of the difference peaks resulting from BChl represent only a few percent (1–3 %) of the absolute absorbance at a particular wavelength, and are considered to be light-induced changes in active center BChl and closely related BChl molecules.

The difference peak at 555 mμ for high light intensity spectra (Fig. 3B) is taken as the α-peak of cytochrome *c-555* (C-555)[9]. The difference peak at 570 mμ is taken as the valley for the 555 mμ change. For the low light intensity changes (Fig. 3A), the difference peak at 552 mμ is taken as the α-peak of cytochrome *c-552* (C-552)[9]. The 525 mμ difference peak is the β-peak of C-555 in the high light intensity spectra and the β-peak of C-552 in the low light intensity spectra. The 472 mμ peak and the 485 mμ valley in the difference spectra are thought to arise from reversible light-induced changes of a small fraction of the carotenoid pigments. The difference peak at 422 mμ with high light intensity illumination is partially the Soret change associated with C-555. Similarly, the difference peak at 423 mμ for low light intensities is partially the Soret peak of light-oxidized C-552. The difference peaks at 452 mμ and 410 mμ are taken as valleys associated with the Soret peaks of oxidized cytochromes. The peaks at 382 mμ, 373 mμ and 362 mμ have not been well studied but appear to be

associated with components which contribute to absorption by BChl at 370 mμ. The wavelength maxima reported were measured at a scan speed of 30 mμ/inch (60 nm/min) and it is estimated that the wavelengths reported are accurate to ± 0.5 mμ.

A number of the small changes observed were not studied further because they were difficult to quantitate. Four changes were studied in detail—those at 883 mμ, 808 mμ and the α- and Soret peaks of the cytochromes. The change centered about 883 mμ was designated Δ883 and the magnitude was measured as the difference in absorbance between 883 mμ and 960 mμ—a wavelength at which no light-induced change in absorbance occurred (isosbestic point).

The change centered around 808 mμ was designated Δ808 and the magnitude was measured as the difference in absorbance between the 808 mμ peak and the 788 mμ valley.

The change in the 550 mμ region was quantitated from the difference in absorbance between the α-peak and the 570 mμ valley. This change was designated Δ555 or Δ552, depending on the component involved.

The cytochrome changes in the 420 mμ region were quantitated from the difference in absorbance between the Soret peak and the valley at 452 mμ. This change was designated Δ422 or Δ423.

Similar results could be obtained from the cytochrome Soret changes by using the difference in absorbance between the Soret peak and the valley at 410 mμ for C-555 and the valley at 408 mμ for C-552.

The cytochrome changes could be expressed on a molar basis by dividing the magnitude of the cytochrome change by the appropriate extinction coefficient. Table I gives the values used.

TABLE I

CYTOCHROME ABSORPTIVITIES

Protein	α (mμ)	$\Delta\varepsilon_{mM}$	*Soret* (mμ)	$\Delta\varepsilon_{mM}$
C-555*	555	23	422	54.6
C-552[14]	552	43	423	156

* The extinction coefficients of *R. rubrum* cytochrome c_2 (ref. 16) have been assumed.

Many of the data are expressed as the fraction of the particular components affected by the light. For example, the fractional change in BChl was determined by dividing Δ883 or Δ808 by the absolute absorbance at 888 mμ or 803 mμ for the particular sample being studied. The fraction of the cytochrome present which was oxidized by illumination was calculated from the μmoles oxidized by light divided by the μmoles determined to be present in the sample[9].

Wavelength dependence

The effect of different wavelengths of exciting light on the light-induced absorbance changes was observed, using 888 mμ, 847 mμ, 803 mμ and 595 mμ actinic light. It was found that neither the magnitude of the light-induced absorption changes nor the wavelength of the peaks were effected by changing the wavelength of actinic light, at intensities of $2 \cdot 10^4$–$3 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}.

Intensity

The effect of various intensities of 888 mμ activation light was investigated over the intensity range $0.2 \cdot 10^4$–$7.6 \cdot 10^4$ ergs·cm^{-2}·sec^{-1} (see Fig. 4). At the lowest intensity used, the BChl region was not appreciably affected, but an appreciable amount of cytochrome with absorption maxima at 552 mμ and 423 mμ was oxidized, indicating that C-552 was the reactive cytochrome. At high light intensities, C-555 was oxidized. Activation of C-552 by low light intensity was initially observed by OLSON AND CHANCE[5]. This result was surprising because all of the C-552 ($E_0' = 10$ mV)[15] would be expected to have become oxidized by exposure to atmospheric O_2 during preparation of the light particles.

At higher light intensities, the C-555 changes were constant in magnitude, indicating that the maximum amount of cytochrome was being oxidized under these conditions. The Soret to α ratio ($\Delta 422/\Delta 555$) for C-555 was found always to fall in the range 4–6; this is in contrast to Soret to α ratios of 2–3 for most *c*-type cytochromes (*Rhodospirillum rubrum* c_2 (ref. 16), horse-heart cytochrome *c* (ref. 17), *etc.*) as determined from oxidized *minus* reduced difference spectra. This observation suggests the presence of a component contributing only in the Soret region. The steady increase in the amount of $\Delta 808$ with increasing light intensity was most likely due to irreversible photo-oxidation of a portion of the 'light harvesting' BChl.

Bacteriochlorophyll concentration

A study of the light reaction as a function of BChl concentration was made to determine the optimum BChl concentration for various light intensities. The extent of cytochrome oxidation is maximum in the range 25–35 μM BChl, while the BChl changes go through a maximum at slightly lower BChl concentration.

