Primary Photochemistry and Electron Transport in *Rhodospirillum rubrum**

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ABSTRACT: The quantum yield for photoxidation of the primary electron donor molecule in bacterial photosynthesis has been determined for a variety of chromatophore fractions obtained from *Rhodospirillum rubrum*. The most accurate value was obtained by measurement of a reaction, the photoxidation of cytochrome c_2, which can be closely coupled to the primary event. The quantum yield for this reaction was 0.85 ± 0.05 as determined with the supernatant fraction. It was demonstrated that for this system the rate of cytochrome c_2 oxidation exactly matches that for the dark conversion of the primary electron donor molecule back into its reduced form. It was also demonstrated that many of these supernatant systems displayed tight coupling so that 75–95% of all primary pigment photoxidized subsequently interacted with reduced cytochrome c_2. Knowing the degree of this coupling and the quantum yield for cytochrome *c* photoxidation, a quantum yield of 0.95 ± 0.05 was determined for production of the primary oxidized species called P 870 or $P_{0.44}$. The extent of the cytochrome *c* photoxidation could be significantly increased by adding horse heart ferrocytochrome *c* and 1.5% Triton X-100 and/or 6 M urea. However, the quantum yield was unchanged. The quantum yield for photooxidation of the primary electron donor molecule was also determined by direct measurement of absorbance change in the near-infrared region for washed chromatophore fractions and using an assumed molar extinction. These results were in good agreement with those of the coupled system. Identical results were obtained for all measurements whether conducted with 887- or 865-mμ exciting light.

The results are discussed with regard to possible mechanisms for the trapping act and the per cent of light energy conserved as chemical potential.

In order to understand the mechanism of the capture of light energy in photosynthesis, it is essential to quantitatively establish the fate of all light energy absorbed. Of central importance is, of course, not only the identification of the molecules at the reaction center(s) which are responsible for the photochemistry, but also a measure of the fraction of the energy which is invested in their change in oxidation state. Considerable evidence has accumulated which bears on the identification of the primary electron donor molecule in bacterial photosynthesis (Duysens, 1952; Duysens *et al.*, 1956; Goedheer, 1959; Arnold and Clayton, 1960; Clayton, 1962a,b, 1963, 1966a,b; Androes *et al.*, 1962; Calvin and Androes, 1962; Vredenberg and Duysens, 1963; Sybesma and Vredenberg, 1963, 1964; Loach *et al.*, 1963; Kuntz *et al.*, 1964; Sistrom and Clayton, 1964; Clayton *et al.*, 1965; Holt and Clayton, 1965; Loach, 1966; Loach and Sekura, 1967; Mauzerall *et al.*, 1967). In *Rhodospirillum rubrum* it has been called P 890 (Vredenberg and Duysens, 1963) or $P_{0.44}$ (Loach *et al.*, 1963) and seems to be a special bacteriochlorophyll molecule in a unique location. Several quantum yield studies have been reported for the photoxidation of this molecule, but the numbers have been sufficiently small (Clayton, 1962b; Vredenberg and Duysens, 1963; Parson, 1967; Ke *et al.*, 1967) or approximate (Clayton *et al.*, 1965; Parson, 1968; Beugeling, 1968) that discrimination between possible mechanisms could not be made. We feel that the data reported herein are accurate enough to allow the first conclusions to be drawn which rule out certain possibilities for the trapping act.

Materials and Methods

R. rubrum was grown, harvested, and subjected to fractionation procedures for the preparation of chromatophore fractions as described previously (Loach *et al.*, 1963). A representative sample of the preparations used in our studies is shown in Figure 1. The cytochrome *c* used was horse heart cytochrome *c*, type III (Sigma Chemical Co., St. Louis, Mo.), and was routinely reduced with sodium borohydride (Metal Hydrides, Inc., Beverly, Mass.). The Triton X-100 is a product of Rohm and Haas. Coenzyme Q_2 was kindly donated by Merck Sharp and Dohme Research Laboratories and coenzyme Q_6 was obtained from Sigma.

Difference spectra were recorded with a Cary Model 14R spectrophotometer (Cary Instruments, Monrovia, Calif.) equipped with a scattered transmission attachment (Model 1462) and an appropriate end-window multiplier phototube as indicated in Table I. Modification of this instrument for admitting a beam of

