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PHOTOCHEMICAL ACTIVITIES OF $K_3Fe(CN)_6$-TREATED CHROMATOPHORES FROM *RHODOSPIRILLUM RUBRUM*

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SUMMARY

1. The bulk of the bacteriochlorophyll from chromatophore particles of *Rhodospirillum rubrum* is irreversibly bleached by $K_3Fe(CN)_6$. The preparations show light-induced absorption changes with maxima at about 305, 325, 365, 435, 550, 700, 790 and 1250 nm and minima at about 270, 385, 605, 810 and 865 nm. The kinetics of the absorption changes at 270, 365, 435, 605, 700 and 865 nm were found to be similar.

2. After extraction with ethanol and reduction with KBH_4, ubiquinone and/or ubiquinol could be identified in the $K_3Fe(CN)_6$-treated chromatophores. The light-induced difference spectrum with a minimum at about 270 nm and a maximum at about 305 nm indicates that the ubiquinone present in the particles is reduced upon illumination.

3. It was found that the amounts of oxidized P_{890} and of reduced ubiquinone involved in the same light reaction, are approximately equal. If it is assumed that these amounts are indeed the same, an absorbtivity of $8.3 \cdot 10^4$ to $9.3 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ can be calculated for the absorption maximum of P_{890} in the bleached chromatophores.

4. The quantum requirement for the photooxidation of P_{890} was estimated to be 0.8 to 1.3. The quantum requirements for the photoreduction of one molecule of ubiquinone and for the photooxidation of one molecule of P_{890}, measured in the same sample, were found to be equal. This indicates that the efficiency for electron transport is twice as high for ubiquinone reduction as for P_{890} oxidation.

5. No light-induced cytochrome oxidation could be demonstrated in the bleached chromatophores. However, added horse-heart cytochrome *c* is oxidized in the light.

6. The action spectrum for the photooxidation of the added cytochrome agrees with that of P_{890} photooxidation. The most plausible explanation is that the oxidation of the cytochrome is caused by photooxidized P_{890}.

Abbreviation: DCIP, 2,6-dichlorophenolindophenol.

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INTRODUCTION

Evidence will be given in this paper to support the hypothesis that in bacterial photosynthesis bacteriochlorophyll is, in one form or another, involved in a primary photochemical oxidation–reduction reaction. The discovery by DUYSENS and co-workers[1–3] of a specialized form of bacteriochlorophyll (P_{890})* that can reversibly be oxidized by light, and the observation of a light-induced cytochrome oxidation[4,5] form the basis of the hypothesis that P_{890} is the primary electron donor in bacterial photosynthesis and that oxidized P_{890} in turn oxidizes a cytochrome. An unknown electron acceptor X is postulated to be reduced when P_{890} is photooxidized.

The treatment of chromatophores of pheophytinized cells of a blue-green mutant of *Rhodopseudomonas spheroides* with Triton X-100 by CLAYTON[6] resulted in particles without accessory pigment, which still showed a reversible photooxidation of P_{890} (in CLAYTON's nomenclature P_{870}). CLAYTON[6] reported that these preparations also show a light-induced cytochrome reaction and a photoreduction of ubiquinone, but no experimental data were given. Earlier, CLAYTON had demonstrated[7], however, that the light-induced difference spectrum of intact *Chromatium* chromatophores exhibited a minimum at 270–275 nm which probably had to be ascribed to a reduction of ubiquinone. He supposed that this quinone might be the primary electron acceptor.

It has been shown[8–11] that treatment of chromatophores from purple bacteria with K_2IrCl_6 also bleaches the bacteriochlorophyll without apparent destruction of the photoactive pigments.

We obtained photoactive preparations by $K_3Fe(CN)_6$ treatment of chromatophores from purple bacteria. In these preparations most of the bacteriochlorophyll and carotenoid is irreversibly bleached. The absorption spectrum of the bleached *Rhodospirillum rubrum* chromatophores resembles that of the Triton X-100- and K_2IrCl_6-treated chromatophores of the *Rps. spheroides* mutant and that of the K_2IrCl_6-treated chromatophores of various other purple bacteria. The light-induced difference spectrum closely resembles that of non-bleached *R. rubrum* chromatophores.

