ABSORPTION CHANGES IN BACTERIAL CHROMATOPHORES

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ABSTRACT The magnitude and kinetics of photo-induced absorption changes in bacterial chromatophores (*R. rubrum, R. spheroides* and *Chromatium*) have been studied as a function of potential, established by added redox couples. No photochanges can be observed above +0.55 v or below −0.15 v. The loss of signal at the higher potential is centered at +0.439 v and follows a one-electron change. The loss of signal at the lower potential is centered at −0.044 v and is also consistent with a one-electron change. Both losses are reversible. A quantitative relationship exists between light-minus-dark and oxidized-minus-reduced spectra in the near infrared from +0.30 to +0.55 v. Selective treatment of the chromatophores with strong oxidants irreversibly bleaches the bulk pigments but appears to leave intact those pigments responsible for the photo- and chemically-induced absorption changes. Kinetic studies of the photochanges in deaerated samples of *R. rubrum* chromatophores revealed the same rise time for bands at 433, 792, and 865 mμ ($t_{\frac{1}{2}}$ = 50 msec.). However, these bands had different decay rates ($t_{\frac{1}{2}}$ = 1.5, 0.5, 0.15 sec., respectively), indicating that they belong to different pigments. Analysis of the data indicates, as the simplest interpretation, a first-order (or pseudo first-order) forward reaction and two parallel first-order (or pseudo first-order) decay reactions at each wavelength. These results imply that all pigments whose kinetics are given are photooxidized and the decay processes are dark reductions. These experiments are viewed as supporting and extending the concept of a bacterial photosynthetic unit, with energy migration within it to specific sites of electron transfer.

Photo-induced absorption changes in biological material were first clearly observed by Duysens (1), who suggested their relationship to photosynthesis. Subsequently, more detailed studies have indicated that the photoinduced absorbance changes in bacterial systems show high quantum efficiencies (2, 3), low temperature reversibility (4), and quenching by strong oxidants (5). These results, together with those concerned with primary events in green plant material (6-8), have substantiated the earlier concepts of photosynthesis as involving the early conversion of light energy into separated oxidants and reductants (9). Since oxidized and

reduced moieties are formed very early in the energy conversion process, a careful systematic study of the redox dependence of light-induced changes might result in the identification of the participating pigments and indicate how they interact with one another.

If some or all of the redox couples composing the photosynthetic apparatus equilibrate with the potential established in the external medium by a suitable redox buffer, the effects of light might be expected to be seen as transient departures from the redox equilibrium. For those systems in which the equilibrium oxidation-reduction ratio is essentially completely in favor of that oxidation state toward which light drives it, the light could not produce a noticeable change. An additional source of information will be the rate of departure from equilibrium and the rate of return.

The present discussion gives the details of first results, published in part earlier (10, 11), and of further studies concerned with the redox dependence of light-induced absorption changes from 350 to 950 mμ.

In principle the kinetics of the light-produced absorption changes should provide a considerable amount of information concerning the type and sequence of reactions in the energy conversion process. In practice, the small signals, the lack of control over the pigment concentrations within a photosynthetic system, and the complexity of the reactions have limited the amount of information readily available. New instrumentation, coupled with control of the redox environment, has permitted a study of some of the kinetic interactions.

EXPERIMENTAL

Materials. Chemicals, special reagents, and the preparation of chromatophores from *Rhodospirillum rubrum, Rhodopseudomonas spheroides,* and *Chromatium* used in these studies have been described previously (12).

Methods. All redox experiments reported in detail have been conducted in the presence of a 10- to 1000-fold molar excess of the external redox couple over the estimated concentration of the photoactive pigments. Values of potential are measured at 22 ± 2°C and pH 7.4. With these large excesses of redox "buffers" the electrodes responded immediately to small changes in potential (0.5 mv), and adjustments to desired values were made quite easily.

For interpretation of the data obtained, it has been assumed that the relatively high concentrations of redox buffers do not complex with the pigments being investigated to such an extent that the pigment properties are changed. Where possible a number of redox couples have been used to cover the same range of potential, and experiments were carried out to determine the effect of reagent concentration and the effects of light on the couples.

For measurements in the absence of air, apparatus and techniques patterned after those designed by Harbury (13) were used with the following modifications. A rectangular, four-sides-clear quartz cuvette was cemented (de Khotinsky's cement) to pyrex tubing, as illustrated in Fig. 1. From standard taper 2 a bridge was connected to a second cuvette which resided in front of the reference beam of a Cary spectrophotometer. The two

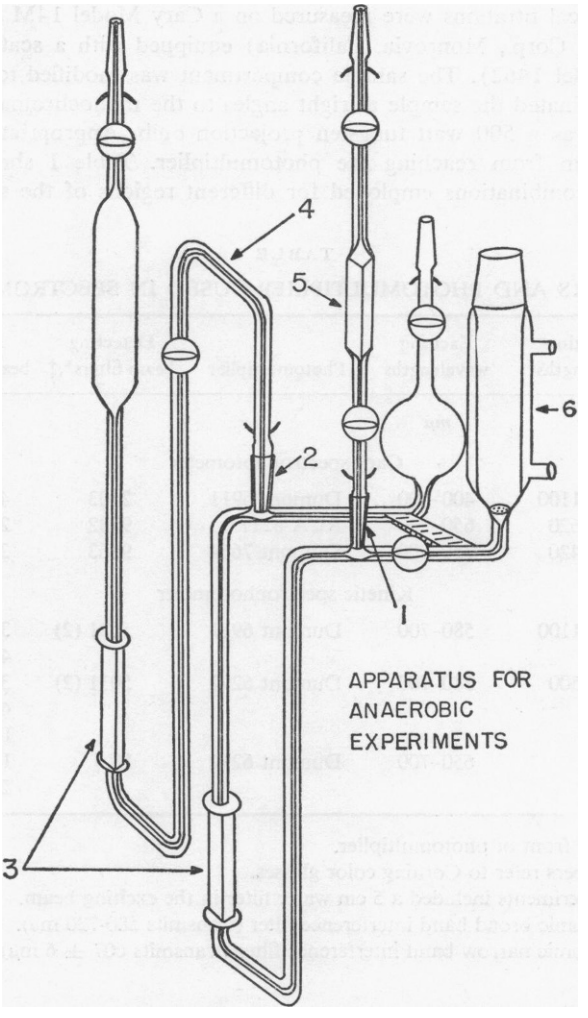

FIGURE 1 Apparatus for anaerobic redox experiments. (1) 10/30 standard taper for sample cell (5) or microburette; (2) 10/30 standard taper for connection to reference cell or for microburette; (3) four-sides-clear rectangular quartz cuvettes; (4) bridge to reference cell; (6) main titration vessel with water jacket.

cuvettes were separated, when placed in the spectrophotometer compartment, by means of a black metal septum. With this apparatus both the reference and the sample material could be adjusted under identical conditions and light-minus-dark spectra observed. Chemical difference spectra could also be obtained with a minimum of manipulation of the sample. Also shown in Fig. 1 is a cell in standard taper 1 which could be deaerated and filled with a sample of the material being investigated. The contents of this cell could then be examined by means of flashing-light spectrophotometry to be described below.

Instrumental. The light-dark difference spectra and the spectral changes at-

tendant to chemical titrations were measured on a Cary Model 14M spectrophotometer (Applied Physics Corp., Monrovia, California) equipped with a scattered transmission attachment (Model 1462). The sample compartment was modified to admit a beam of light which illuminated the sample at right angles to the monochromator beam. The excitation source was a 500 watt tungsten projection bulb. Appropriate filters prevented the exciting beam from reaching the photomultiplier. Table I shows the filter and photomultiplier combinations employed for different regions of the spectrum.

TABLE I

FILTERS AND PHOTOMULTIPLIERS USED IN SPECTROMETERS

Detecting wavelengths	Exciting wavelengths	Photomultiplier	Detecting beam filters*,‡	Exciting beam filters‡,§
mμ	*mμ*			
		Cary spectrophotometer		
650–1100	400–500	Dumont 6911	2403	4600, 9782
380–620	650–900	RCA 6217	9782	2403
260–420	750–900	Dumont 7664	9863	2600
		Kinetic spectrophotometer		
750–1100	580–700	Dumont 6911	5031 (2)	3480 4600 (2), IF‖
350–500	580–700	Dumont 6292	5031 (2)	3480 4600 (2) IF‖
607	650–700	Dumont 6292	IF¶	IF‖ 2030

*Placed in front of photomultiplier.
‡All numbers refer to Corning color glasses.
§Cary experiments included a 5 cm water filter in the exciting beam.
‖Baird-atomic broad band interference filter (transmits 580-720 mμ).
¶Baird-atomic narrow band interference filter (transmits 607 ± 6 mμ).