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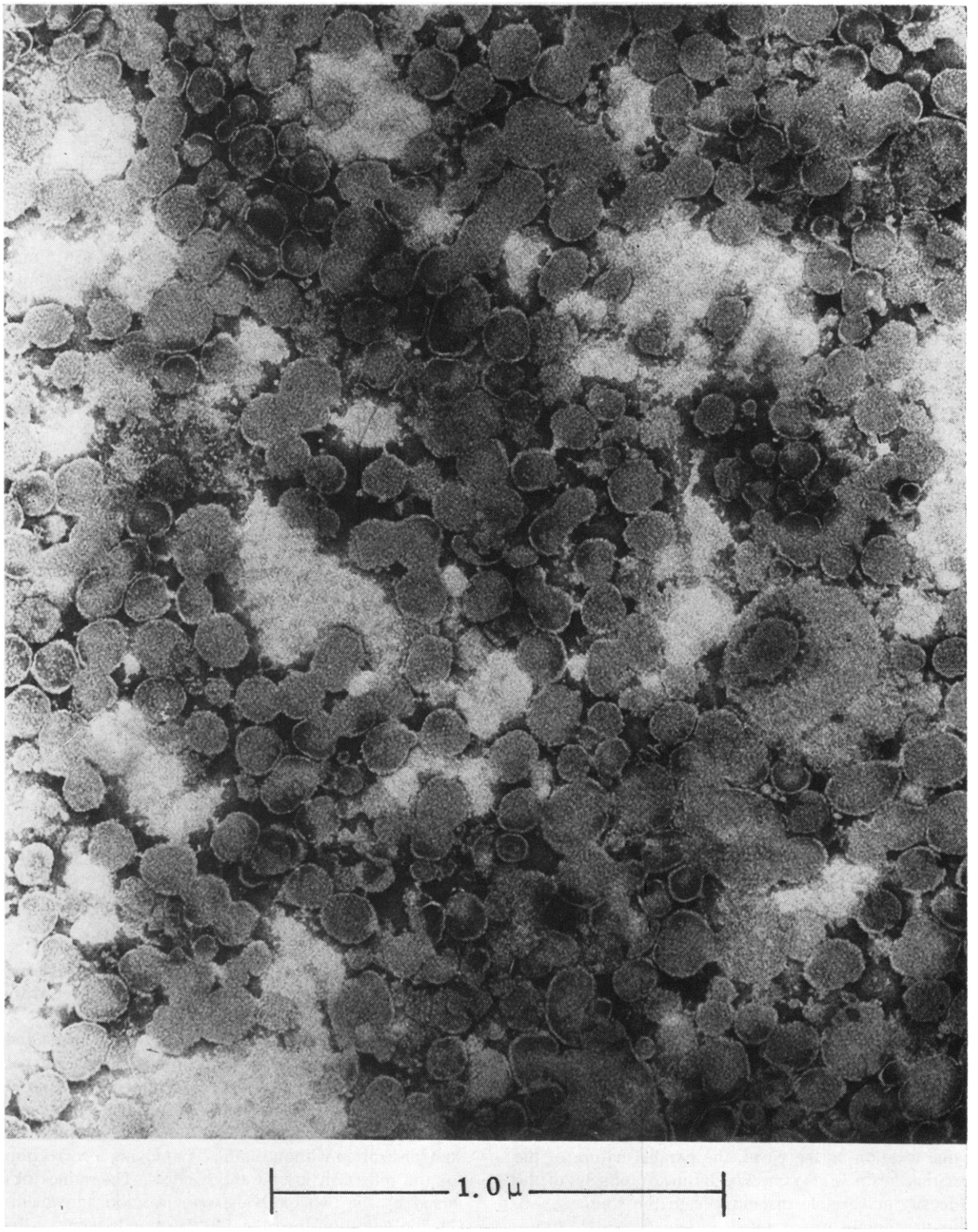

FIGURE 1: Electron micrograph of a chromatophore sample prepared from *R. rubrum*. The preparation was negatively stained with 2% phosphotungstic acid adjusted to pH 7.0. A Hitachi Hu-11A electron microscope was used at an accelerating voltage of 75 kV. Magnification was 30,000.

exciting light, and details of the spectrometer used for measuring kinetics in the millisecond region, have been reported (Loach and Loyd, 1966). The amount of oxygen consumed in some experiments was measured with a YSI Model 53 biological oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio). Measurement of light intensity was conducted using either an Eppley thermopile with a 12-junction bismuth–silver linear-type surface coated with lampblack (basic sensitivity is 0.066 $\mu V/\mu W$ per cm^2) or an Eppley

TABLE I: Filter and Multiplier Phototube Combinations Used for Measuring Absorbance Photochanges.

Detecting Wavelength (mμ)	Phototube	Detecting Beam[a] Filters	Exciting Beam Filters[c]
433	Dumont 6911 or RCA 6342A	NBP[d] or 9782[b]	NBP[d] transmitting maximally at 886.5 mμ
550	RCA 6342A	9782	NBP[d] transmitting maximally at 886.5 mμ
605	RCA 6342A or Dumont 6911	9782	NBP[d] transmitting maximally at 886.5 mμ
792	Dumont 6911	NBP	NBP[d] transmitting maximally at 886.5 mμ
810	Dumont 6911	NBP	NBP[d] transmitting maximally at 886.5 mμ
855	Dumont 6911	NBP	NBP[d] transmitting maximally at 886.5 mμ