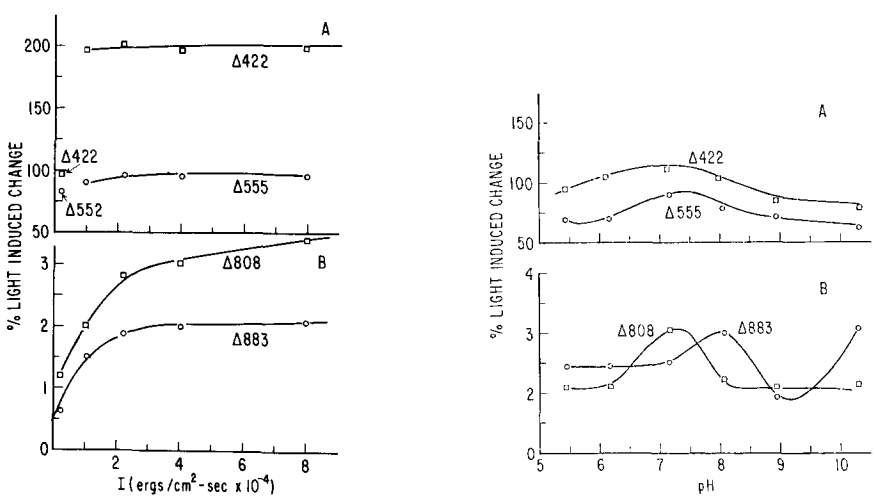

Fig. 4. Light-induced absorbance changes *vs.* light intensity. A. α and Soret changes. B. BChl changes. BChl concentration 35 μM, anaerobic conditions, pH 7.5 (PS buffer).

Fig. 5. Light-induced absorbance changes *vs.* pH. A. α and Soret changes. B. BChl changes. BChl concentration 35 μM, $I = 2.2 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}, anaerobic conditions (0.1 M Tris–0.1 M sodium acetate–0.1 M potassium phosphate).

Buffers

The effect of light on the light particles suspended in various buffers (Tris, potassium phosphate and glycylglycine) at pH 7.5 was studied. No effect of a particular buffer ion was noted, although it was found that at buffer concentrations less than 0.10 M the light-induced absorbance changes were slightly diminished relative to the constant changes at higher concentrations.

pH optimum of the light-induced changes

The light-induced absorbance changes were measured over the pH range 5–10. As illustrated in Fig. 5, it can be seen that the maximum $\Delta 808$ and cytochrome changes were between pH 7.0 and 7.5 and that the $\Delta 883$ change was maximal at pH 8.0.

The particles, if treated at pH 5 and then adjusted to higher pH, showed the usual light reaction. However, if the particles were first treated at pH 10 and then taken to a lower pH, the light-induced changes were greatly reduced in magnitude. The experiment shown in Fig. 5 was performed with particles which had been stored in the presence of oxygen and therefore exhibited less than maximal light-induced absorbance changes. It is assumed that all responses were equally affected on prolonged exposure to air.

Optimum conditions

On the basis of the above studies, conditions were chosen which gave the largest magnitude of reproducible light-induced absorbance changes. Thus, the light particles were routinely suspended in PS buffer, at a BChl concentration of 30–40 μM. The particles were ordinarily stored anaerobically as described above. The intensity of actinic light (generally 888 mμ) incident on the reaction cuvette was $2.2 \cdot 10^4$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}.

Effect of NADH

The effect of NADH on the light reaction was tested over the concentration range 0.66 μM–0.74 mM. No effect on the magnitude of the light reaction was found but there was an increase in the reversibility of the light reaction at NADH concentrations as low as 7 μM. Without NADH, the magnitude of light-induced absorbance changes decreased after two cycles of 10-min illumination, followed by 5-min dark periods. With added NADH, six cycles could be followed.

This effect may reflect interaction of NADH with photo-sensitive components in the electron transfer chain, mediated by residual NADH–heme protein reductase[9].

Effect of succinate

The effect of succinate on the light reaction was observed over the concentration range, 0.66 μM–0.74 mM. In general, the higher the succinate concentration, the smaller the magnitudes of the light reactions for both BChl and cytochrome, although the light reactions were readily reversible. The BChl change was depressed as much as 40 % below the usual value at succinate concentrations of 5–10 μM.

These results suggest that succinate couples though succinate dehydrogenase[9] to the electron transfer system with the consequent decrease in the steady-state oxidation level of the reactive components.

Effect of oxygen

Although a detailed study of the light reaction in the presence of oxygen was not undertaken, a clear picture emerged as to the effect of oxygen. Particles which were stored in the presence of air gave a light reaction after deaeration which was of smaller magnitude than that for particles stored in the presence of an inert gas. This deterioration continued steadily with time, and could be accelerated by diluting the particles and allowing them to stand in the presence of air. The magnitude of the light reaction was decreased approx. 50 % by standing in air (4°) for 2 h at a BChl concentration of 30 μM. Further incubation resulted in a further reduction of the light reaction.

Apparently, oxygen slowly inactivated some component(s) involved in the light reaction. This inactivation was only partly (approx. one-half) reversed by the addition of small amounts of reductant (ascorbate, $Na_2S_2O_4$).

Carbon monoxide

The light reaction of a deaerated suspension of light particles which had been bubbled with deoxygenated CO (Matheson Co. C.P. grade, 99.5 % minimum purity) for 20 min was tested. Both the BChl and cytochrome changes were decreased by approx. 40 %. The inhibition was not reversed after prolonged bubbling with N_2.

The responses to CO were similar to those observed with oxygen. The component being affected could not have a heme protein because of the previous demonstration[9] that the heme proteins bound to the light particles did not interact significantly with CO. The nature of this CO-reacting material remains to be determined.

Iron proteins

The effect of high potential iron protein[18], cytochrome *cc'* (ref. 15) and cytochrome *c-552* (ref. 15) was studied. Neither the oxidized nor reduced forms of these proteins had any influence on the magnitude, or form, of the light reaction over the concentration range 0.1–100 μM. Furthermore, the proteins themselves were unaffected by illumination in the presence of the light particles.