An expanded scale slide-wire (0 to 0.2 OD unit full deflection) was as routine used for measuring difference spectra. Instrument controls ("slit control" and "dynode setting") were adjusted to minimize the noise level, consistent with the resolution requirements of the experiments. Slit widths of up to 1 mm were found acceptable.

The peak-to-peak noise under normal operating conditions was 0.0005 OD unit when using the blue-sensitive photomultipliers. An increase in the noise (0.002 to 0.003 OD unit) and a base line shift (0.001 to 0.010 OD unit) occurred as the exciting light was turned on for measurements above 650 mμ. These effects were not observed at low light intensities and probably result from infrared fluorescence or scattering of the actinic light. Since the base line shift is independent of wavelength, it was easily corrected for by selecting a wavelength at which no absorption change occurs. The precision of these experiments was consistent with the noise levels described.

We performed the kinetic measurements on a rapid-response spectrophotometer constructed specially for this purpose. The instrument employed the technique of continuous

averaging to provide both fast response and high sensitivity. This method has been discussed in the recent literature (14).

Fig. 2 shows a block diagram of the kinetic spectrophotometer. It operates as follows: The light from the monochromator passes through the sample and falls on a photomultiplier. A modulated actinic beam (a neon light) illuminates the sample. Reversible changes in the absorption of light by the sample thus appear as a modulation on the transmitted intensity of the detecting beam. The photomultiplier output, then, consists of a DC signal

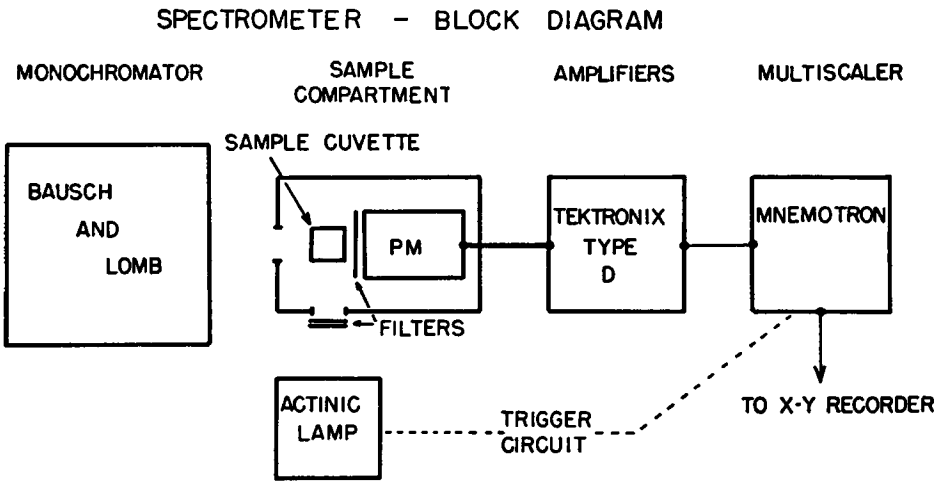

FIGURE 2 Block diagram for rapid-response spectrometer.

(the transmitted intensity) and a small AC signal (repetitive absorption change). The alternating signal is amplified while the DC signal is removed by AC coupling or DC suppression. The amplified signal now consists of a pulse train whose voltages are proportional to the periodic fluctuations in the transmitted intensity from the sample. For small changes in transmitted intensity the fluctuations are also proportional to the changes in the optical density ($\triangle$OD).

The pulses are fed into a multiscaler unit with a built-in voltage-to-frequency converter. The time base of the multiscaler is held in a fixed (but adjustable) phase relation to the incoming signal. After sufficient repetition to improve the signal-to-noise ratio the process is stopped and the stored signal is read out on an X-Y recorder.

Further details of the components and of the operation of the spectrometer are given below:

1. *Monochromator:* A Bausch & Lomb grating monochromator (model 33-86-45) using a 500 watt tungsten projection bulb was operated with a 3 to 10 mμ bandpass. The detecting beam is adjusted to a low enough intensity to prevent appreciable excitation of the sample.
2. *Actinic lamp:* The neon lamp used to induce the photoabsorption changes was kindly made available for our use by Dr. L. Piette of Varian Associates, Palo Alto. Most of the details of its circuitry are in the literature (15). Its ease of modulation, rapid rise and decay times (~5 μsec.), and convenient spectral lines make it a very useful source. One disadvantage is the relatively low intensity (*ca.* 10^{16} quanta/sec.-cm^2).

3. *Photomultipliers and filters:* The filters and photomultipliers used for the kinetic measurements are given in Table I. The filters were chosen to supply the same incident excitation wavelengths and intensity for the detection wavelengths of greatest interest. All the photomultipliers used are of the 2 inch end-window variety. They have conventional base circuitry with approximately microsecond response time.
4. *Amplifiers:* Tektronix type D high-gain preamplifiers were employed. They were powered by a Tektronix type 127 power supply and required a line regulator for best performance. Typical gains were 100 to 300, providing the 6 v (maximum) input to the multiscaler. The preamplifiers were ordinarily DC-coupled to avoid distortion of the rather slow waveforms. A simple battery circuit was used to bias out any DC voltages at the input to the preamplifiers.
5. *Multiscaler unit:* A commercial unit, the Computer of Average Transients (Mnemotron Corp., Pearl River, New York), model 400, satisfactorily met our requirements of millisecond response time and 1 per cent accuracy. Internal noise, although not negligible, was usually well below the noise at the input. At times, coherent internal noise proved troublesome. We employed an external triggering unit to remove this difficulty. The final trigger circuit used is shown in block form in Fig. 3.

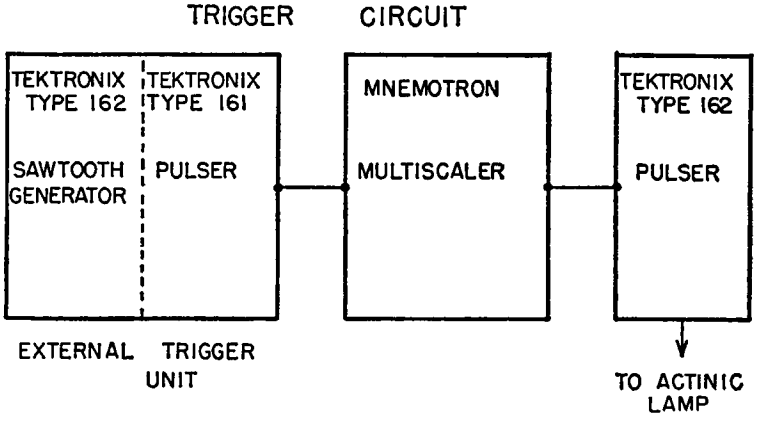

FIGURE 3 Trigger circuits used in rapid-response spectrometer. The external trigger unit produces a −1.5 v, 0.1 msec. square pulse to start the multiscaler sweep. The multiscaler then triggers a second Tektronix type 162 unit which pulses on the actinic lamp. The lamp remains on for the duration of the pulse (100 μsec. to 10 sec.).

Performance. This instrument has an over-all rise time faster than 0.1 msec. The major noise source (the shot noise in the detecting beam) produces about 10^{-4} OD units of noise for typical operating conditions, that is, 2 to 10 minute experiments. Repetition rates were determined by the decay time of the biological material. Rates of 4 cps to 0.03 cps have been used. An interesting feature of the system is that a moderate amount of fluorescence can be conveniently compensated for by turning off the detecting beam (thus removing all of the absorption changes but not the fluorescence) and running the multiscaler for an equivalent length of time in the subtract mode.

The over-all performance of the spectrometer is limited by the reproducibility of the biological responses. Magnitudes of the absorption changes were reproducible to a few per cent. The rates and rate constants given are probably accurate to 20 to 30 per cent.

RESULTS

Magnitude of Reversible Photoabsorption Changes as a Function of Redox Potential.

1. *High Potential.* Samples of the deaerated chromatophores were treated with mixtures of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ to cover the potential range of +0.30 to +0.55 v.[1] Representative absorption spectra and light-dark difference spectra for control samples (E_h = +0.35 v)[2] are shown in Figs. 4 to 6. These results are in relatively good agreement with those of other workers (1, 3, 17).

