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THE ABSOLUTE QUANTUM EFFICIENCY OF BACTERIOCHLOROPHYLL PHOTOOXIDATION IN REACTION CENTRES OF *RHODOPSEUDOMONAS SPHEROIDES*

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SUMMARY

The widely assumed correspondence between fluorescence and photochemistry in photosynthetic systems has recently been challenged by observations on the triplet state of bacteriochlorophyll in reaction centres of *Rhodopseudomonas spheroides*. In order to check this assumption we have conducted a precise determination of the quantum efficiency of bacteriochlorophyll photooxidation in reaction centres at room temperature. We find a quantum efficiency of 1.02 ± 0.04 in contrast to a value of about 0.7 predicted from the variations in fluorescence yield.

INTRODUCTION

An electron spin resonance (ESR) signal suggesting formation of the excited triplet state of bacteriochlorophyll has been observed at liquid He temperature in chromatophores and sub-chromatophore preparations of several species and strains of photosynthetic bacteria [1]. This light-induced signal originates from the reaction centres and is only observable when normal photochemistry is inhibited by reduction of the primary electron acceptor. Photochemically active preparations show, instead, the light-induced signal attributed [2] to oxidized bacteriochlorophyll. The triplet could arise in one of two ways: as a degradative side pathway for de-excitation of the singlet state, or as an essential precursor of photochemistry. In experiments with reaction centre particles from *Rhodopseudomonas spheroides* subjected to laser flash excitation at liquid He temperature, comparisons of light–saturation curves showed that the quantum efficiency for presumed triplet formation in inactive reaction centres is as great as that for bacteriochlorophyll oxidation in active reaction centres [3].

These experiments indicated that the conversion of reaction centres to the photochemically inactive state causes a large (≥ 20-fold) increase in the yield of triplet bacteriophlorophyll. In contrast we have found that the yield of bacteriochlorophyll fluorescence rises about 3-fold when reaction centres are made inactive [4]. Following the widespread assumption (see e.g. refs 5 and 6) that photochemical electron transfer is initiated from the singlet excited state, a 3-fold rise in fluorescence is consistent with a photochemical quantum efficiency of 0.7 in the active state; a significant yield of the triplet would also be expected in the active state but this is not observed. If we assume instead that the photochemistry is mediated by the triplet state we account easily for the high triplet yield in inactive reaction centres, but the variations in fluorescence must be ascribed to fortuitous changes in the rate of intersystem crossing.

Using newly derived optical extinction coefficients for reaction centre particles [7], a recalculation of earlier data [2, 4, 7] did indeed give values near 0.7 for the quantum efficiency of bacteriochlorophyll oxidation. However, a scrutiny of the literature shows that none of the reported, direct quantum yield determinations were of high precision and many gave very low values [8, 9]. Loach and Sekura [10] avoided the problem of the uncertainty of the extinction coefficient for the P870 component of bacteriochlorophyll by employing the indirect method of measuring the oxidation of added cytochrome *c* by a subcellular preparation of *Rhodospirillum rubrum*. Their technique was precise and gave a result close to unity, as have determinations of cytochrome oxidations in situ in *Chromatium* [11, 12].

Faced with this problem we were moved to redetermine, as carefully as possible the absolute quantum efficiency of bacteriochlorophyll oxidation in active reaction centres from *R. spheroides*. Using a ferrioxalate actinometer [13] as a standard and basing our calculations on the new extinction coefficients of Straley et al. [7], we obtained an efficiency of 1.02 ± 0.04. This is not consistent, in a simple model for direct photochemical quenching of the singlet state, with a 3-fold rise in fluorescence. The fluorescence rise should, therefore, be taken not as a quantitative component of a simple theory, but only as an empirically justified probe of the states of the reaction centres.

MATERIALS AND METHODS

Materials

Reaction centres were prepared from the carotenoidless strain R-26 of *R. spheroides* by treatment of a chromatophore suspension with 1 % lauryl dimethylamine oxide and subsequent purification of the reaction centre fraction. Two preparations were used in these experiments. One, purified by the method of Clayton and Wang [14] using $(NH_4)_2SO_4$ fractionation, was suspended in 0.3 % lauryl dimethylamine oxide (Preparation 1). The other was separated on a DEAE-cellulose column and brought off in 0.25 M NaCl, 0.01 M Tris–HCl (pH 8.0) containing 0.05 % Triton X-100 (Preparation 2).

For kinetic measurements the reaction centres were diluted, to a concentration in the range 1–3 μM, in 0.01 M Tris–HCl (pH 8.0), plus 0.05 % lauryl dimethylamine oxide or Triton X-100 and 15 μM ubiquinone (Coenzyme Q_6 from Sigma Chemical Co., St. Louis, Mo.). The ubiquinone slowed the back reaction of oxidized P870, allowing a greater steady state amplitude of the absorbance change at 865 nm under a given light intensity. This facilitated measuring the initial slope of the trace of absorbance versus time.