High potential iron protein has previously been shown to be photo-oxidized by *Chromatium* chromatophores[18]. However, these results were obtained with samples that were not as vigorously deaerated as in this work. Residual oxygen may have served as an electron acceptor to drive this oxidation. The particles used in this work could photo-oxidize high potential iron protein in the presence of air.

The lack of interaction with the soluble proteins implies that there are no sites in the particulate matrix available for the added proteins or that the added proteins are modified on solubilization and can no longer interact with the chromatophores.

Effect of ascorbate

Ascorbate has been used extensively to fix the redox potential of *R. rubrum*[19,20] and *Chromatium*[21] chromatophores to obtain optimum photophosphorylation. Results of studies on the light reaction are given in Table II.

Two dramatic changes took place on increasing the ascorbate concentration.

1. A shift of the 883 mμ peak to 900 mμ at high ascorbate concentration (approx. 100:1 ascorbate/BChl).

2. A shift in the light reacting cytochrome from C-555 to C-552 with increasing ascorbate concentration.

TABLE II

EFFECT OF ASCORBATE ON THE LIGHT REACTION

This experiment was performed on a sample which had been stored in air, thus the magnitude of the changes was not maximal. The % light affected for $\Delta 808$ or $\Delta 883$ was determined by dividing $\Delta 883$ or $\Delta 808$ by the absolute absorbance at 888 and 805 mμ, respectively. The % of cytochrome light affected was determined from the amount detected divided by the amount previously[9] determined to be present.

Light-induced change	*% Light affected*				
	Ascorbate (μM)	*0*	*3.3*	*33*	*320*	*3100*
$\Delta 883$		2.0	1.8	2.0	1.8	1.2*
$\Delta 808$		2.5	2.5	2.7	2.5	1.9
$\Delta 555$		33	33	55	**	61***
$\Delta 422$		80	93	110	§	78§§

* The 883 mμ peak has shifted to 900 mμ.
** The α-peak is at 554 mμ.
*** The α-peak is at 552 mμ.
§ The Soret peak is between 422 and 423 mμ.
§§ The Soret peak is at 423 mμ.

The reason for the shift in the BChl region is not apparent; however, it suggests that modification of the 'active center' may have taken place.

The shift in the light reacting cytochrome from C-555 to C-552 on increasing ascorbate concentration points out the widely different redox properties of these two components.

Reductases

Two NADH–dye reductases have been isolated[21] from photo-heterotrophically grown *Chromatium* in a medium containing succinate as the main substrate. These enzymes have been designated Reductase-1 and Reductase-2 and are easily separated during column chromatography on DEAE-cellulose (Reductase-1 is eluted with 70 mM NaCl–10 mM Tris (pH 7.8), and Reductase-2 is eluted with 120 mM NaCl–10 mM Tris (pH 7.8)).

These proteins have quite different properties. Reductase-1 in the presence of NADH will reduce both dyes (2,6-dichlorophenolindophenol, methylene blue) and heme proteins (horse-heart cytochrome *c*, cytochrome *cc'*, cytochrome *c-552*). Reductase-2 will reduce dyes but not the heme proteins tested. The two proteins have different electrophoretic mobilities on cellulose acetate strips. Reductase-1 is activated by FMN (100 μM) while Reductase-2 requires no added flavin cofactor.

The effect on the light reaction by these proteins, purified 100-fold over their specific activities in the crude extract, are given in Table III.

The results shown were obtained with particles which were stored in the presence of air and thus the magnitude of the light reaction was decreased. Reductase-1 partially reversed this oxygen inactivation without any change in the form of the light reaction. Further, in the presence of Reductase-1, the Soret to α ratio for C-555 was 3.6 compared to 5.1 for the control, suggesting that in the presence of Reductase-1 the number of components affected by light was reduced.

TABLE III

EFFECT OF THE REDUCTASES ON THE LIGHT REACTION

The % light affected for $\Delta 808$ or $\Delta 883$ was determined by dividing by the absolute absorbance at 888 and 805 mμ, respectively. The % of cytochrome light affected was determined from the amount detected divided by the amount previously[9] determined to be present.

Sample	*Wavelength of change (mμ)*	*% Light affected*
Complete (particles + NADH)	883	1.2
	808	2.0
	555	34.2
	422	69.5
Complete + Reductase-1	883	1.2
	808	1.8
	555	50.0
	422	76.8
Complete + Reductase-2	883	1.1
	808	1.8
	552	71.0
	423	58.0

In the presence of Reductase-2, C-552 was photo-oxidized with a Soret to α ratio of 2.9. This result could be interpreted in two ways, *viz.*:

1. In the presence of Reductase-2 and NADH, both C-552 and C-555 were reduced with C-555 being reduced so rapidly that there was no net steady-state oxidation, whereas C-552 was reduced at a rate which permitted net steady-state oxidation.

2. In the presence of Reductase-2 and NADH, C-555 was uncoupled from the active center and no longer photo-oxidized.

Effect of oxidation–reduction potential on the light reaction

There have been several discussions of the effect of redox potential[10,23] on photosynthesis. However, for the most part, redox potential has not been controlled in the study of light-induced electron transport.

In these experiments, potentials were measured with the calibrated Ag–AgCl–Pt electrode couple as described. In this operation, the redox buffers served to couple efficiently between the electron carriers and electrodes. It is helpful to describe a typical redox titration.

Fifteen ml of the appropriate redox buffer were placed in the sample cuvette, and deaerated by bubbling with oxygen-free N_2 until a stable potential was obtained. The appropriate amount of light particles was then added to the sample cuvette from an anaerobic concentrated stock solution.

The sample was stirred for several minutes, and then the potential was adjusted to the desired level by addition of sodium dithionite (1 % solution in 10 mM NaOH) or ferrous ammonium sulfate (1 mM). The two reductants gave identical results above 100 mV. Over the redox potential range (400 to 50 mV) the presence of a potent endogenous reductant made unnecessary the addition of reductant. In this potential range, the system redox potential dropped slowly during the recording of light-induced absorb-

ance changes as well as in the dark; therefore, the average value of the redox potential during the light-on period was recorded. The variation around the mean potential was usually $\pm$ 3 mV.