The present investigation includes a comparison of the kinetics of light-induced absorbance changes in the $K_3Fe(CN)_6$-treated chromatophores at various specific wavelengths and for different intensities of actinic light. Evidence is given that the light-induced difference spectrum of these chromatophores in the ultraviolet wavelength region may be due to ubiquinone reduction. Quantum requirements of the light-induced bleaching of P_{890} and of ubiquinone reduction were determined.

No light-induced cytochrome oxidation could be demonstrated in the $K_3Fe(CN)_6$-treated chromatophores. However, addition of horse-heart cytochrome *c* led to a photooxidation of this compound. In order to ascertain whether this oxidation could be the result of a supposed reaction of the cytochrome with oxidized P_{890} or with another pigment, action spectra for the photooxidation of P_{890} and of cytochrome oxidation were determined.

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* Although for different species of purple bacteria and for chemically bleached chromatophores the maxima of the absorption bands of the specialized bacteriochlorophyll are situated at somewhat different wavelengths, we shall continue to call this pigment P_{890}.

METHODS AND MATERIALS

Preparation of chromatophore particles from R. rubrum

R. rubrum, strain 1 (van Niel) was cultured in stoppered flasks of 1 l at 25° in a medium containing pepton–NaCl (see ref. 12). The organisms were illuminated at an intensity of about 20000 lux from fluorescent tubes and electric bulbs. Generally, the bacteria were harvested after 4 to 10 days by centrifugation for 5 min at 10000 × *g* in a cooled centrifuge. The following procedures were also carried out at about 0°: The sediment was washed with 0.05 M Tris–HCl buffer (pH 7.4). Thereafter the cells were centrifuged again. This treatment was repeated twice. A slightly viscous suspension of cells in Tris buffer was sonicated for 20 min at 10 kcycles/sec, using a Raytheon sonic oscillator (model DF 101). Unbroken cells and cell debris were centrifuged at 10000 × *g* for 5 min and the supernatant was centrifuged for 20 min at 40000 × *g*. The sediment was discarded and the remaining supernatant centrifuged during 2 h at 40000 × *g*. Finally the crude chromatophores were suspended in Tris buffer with sucrose (15 %, w/w) and stored at −20°.

$K_3Fe(CN)_6$ treatment of chromatophores

About 8 ml of a suspension of chromatophore particles ($E_{1\ cm}$ approx. 45 at 880 nm) were dialyzed against 500 ml of a 0.1 M $K_3Fe(CN)_6$ solution in water at a temperature of 25–29° for at least 50 h in diffuse daylight (or in light from fluorescent tubes at an intensity of about 10000 lux). Thereafter the suspension was dialyzed against water at 0° in the dark. The bleached green chromatophores were centrifuged for 45 min at 36000 × *g* in a cooled centrifuge and the sediment was resuspended by sonication in 16 ml of Tris buffer. In most cases the order of magnitude of $E_{1\ cm}$ at 800 nm was 1.

Qualitative determination of ubiquinone and/or ubiquinol

About 2 ml of a suspension of chromatophores or 5 ml of the bleached chromatophores were centrifuged for 45 min at 36000 × *g*. The sediment was extracted with about 4.5 ml ethanol or ethanol–isooctane (1:1, v/v). After recording of the ultraviolet spectrum (1-cm light path) some grains of KBH_4 were added to the solution and the spectrum was recorded again.

Measurements

The light-induced absorbance changes were measured with a split-beam differential spectrophotometer[13]. The absorbance changes were brought about by actinic illumination from a dc slide projector. Colour filters (Schott) combined with interference filters (Schott and Balzers) were placed in the actinic beam for the selection of the appropriate wavelengths.

Intensities of actinic illumination were measured by means of a calibrated photocell and are given in Einstein·cm^{-2}·sec^{-1}. The intensity 'seen' by a suspension in a 1-mm vessel in the apparatus is 0.48 times the intensity of the actinic beam. The photomultiplier used for the ultraviolet region was a Dumont 7664 (S-13 type). For the visible region an RCA 5819 (S-11 type) and an RCA 7326 (S-20 type) photomultiplier were used and for the near-infrared region a Dumont 6911 (S-1 type). In the ultraviolet region (< 350 nm) a Corning 7-54 filter, 3 mm thick, was placed in

front of the photomultiplier. In the other cases Schott colour filters or a combination of interference filters and colour filters were used.