Potassium ferrioxalate was prepared by mixing stoichiometric amounts of potassium oxalate and $FeCl_3$, as described by Hatchard and Parker [3]. The product was recrystallized twice from hot water. All handling of potassium ferrioxalate was done in the dark or under a red safelight.

Methods

(*1*) *General optical arrangement.* The optical arrangement is shown schematically in Fig. 1. The light-induced oxidation of bacteriochlorophyll in reaction centres was measured by the change of absorbance at 865 nm (bleaching of P870), using a splitbeam spectrometer described earlier [5]. Samples were held in silica cuvettes of inside dimensions 1 cm × 1 cm × 3 cm. For measuring actinic light intensities, the reaction centre sample was replaced by actinometer solutions held in exactly the same optical configuration. This procedure compensated automatically for any unsuspected reflections or inhomogeneities of the actinic beam in the sample.

The actinic lamp was a 650-W tungsten–iodine lamp (Sylvania Type DWY). The beam was collimated by an appropriate lens, and passed through 2.5 cm of water in a 6 inch × 6 inch glass cell and then through a combination of filters including a second liquid cell (2 cm path) just outside the sample chamber. The second cell contained $CuSO_4$ in water (absorbance 8 at 800 nm) for excitation with blue or near ultraviolet light, and water for excitation at 600 nm and greater wavelengths. Next to this cell was a 2 inch × 2 inch interference filter (Baird Atomic, Inc.) or a Corning glass filter (see Table I). A manually operated shutter in the path of the actinic beam was made from three layers of cardboard separated by about 1 cm of air; the sides

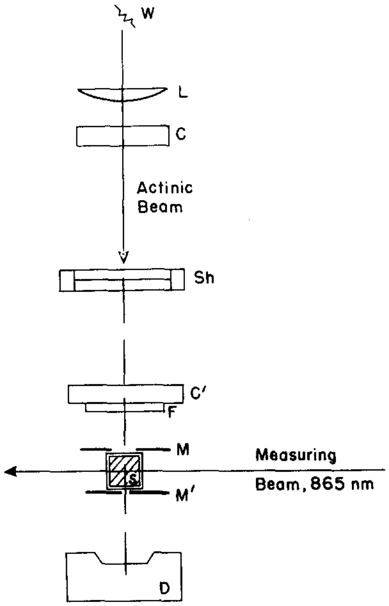


Fig. 1. Optical arrangement for measuring light-induced oxidation of bacteriochlorophyll in reaction centres and for irradiating actinometer solutions. Parts are not drawn to scale; see the text for important dimensions. S, sample in cuvette, in sample compartment of split-beam absorption spectrometer; D, detector (thermopile or photomultiplier); M′, mask with 2 mm × 10 mm aperture, present only during measurements of fractional absorption of the actinic light, using the photomultiplier; M, mask to prevent actinic beam from striking the edges of the cuvette; M,M′ and cuvette are all removed during measurements with the thermopile; F, interference filter or Corning glass filter; C′, liquid cell containing $CuSO_4$ solution or water; Sh, shutter; C, liquid cell containing water; L, collimating lens; W, tungsten–iodine lamp.

TABLE I

CHARACTERISTICS OF ACTINIC FILTER COMBINATIONS

Filter type	Peak wavelength (nm)	Band width at 50 % of peak transmission (nm)
Corning 7-39	363 (nominal)	50
	371 ("effective")*	
Corning 7-51	367 (nominal)	60
	377 ("effective")*	
Baird–Atomic interference filter	404	7
	436	6
	600	8
	760	10
	800	10
	880	10

* See Materials and Methods, part 4.

facing the sample were painted black and those facing the light source were painted white to minimize heat radiation which might give a large "dark" reading in thermopile measurements.

Because the measurements with reaction centres and with actinometer solutions were not made in a single experimental session, a thermopile was used to measure the actinic beam intensity in each case. We regarded the thermopile as a device for correlating light intensities in different measurements, and the ferrioxalate actinometer as our primary standard of radiation intensity.

Because of its size, the thermopile had to be situated in the actinic beam about 5 cm behind the position of the sample cell. We were interested in the absolute intensities indicated by the thermopile, so we took care to compare the average intensity over its sensor (about 1 cm in diameter) with that over the illuminated region of the sample cell. For this comparison we used a fiber optics probe leading to a photomultiplier to scan the plane perpendicular to the axis of the actinic beam at 2-mm intervals horizontally and vertically. The maximum variations of intensity over the illuminated areas of the cuvette face and the thermopile sensor were ± 4 % and ± 2 %, respectively (with standard deviations 2.5 % and 1 %). Since the actinometer was our primary standard, these inhomogeneities were inconsequential. The mean intensity over that part of the cuvette face exposed to actinic light was 1.016 times the mean intensity over the thermopile sensor. In measuring absorbance changes, only the central 12 mm (vertically) of the actinic beam intersected with the measuring beam and the mean intensity over this central part at the cuvette face was 1.033 times that over the thermopile sensor.