The light reaction was studied over the redox range (520 to 350 mV) using Buffer A. Light-induced absorbance changes observed above 400 mV were limited to those associated with BChl and carotenoids. Fig. 6 shows the magnitude of $\Delta 883$ and $\Delta 808$ plotted against the imposed redox potential. The solid lines in Fig. 6 are the theoretical curves for a one-electron change, and the dashed lines are for a two-electron change through the designated midpoint potential. $\Delta 883$ (E_m = 488 mV) and $\Delta 808$ (E_m = 490 mV) fitted curves for a one-electron change.

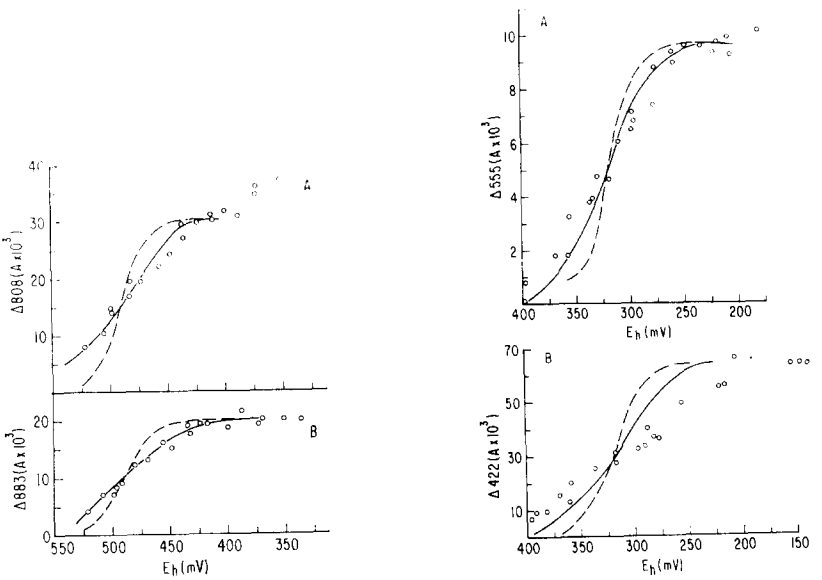

Fig. 6. Redox titration, light-induced absorbance changes BChl region (520 to 350 mV). A. $\Delta 808$ *vs.* redox potential. B. $\Delta 883$ *vs.* redox potential. ———, calculated one-electron change with E_m = 489 mV; – – – –, calculated two-electron change with E_m = 489 mV. BChl concentration 35 μM, $I = 2.2 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}, anaerobic conditions (pH 7.5) (Buffer A).

Fig. 7. Redox titration, light-induced absorbance changes α and Soret regions (400 to 150 mV). A. α changes. B. Soret changes. ———, calculated one-electron change with E_m = 319 mV; – – – –, calculated two-electron change with E_m = 319 mV. BChl concentration 35 μM, anaerobic conditions, $I = 2.2 \cdot 10^4$ ergs·cm^{-2}·sec^{-1} (pH 7.5) (Buffer B).

Light-induced absorbance changes in the cytochrome α and Soret region appeared below 400 mV. Fig. 7A shows the light-induced absorbance changes in the α region ($\Delta 555$) as a function of redox potential. The solid line is the theoretical curve for a one-electron transition and the dashed line for a two-electron change, with E_m = 319 mV. The light-induced absorbance changes centering about 555 mμ fitted a one-electron transition very well, which indicated that only one component was involved in the light reaction in this redox range (400 to 200 mV). The midpoint potential (319 mV) agreed with the chemical titration of C-556 using autotrophic *Chromatium* chromatophores (E_m = 330 mV) by R. G. BARTSCH AND J. M. OLSON (personal communication).

A similar plot for the light-induced Soret changes over the redox potential range (400 to 150 mV) is given in Fig. 7B. These results show that the Soret changes, unlike the α changes, did not arise from a single component, as judged by the lack of agreement with the theoretical curves.

Below 200 mV, the light-induced absorbance changes did not appear to be amenable to use of a single redox buffer, because of lack of clear resolution of the changes over a narrow redox region. This lack of resolution necessitated the use of several overlapping redox buffers. Fig. 8 shows Δ883 and Δ808 plotted as a function of redox potential over the range 500 to −100 mV.

The various redox buffers gave results which overlapped quite well. The magnitude of the absorbance changes rose steadily from the plateau at 400 mV to a maximum at 300 mV, a minimum at 200 mV and another maximum around 125 mV.