The oxidized-minus-reduced and the light-minus-dark signals at 792, 810, and 865 mμ were measured at each potential. As shown in Fig. 7 and Table II, raising

TABLE II

QUANTITATIVE COMPARISON OF LIGHT-DARK AND CHEMICALLY-INDUCED SPECTRAL CHANGES*

	$\Delta OD_{792} + \Delta OD_{810}$			ΔOD_{865}		
E_h	Photooxidizable	Chemically oxidized	Total active pigment	Photooxidizable	Chemically oxidized	Total active pigment
0.37_7	0.051	0.005‡	0.056	0.041	0.004‡	0.045
0.39_6	0.043	0.014	0.057	0.032	0.009	0.041
0.41_1	0.036	0.019	0.055	0.032	0.016	0.048
0.43_2	0.032	0.026	0.058	0.026	0.022	0.048
0.46_9	0.016	0.039	0.055	0.015	0.036	0.051
0.49_1	0.008	0.045	0.053	0.008	0.036	0.044

*Data not corrected for irreversible selective or general bleaching.
‡Arbitrary zero assigned on basis of 90 per cent light changes at 0.38 v.

the potential through the region of +0.4 v removes the light-dark signals, replacing them with the same bands in the oxidized-reduced spectra. The samples were then reduced in a stepwise fashion with sodium dithionite to demonstrate the reversibility of the effects. The midpoint of both the disappearance of the photochanges and the chemical titrations for each wavelength studied was +0.439 v. Fig. 7 also shows the theoretical curves for one- and two-electron transitions with E_m = +0.439 v. The data are consistent with a one-electron transition occurring in a key pigment(s) with a midpoint of +0.439 v. Within the limits of the experiment there is quantitative agreement between the amount of material available for

[1] In the absence of externally added redox couples, the oxidation-reduction potential of each sample could be measured and the system seemed to be somewhat buffered at the measured value, but the couple(s) equilibrating with the electrode is unknown.
[2] The symbols E_h and E_m are used as suggested by Clark (16).

reversible photochemistry and that available for reversible oxidation (Table II, columns 4 and 7).

In the above experiment potassium iridic chloride could replace potassium ferricyanide as oxidant, and potassium ferrocyanide could replace sodium dithionite as reducing agent. Results to be described below show that changes in the kinetics are not responsible for the loss of signal.

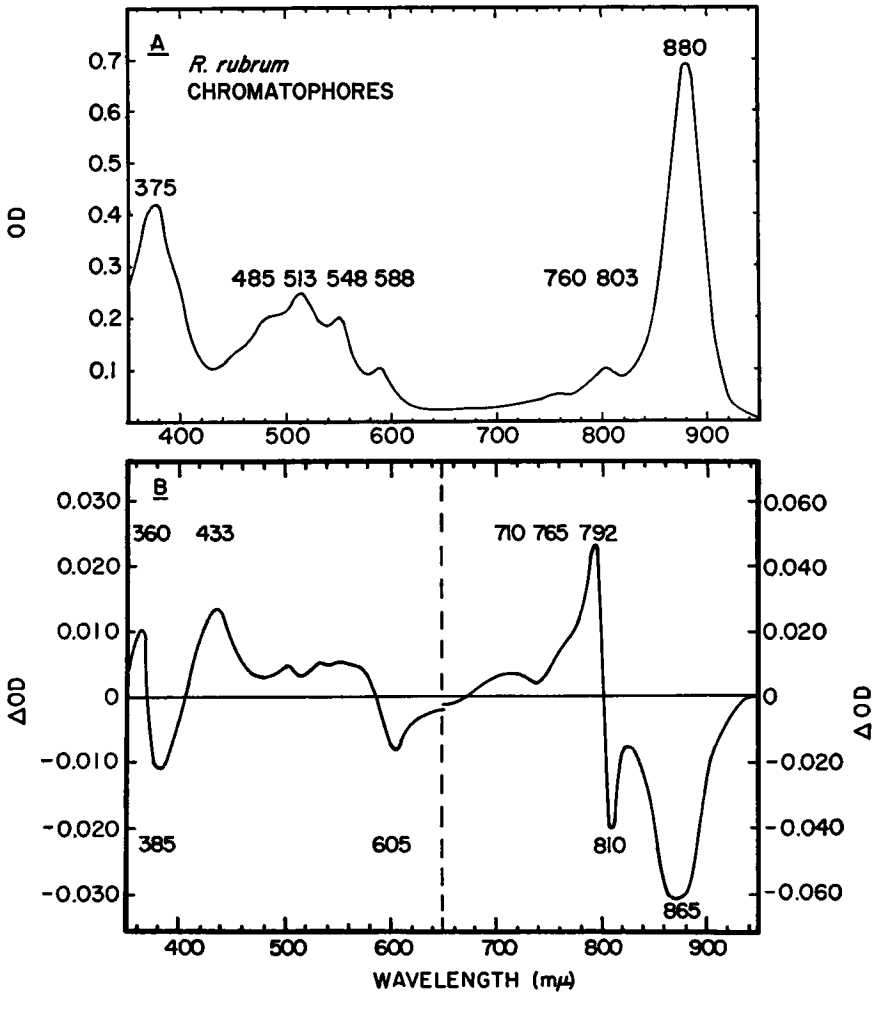

FIGURE 4*A* Absorption spectrum of *R. rubrum* chromatophores suspended in 0.01 M phosphate buffer, pH 7.13; 1 cm cuvette.

FIGURE 4*B* Light-induced absorption changes in *R. rubrum* chromatophores whose absorbance was 2.2 at 880. Note that different excitation wavelengths were used above and below 650 mμ (Table I). The absorption scale below 650 mμ is expanded twofold. Air removed; 1 cm cuvettes.

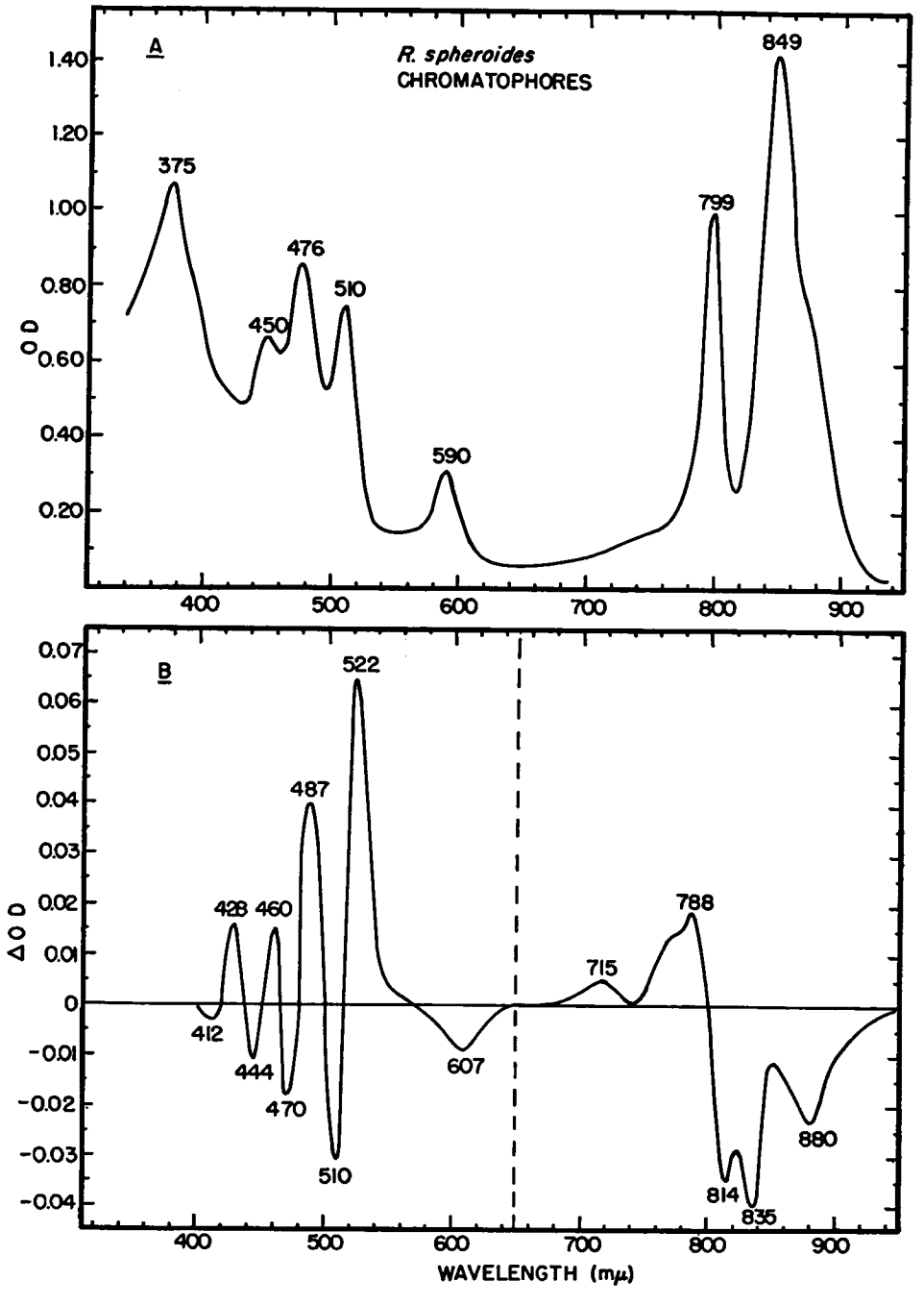

FIGURE 5A Absorption spectrum of *R. spheroides* chromatophores suspended in 0.05 M phosphate buffer, pH 7.4; 1 cm cuvette.