To measure the fraction of incident actinic light absorbed by the sample (reaction centres or actinometer solution) we replaced the thermopile by a photomultiplier, with opal glass forming a 1.5 inch circular window in the housing. A 2 mm × 10 mm slit placed just behind the cuvette ensured that the photomultiplier window collected all of the light passing through the system. The photomultiplier was Radio Corp. of America (Lancaster, Pa.) Type 1P28 for measurements in the visible and ultraviolet and Type 7102 for the near infrared. The linearity of these photomultipliers, operated with an Electrometer Amplifier (Model 201c, E-h Research Labs, Oakland, Calif.), was confirmed in measurements with neutral filters to within 1 % for each doubling of intensity.

(*2*) *Use of the thermopile.* The thermopile was an 8-junction Bi-Cu instrument from Eppley Laboratories, Newport, R.I. We covered the shiny parts of its case with a matt black mask, separated from the case by 2 mm of air, to suppress reflections. The thermopile was connected to a Beckman Model 14 Breaker Amplifier (Beckman Instruments, Fullerton, Calif.) and the output registered on a Bausch and Lomb (Rochester, N. Y.) Model VOM6 Recorder (2.5 mV). The amplifier had an internal voltage reference provided by a mercury battery which we calibrated with known input voltages in place of the thermopile, using circuits that presented the same input resistance as the thermopile. Our primary standard in this calibration was a pair of Weston Standard Electrolytic Cells, 1.02 V (Central Scientific Co., Chicago, Ill.).

The actinic intensity incident on the thermopile sensor, with all masks and cuvette removed, was measured as the average of several readings taken by opening and closing the shutter at about 1-min intervals, sufficient to let the signal reach a steady state. A quartz window over the sensor eliminated large fluctuations due to thermal convection. Radiant energy flux was computed from the photovoltage, using a calibration kindly provided by Dr H. H. Seliger, who compared our thermopile with some of his own which had been calibrated at the National Bureau of Standards. A correction (transmission) factor for the quartz window was determined with a photomultiplier for each actinic filter combination; this factor varied no more than ± 1.5 % between 365 and 880 nm. From the intensity at the thermopile sensor we could compute the average intensity at the cuvette face as discussed earlier.

We assume that the thermopile sensor, which bore an unmarred coating of lamp-black, had a sensitivity independent of wavelength over the range of our experiments. This is an important assumption because our most significant measurements with reaction centers were made with 880 nm actinic light and compared, via the thermopile, with actinometer measurements using blue and near ultraviolet light. Lee and Seliger [16] have previously found lamp-black to be reliable in this respect, whereas the usually thinner gold-black coatings may exhibit an optical window in the far infrared.

(*3*) *Determining the fraction of light absorbed by the samples.* Analysis of the effects of multiple reflections at the surfaces of the cuvette (see Appendix I) showed that three measurements are necessary to compute the fractional absorption: one with no cuvette (I_{00}), one with a cuvette containing solvent only (I_0), and one with the cuvette containing the sample (I). The fraction of the incident intensity I_{00} absorbed by the sample is given to a close approximation by

$$A = (I_0 - I)/\sqrt{I_0 I_{00}}$$

The rate of absorption by the sample is AI_{00}, with I_{00} determined through the thermopile measurement but referred ultimately to the actinometer.

We found that reflections from the cuvette can interact significantly with reflections from the metallized surfaces of an interference filter in the actinic beam. This was seen when we placed a narrow slit either in front of or behind the cuvette; more light was reflected to the photomultiplier in the latter case, but only if both the cuvette and an interference filter were in the path. This effect disappeared when the interference filter was replaced by a Corning glass filter transmitting in approximately the same wavelength region. We found that the effect could be eliminated by facing the most obviously reflective surface toward the light source.

To ensure no photochemical effect of the actinic beam on the sample during the measurements of fractional absorption, the actinic light source was attenuated 10-fold with a neutral density filter. This filter, placed between the two liquid cells, showed no reflective interactions with the cuvette. Exposure of the actinometer solution to the actinic beam was for only a few seconds in these measurements and even without a 10-fold attenuation the effect on the sample was negligible. With reaction centers the fractional change of absorbance caused by continuous illumination at these actinic intensities was also negligible, as measured with the spectrometer. Repeated measurements showed that fractional absorption could be determined with precision better than 1 % with our samples.

(*4*) *Converting energy flux to photon flux.* When narrow-band interference filters were used there were no complications associated with the conversion of incident energy into photon flux. The thermopile measurement, in $\mu W \cdot cm^{-2}$ was converted to $nEinsteins \cdot cm^{-2} \cdot s^{-1}$ using the peak wavelength value of the filter as determined on a Cary 14R Spectrophotometer, calibrated with the emission lines of mercury and hydrogen arcs. However, in the case of ultraviolet actinic light the comparatively broad spectral nature of the Corning glass filters, 7-39 and 7-51, made it necessary to determine an "effective" wavelength. This was done by multiplying the transmission curve of the filter by the black body radiation curve for the actinic lamp and by the percent absorption of the actinometer pyrolyte sample. The black body radiation curve of the lamp was calculated from an estimated colour temperature of the lamp (rated at 3400 °K at 120 V). The resulting filter spectrum was used to derive a median wavelength. The correction to the nominal peak wavelength was not large (< 5 %). Calculation of an "effective" wavelength for the narrow-band interference filters in the blue spectral region (404 and 436 nm) did not give a value significantly different from the measured peak value (< 0.2 %).