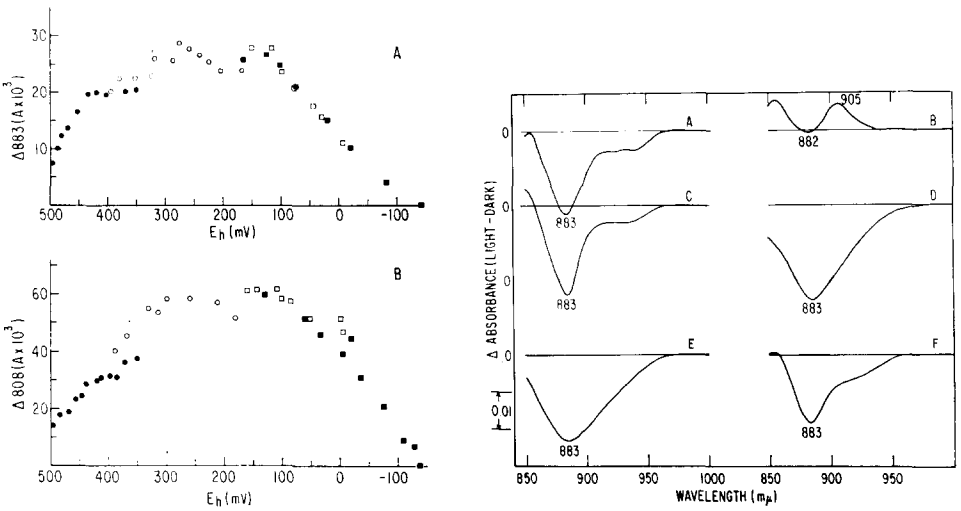

Fig. 8. Redox titration, light-induced absorbance changes BChl region (520 to −200 mV). A. Δ883 *vs.* redox potential. B. Δ 808 *vs.* redox potential. BChl concentration 35 μM, $I = 2.2 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}, anaerobic conditions (pH 7.5) (●, Buffer A; ○, Buffer B; □, Buffer C; ■, Buffer D).

Fig. 9. Δ883 and P905 under various conditions. BChl concentration 35 μM, $I = 2.2 \cdot 10^4$ ergs·cm^{-2}·sec^{-1} (pH 7.5). A. Anaerobic conditions (PS buffer). B. E_h = −100 mM (Buffer D). C. E_h = 200 mV (Buffer B). D. Aerobically stored particles (anaerobic conditions, PS buffer). E. E_h = 400 mV (Buffer A). F. Incubated for 3 h in air, anaerobic conditions (PS buffer).

Below 100 mV there was a steady decrease in the magnitude of the light-induced absorbance changes in the BChl regions.

The significance of this pattern is not apparent. However, two sources of difficulty in the explanation of low-potential changes should be noted. The first is that with different samples the slope of the decrease below 100 mV varies and the light reaction in the BChl region stops completely at different potentials ranging between −50 and −100 mV.

The second is that the detailed spectrum of the Δ883 change is modified as the potential is lowered. This is shown in more detail in Fig. 9 where Δ883 is shown under a variety of conditions. It can be seen that as the potential is lowered (compare E,

C and B), a shoulder centered about 905 mμ develops and at —100 mV is manifested as a discrete positive peak. This absorbance change will be tentatively termed 'Δ905' and assumed to be associated with a pigment termed P905. P905 is extremely sensitive to oxygen (compare A and F or A and D in Fig. 9).

Fig. 10A gives a plot of the light-induced changes in the α region as a function of imposed redox potential. Through the region (400 to 125 mV) the α change is centered about 555 mμ. In the redox range (125 to 0 mV) the α change is between 555 and 552 mμ, and below 0 mV it is centered about 552 mμ.

Based on the observed absorption maximum in the α region, C-555 appears the only component involved over the redox range 400 to 200 mV and C-552 the only component below 0 mV. The complex curve of Fig. 10A can be analyzed by assuming that the α change at —100 mV is the maximum change of C-552 and further that particle-bound C-552 has a midpoint potential identical to the soluble form (E_m = 10 mV). Then, the contribution of C-552 can be calculated by use of the Nernst equation (Fig. 10A, dashed line). If this contribution is subtracted from the overall curve, the solid line shown in Fig. 10A is obtained. This analysis indicates some component other than C-555 and C-552 may contribute to the α change between 200

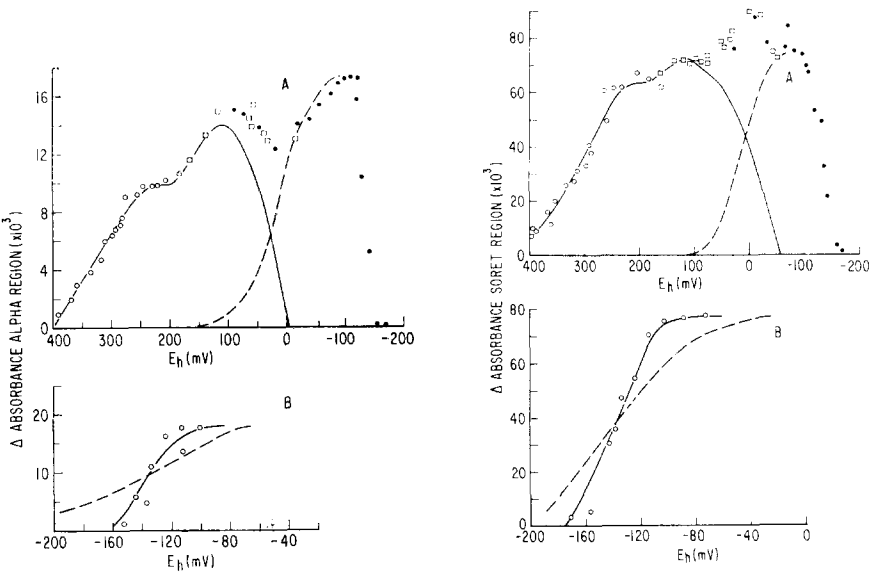

Fig. 10. A. Redox titration light-induced absorbance changes α region (400 to —200 mV). BChl concentration 35 μM, $I = 2.2 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}, anaerobic conditions (pH 7.5) (○, Buffer B; □, Buffer C; ●, Buffer D). – – – –, calculated one-electron change with E_m = 10 mV, assumed to be contribution of C-552. ———, redox titration light-induced α change (400 to —100 mV) with contribution of C-552 subtracted. B. Redox titration light-induced α change (552 mμ) (—100 to —160 mV). ———, calculated two-electron change with E_m = —135 mV; – – – –, one-electron change with E_m = —135 mV.