FIGURE 5B Light-induced absorption changes in *R. spheroides* chromatophores whose absorption is given above. Air removed; 1 cm cuvettes.

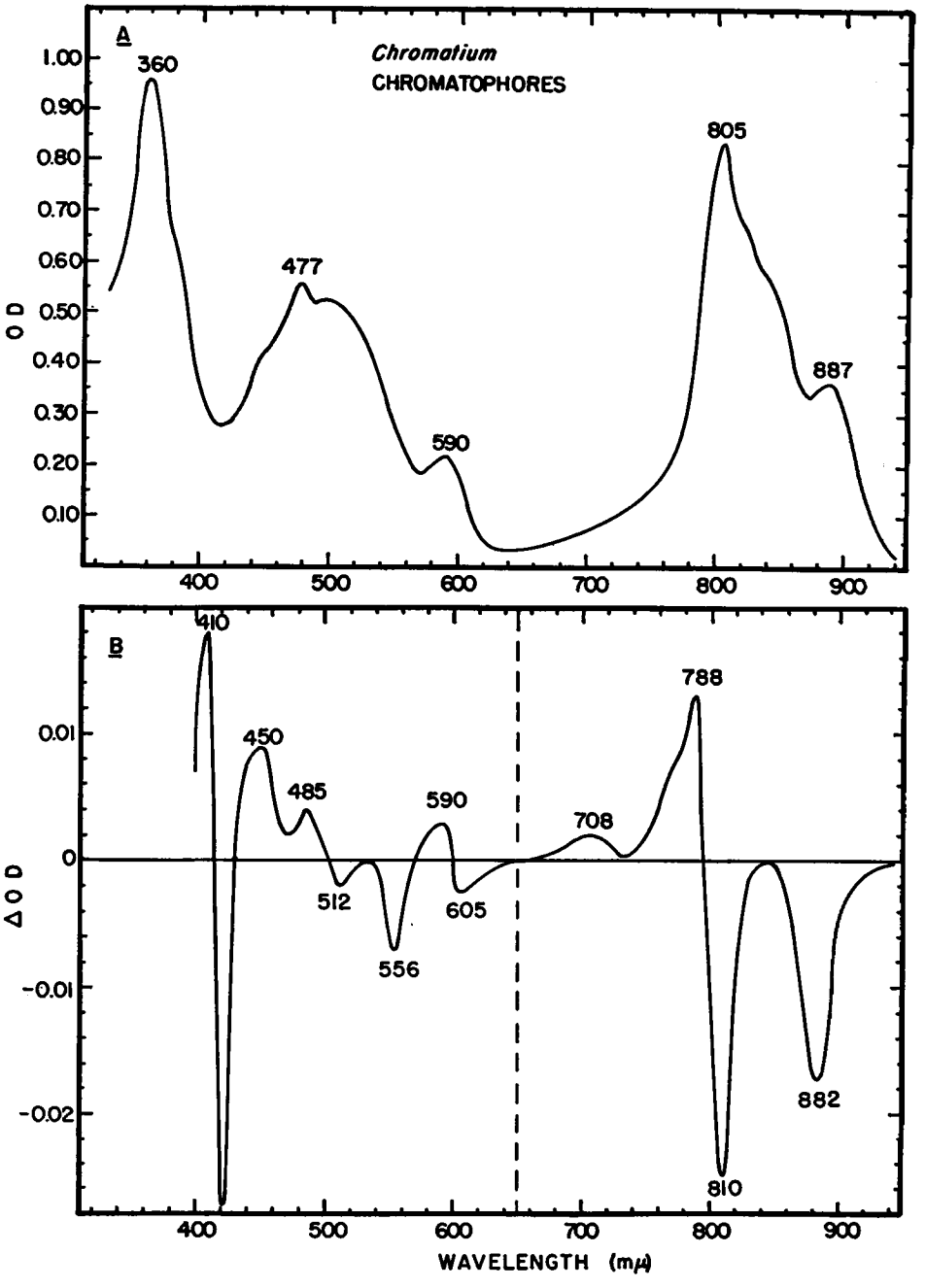

FIGURE 6A Absorption spectrum of *Chromatium* chromatophores suspended in 0.05 M phosphate buffer, pH 7.4; 1 cm cuvette.

FIGURE 6B Light-induced absorption changes in *Chromatium* chromatophores whose absorption is given above. Air removed; 1 cm cuvettes.

2. Intermediate Potential. Experiments conducted through the range of potential from +0.05 to +0.35 v showed no variation in the spectra of absorbance changes.

3. Low Potential. The reversible light-induced absorbance changes in *R. rubrum* chromatophores are not observed if the redox level of the solution is below −0.15 v. Fig. 8 shows the effect of lowering the potential through the region of +0.02 v to −0.1 v. The oxidized forms of indigo tetra- and trisulfonic acid were present at 2×10^{-5} M as "buffers" in the potential ranges +0.02 to −0.06 and −0.06 to −0.10 v, respectively. The potential was lowered slowly by the addition of reducing agent, reduced indigodisulfonic acid, prepared separately by 95 per cent re-

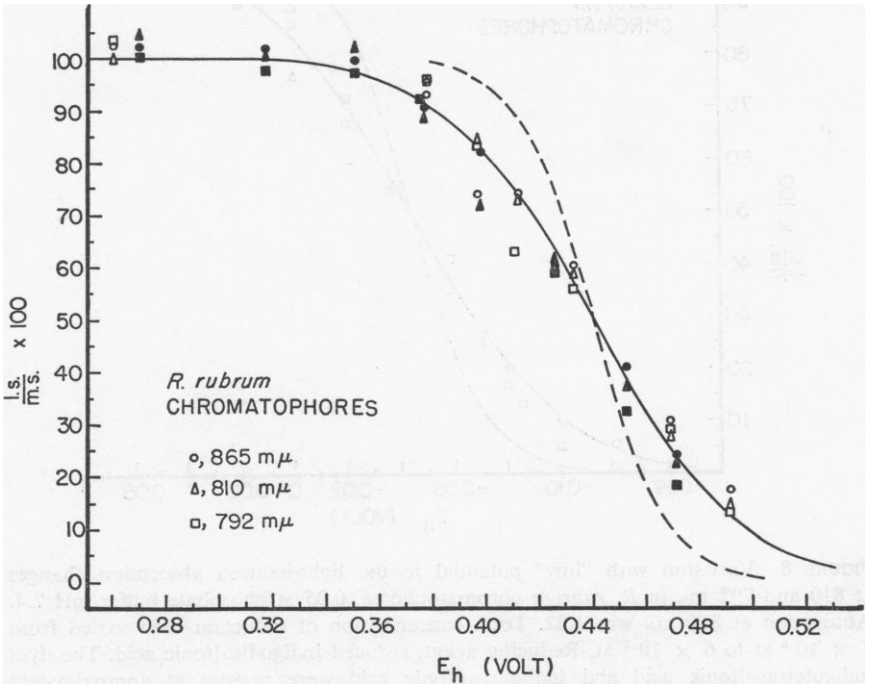

FIGURE 7 Variation with "high" potential of the light-induced absorption changes at 865, 810, and 792 mμ in chromatophores from *R. rubrum*. 0.05 M phosphate buffer, pH 7.4. Absorption at 880 mμ was 2.0. Total concentration of ferricyanide and ferrocyanide varied from 5×10^{-6} M to 2×10^{-4} M. Reducing agent, 0.01 M $Na_2S_2O_4$. After removal of oxygen, enough $K_4Fe(CN)_6$ was added to make it 5×10^{-6} M. ○, △, □, experimental points taken at 865, 810, and 792 mμ after increasing $K_3Fe(CN)_6$ concentration to increase potential. ●, ▲, ■, experimental points taken at 865, 810, and 792 mμ upon addition of increasing amounts of $Na_2S_2O_4$ to lower the potential, beginning after all $K_3Fe(CN)_6$ had been added. The solid and dashed lines were obtained by use of the equation $E_h = E_m + RT/n\mathcal{F} \ln(m.s. - l.s.)/l.s.$ with $n = 1$ and 2, respectively, and $E_m = +0.439$ v, $m.s.$ = maximum light-induced signal, observed between +0.35 to +0.30 v, $l.s.$ = light-induced signal observed at potential reported.