[a] Placed in front of the phototube window. [b] Corning color glass. [c] A 5-cm water filter was always placed between the exciting light source and the sample. [d] Baird-Atomic B-9 narrow band pass interference filters transmitting maximally at the detecting or exciting wavelength indicated.

thermopile with an 8-junction bismuth–silver circular-type surface coated with lampblack (basic sensitivity is 0.118 $\mu V/\mu W$ per cm^2 with quartz window). Both thermopiles were originally calibrated at Eppley Laboratories (Newport, R. I.) but were also periodically checked against each other and also against two Eppley carbon filament lamps (E6327 and E-6629) used as standards of total radiation according to the procedure outlined by the National Bureau of Standards. The calibration of these units has not changed more than 5% over a 2-year period of usage. A Keithley millimicrovoltmeter, Model 149 (Keithley Instruments, Cleveland, Ohio), has been used to measure the small voltages produced at the thermopile junctions. The voltages used in calculations were the average of several measurements as recorded on a Mosley Autograf X–Y recorder (Model 7001A).

Since the accuracy of the quantum yield measurement depends most importantly in knowing the amount of light absorbed at a particular wavelength during some convenient unit of time, we have taken considerable care to measure the homogeneity of our excitation beam. Over the cross-sectional area of the beam employed for exciting the sample, variation in intensity from one region to another was less than 2%. Although we have mechanically designed our cell compartment so that the thermopile may be placed in exactly the same location as the cuvet, the parallel nature of the exciting beam is also checked by measurements of the intensity at variable distances from the source. Also, the thermopile was rotated systematically to ensure that it demonstrated a maximal response, indicating that it was truly perpendicular to the exciting beam.

Before and after each set of quantum yield measurements, a ferrioxalate actinometer was run as a control on the entire system used. Crystalline ferrioxalate was prepared by the method of Hatchard and Parker (1956). Ferrous ion formed as a result of light exposure at 365 mμ (exciting light from a tungsten projection bulb was passed through either a Corning 7-39 or 7-51 color filter and a 5-cm path of 4% $CuSO_4$ in 0.01 N H_2SO_4) was measured by the *o*-phenanthroline assay. We have found that the actinometer check is an essential one to conduct in order to ensure good accuracy, since, particularly in setting up our quantum yield measuring apparatus, many sources of error (large enough to cause 30–50% errors) could escape visual detection.

The absorbancy of all systems used for quantum yield measurements was adjusted so that greater than 98% of the light entering the cuvet at the wavelengths of excitation was absorbed. Since the detecting beam passed through the center of the cuvet, each solution had to be stirred rapidly enough to ensure complete mixing in order that the measurement was valid for the entire volume of the sample. Model car motors have proven useful for such mixing when the shaft of the motor is equipped with a glass extension to act as an agitator. This shaft is positioned in a corner of the cuvet so that it does not interfere with the measuring beam. A variety of stirring speeds can be achieved by varying the voltage which runs the motor.

Several corrections were applied to the measured thermopile voltages in order to obtain the quantity of light actually absorbed by the sample. These consisted of the following. (1) The sensitivity of the thermopile without its quartz window was determined both by standardization without it and by applying a correction for the reflection loss at the window. The values obtained by the two methods were in good agreement. (2) The reflection losses at the thermopile window for the specific wavelengths of excitation were determined by measuring the percentage light transmitted by the window when placed in the sample beam of a Cary spectrophotometer at the specified wavelength. (3) A total value of 6.4% was used to account for the reflection losses at the quartz–air and quartz–solvent interfaces of the quartz cuvet employed.

All experiments were conducted at room temperature (25 ± 1°) except where especially noted. The

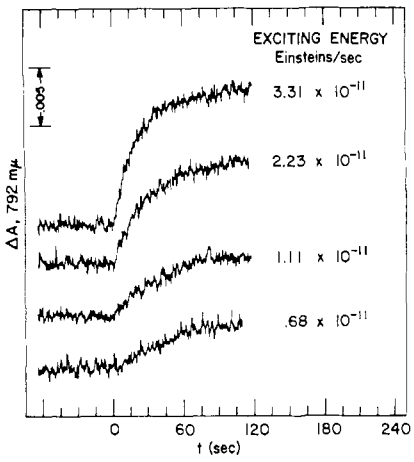

FIGURE 2: Time dependence for the onset of $P_{0.44}$ absorbance change with varying intensities of 887-mμ exciting light. The light energies shown are for absorbed energy. Absorbance of the sample at 880 mμ is 6.0. Light was allowed to fall on the sample at time zero. The sample was that of washed chromatophores suspended in 0.01 M $K_4Fe(CN)_6$ and 0.1 M glycylglycine buffer at pH 7.5. The cuvet contents were thoroughly stirred. The exciting light passed through a 5-cm water filter and an 887-mμ Baird-Atomic B-9 narrow band pass filter before falling on the sample.