Absorbance measurements were carried out in a Zeiss PMQ II spectrophotometer; opal glass was placed behind both the sample and reference vessels to minimize the effects of scattering[14]. For the comparison of the absorbance at 800 nm with the absorbance at 860–865 nm or with the absorbance change at 860–865 nm, a further correction for scattering was made by subtracting the apparent absorbance at 970 nm from the measured value. Absorption spectra were recorded with a Beckman DK 2 spectrophotometer and a Unicam SP 700 spectrophotometer.

RESULTS AND DISCUSSION

The absorption spectrum of the $K_3Fe(CN)_6$-treated *R. rubrum* chromatophore particles in the reduced state is given in Fig. 1. Nearly all the bacteriochlorophyll has disappeared. The absorption maxima at 590, 800 and 865 nm are presumably due to two bacteriochlorophyll types (P_{800} and P_{890}), which are supposed to play a part in bacterial photosynthesis[10,11]. The absorption band at 690 nm, apparently caused by an oxidation product of bacteriochlorophyll, is higher than the band found at about 690 nm in Triton X-100-treated *Rps. spheroides* chromatophores[6] and in K_2IrCl_6-treated chromatophores of *R. rubrum*, *Rps. spheroides* and *Chromatium*[8,11].

As can be seen from Fig. 1, addition of an oxidizing agent (such as $K_3Fe(CN)_6$) to the treated chromatophores results in a bleaching of the 865 nm band due to P_{890} oxidation and a shift to shorter wavelengths of the 800 nm band (P_{800}). Chemical oxidation also results in an absorption increase at 1250 nm and an absorption decrease at about 600 nm. Similar changes produced by chemical oxidation have been reported for intact chromatophores of various purple bacteria[15,16].

The light-induced near-infrared difference spectrum of the $K_3Fe(CN)_6$-treated chromatophores in Fig. 2 shows a bleaching of P_{890} and a shift to shorter wavelengths of P_{800}. As compared to the difference spectrum of intact chromatophores the minimum of the band of P_{890} in the bleached chromatophores is situated at a shorter wavelength (865 nm). This phenomenon probably results from a structural change of the protein and/or a minor change in the pigment molecule. The absorbance changes at

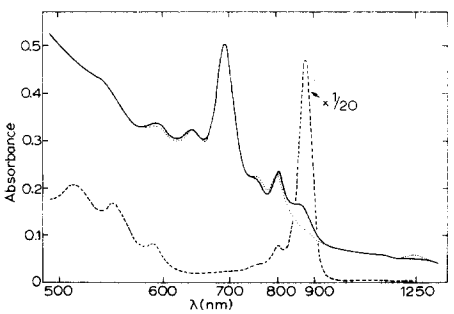

Fig. 1. ——, absorption spectrum of untreated *R. rubrum* chromatophore particles. ———, absorption spectrum of $K_3Fe(CN)_6$-treated chromatophore particles of *R. rubrum* in the reduced state. ....., absorption spectrum of these particles in the oxidized state. Oxidation was brought about by the addition of 6 mM $K_3Fe(CN)_6$. The absorption spectrum of the intact chromatophores has been reduced 20 times.

790 and 810 nm relative to that of the minimum of P_{890} are about 0.6 times as high as those in whole cells or chromatophores of *R. rubrum*[16,17]. It is possible that in intact chromatophores a part of the surrounding bacteriochlorophyll that absorbs around 800 nm (B_{800}) is also shifted to shorter wavelengths.

In Fig. 2 are also shown the light-induced difference spectra of the bleached chromatophores in the regions of 340–470 nm and of 500–660 nm. We found similar difference spectra in *R. rubrum* cells, suspended in a mixture of potassium glycerophosphate and glycerol (unpublished results). However, in the last case, additional decreases can be seen at about 420, 520 and 550 nm, even under aerobic conditions due to cytochrome oxidation. In the bleached chromatophores neither light-induced