duction with sodium dithionite. The absorbance changes at 792 and 865 mμ disappeared in the same relative proportions as the potential was lowered. Unlike the ferricyanide treatment, this reduction does not replace the light-induced signals with chemically-produced counterparts. The only pronounced change in the visible or near infrared spectrum is the reduction of an iron prophyrin complex (probably the RHP (*Rhodospirillum* heme protein) of Kamen and Bartsch, 18). The data of Fig. 8 are compared with theoretical curves representing one- and two-electron transitions. The data are consistent with a one-electron change with a midpoint of

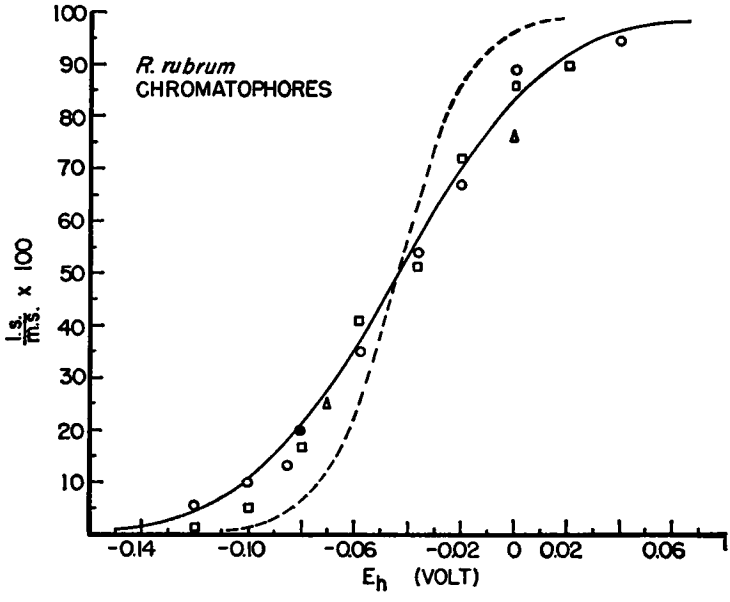

FIGURE 8 Variation with "low" potential in the light-induced absorption changes at 810 and 792 mμ in *R. rubrum* chromatophores. 0.05 M phosphate buffer, pH 7.4. Absorption at 880 mμ was 1.22. Total concentration of reductant used varied from 5×10^{-6} M to 6×10^{-5} M. Reducing agent, reduced indigodisulfonic acid. The dyes indigotetrasulfonic acid and indigotrisulfonic acid were present at approximately 2×10^{-5} M concentrations to act as redox buffers. ○, experimental points obtained from the sum of △ OD at 792 and 810 mμ as recorded by the Cary spectrophotometer using very high light intensity; points were recorded sequentially as the potential was lowered. ●, experimental point obtained as circles except $K_3Fe(CN)_6$ was added to −0.12 v sample. △, experimental points taken from a separate experiment in which indigotetrasulfonic acid (1×10^{-5} M) was the only dye present. □, experimental points obtained from steady-state values at 792 mμ using the kinetic spectrometer; these data have been corrected for changes in decay rate over this potential range (Fig. 16) as noted in the text. The solid and dashed lines were obtained by use of the equation $E_h = E_m + RT/n\mathcal{F} \ln l.s./(m.s. - l.s.)$ with $E_m = -0.044$ v and $n = 1$ and 2, respectively. As in Fig. 7, *m.s.* = maximum signal observed between + 0.35 and +0.30 v and *l.s.* = the light-induced signal observed at the potential reported. Anaerobic conditions.

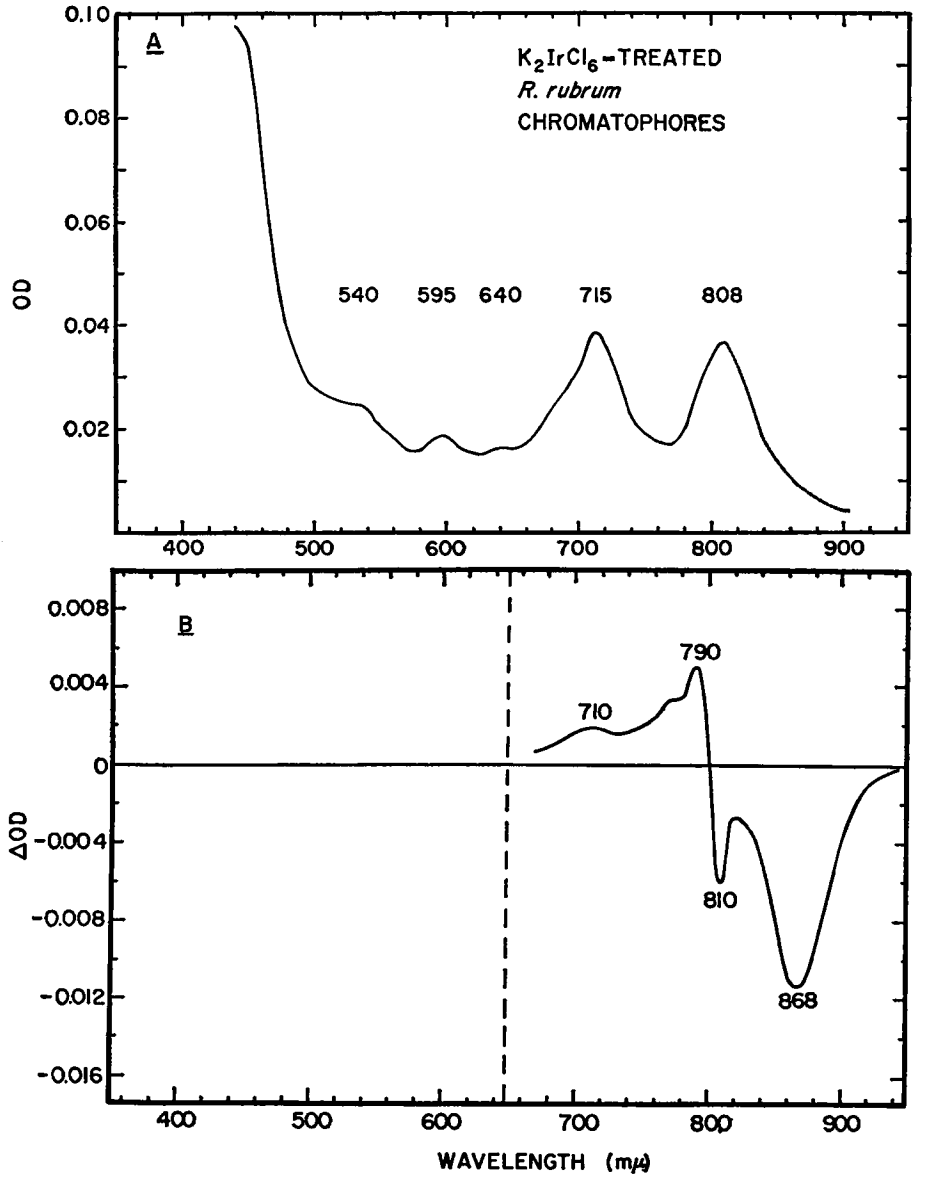

FIGURE 9*A* Absorption spectrum of K_2IrCl_6-treated *R. rubrum* chromatophores suspended in 0.05 M phosphate buffer, pH 7.4; 1 cm cuvette.
FIGURE 9*B* Light-induced absorption changes in the sample whose absorbance is given above; 1 cm cuvettes.