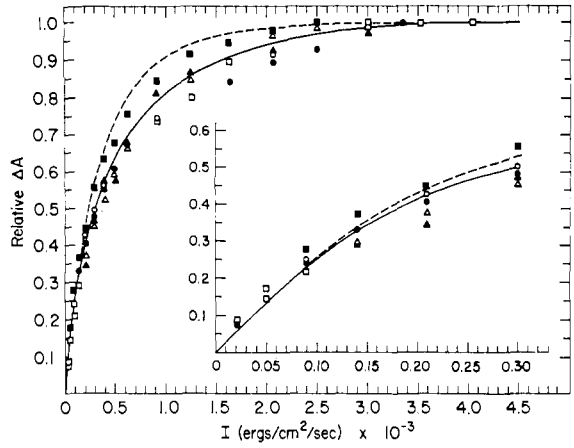

FIGURE 3: Light saturation of the steady-state absorbance changes of washed chromatophores at 430 (△), 605 (▲), 792 (○), 810 (●), and 855 mμ (■). Absorbance at 880 mμ is 3.0. The sample was in 0.1 M glyclyglycine buffer at pH 7.5. The light intensity indicated is the energy incident upon the cuvet surface. The exciting light passed through a 5-cm water filter and an 887-mμ Baird-Atomic B-9 narrow-band pass filter. Changes at all five wavelengths were recorded at each light intensity for which data are given. The open squares (□) in the figure represent overlapping data for two or more of the above wavelengths. The solid line is merely drawn through the middle of the data. The dashed line represents data for cytochrome *c* photoxidized as explained in the text.

volume of the sample was always 3.0 ml and the pH of chromatophore samples was maintained at 7.5 with 0.05–0.10 M glycylglycine buffer.

Results

Measurement of the rate of absorbance change *vs.* the rate of light energy absorption can be readily accomplished with the Cary 14R spectrophotometer equipped with the 0–0.1 slide wire and appropriately modified to admit a beam of exciting light. Figure 2 shows typical absorbance changes resulting from excitation with light of 866.5 mμ. These measurements were conducted in a 1-cm path-length, four-sides-clear cuvet with sufficient stirring to ensure that mixing was not limiting the observed rate. Although it is possible to make satisfactory measurements at 865 mμ even though the excitation wavelength is 887 mμ, other wavelengths of absorbance change are equally valid since, as shown in Figure 3, they are all saturated by the same light intensity. The saturation curve for the steady-state change recorded in Figure 3 does of course depend upon the dark decay time for the process being observed, as well as the absorbed light intensity. The identity of these dark decay times at each wavelength examined has been established for the specific material studied herein (Loach and Sekura, 1967).

From the data of Figure 2 one could calculate the quantum yield, providing an accurate value for the molar absorptivity of $P_{0.44}$ were available. The more recent values of Clayton (1966a; ϵ_{mM} 113), if corrected to a differential millimolar absorptivity according to the data of Reed and Clayton (1968; ϵ_{mM} 90), and the data of Parson (1968; ϵ_{mM} 90) and Beugeling (1968; ϵ_{mM} 90), are applicable although the magnitude of the plus or minus attached to the value may be a limitation on interpretation of the data. We have been attempting simplification and purification of the complex involving the $P_{0.44}$ molecule with the hope that we can eventually directly titrate it in a redox reaction and establish its molar extinction value with greater accuracy. However, this has not yet been achieved. The next best route toward obtaining the desired information would be to examine a secondary reaction which could be demonstrated to be sufficiently closely coupled to the primary event that all $P_{0.44}$ photoxidized would react directly to oxidize this second molecule. We are fortunate in this respect to have such a secondary molecule which, in itself, has a fairly well established extinction coefficient. This molecule is cytochrome *c*.

We have found that the best system with which to conduct cytochrome *c* photoxidation is the supernatant fraction (see Figure 8 and Table II) obtained after a 50-min centrifugation of sonicated bacteria at 144,000*g*. Although such a supernatant fraction is not, by definition, a chromatophore fraction, electron micrographs show that it also contains large quantities of cup-shaped units approximately 500 Å in diameter quite similar to those shown in Figure 1. As a distinction from a chromatophore fraction the supernatant has a greater preponderance of smaller membrane fragments and also, of course, all the soluble proteins. A light minus dark difference spectrum is shown in Figure 4. It is clear that ferrocytochrome *c* is being oxidized as a result of light absorption. If the coupling of reduced cytochrome *c* oxidation to oxidized $P_{0.44}$ reduction is not sufficiently tight, this secondary reaction would not be very useful for quantum yield determinations. It is important, then, that we can