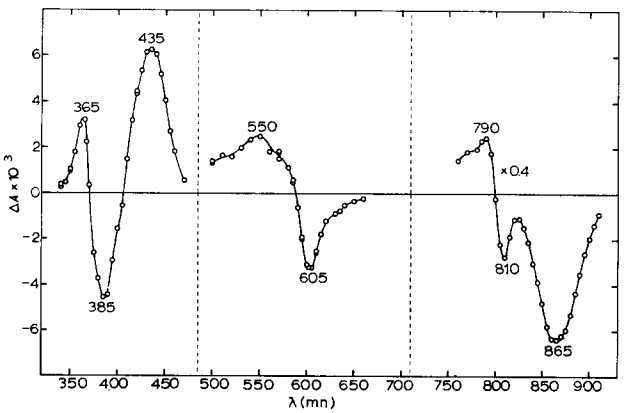

Fig. 2. Light-induced difference spectrum of $K_3Fe(CN)_6$-treated *R. rubrum* chromatophores. The spectra in the regions of 340 to 470 nm and 500 to 660 nm were obtained with actinic light of wavelength 800 nm and intensity of 1.9 nEinstein·cm^{-2}·sec^{-1}. The spectrum in the near-infrared region was obtained with actinic light of wavelength 593 nm and intensity of 1.8 nEinstein·cm^{-2}·sec^{-1}. The last spectrum has been reduced 2.5 times. The three spectra were obtained with different samples.

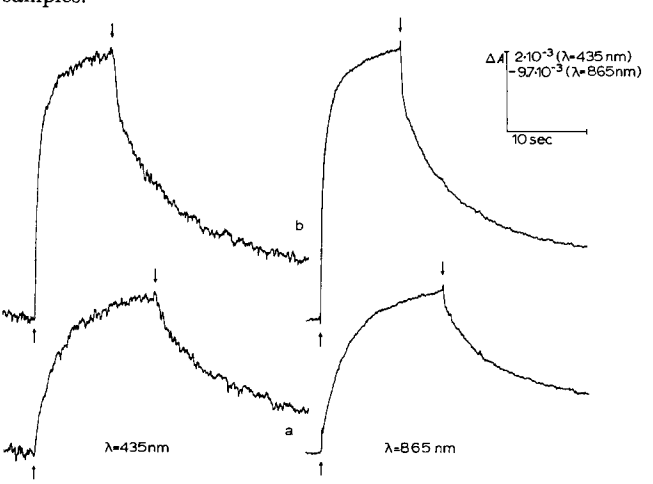

Fig. 3. A comparison of the kinetics of absorbance changes at 435 nm (left) and at 865 nm (right) of $K_3Fe(CN)_6$-treated *R. rubrum* chromatophores. The intensities of the actinic light, λ = 800 nm, were 0.7 nEinstein·cm^{-2}·sec^{-1} (a) and 3.5 nEinstein·cm^{-2}·sec^{-1} (b). The switching on and off of the actinic light is indicated by upward and downward pointing arrows, respectively.

nor chemically induced changes in the oxidation state of cytochrome could be detected.

The time courses of the light-induced absorbance changes for the maxima at 365 and 435 nm and the minima at 385, 605 and 865 nm were practically the same. Fig. 3 shows the time courses at 435 and 865 nm for two different intensities of the actinic light. In Table I, the ratio of the steady states for two intensities is given. The steady state was nearly saturated at the highest intensity of the actinic light. The kinetics of the absorbance change at 700 nm were compared with those at 865 nm in a separate experiment. Also in this case we could not detect any difference.

The kinetics of the absorbance changes in the treated chromatophores at the wavelengths of 790 and 810 nm were not compared with those at 865 nm. However, in cells of *R. rubrum*, suspended in the glycerol medium mentioned above and in intact chromatophores of *R. rubrum* suspended in Tris buffer, we were unable to detect any differences in the kinetics of the absorbance changes at 790, 810 and 883 nm (unpublished results).

The ratios of the steady states at 865 and 435 nm in different bleached preparations varied and were lower than the value of 4.9 derived from Table I. The lowest ratio was 3.8. This suggests that at least two different compounds are present causing the visible and near-infrared absorption changes. However, it is possible that there is

TABLE I

RELATIVE SIZE AND INTENSITY DEPENDENCE OF LIGHT-INDUCED ABSORBANCE CHANGES AT VARIOUS WAVELENGTHS

Wavelength (nm)	Δ_2/Δ_1*	Δ_{rel}**
365	1.65	1.00
390	1.64	—1.4
435	1.66	2.0
605	1.62	—1.2
865	1.65	—9.8

* Δ_2/Δ_1 is the ratio of the absorbance changes in the steady state at actinic intensities of 3.5 and 0.7 nEinstein·cm^{-2}·sec^{-1} (λ = 800 nm).