(*5*) *Actinometry.* Depending on the actinic wavelength, the concentration of ferrioxalate was adjusted to give fractional absorption ranging from about 45 to 95 %. Illumination times were chosen to produce about 200 nmoles of Fe^{2+} per 3 ml sample. Fe^{2+} was assayed as the orthophenanthroline complex, as described by Hatchard and Parker [13]. The absorbance of the complex was measured at 510 nm with the Cary 14R Spectrophotometer; with 200 nmoles the accuracy of this measurement was better than ± 1 %. The illumination times ranged from 100 to 1000 s and the actinic intensity was monitored with a photomultiplier to certify its constancy throughout these long exposures.

(*6*) *Measuring the absorbance change in reaction centres.* In the absorption spectrometer, monochromatic light modulated at 1000 Hz was split into a sample channel and a reference channel and directed onto two photomultipliers. The difference signal was processed by a preamplifier (Model CR-4, Princeton Applied Research Princeton, N. J.) and a phase-sensitive amplifier (Princeton Applied Research Model JB-5) and the demodulated output, proportional to changes of sample measuring beam intensity, was recorded by any or all of three means: an Esterline–Angus Instrument Co. (Indianapolis, Ind.) Model O-293 Recorder, a Siemens Corp. (Iselin, N. J.) Oscillomink P Recorder, and a Hewlett–Packard (Berkely Heights, N. J.) Model

141A Storage Oscilloscope. These devices had time constants of 10 ms, 1 ms, and <1 μs, respectively. The time constant of the amplifying system was about 20 ms when the output filter was set nominally at 10 ms. At this setting, using the storage oscilloscope, the traces of absorbance vs time showed no response time limitations on the time scale of our measurements with reaction centres. The responses of the chart recorders were checked against the storage oscilloscope to detect any spurious instrumental response limitations. We compared initial slopes and step-changes, recorded simultaneously on all three instruments. For slopes near 45 ° at chart speeds of 20 or 25 $\mathrm{mm \cdot s^{-1}}$, representative of most of our measurements, the Siemens Recorder agreed exactly with the oscilloscope. The Esterline–Angus Recorder gave slopes that were 9 % lower than the others in comparison with step-changes. Data obtained with this recorder were corrected accordingly, but we came to rely on the Siemens for our most exacting measurements.

The output of the system was proportional, of course, to the photovoltage chosen to represent 100 % of the beam intensity. Great care was taken with this setting, which was potentially a major source of inaccuracy. Once this had been set, the responses to small changes in the sample beam were calibrated directly by interchanging sample cuvettes containing water and ones containing standardized solutions of $CuSO_4$ in dilute H_2SO_4. The standard $CuSO_4$ solution (see ref. 17) was prepared and donated by Dr J. R. Bolton. Undiluted, its absorbance at 800 nm was confirmed to be 0.952 for 1 cm path by direct transmittance measurements of a 3-fold dilution, using our system for measuring fractional absorption by samples.

The wavelength of the measuring beam was set at 865 nm to record the light-induced bleaching of P870 in reaction centres. Five to ten traces were recorded at each setting; an example of one is shown in Fig. 2. On the time scale employed, the manual opening of the shutter caused no limitation, as can be seen by the sharp break in the trace at the onset of illumination. Both of us independently measured and averaged the initial slopes, using a front–surface mirror as suggested by Bolton et al. [2].

The reaction centre samples were usually adjusted to an absorbance of about 0.2 at the actinic wavelength. In some cases, as with 436-nm excitation, a lower value was needed in order to avoid excessive absorbance at 865 nm. Too high an absorbance at the actinic wavelength can cause errors due to non-uniform distribution of actinic intensity across the zone of the measuring beam [6]; we show in the next Section that this problem was not apparent in the absorbance range used.

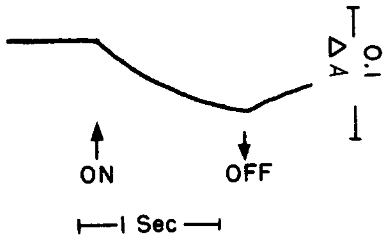


Fig. 2. Photograph of a trace of absorbance at 865 nm vs time, showing the light-induced oxidation of bacteriochlorophyll in a suspension of reaction centres from *R. spheroides* R-26. The trace was made with a Siemens Oscillomink Recorder. Actinic light was 2.17 nEinsteins $\cdot \mathrm{cm^{-2} \cdot s^{-1}}$ at 880 nm; 34 % of the light incident on the cuvette was absorbed by the sample. The width of mask M (see Fig. 1) was 8.45 mm.