Fig. 11. A. Redox titration, light-induced absorbance changes Soret region (400 to —200 mV). Conditions as in Fig. 10A. – – – –, calculated one-electron change with E_m = 10 mV, assumed to be contribution of C-552 to Soret region. ———, redox titration light-induced Soret change (400 to —100 mV) with contribution of C-552 subtracted. B. Redox titration light-induced Soret change (423 mμ) (—100 to —160 mV). ———, calculated two-electron change with E_m = —135 mV; – – – –, one-electron change with E_m = —135 mV.

and 0 mV. The decrease in the steady-state absorbance changes in the region 100 to 0 mV (solid line) may result because C-555 and possibly other components are reduced faster by the environment than they can be oxidized by light.

In Fig. 10B, we present the absorbance change in the α region at redox potentials below —100 mV. Assuming that only C-552 is reactive in this region, it appears that a two-electron transfer component with $E_m = -135$ mV acts as an electron acceptor for C-552. It is suggested that some component which accepts electrons from light-oxidized C-552 becomes reduced below —100 mV and thus blocks the light-oxidation C-552. This component will be termed P_{-135}.

The light-induced changes in the Soret region are shown in Fig. 11A. The overall picture seems similar to that observed in the α region, with a transition from 422 to 423 mμ taking place between 125 and 25 mV. The Soret changes can be analyzed in the same manner as the α changes by subtracting out the contribution of C-552. This result shows that the contribution of unidentified component(s) extends below 0 mV, in contrast with the results observed in the α region.

Fig. 11B is a plot of the light-induced changes in the Soret region below —100 mV. This result is identical to that observed in the α region, with the slope of the Soret change over the redox potential range —40 to —160 mV, fitting a two-electron change with $E_m = -135$ mV.

These results indicated the presence of several components affected by light in the cytochrome region. To identify the components involved with more certainty, the data obtained were re-plotted in the form of the ratio of Soret to α absorbance changes as a function of imposed redox potential as shown in Fig. 12.

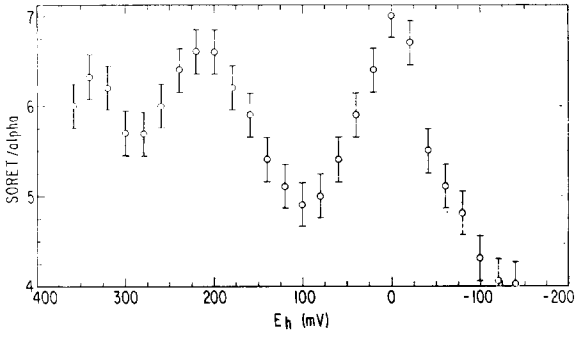

Fig. 12. Soret to α ratio *vs.* redox potential. Conditions were as in Fig. 10.

From the various peaks and valleys shown in Fig. 12, at least six components may be present. The components identified in Figs. 10–12 are summarized in Table IV, along with redox range in which each component is active.

A component (Component-1) may be present on the basis of its contribution to the Soret to α ratio (Fig. 12) in the redox range 400 to 300 mV. However, the Soret absorbance is very small in this region (0.02–0.04 A); thus, it may be an artifact.

C-555 is identified on the basis of its α absorbance (555 mμ) and the fact that the magnitude of the α change as a function of the imposed redox potential fits a theoretical curve for a one-electron change with $E_m = 319$ mV. It appears that the C-555 α change is associated with a Soret change at 422 mμ. However, the high Soret

TABLE IV

TENTATIVE IDENTIFICATION OF CYTOCHROME COMPONENTS INVOLVED IN LIGHT REACTIONS

Component	*Redox region component identified (mV)*	*α contribution (mμ)*	*Soret contribution (mμ)*
1	400–300	?	422 (?)
C-555	400–0	555	422
2 (cytochrome *cc'*)	275–125	?	422 (?)
3	200–0	555–552	422–423
4	100–(–50)	None	423 (?)
C-552	150–(–150)	552	423

to α ratio (4–6) observed consistently with C-555 would suggest that its light-oxidation is associated with a component which contributes only to the Soret region. This is in contrast to the Soret to α ratio (3.6) for C-555 observed in the presence of Reductase-1.

Component-2 is identified on the basis of its contribution to the Soret to α ratio in the redox region 275 to 125 mV (Fig. 12). The contribution of Component-2 may extend over a much wider redox range which is masked by overlapping components.

Component-3 is identified by its contribution to both the α and Soret regions, as shown in Figs. 10 and 11. The number of components which are light-oxidized while Component-3 is undergoing reaction prevents an accurate determination of the wavelength maxima of the contribution of this component.

Component-4, which is light affected in the redox region 100 to —50 mV, is identified by its large contribution to the Soret to α ratio in this region (Fig. 12).

C-552 is identified on the basis of the similarity of the light-oxidized form to soluble C-552 in wavelength maxima and the redox region in which this protein is involved in the light-induced absorbance changes.

Steady-state measurements do not permit further resolution of the components involved, unless the wavelength maxima or some other primary properties are widely different. Within the limits of detection, C-555, C-552 and Components 2, 3 and 4 are all involved in the light-induced absorbance changes in the transition redox region from 125 to 0 mV.

Endogenous reductant

The presence of a potent endogenous reductant in the light particles was a continuous source of difficulty in studying the effect of redox potential on the light reaction. The endogenous reductant was capable of reducing any of the redox buffers used over the range 400 to 50 mV. A similar phenomenon has been observed with *R. rubrum*[20] and *Rhodopseudomonas spheroides*[10].

The particle-bound reductant associated with 35 mμmoles of BChl could reduce between 1 and 5 μmoles of $K_3Fe(CN)_6$. Thus, the reducing equivalents present were 20–100 times that of the BChl. The action of the endogeneous reductant was complex, as judged by its behavior when titrated with an oxidant. It was found that upon the addition of oxidant (ferricyanide) the measured redox potential jumped to a high level (300–400 mV) and then slowly drifted back to 200–250 mV. Only when the endogenous reductant was almost completely titrated would the redox potential begin to

rise. The endogenous reductant apparently interacted very slowly with oxidants, in contrast to most metal–ligand complexes which reach a stable new potential immediately after the addition of oxidant or reductant.