** Δ_{rel} is the relative value of the steady state at an actinic intensity of 3.5 nEinstein·cm^{-2}·sec^{-1} (λ = 800 nm). The value at 365 nm is chosen 1.00.

TABLE II

RELATIVE AMOUNTS OF P_{890} AND P_{800} AND QUANTUM REQUIREMENTS OF P_{890} OXIDATION

The numbers in the second column were calculated from the maximum light-induced absorbance change at 860–865 nm and the corrected absorbance at 800 nm; the numbers in the third column were calculated from the corrected absorbances at 860–865 nm and 800 nm. It was assumed that the extinction coefficients of P_{890} and of P_{800} are the same. φ P_{890} is the quantum requirement of the light-induced bleaching of P_{890} measured at 865 nm with actinic light of 800 nm.

Sample No.	P_{890} *(bleached)*$/P_{800}$	P_{890} *(total)*$/P_{800}$	φ P_{890}
1	0.45	0.71	1.1–1.3
2	0.34	0.59	0.8
3	0.30	0.63	0.9–1.1
4	0.25	0.59	—

only one pigment that causes the absorption changes and that this occurs in a slightly different form in the various preparations.

Table II gives the relative amounts of P_{890} and P_{800} for 4 different preparations. The numbers in the second column are based on the maximum light-induced absorbance change around 865 nm and those in the third column on the corrected total absorbance at this wavelength. It was assumed that oxidized P_{890} does not absorb at 865 nm (see ref. 6).

The fact that the amount of P_{890} that can be bleached appears to be lower and varies more strongly than the total amount of P_{890} present can be explained in various ways. At saturating intensities of the actinic light a biphasic reaction can be seen at 865 nm. There is a fast absorbance decrease due to the oxidation of P_{890} and a slower absorbance increase of unknown origin that probably influences the steady state also in the beginning of the light-reaction. We observed a similar absorbance increase in the treated chromatophores which were extracted with ethanol. These preparations, however, did not show a light-induced bleaching of P_{890}. Secondly, the low ratios in Column 2 may be due to an inactivation of P_{890} caused by the treatment with $K_3Fe(CN)_6$. Finally, the actual amount of P_{890} may be lower than that calculated from the absorbance at 865 nm because of overlap of the P_{800} band. Taking into account these uncertainties it may be stated that the working hypothesis of CLAYTON[11] that one molecule of P_{890} and two molecules of P_{800} are associated jointly with a photosynthetic reaction center is compatible with our results.

In Table II the quantum requirements of P_{890} bleaching are also given. The actinic light used had a wavelength of 800 nm. The extinction coefficient for P_{890} bleaching at 865 nm was assumed to be $10^5\ M^{-1}\cdot cm^{-1}$ which is approximately the extinction coefficient, in the near-infrared spectral region, for bacteriochlorophyll in purple bacteria[18]. The values are lower than those measured for intact cells or unbleached chromatophores of purple bacteria[19–21]. In view of the possible errors in the measurement of the light intensity and the fraction of light that is absorbed by the preparation and the uncertainty in the extinction coefficient applied, it may be stated that the calculated values are consistent with a quantum requirement of 1 for P_{890} oxidation.

The energy transfer from P_{800} to P_{890} apparently is very efficient. This is in agreement with the results of CLAYTON AND SISTROM[10], who showed that energy transfer from P_{800} to P_{890} in chromatophores of *Rps. spheroides*, strain R. 26, is more efficient than that to the bulk bacteriochlorophyll.