RESULTS

Controls

(*1*) *Width of actinic beam aperture.* Lee and Seliger [16] reported that when the actinic beam overlapped the edges of the sample cuvette of a ferrioxalate actinometer an anomalous increase in the quantum yield was found. We used masks with apertures 2.92–8.45 mm wide so as to restrict the actinic beam within the 10 mm internal dimensions of the cuvette. We observed that for both the ferrioxalate reaction and the oxidation of reaction centres, the photoproduct formed per s was proportional to the aperture width when other factors were kept constant. The computed quantum efficiency was then independent of aperture width. Thereafter, we used the mask with the widest aperture, 8.45 mm.

(*2*) *Reaction centre photochemistry: absence of significant excitation by the measuring beam. Sufficiency of spectral definition.* In most measurements the slits of the measuring beam monochromator were set at 0.2 mm to give a band width (at half-maximum) of 10 nm at the measuring wavelength of 862 nm. When the slits were closed to 0.1 mm, so as to reduce the measuring beam intensity 4-fold and its band width 2-fold, there was no change in the observed rate of P870 bleaching at a given intensity of actinic light. Thus we conclude that the measuring beam was not significantly actinic and that spectral resolution was sufficient. This test was performed with actinic light of 880 nm at about 0.5 nEinsteins · $cm^{-2} \cdot s^{-1}$.

(*3*) *Reaction centre photochemistry: freedom from shading artifact.* The measuring beam was about 4 mm wide as it passed through the center of the sample cuvette. If the absorbance of the sample at the actinic wavelength had been too great, an error would have resulted from the non-uniform distribution of actinic intensity through the zone of measurement. We measured the quantum efficiency of bacteriochlorophyll oxidation in reaction centres, using 880-nm actinic light at about 0.3 nEinsteins · $cm^{-2} \cdot s^{-1}$, with three concentrations of reaction centres: $A_{880\ \mathrm{nm},\ 1\ \mathrm{cm}} = 0.12$, 0.24, and 0.36. No significant or systematic differences were observed in the relative quantum yields showing that the foregoing source of error was negligible. In most of our measurements the absorbance at the actinic wavelength was 0.2.

(*4*) *Reaction centre photochemistry: dependence of apparent quantum efficiency on actinic light intensity.* In preliminary experiments we found that if the rate of quantum

TABLE II

REACTION CENTRES, ABSORBANCE = 0.2 AT THE ACTINIC WAVELENGTH OF 880 nm

Each datum is the average of five to ten measurements; slopes were evaluated by each of us and averaged together.

Absorbed actinic intensity AI_{00} (nEinsteins · $cm^{-2} \cdot s^{-1}$)	Apparent quantum efficiency for bacteriochlorophyll oxidation
0.059	0.83
0.125	0.87
0.245	0.98
0.41	0.95
0.60	0.98
0.83	0.97
1.10	1.01

absorption was less than about 0.2 nEinsteins $\cdot$ $cm^{-2} \cdot s^{-1}$ the quantum efficiency was anomalously low. This is shown in Table II for reaction centres in lauryl dimethylamine oxide. At values of AI_{00} greater than about 0.2 nEinsteins $\cdot$ $cm^{-2} \cdot s^{-1}$, the measured quantum efficiency showed no systematic variation with actinic intensity. We have not identified the cause of the deficiency at low actinic intensities.

Quantum efficiency of bacteriochlorophyll oxidation: outline of computation

The procedures described above gave the following data for each experimental point: (a) An initial slope of the absorbance change, averaged over 5–10 separate measurements. The slopes were independently determined by both of us. This was converted to absorbance $\cdot$ s^{-1} via the calibration with standard $CuSO_4$ solution. (b) The fractional absorption, A, of the actinic wavelength by the sample determined before and after the actinic measurements. (c) A thermopile measurements, in μV, of the actinic light intensity in the absence of cuvette and mask (I_{00}). This was converted to $\mu W \cdot cm^{-2}$ by means of the nominal thermopile calibration (intensity dependent), the quartz window factor (wavelength dependent) and the sensor position to cuvette face intensity ratio (1.033). Conversion to nEinsteins $\cdot$ $cm^{-2} \cdot s^{-1}$ was by the appropriate factor for the "effective" actinic wavelength.

Expressing the differential extinction coefficient $\Delta\varepsilon$ in $cm^2 \cdot nmole^{-1}$, absorbed intensity AI_{00} in nEinsteins $\cdot$ $cm^{-2} \cdot s^{-1}$ and width of the actinic beam aperture x in cm, the quantum efficiency is given (see ref. 6) by

$$\Phi_P' = \frac{\Delta \text{ absorbance/s}}{x(AI_{00})\Delta\varepsilon}$$

From Straley et al. [7], $\Delta\varepsilon = 112\ mM^{-1} \cdot cm^{-1} = 0.112\ cm^2 \cdot nmole^{-1}$.