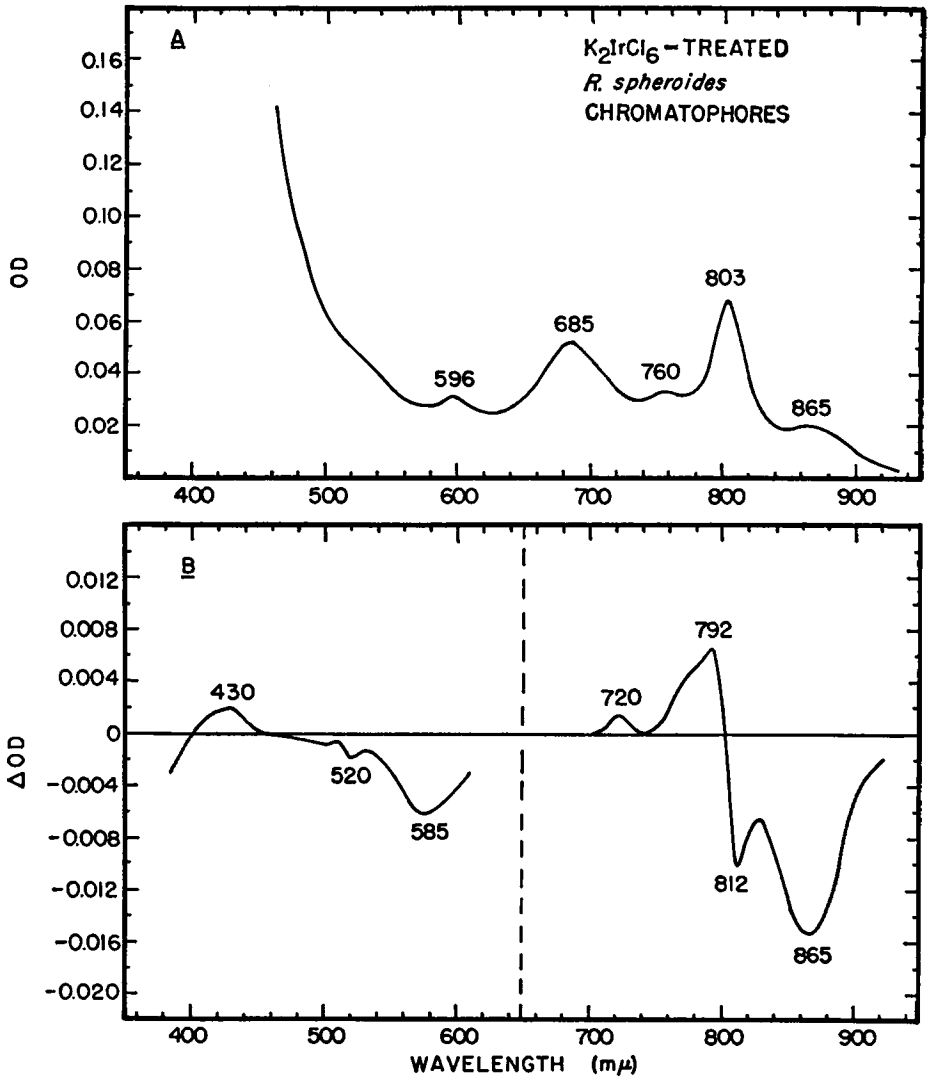

FIGURE 10*A* Absorption spectrum of K_2IrCl_6-treated *R. spheroides* chromatophores suspended in 0.05 M phosphate buffer, pH 7.4. Excess $K_4Fe(CN)_6$ present (approximately 0.05 M); 1 cm cuvette.

FIGURE 10*B* Light-induced absorption changes in the sample whose absorbance is given above; 1 cm cuvettes.

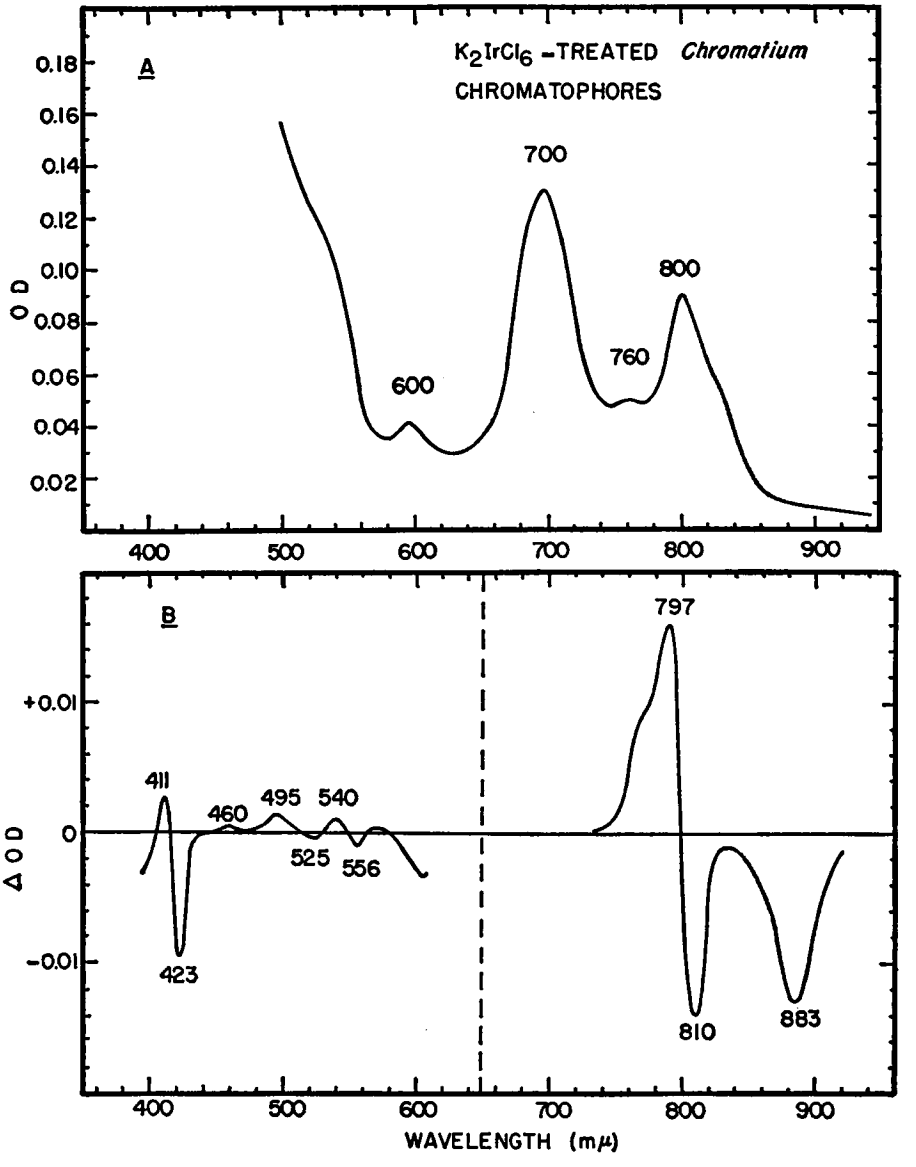

FIGURE 11*A* Absorption spectrum of K_2IrCl_6-treated *Chromatium* chromatophores suspended in 0.05 M phosphate buffer, pH 7.4. Excess $K_4Fe(CN)_6$ present (approximately 0.05 M); 1 cm cuvette.

FIGURE 11*B* Light-induced absorption changes in the sample whose absorbance is given above; 1 cm cuvettes.

−0.044 v. Again, results to be described below show that changes in the kinetics are not alone responsible for the loss of signal.

The effects of 10^{-5} M concentrations of other strong reducing agents (sodium dithionite, semiquinone of methylviologen, and reduced phenosafranine) were each examined on separate samples of well deaerated *R. rubrum* chromatophores. In each case, no light-induced signals could be observed in the presence of these reducing agents.

Separation of Photoactive Pigments from Bulk Pigments by Use of Strong Oxidants

Irreversible oxidation of large amounts of the long wavelength absorbing pigments in the presence of an excess of strong oxidant (12) leaves a material which still has absorption changes of the same magnitude as the original samples. Figs. 9 to 11 compare the light-dark changes and absorption spectra of K_2IrCl_6-treated chromatophores prepared from *R. rubrum, R. spheroides,* and *Chromatium.* The similarity of the absorbance of these materials in the near infrared is as striking

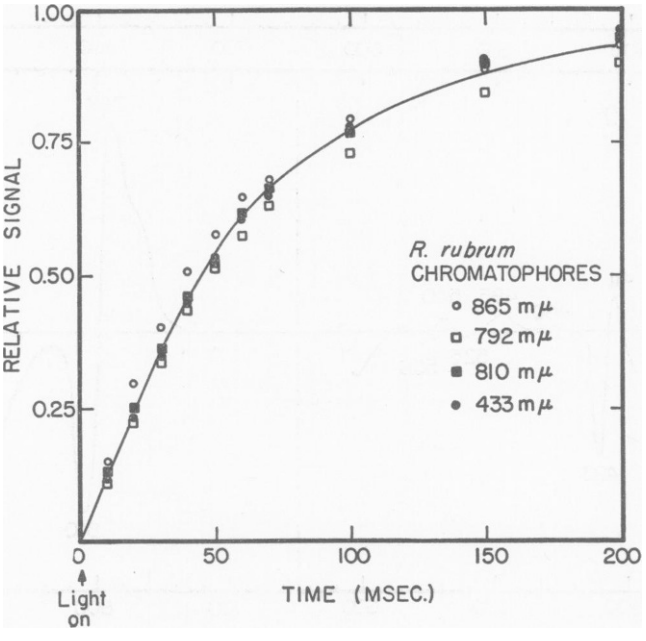

FIGURE 12 Variation with wavelength in the rise kinetics of the light-induced absorption changes in *R. rubrum* chromatophores at 433, 792, 810, and 865 mμ. For purposes of comparison signal heights are normalized and all signals are shown as positive. The actinic lamp was on for 0.4 sec. and off for 3.6 sec. Anaerobic conditions; 10^{-4} M $K_4Fe(CN)_6$ present to keep $E_h = +0.35$ v; 1 cm cuvette; 0.01 M phosphate buffer, pH 7.4; OD at 880 mμ, 0.70.