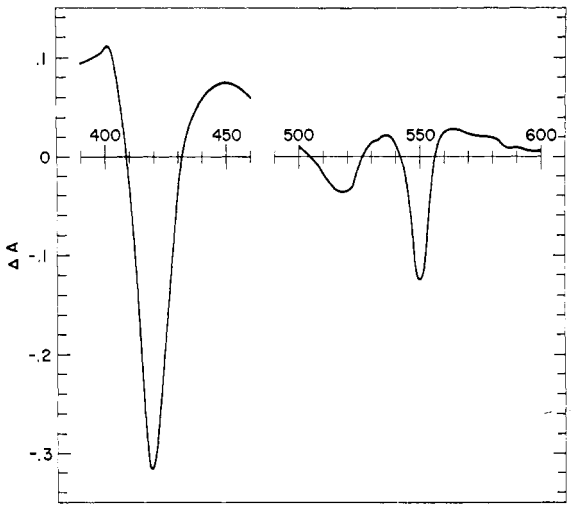

FIGURE 4: Photoxidation of ferrocytochrome *c* by the supernatant fraction from a preparation of chromatophores of *R. rubrum*. Absorbance at 880 mμ is 1.8 in a 1-cm cuvet. Triton X-100 at 1.5% is present. Exciting light intensity was approximately 10^4 ergs/cm² per sec after passing through a Corning 7-69 color filter and a 5-cm water filter; 0.01 M glycylglycine buffer (pH 7.5). Added cytochrome *c* is 2.5×10^{-5} M. Coenzyme Q_6 is 5×10^{-5} M.

routinely obtain systems where 75–95% of all $P_{0.44}$ oxidized by light decays through cytochrome *c*. Part of the data for this conclusion is shown in Figure 5. Of the two curves plotted, the solid one is for the onset of photoxidation of cytochrome *c* as measured by a decrease at 550 mμ. The dashed curve represents the recovery of $P_{0.44}$ as measured at 605 mμ. This latter change is an increase in absorbance but it has been inverted in the figure so that it can be more readily compared with the 550-mμ change. Onset of $P_{0.44}$ changes are instantaneous with the flash which is less than 0.1 msec. Since in the uncoupled system (washed chromatophores as in Figure 2), $P_{0.44}$ decays with a

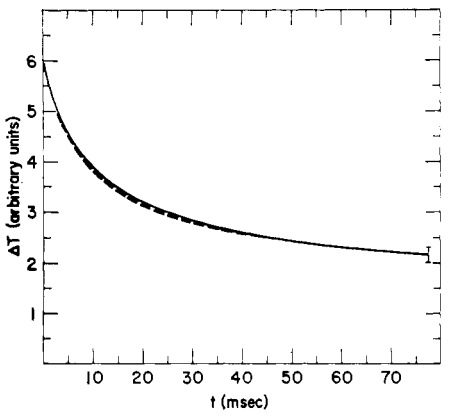

FIGURE 5: Rates of dark reduction of photoxidized $P_{0.44}$ (605 mμ) and of dark oxidation of ferrocytochrome *c* (550 mμ). Supernatant fraction from chromatophore preparation. Absorbance at 880 mμ is 3.6 in a 1-cm cuvet. Exciting light was supplied by a xenon flash lamp whose decay is >99% complete in 0.5 msec. The exciting beam was passed through a 5-cm water filter and a Corning 7-69 color filter. Added cytochrome *c* is 1×10^{-5} M. (——) Decrease at 550 mμ. (– – – –) Increase at 605 mμ.

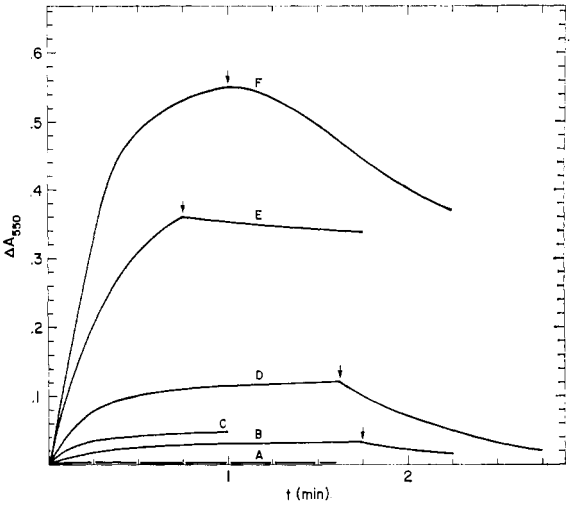

FIGURE 6: Photoxidation of cytochrome *c* by various fractions from a chromatophore preparation. (A) Washed chromatophores; added cytochrome *c* is 1×10^{-6} M; absorbance at 880 mμ is 4.0. (B) Supernatant fraction; added cytochrome *c* is 1×10^{-5} M; absorbance at 880 mμ is 2.0. (C) Washed chromatophores in 1.5% Triton X-100; absorbance at 880 mμ is 3; cytochrome *c* is 2.5×10^{-5} M. (D) Supernatant fraction in 1.5% Triton X-100; absorbance at 800 mμ is 3.8; 5×10^{-5} M cytochrome *c*. Anaerobic conditions. (E) Washed chromatophores in 1.5% Triton X-100; absorbance at 880 mμ is 3.0; cytochrome *c* is 2.5×10^{-5} M; coenzyme Q_6 is 5×10^{-5} M. (F) Supernatant fraction in 1.5% Triton X-100; anaerobic conditions; absorbance at 880 mμ is 1.9; cytochrome *c* is 5×10^{-5} M; coenzyme Q_6 is 1×10^{-4} M. For A, C, E, and F the exciting light was approximately 10^4 egs/cm² per sec after passing through a 5-cm water filter and a Corning 7-69 color filter. The exciting light intensity was about 10^3 ergs/cm² per sec for B and D. Light was allowed to fall on the sample at $t = 0$ and turned off at the arrow. No stirring.