DISCUSSION

Proper preparation and storage of particles are critical for the accurate determination of light-induced absorbance changes. When light particles are extensively exposed to air, a substantial portion of the light-induced absorbance changes in both the BChl and cytochrome region are irreversibly lost. This inactivation by oxygen has been observed by OLSON AND STANTON[24] when studying fluorescence emission spectra and possibly explains the difficulties encountered in the determination of pyridine nucleotide photo-reduction by *Chromatium* chromatophores[25].

CLAYTON has recently proposed that the molecular ratio of P800 and P890 is 2:1, and that the light-induced changes of P800 are not directly involved in the generation of a high energy electron, but are associated with an accessory pigment in a specialized environment close to the P890 molecule. The results presented support the notion that P800 and P890 are closely related on the basis of redox potential. Further, the different responses of the light-induced absorbance changes of P890 and P800 as a function of pH support the view that P890 and P800 are separate molecules in separate environments.

As the oxidation–reduction potential of the environment of the light particles is lowered below 250 mV, a shoulder on the long wavelength side of the Δ883 appears. At −100 mV, this shoulder is resolved into a peak at 905 mμ. VREDENBERG AND AMESZ[26] have observed an identical component by illuminating intact *Chromatium* cells with 'moderate' light intensities. This peak was interpreted as a long wavelength shift of Δ883.

CLAYTON[27] found that freshly harvested *Rhodopseudomonas spheroides* gave a light-induced peak at 880 mμ (normal BChl bleaching is at 870 mμ in this organism). CLAYTON termed the component responsible for this peak 'B^-' and considered it to represent a reduction, in contrast to the oxidation of P870. He found that the 'B^- light reaction' was enhanced by mild reductants (E_h = 100 mV) and inhibited by oxygen.

The results presented in this work could be interpreted similarly—P890 being bleached at high redox potential where reduced P905 cannot accumulate, both P890 and P905 being light affected at intermediate redox potentials between 250 and −50 mV, and P905 being reduced at low potentials where bleached P890 cannot accumulate. At moderate light intensitites, using intact cells where the endogeneous redox potential should be in the intermediate redox range, it may be that only P905 is affected, as observed by VREDENBERG AND AMESZ[26].

Further attempts to study the P905 component were hampered by inability to resolve P890 and P905 at intermediate redox potentials, and by the variations in the potential at which P905 was light-affected. It could not be determined with certainty that Δ905 was a reduction nor, for that matter, that Δ883 was an oxidation; however, it was clear that these two light-induced absorbance changes had quite different properties. The simultaneous occurrence of Δ883 and Δ905 between 250 and −50 mV argue that P905 and P890 are separate pigment molecules.

In order to interpret the various absorbance changes in the cytochrome region, it is useful to review previous results with intact cells and relate them to the results presented in this work.

As mentioned in INTRODUCTION, OLSON AND CHANCE[5] found that with photoautotrophically grown *Chromatium*, four light-oxidized cytochromes could be observed kinetically. Upon illumination, C-423.5 was seen in a fast phase and the remaining components in a second slower phase. Upon cessation of illumination, C-422 and C-430 were reduced in a fast phase, C-426 in a second slower phase and C-423.5 in a very slow third phase.

OLSON AND CHANCE found aerobic suspensions of *Chromatium* showed only C-422 and C-430 being light-oxidized, with C-426 and C-423.5 air-oxidized. Further, C-426 interacted with CO, and the light-oxidation of C-426 was inhibited by phenylmercuri-acetate.

The work of OLSON AND CHANCE[5] was confirmed by MORITA, EDWARDS AND GIBSON[6] and extended by showing that with sulfur-starved *Chromatium* only C-422 and C-430 remained reduced and therefore reactive upon illumination. On the addition of substrate ($S_2O_3^{2-}$, S^{2-} or H_2), C-423.5 and C-426 were reduced in the dark.

C-555 can be equated to C-422 and C-552 to C-423.5 on the basis of absorption spectra and redox potential. In the work of OLSON AND CHANCE[5], C-430 had a Soret peak at 430 mμ and a very broad α- peak at 560 mμ. The Soret to α ratio for C-430 was 10–11 and the light-oxidation of C-430 was closely associated with C-555. Based on these facts, C-430 can be equated with Component-2 in this work. Component-2 makes no appreciable contribution to the α region as observed in the steady state and is closely associated with C-555.

As shown by OLSON AND CHANCE[5], the Soret peak of C-430 agrees perfectly with the Soret peak of an oxidized *minus* reduced difference spectrum of cytochrome *cc'* (ref. 16). In the α region, the data of OLSON AND CHANCE[5] agree within experimental error with that of an oxidized *minus* reduced spectrum of cytochrome *cc'*. Further, the Soret to α ratio for cytochrome *cc'* (11.6)[16] is in excellent agreement with that found for C-430. Thus, we equate Component-2 with cytochrome *cc'*. The only difficulty with such an interpretation is that the redox region in which Component-2 is active is approx. 350 to 0 mV, whereas the midpoint potential of soluble cytochrome *cc'* is −5 mV (ref. 15). The lack of interaction with CO by bound cytochrome *cc'* (ref. 9) indicates that the environment of the heme has been changed relative to the soluble form, and therefore, possibly also the redox properties.

C-426, as identified by OLSON AND CHANCE[5], does not appear to function in the light particles. This conclusion follows from the lack of interaction with CO by particle-bound heme proteins. However, C-426 is apparently present in whole cells where it can interact with O_2, CO and light.

If the wavelengths of the absorption maxima and minima are compared for CO-reduced *minus* reduced difference spectra of C-426, C-552 and cytochrome *cc'*, as shown in Table V, it can be seen that C-426 can be accounted for as a mixture of mostly unbound C-552 and some unbound cytochrome *cc'*. The fact that soluble cytochrome *cc'* and C-552 do not interact with the light particles requires the postulation of a 'coupling factor' to explain this interaction in intact cells. The inhibition of the light-oxidation of C-426 by phenylmercuri-acetate suggests that the coupling factor may be an enzyme.