This value for the quantum efficiency is based on the nominal sensitivity of the thermopile. Using this nominal sensitivity we obtained an average quantum efficiency for the ferrioxalate actinometer that was 1.01 times the accepted value as given by Lee and Seliger [16] (see later). Taking the accepted quantum efficiency of the ferrioxalate actinometer as our primary standard, we obtain $\Phi_P = \Phi_P'/1.01$.

Excluding uncertainties in the accepted values of $\Delta\varepsilon$ for P870 bleaching and of the quantum efficiency of the ferrioxalate actinometer, we estimate uncertainties in the measurements with reaction centres as follows:

Calibration with $CuSO_4$ solution	±0.7 %
Setting measuring beam photocurrent	±1.5 %
Measuring slopes	±3 %
Precision of thermopile measurements	±0.7 %
Measurement of fractional absorption, A	±1 %
Square root of sum of squares of the above	±3.6 %

The quantum efficiency of the ferrioxalate actinometer was determined in three wavelength regions; near ultraviolet (371–377 nm), 404 nm and 436 nm, using a variety of concentrations of pyrolyte (0.0015 M, 0.006 M and 0.012 M). No significant dependence on concentration was noted in the range used and all determinations were included in the final averaging of 31 measurements.

Accountable sources of error for the quantum efficiency of the ferrioxalate actinometer include the following:

Pipetting of sample	±1 %
Pipetting of assays and spectrophotometric estimation of Fe^{2+}	±1 %
Measurement of fractional absorption, A	±0.3 % in ultraviolet ±0.7 % at 404 nm ±1 % at 430 nm
Thermopile window factor	±1 %
Precision of thermopile measurement	±2 %
Square root of the sum of the squares	±2.9 %

Factors contributing to the operation of the thermopile as a device for measuring absolute energy fluxes have been omitted from this list, since they are not applicable to assessment of the overall error for the quantum efficiency of bacteriochlorophyll oxidation. Such factors include thermopile calibration, calibration of the National Bureau of Standards radiant energy standard lamp and conversion of energy incident on the thermopile to that incident on the sample. From Lee and Seliger's data [16] and from consideration of our particular experimental set up we estimate the total uncertainty for the quantum efficiency of the ferrioxalate actinometer to be ± 3.4, which is in good agreement with the observed standard deviations (see Table III).

TABLE III

SUMMARY OF MEASUREMENTS OF THE QUANTUM EFFICIENCY OF THE FERRIOXALATE ACTINOMETER

Wavelength (nm)	ϕ*	Number of determinations	Literature value of ϕ (refs 6 and 14)	Percent deviation from literature value
371–377	1.26 ± 0.03	6	1.24–1.25	+1.5
404	1.155 ± 0.06	15	1.14	+1.3
436	1.105 ± 0.03	10	1.11	−0.5
			average:	+0.8

* The uncertainty value is the standard deviation of the experimental determinations.

Summarized quantum efficiency measurements. Our determinations of the quantum efficiency of the ferrioxalate actinometer are summarized in Table III.

The average values referred to our thermopile are 1.01 times the accepted values of Hatchard and Parker [13] and Lee and Seliger [16]. This factor is used in computing the quantum efficiency of photochemistry in reaction centres.

In Table IV we present all of our determinations (except for a few preliminary measurements) of the quantum efficiency of bacteriochlorophyll oxidation in reaction centres with lauryl dimethylamine oxide, using 880-nm actinic light. We present only the values for which $AI_{00} > 0.2$ nEinsteins · $cm^{-2} \cdot s^{-1}$ so that the measured efficiency did not vary with actinic intensity (see Table II).

Combination of the attributable errors described above for the measurements

TABLE IV

THE QUANTUM EFFICIENCY OF BACTERIOCHLOROPHYLL OXIDATION (Φ_P)

Reaction centres with 0.05 % lauryl dimethylamine oxide; actinic light, 880 nm. Data represent three experimental sessions; further details in text.

Expt	ϕ_P
1	1.00
	1.03
	1.01
	1.03
2	1.02
	1.04
3	1.00
	1.03
average	1.02 ± 0.035*

* Deviation computed from individual measurements.

TABLE V

COMPARISON OF QUANTUM EFFICIENCIES OF BACTERIOCHLOROPHYLL OXIDATION IN TWO TYPES OF REACTION CENTRE PREPARATION

Actinic wavelength	Actinic intensity, I_{00} (nEinsteins · cm^{-2} · s^{-1})	Reaction centres with 0.05 % lauryl dimethylamine oxide		Reaction centres with 0.05 % Triton X-100	
		A	ϕ_P	A	ϕ_P
880	1.04	0.33	1.00	0.345	0.98
	2.17	0.33	1.03	0.345	1.06
760	0.95	0.42	0.82	0.39	0.83
	2.17	0.42	0.83	0.39	0.90

TABLE VI

DEPENDENCE OF THE QUANTUM EFFICIENCY OF BACTERIOCHLOROPHYLL OXIDATION ON ACTINIC WAVELENGTH

Each datum is the average of five to ten measurements.