as the dissimilarity among the absorbance of the original chromatophores. Note also the loss of the light-dark carotenoid change in *R. spheroides* (compare changes at 444, 460, 470, 487, 510, and 522 mμ in Figs. 5*B* and 10*B*), and the participation of what is probably an iron prophyrin complex in the light-induced changes of *Chromatium* (compare similarities at wavelengths 410, 423, and 556 mμ in Figs. 6*B* and 11*B*). Higher incident light intensity is required to produce the same size absorption change in these K_2IrCl_6-treated systems as in the unmodified material; this seems to reflect the loss in absorption rather than a major change in quantum efficiency.

If the K_2IrCl_6-treated material from *R. rubrum* is chemically titrated in the dark with ferricyanide, absorbance changes identical to those obtained by the action of light can be observed. Thus, it appears that the bulk pigments are not essential components in either the light-produced absorption changes or the chemically-induced changes.

Kinetics

Rise and decay times were studied as a function of wavelength, light intensity, and potential.

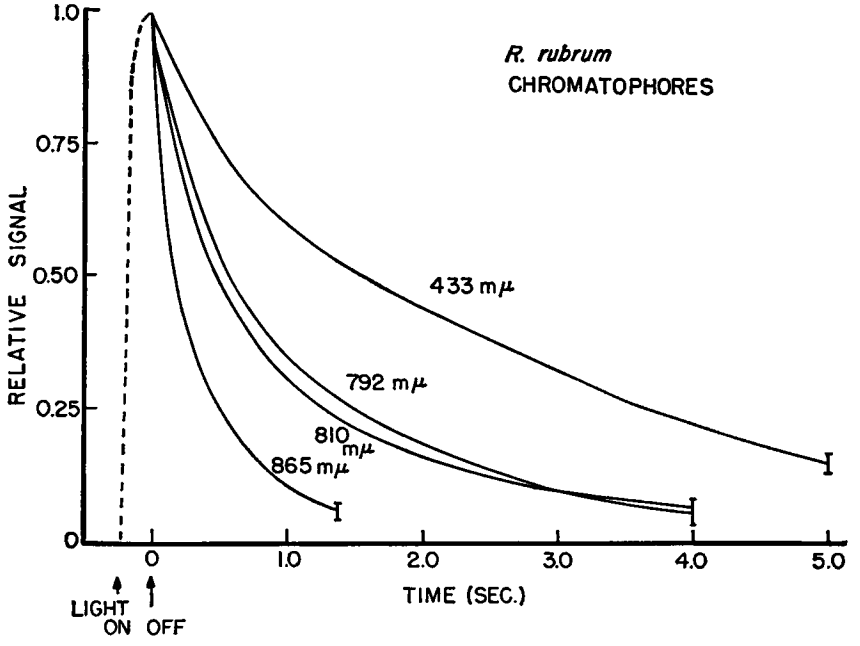

FIGURE 13 Variation with wavelength in the decay kinetics of the light-induced absorption changes at 433, 792, 810, and 865 mμ in *R. rubrum* chromatophores. Conditions as in Fig. 12. The bars represent the total estimated uncertainties including base line drift and sample variability. The actinic lamp was on 0.5 sec. and off 7.5 sec.

Variation of Kinetics with Wavelength. Typical rise and decay curves at 433, 792, 810, and 865 mμ for deaerated samples ($E_h \cong +0.35$ v) are shown in Figs. 12 and 13. The rise times are inversely related to the absorbed intensity from the actinic source. Hence, different sources, high optical densities, or the presence of colored reagents can change the rise constants. The decay constants are not particularly sensitive to these effects. Although the time for half-rise is nearly the same for each band measured, significant differences occur in the decay kinetics. The rise and decay kinetics are complex. The simplest interpretation of the data is a first-order (or pseudo first-order) forward reaction and two parallel first-order (or pseudo first-order) decay (or back) reactions (Figs. 14 and 15). Rate constants for the first-order reactions are compared in Table III.

These different decay kinetics at different wavelengths clearly indicate that the absorption changes studied belong to at least three different pigments (Fig. 13).

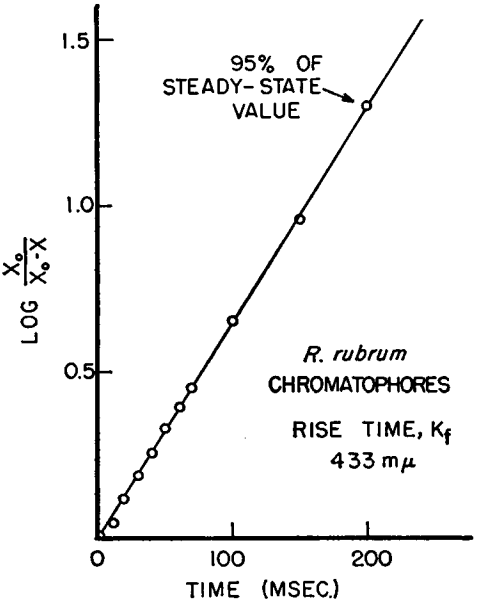

FIGURE 14 Analysis of rise kinetics for 433 mμ as shown in Fig. 12. X_0 = maximum observed steady-state absorption change, X = absorption change at any given time. The solid line is the theoretical plot for a first-order reaction with $K_f = 16$ sec^{-1}. Other wavelengths show similar behavior.

Variation of Kinetics with Light Intensity. A further study of the kinetic order of the decay reactions involves changing the steady-state level of photoproducts by variation of the incident light intensity. A fourfold change in the steady-state level produced less than a 20 per cent change in the time for half-decay. This

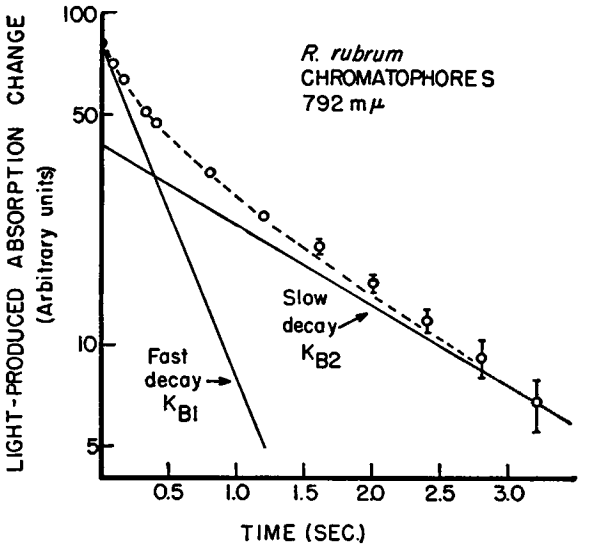

FIGURE 15 Analysis of decay kinetics of the light-induced absorption changes at 792 mμ in *R. rubrum* chromatophores shown in Fig. 13. The vertical scale is logarithmic. Two decay processes are shown by solid lines (K_{B1} and K_{B2}). The computed decay pattern (dotted line) was obtained from

$$X_{calc} = \alpha \exp(-K_{B1}t) + \beta \exp(-K_{B2}t)$$

where X = concentration of photoproduced species, K_{B1} and K_{B2} are first-order decay constants 2.0 and 0.46 sec.$^{-1}$, respectively. β is the extrapolated Y intercept for the slow decay process. α = total initial signal minus β. t is time measured from when the lamp went off.

observation strongly supports the assignment of one or more first-order or pseudo first-order decay reactions to each of the three different pigments.