half-time of 5 sec, these two curves with half-times near 10 msec indicate good coupling for this particular sample. The dashed line in Figure 3 shows the light saturation curve for the steady-state amount of cytochrome *c* photoxidized. Since for this comparison the light intensities for the cytochrome *c* data were multiplied by the ratio of the half-time for pseudo-first-order decay of cytochrome *c* *vs.* that of $P^+_{0.44}$, the two curves would be expected to be identical provided they have the same efficiencies for utilizing the light energy absorbed. Experiments at lower temperatures (5°) resulted in much slower dark decay times, but the rate of the light-induced cytochrome photoxidation was identical with that at higher temperatures (25°). Therefore, interaction of ferrocytochrome *c* with $P^+_{0.44}$ is not rate limiting under the conditions of low light intensities employed. The high efficiency of photooxidation and the speed of the reaction (Figure 5) suggest that cytochrome *c* is held specifically in some special site near $P_{0.44}$.

In all experiments except those where both 6 M urea and 1.5% Triton X-100 were present (Table II), all cytochrome *c* photoxidized was quantitatively reduced in the subsequent dark period. This result suggests that pools are available for storing reducing equivalents and that they can be quantitatively mea-

TABLE II: Quantum Yield Values.

Photooxidation	ϵ_{mM}	Φ
Chromatophore Supernatant		
Fe^{2+} cytochrome c_2 (endogenous) or added Fe^{2+} cytochrome c	20 (550)	0.85 ± 0.05[a]
$P_{0.44}$	90 (865)	0.1 ± 0.05
Sum		0.95 ± 0.05
Added Fe^{2+} cytochrome c (1.5% Triton)		0.85 ± 0.05[a]
Added Fe^{2+} cytochrome c (1.5% Triton + 6 M urea)		0.85 ± 0.05
Washed Chromatophores		
Fe^{2+} cytochrome c_2 (endogenous)		<0.01
$P_{0.44}$	90 (865)	1.0 ± 0.2[b]
Added Fe^{2+} cytochrome c (1.5% Triton)		0.2
Added Fe^{2+} cytochrome c (1.5% Triton + CoQ_6)		0.2
0.25 M $K_4Fe(CN)_6$	1.00 (420)	0.25 ± 0.05
0.01 M $K_4Fe(CN)_6$		<0.005
Ferrioxalate Actinometer		
Measured		1.33 ± 0.05
Literature (Hatchard and Parker, 1956)		1.26 ± 0.03

[a] Total variation over approximately a 1-year period ranged from 0.45 to 0.95. The value given is the average of results obtained with particular supernatant fractions in which the coupling was high. Approximately 20 separate determinations are represented. Nearly identical results were obtained for excitation at either 865 or 890 mμ. No variation of quantum yield was observed over a tenfold range of light intensities. [b] Total variation over approximately a 2-year period ranged from 0.8 to 1.2. The value given represents the average of all these determinations.

sured for a given set of conditions by determining the total cytochrome c photooxidizable. Quinones seemed to be the most likely candidates for the pool of secondary electron acceptors since they are present in significant concentrations (Lester and Crane, 1959), and also, there is evidence for quinones serving as secondary, and possibly primary, electron acceptors (Clayton, 1962c; Ke *et al.*, 1967) at least in fractionated systems. However, absorbance change at 275 mμ has not always been observed for the supernatant fractions studied herein.

The extent of the cytochrome c photooxidation could be increased in two significant ways. First, as reported by others (Zaugg, 1963), added coenzyme Q_6 was

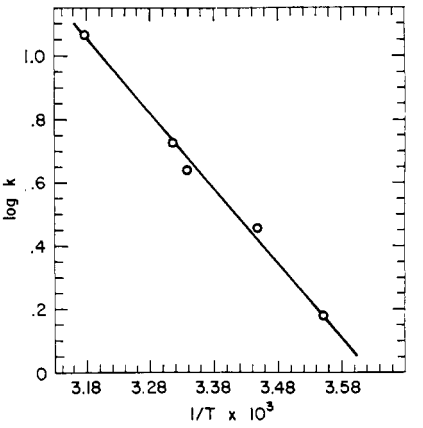

FIGURE 7: Arrhenius plot for dark reduction of photooxidized $P_{0.44}$. Chromatophores of *R. rubrum* in the presence of 0.25 M $K_4Fe(CN)_6$ concentration. k is the rate constant for the pseudo-first-order process and T is the absolute temperature. Absorbance at 880 mμ = 2; 0.05 M phosphate buffer (pH 7.4). $\Delta H^{\ddagger} = +11.0 \pm 0.5$ kcal/mole and $\Delta S^{\ddagger} = -25 \pm 5$ cal/deg per mole.