Actinic wavelength (nm)	Relative* ϕ_P
880	1.00
800	0.93
700	0.84, 0.81, 0.85
600	0.96
404	0.89, 0.97, 0.85, 0.77
365	0.93

* Relative to ϕ_P at 880 nm normalized to 1.00.

of bacteriochlorophyll oxidation and for the ferrioxalate actinometer leads to an expected deviation for the quantum efficiency of bacteriochlorophyll oxidation of ± 4.6 %. This is reasonably close to the observed deviation of ± 3.5 %.

The reaction centres with 0.05 % Triton X-100 gave essentially the same results as those with 0.05 % lauryl dimethylamine oxide, as shown in Table V. The quantum efficiency varied slightly with wavelength (see Table VI). Many of these measurements involved low rates of quantum absorption for which the measured efficiency was anomalously low; we show here only relative values, but these are all based on comparisons made at approximately equal rates of absorption.

DISCUSSION

The quantum efficiency of bacteriochlorophyll photooxidation in reaction centres has been found to be equal to 1.02 ± 0.04. Values of greater than unity may be questioned on theoretical grounds and we consider that for practical purposes our result does, in fact, indicate a quantum efficiency of greater than 0.98 for actinic light at 880 nm.

The determination of the quantum efficiency of bacteriochlorophyll oxidation which we have presented here is of high precision, with an expected uncertainty of less than 5 %. This uncertainty is purely experimental and does not include the dependence of the calculated efficiency on the values we have used for the extinction coefficient for P870 bleaching or for the quantum efficiency of the ferrioxalate actinometer. Thus, should either of these parameters undergo radical revision, the quantum efficiency we have determined can be readily re-evaluated. We do not consider this event to be very likely, especially in the case of the quantum efficiency of the ferrioxalate actinometer.

The wavelength dependence of the quantum efficiency (Table VI) prompts some comment. The efficiency at 800 nm is 7 % lower than that at 880 nm. This could be due to the presence of some inactive reaction centres, already bleached at the long wavelength peak; inactivation due to prior reduction of the primary acceptor could not account for this discrepancy since the efficiency at 880 nm would be similarly affected. However, addition of dithionite to the samples caused an increase in the 865-nm peak height which was less than 3 %. A second possibility is that some overlap with the bacteriopheophytin absorption band occurs at 800 nm, and the lower efficiency of light absorbed by this pigment contributes to an apparent lowering of the efficiency for P800. However, the efficiency of light absorbed at 600 nm, where both P800 and P870 absorb but not bacteriopheophytin, is intermediate between those at 800 nm and 880 nm. This indicates that the discrepancy is real and leads one to consider the possibility that excitation energy transfer from P800 to P870 is significantly less than 100 %. This is speculative and further work is required to test this possibility, but it is of considerable interest since it would furnish information on the degree of coupling between the four bacteriochlorophyll molecules in the reaction centre and on the "separate molecule" approximation widely used for the two bacteriochlorophyll species.

The lower efficiency of light absorbed at 760 nm is not unexpected since there is the possibility of absorption by non-essential bacteriopheophytin. The ratio of the 760 nm peak to that at 865 nm in pure reaction centres is not precisely known and fluctuates by about 10 % in different preparations. The ratios of absorbance ($A_{760\text{ nm}}/A_{865\text{ nm}}$) in the two preparations used here were 1.09 (Preparation 1) and 0.96 (Preparation 2), the latter being the lowest we have obtained. The quantum efficiencies at 760 nm in the two cases did not differ by more than 5 % and it is probable that the efficiency of light absorbed by "essential" bacteriopheophytin is indeed some 15 % lower than that for 880-nm light.

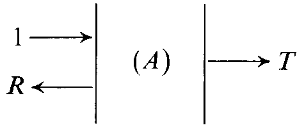
It is well established that in the presence of electron donors the fluorescence yield of reaction centres increases about 3-fold as the primary electron acceptor becomes reduced [4]. If the photochemistry were singlet-mediated, a simple interpretation would suggest that the quantum yield of photochemistry is about 0.7, as given by $\Phi_P = 1-\Phi_f^{\min}/\Phi_f^{\max}$ (see, for example, ref. 6). Although such a simple model requires many implicit assumptions, such as constancy of intrinsic rate constants of deexcitation, in the absence of strong evidence to the contrary this description of events in photosynthetic systems has been widely utilized.