TABLE V

ABSORPTION MAXIMA AND MINIMA OF CO-REDUCED *minus* REDUCED DIFFERENCE SPECTRA OF C-426, C-552 AND CYTOCHROME *cc'*

Component	*Wavelength maxima* (+) *and minima* (—)			
C-426 (ref. 5)	414 (+)	428 (—)	540–545 (+)	552.5 (—)
C-552 (ref. 16)	414 (+)	424 (—)	535 (+)	552 (—)
Cytochrome *cc'* (ref. 16)	418 (+)	437 (—)	534 (+)	550 (—)

Only Components 1, 3 and 4 have not been related to known or previously identified compounds. Component-1, because of its minor contribution and tenous identification, need not be considered further. Component-3 appears to be a *c*-type cytochrome with both α and Soret contributions, although the wavelength maximum cannot be identified since Component-3 has not been seen alone. Based on the contribution of Component-3 to Fig. 10A, its midpoint potential can be estimated to be approx. 150 mV.

The light-oxidation of Component-4 seems to be closely related to the light-oxidation of C-552. Component-4 does not appear to make any appreciable contribution to the α region, again suggesting possible involvement of cytochrome *cc'*.

It seems reasonable to postulate two pathways for electron transport, one includes P890, C-555, cytochrome *cc'* and possibly Component-3; the second includes P905, C-552, Component-4 and P_{-135}. These two reaction centers overlap with P905 being observed up to 250 mV and all components being affected by light between 150 and 0 mV.

The correlation of P890 and C-555 has previously been made by both BEUGELING AND DUYSENS[28] and CLAYTON[29]. CLAYTON demonstrated that C-555 and P890 oxidation could be correlated with the reduction of CoQ_7 in chromatophores with a 1:1:1 stoichiometry. We have repeated this work with the light particles and obtained identical results. Thus CoQ_7 can be included in the P890 reaction center.

Two observations remain to be discussed:

(1) Anaerobic suspensions of light particles ($E_h \cong 200$ mV) show a light-induced oxidation of C-552 at low light intensities (approx. $2 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}). These intensities are similar to those used by VREDENBERG AND AMESZ[26] (approx. $1.7 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}) to observe Δ905. It is plausible that at these light intensities the the P890 reaction center is being affected by light but that the dark reductions proceed at such a rate that there is no net steady-state oxidation.

(2) Anaerobic suspensions of light particles when illuminated with high light intensitites ($\geqslant 2 \cdot 10^4$ ergs·cm^{-2}·sec^{-1}) show only C-555 in the α region, not C-552. The failure to observe C-552 photo-oxidation at high light intensities is not understood; it may result from an interaction between the two reaction centers in which P890 funnels away electrons from the pathway mediated by P905.

Several other properties of the light reaction should be summarized. Both NADH and succinate can interact with C-555. The action of the two dye reductases demonstrates that C-555 and C-552 have definite specificities for these enzymes, and, through the reductases, C-555 and C-552 are able to interact with electron donors in the vicinity of the electron transport chain.

Before proceeding to summarize the results in terms of a model, it is helpful to state two results from a following publication[30] concerned with photophosphorylation by the light particles. First, the cyclic formation of ATP in the light is correlated with the electron transport pathway mediated by P890. Second, the pathway mediated by P905 is not involved in photophosphorylation. The inclusions of C-555 in a cyclic system is consistent with the work of MORITA, EDWARDS AND GIBSON[6] and CHANCE *et al.*[31].

With data presented, a model for electron transport in *Chromatium* has been constructed and is given in Fig. 13. Obviously, this is not to be considered final but simply a working scheme. The principal of two electron transport chains can be expressed by a model in which the two chains interact at some common point. This common point could be BChl where P890 and P905 are the same molecule, only reactive under different conditions. Another possibility is that cytochrome *cc'* is the common point of entry and that Component-2 and Component-4 are equivalent. The model shown in Fig. 13 emphasizes that there are two functionally, if not chemically, different pathways present. Component-3 has been placed on a side path because of the narrow redox region in which it acts and because its inclusion in the cyclic system would not permit any one electron transfer step to have sufficient potential span to generate a high energy phosphate bond.

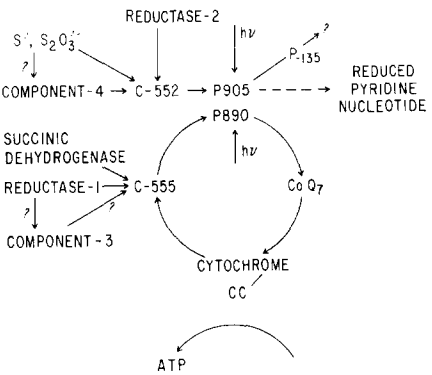

Fig. 13. Model for electron transport in *Chromatium* light particles (see text).

It is suggested that the two reaction centers serve different functions, the P890 center for cyclic photophosphorylation and the P905 center for the production of reduced pyridine nucleotide. In contrast to the green plants where two photo-systems are activated, each by a different wavelength of light, the reaction centers in *Chromatium* are activated by different endogenous redox potentials. It might be expected that when *Chromatium* is grown photoautotrophically in the presence of sulfide, or thiosulfate, and CO_2, that the endogenous redox potential at which the cell functions will be quite low (below 0 mV) and under these conditions much NADH is required for the reduction of CO_2 to carbohydrate. Thus, the P905 reaction center would be dominant. When grown photo-heterotrophically with succinate, the endogenous redox potential should be much higher than under autotrophic conditions, and because a source of fixed carbon is already present, much less NADH would be required and the cyclic system for the production of ATP would dominate.

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