effective with washed chromatophore fractions either in the presence or absence of air, but not without added Triton. The supernatant fraction also exhibited a response to added coenzyme Q_6 in the presence of Triton. Figure 6 shows the extent of some of these reactions. The second method for increasing the extent of cytochrome c photooxidation consists of changing the reaction centers sufficiently to allow oxygen to act as the electron acceptor. Of course, the cytochrome photooxidation reaction is irreversible in this case and does not proceed in the absence of air. That oxygen is in fact taken up by such systems has been verified by measurements with an oxygen electrode. However, the quantity of oxygen consumed is often several times greater than an amount equivalent to cytochrome c oxidized. It was reported previously (Loach and Sekura, 1967) that the reaction center activity could also be affected by increasing the ionic strength with $K_4Fe(CN)_6$. In this case oxygen is taken up in amounts stoichiometrically equivalent to ferrocyanide converted into ferricyanide. At lower ionic strengths no oxygen was consumed and $K_4Fe(CN)_6$ was only very inefficiently converted into ferricyanide (see Table II). Consistent with this is the fact that the decay rate of $P^{+}_{0.44}$ in 0.25 M $K_4Fe(CN)_6$ is temperature dependent (Figure 7) while no dependency on temperature (5–40°) is observed in 0.01 M $K_4Fe(CN)_6$.

Representative sets of data used for quantum yield determinations are shown in Figure 8 and the averages of values calculated for many systems are summarized in Table II. In the table, note the $\Delta\epsilon_{mM}$ assumed for cytochrome c is an average of many values found in the literature. The value assumed for $P_{0.44}$ at 865 mμ is 90 mM^{-1} cm^{-1} (Reed and Clayton, 1968; Parson, 1968; Beugeling, 1968). The value of the quantum yield for $P_{0.44}$ listed immediately below the cytochrome c number is relatively small and is added to the cytochrome c number to get a sum. In each of our preparations this sum has been close to 1.0. Therefore, the sum represents the idealized value for the case

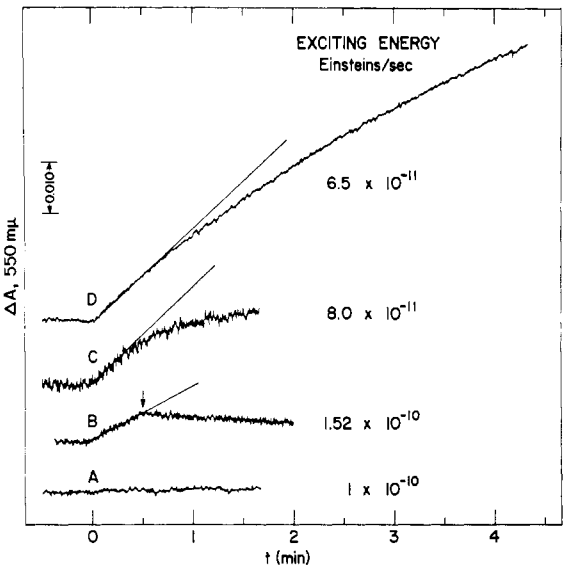

FIGURE 8: Rate of ferrocytochrome *c* photoxidation by various fractions from the preparation of chromatophores of *R. rubrum*. Light energy listed is that absorbed by the samples. (A) Washed chromatophores; absorbance at 880 mμ is 4.0; added cytochrome *c* is 1×10^{-5} M. (B) Washed chromatophores in 1.5% Triton X-100; absorbance at 880 mμ is 3; cytochrome *c* is 2.5×10^{-5} M; coenzyme Q is 5×10^{-5} M. (C) Supernatant fraction; absorbance at 880 mμ is 4; added cytochrome *c* is 1×10^{-5} M. (D) Supernatant fraction in 1.5% Triton X-100; absorbance at 880 mμ is 4.0; cytochrome *c* is 1×10^{-5} M. The exciting light was allowed to fall on the sample at $t = 0$ and the arrow for B indicates when the light was turned off.

where all sites would be tightly coupled. The washed chromatophore sample is a residue fraction in which cytochrome *c* is not present. The very bottom part of the table shows results for various ferrioxalate actinometers and is a most important part of the data. The experimental value is the average of many separate experiments conducted before and after the experiments involving chromatophore fractions. For these standard systems, only the narrow band pass excitation filter, and, of course, the cuvet contents were changed. All other parameters remained constant.