There are now two powerful, independent reasons for questioning this model; one is the result of this work which establishes the quantum efficiency of photochemistry to be 1.00, within experimental error, rather than 0.7. The other is that a recent study on the triplet state of bacteriochlorophyll in reaction centres has shown that the relative quantum efficiency of triplet formation, at low temperature, increases at least 20-fold as the traps become inactive [3], in conflict with the 3-fold increase in fluorescence. Either one of these results would render the relationship between Φ_P and Φ_f invalid. However, by assigning separate fluorescence yields to active and inactive traps, fluorescence may be retained as a proportional indicator of photochemical activity. With this support for the reactive singlet model of photosynthesis undermined, it is appropriate to reconsider the possibility for a triplet role in the primary photochemistry.

APPENDIX

Computing the fraction of light absorbed by the sample

Consider an intensity of light, normalized to unity, incident on the cuvette. Let the fractions reflected, transmitted and absorbed be denoted R, T and A, respectively.



$$R+T+A = 1 \qquad [1]$$

If the cuvette contains only a non-absorbing solvent*, $A = 0$ and T is designated T_0. In practice we measure the intensities I_{00} with no cuvette (here normalized to unity), I_0 transmitted through the cuvette with solvent, and I through the cuvette with sample. Then $T = I/I_{00}$ and $T_0 = I_0/I_{00}$. We wish to evaluate A from these measurements.

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* A detailed empirical study showed that negligible error is introduced by solvent absorbance up to 0.05. If the ensuing formulae are applied as if the solvent were non-absorbing one obtains the correct rate of absorption by the solute in the presence of the absorbing solvent.

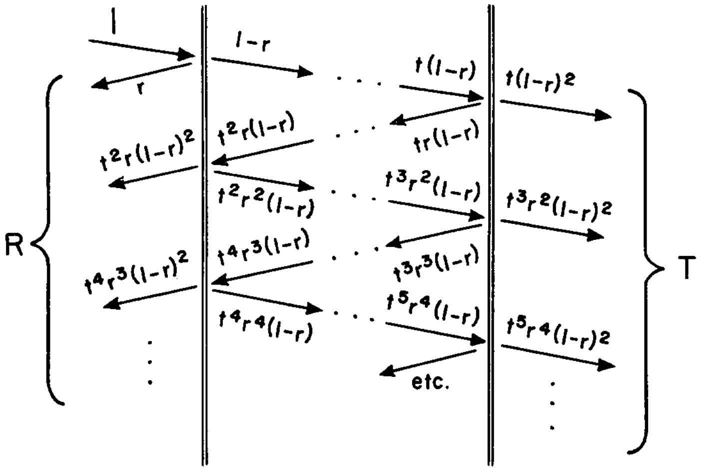

Fig. 3. Showing the relative intensities of successive multiple-reflected and transmitted components of light passing through a cuvette. A fraction r is reflected at each wall and a fraction t is transmitted in one traverse of the cell contents. The total transmitted and reflected intensities, normalized to unity incident on the cell, are T and R.

Imagine the individual reflections and absorptions that attend single traverses of the cuvette; let r = fraction reflected at one wall (both surfaces); t = fraction transmitted in one traverse of the contents; $1-t$ = fraction absorbed in one traverse. The relative intensities of successive multiply reflected and transmitted components are shown in Fig. 3. Adding all transmitted components as the sum of a geometric progression gives

$$T = t(1-r)^2(1+t^2r^2+t^4r^4+\ldots) = t(1-r)^2/(1-t^2r^2) \qquad [2]$$

and for the reflected components,

$$R = r+rt^2\,(1-r)^2/(1-t^2r^2) \qquad [3]$$

Combining Eqns 1–3 and rearranging gives

$$A = (1-t)\,(1-r)/(1-rt) \qquad [4]$$

Now we must express r and t in terms of the measured quantities T and T_0. With (non-absorbing) solvent only, $t = 1$ and Eqn 2 reduces to $T_0 = (1-r)/(1+r)$ or

$$r = (1-T_0)/(1+T_0) \qquad [5]$$

Substituting this in Eqn 4 gives

$$A = 1-T-(1+tT)\,(1-T_0)/(1+T_0) \qquad [6]$$

Next we substitute Eqn 5 in Eqn 2 and solve for t, obtaining

$$tT = [\sqrt{4T_0^4+T^2(1-T_0^2)^2}-2T_0^2]/(1-T_0)^2 \qquad [7]$$

For T_0 greater than about 0.9, the second term under the radical sign is much smaller than the first. A binomial expansion then gives, to a very close approximation,

$$tT = T^2(1+T_0)^2/4T_0^2 \quad [8]$$

and Eqn 6 becomes

$$A = 1-T-[1+T^2(1+T_0)^2/4T_0^2]\,(1-T_0)/(1+T_0) \quad [9]$$

Empirically, for T_0 about 0.9 or greater, we find that the alternative simpler expression

$$A = (T_0-T)/\sqrt{T_0} = (I_0-I)/\sqrt{I_0 I_{00}} \quad [10]$$

agrees within 1.5 % with the exact expression, for all A between 0.1 and 1. Eqn 10 is the formula that would result if only one reflection at each wall were considered.

With incident intensity I_{00}, the rate of absorption by the sample is then AI_{00}.

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