Variation of Kinetics with Potential. While the rate constant for the forward reaction, K_f, is nearly independent of potential in the regions studied, the decay constant, K_{B1}, is a function of redox potential. Fig. 16 shows the variation of the first-order decay constant K_{B1} for the changes in the 865 mμ band in the

TABLE III

RATE CONSTANTS FOR PHOTOABSORPTION CHANGES*
(assuming a first-order forward reaction (K_f) and two parallel first-order back reactions (K_{B1}, K_{B2}))

Wavelength	K_f (sec.$^{-1}$)	K_{B1} (sec.$^{-1}$)	K_{B2} (sec.$^{-1}$)
433	16.3 ± 0.3	0.9 ± 0.2	0.2 ± 0.06
792	15.9 ± 0.3	1.7 ± 0.3	0.4 ± 0.1
810	16.1 ± 0.3	1.9 ± 0.3	0.4 ± 0.1
865	16.3 ± 0.3	4.8 ± 0.6	0.9 ± 0.2

*Deaerated samples, $E_h \sim +0.35$ v.

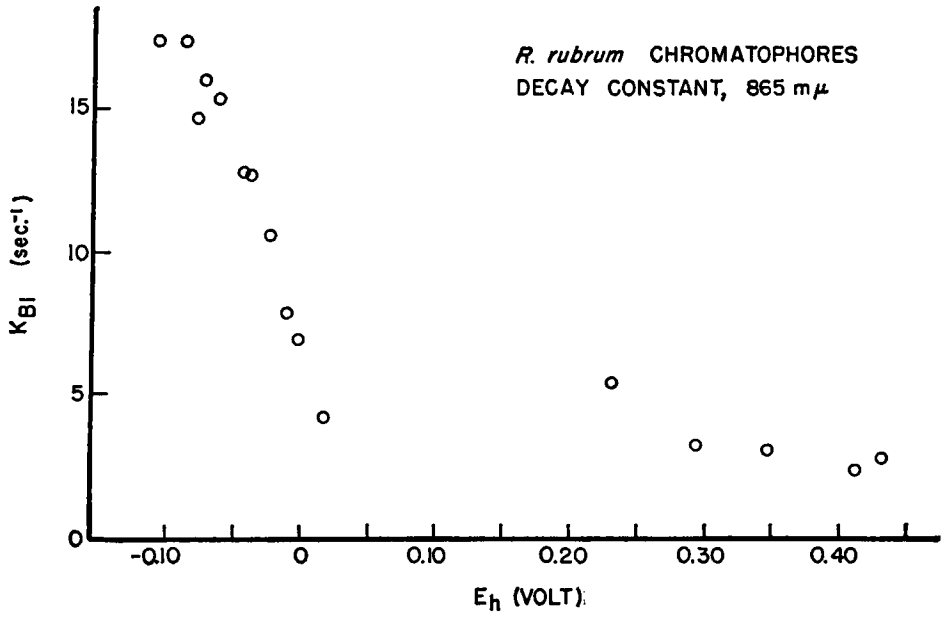

FIGURE 16 Variation with potential of the decay rate constant (K_{B1}) of light-induced absorption changes in *R. rubrum* chromatophores. Experimental conditions as given in Figs. 7 and 8.

potential range of −0.15 to +0.5 v. The rate constant for the 792 mμ band showed similar behavior. Generally, the faster of the two decay constants increases with decreasing potential. The relative amount of material decaying by the fast route also increases as the potential is lowered. The difficulty in measuring the constant for the slower decay process K_{B2} precludes a generalization of its behavior. The rapid change in K_{B1} in the vicinity of −0.05 v suggests the reduction of a pigment which can then interact with the photooxidized materials.

The decay rates never become rapid enough to explain the loss in signals at low potential (or, for that matter, at high potential). For the reactions described here the steady-state concentration of the photooxidized species is given by

$$\frac{P^+}{Po - P^+} \cong \frac{K_f}{K_B} \quad \text{or} \quad P^+ \cong \left(\frac{K_f/K_B}{1 + Kf/K_B}\right) Po$$

where *Po* is the initial concentration of the oxidizable species, P+ is the concentration of the oxidized material, K_f is the first-order forward rate constant, K_B is the fast back reaction first-order rate constant. $K_f = 16$ sec.$^{-1}$, K_B varies from 2 to 14 sec.$^{-1}$ (at 792 mμ). Thus, the steady-state value of *P*+ would fall from 89 per cent of *Po* to 53 per cent of *Po* because of the increase in decay constant from 2 to 14 sec^{-1}. Experimentally the steady-state level of *P*+ fell to lower than 2 per cent for $K_B = 14$ sec.$^{-1}$ (at $E_h = -0.12$ v). These findings support the assign-

ment of the losses in the steady-state signal to the titration of pigments required for the appearance of the light signal rather than those hastening its disappearance.

It is of interest to note that the decay rates tend to approach the same high value ($K_B^1 \cong 14$ sec.$^{-1}$) for each of the three wavelengths studied at high concentrations of $K_4Fe(CN)_6$ (> 0.1 M).

Variation of Kinetics with Oxygen Pressure. The effect of oxygen on the rise and decay kinetics was examined at two fixed potentials, +0.44 v and +0.30v. No significant effect was observed on any of the bands studied.

DISCUSSION

The experiments presented here are interpreted as characterizing a photoactive unit composed of at least two essential pigments. Photosynthetic activity (as measured by the typical light-induced absorption changes) in chromatophores from *R. rubrum, R. spheroides,* and *Chromatium* is dependent upon the reduced form of a pigment, $P_{0.44}$ and the oxidized form of a pigment $P_{-0.04}$ which appear to be capable of undergoing a one-electron oxidation or reduction, respectively.[3] Thus, $P_{0.44}$ must be the primary electron donor, or kinetically indistinguishable from it, and $P_{-0.04}$ may be the primary electron acceptor, or kinetically indistinguishable from it in these bacterial systems.[4] Other interpretations of the loss of absorption changes below 0 v, such as further reduction of $P_{0.44}$ or complex formation with the added redox couples, cannot be ruled out at the present time.

A fundamental question is that of the relationship of $P_{0.44}$ to the oxidized-minus-reduced and light-minus-dark difference spectra. The available evidence cannot support a rigorous assignment of absorption bands to $P_{0.44}$ but some deductions can be made. The agreement between oxidized-minus-reduced and light-minus-dark spectra in the near infrared demonstrates that the photoabsorption changes at 792, 810, and 865 mμ are associated with electron donors. Thus, one or more of these bands could be due to $P_{0.44}$. The difference in decay kinetics for the photoabsorption changes at 433, 792, 810, and 865 mμ (Fig. 13) clearly indicates that there are at least three different molecules affected by the light. It follows that $P_{0.44}$ is most likely to absorb strongly at only one of these wavelengths (if at all).

A tentative working hypothesis assigns to $P_{0.44}$ an 865 mμ band in the reduced form. This probably is only one of several reduced bands, and no bands have been identified with the oxidized form since no photoinduced increases in absorbance between 350 and 950 mμ show kinetics to correspond with the 865 decrease.

The emerging picture of a discrete active unit is nicely supported by the iridic

[3] This notation is used to stress the midpoint potential (+0.44 or −0.04 v) for the redox changes and to stress our lack of knowledge about the complex.

[4] A midpoint of +0.43 v for the light-induced absorption loss at 700 mμ in chloroplasts has been reported by Kok (19). The similarity of the potentials in the two systems is probably not accidental.

chloride experiments and Clayton's observations with pheophytinized *R. spheroides* chromatophores (17). The removal of the bulk pigment absorption produces only small changes in the IR spectra of the photosignals and does not change their magnitude. It thus appears that the 880 mμ pigment in *R. rubrum*, the 850 mμ and part of the 800 mμ pigment in *R. spheroides*, and the 880 and 800 mμ pigments in *Chromatium*, which can be irreversibly removed by chemical oxidation, are not essential components in either the light-produced absorption changes or the light-produced ESR signals (12), both of which may be produced qualitatively and quantitatively in the same fashion in their absence as in their presence. Thus, the bulk pigments can only act as energy-gathering and energy-transfering systems which may supply the photoactive unit. This clean-cut separation of the active and bulk pigments offers considerable support to the concept of a "photosynthetic unit" (20, 1, 17, 3).

The authors wish to thank Mr. Bill Hart for construction of all glass apparatus used for anaerobic measurements and Mr. Roland Thompson for his expert electronic advice and assistance in the construction of the kinetic spectrophotometer. We are indebted to Dr. Kenneth Sauer for his many helpful discussions, advice, and encouragement.

Irwin D. Kuntz, Jr., was a National Science Foundation Fellow, 1961-1963. Paul A. Loach was a Fellow of the National Academy of Sciences-National Research Council, supported by the Air Force Office of Scientific Research of the Office of Aerospace Research, 1961-1963. The work described in this paper was sponsored, in part, by the United States Atomic Energy Commission.

Received for publication, July 16, 1963.

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