Discussion

Although it is less direct to measure the quantum efficiency of light capture by the primary electron donor molecule, $P_{0.44}$, by following a secondary reaction, there are distinct advantages which can be counted. (1) The secondary reaction can be run to such a bulk extent that there need be no concern about possible contributions to the reaction by previously formed high-energy intermediates (which could give rise to a false economy) or by other absorbance changes at the wavelength of measurement that might have significantly added to, or subtracted from, the change of interest. (2) Because more reactions are necessarily involved from the trapping act to the accumulated products, more information is potentially available. For example, it is clear in this system that cytochrome *c* oxidation is a tightly coupled "dark" reaction immediately following the trap redox change (Figure 5). Ample evidence is now accumulating (Parson, 1967, 1968; DeVault, 1967; Chance *et al.*, 1967; Ke and Ngo, 1967) to indicate that the same sequence occurs in the intact cell according to the original suggestions of Duysens (Duysens *et al.*, 1956; Vredenberg and Duysens, 1964; compare with Chance, 1963), and that cytochrome *c* photooxidation can be very efficient (Olson, 1962; Vredenberg and Duysens, 1964).

From the values listed in Table II the following conclusions seem warranted. Using the best data, that for cytochrome *c* photooxidation, and knowing the degree of coupling to $P_{0.44}$, the quantum yield for $P_{0.44}$ photooxidation can be calculated. This is shown as a sum in Table II and has the average value of 0.95 ± 0.05. Since some small amount of absorbed energy is lost to photochemistry because of reemission as fluorescence, these results are rather convincing that one molecule of $P_{0.44}$ is oxidized as a result of trapping one quantum of light energy. Moreover, it is the same oxidizing moiety that later shows up as ferric cytochrome *c*. Also from Table II the quantum yield for $P_{0.44}$ photooxidation in washed chromatophore fractions is seen to be in good agreement (1.0 as compared with 0.95) with data obtained from the coupled reaction.

It had been previously suggested (Loach, 1966) that in order to explain the redox dependency shown by bacterial systems, two molecules of $P_{0.44}$ might be involved per trapping act. This possibility can be eliminated as a result of the data listed in Table II.

Certain restrictions might be cited with respect to the reducing equivalent that is initially formed. Since the photochemical trap has just one molecule of $P_{0.44}$ as primary electron donor, it therefore would seem to have some as yet unidentified molecule as a *one*-electron acceptor. However, the apparent two-electron redox dependency shown by bacterial systems in the region of zero volts (Loach, 1966) and the lack of any electron paramagnetic resonance signal ascribable to the primary electron acceptor molecule are difficult to relate to a one-electron acceptor. It is of course possible that other molecules are also involved in the primary redox chemistry and they have not yet been discovered simply because they lack easily distinguishable absorbance or electron paramagnetic resonance changes; *e.g.*, some functional group (or perhaps a metal ion like Cu^+) could be located in the trap and provide a second electron to the acceptor molecule after $P_{0.44}$ is converted to $P^+_{0.44}$. Thus two electrons could be used to reduce the electron acceptor molecule per quantum of light absorbed.

Other chemistry which is not photoinduced may also explain the redox dependency at low potential. For example, a structural component such as a protein disulfide bond would, when reduced, result in a structural change in the trap so that it could not function. Such a redox change would thus have nothing to do with the primary photochemistry but could constitute an interesting control on photosynthesis based on the over-all redox level of the cell.

However, nearly all experimental data can presently be explained without recourse to such additional

unknown molecules or mechanisms. The electron acceptor molecule could either be a one-electron acceptor (*e.g.*, a transition metal complex of low optical extinction) or a group capable of either a one- or two-electron reduction. In the latter case, a one-electron conversion would be the light-driven result while a thermodynamically equilibrated system, such as exists during a redox titration (Loach, 1966), would show a two-electron dependency. In this instance the absence of a photoproduced electron paramagnetic resonance absorbance could be explained by an exceptionally fast secondary reaction leading to an "electron pool." As a result, the steady-state concentration of a one-electron-reduced species would be below normal levels of detectability. Evidence for the existence of such pools is ample; the data given in this paper for the extent of cytochrome *c* photoxidation in those cases where oxygen uptake is either not observed or impossible (anaerobic) clearly dictate that pools of reductant do accumulate.

The data presented in this paper have established that the absorption of one quantum of light between 865 and 880 mμ in *R. rubrum* leads to the oxidation of one molecule of $P_{0.44}$ and, very likely, its subsequent interaction with one molecule of ferrocytochrome *c*. An ideally high yield of one molecule changed per quantum absorbed is thus observed. However, because of uncertainty about the chemical properties of the primary electron acceptor molecule, it is not yet possible to ascertain the fraction of the energy of each quantum which is actually conserved as chemical potential. Approximation of the minimal efficiency at 35% seems valid on the basis of a minimal potential span of nearly 0.5 V (Loach, 1966) for a one-electron change. Several calculations (Duysens, 1958; Ross and Calvin, 1967) have placed the maximum possible theoretical energy conversion at about twice this magnitude.

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