In Fig. 4 the light-induced difference spectrum of the $K_3Fe(CN)_6$-treated *R. rubrum* chromatophores in the ultraviolet region is given. This spectrum is similar to the difference spectrum of intact chromatophores of *Chromatium*, measured by CLAYTON[7]. He attributed this difference spectrum to a reduction of ubiquinone. In order to determine if ubiquinone was present in the $K_3Fe(CN)_6$-treated chromatophore preparations, ethanol and ethanol–isooctane extracts of intact and bleached *R. rubrum* chromatophores were made and the spectra recorded before and after addition of KBH_4. As Figs. 5a and b show, both extracts showed a large absorbance decrease at 270–275 nm upon reduction. Comparison of the shapes of the spectra indicates that the bleached chromatophores still contain ubiquinone too. The high extinction values of the spectra indicate that other lipid material is also dissolved by the extraction. This may also be the reason why the whole spectrum is lowered after

reduction with KBH_4. From difference spectra as shown in Fig. 5c, the total amount of ubiquinone in the bleached chromatophores could be estimated. We assumed that the isosbestic point was situated at 291 nm. The amount was 1.0 to 1.3 times the maximal amount of ubiquinone that could be reduced by light in the same sample.

Fig. 6 shows that at two intensities of actinic light the kinetics of the light-

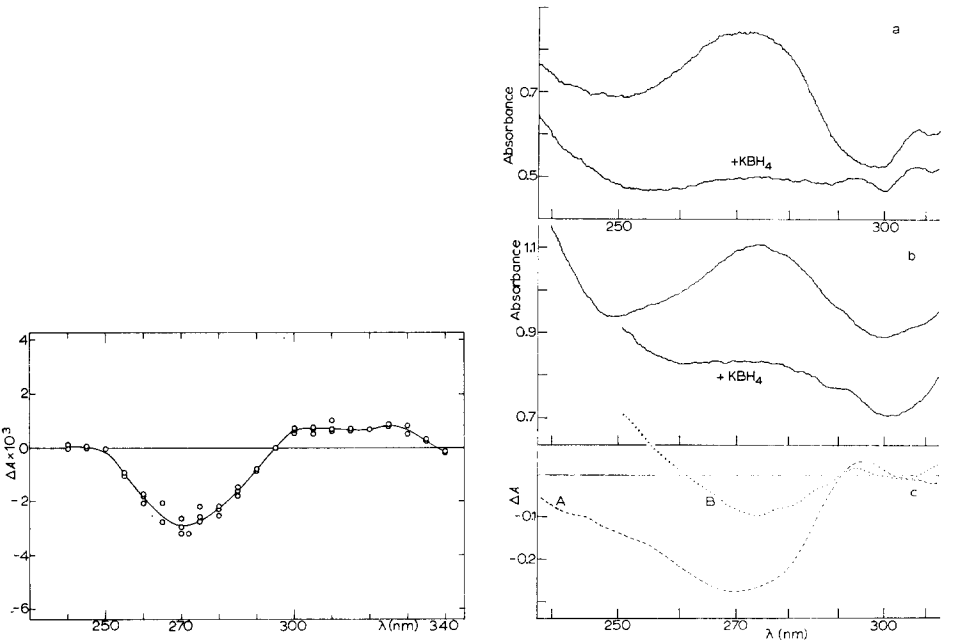

Fig. 4. Light-induced difference spectrum in the ultraviolet region for $K_3Fe(CN)_6$-treated *R. rubrum* chromatophores. The intensity of the actinic light, $\lambda = 593$ nm, was 3.9 nEinstein·cm^{-2}·sec^{-1}.

Fig. 5. a. Ultraviolet spectrum of an ethanol–isooctane extract of *R. rubrum* chromatophores before and after reduction with KBH_4. b. Ultraviolet spectrum of an ethanol extract of $K_3Fe(CN)_6$-treated *R. rubrum* chromatophores before and after reduction with KBH_4. c. Oxidized-*minus*-reduced difference spectra from a (A) and b (B).

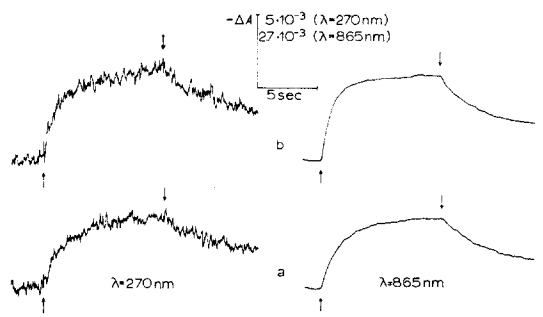

Fig. 6. A comparison of the kinetics of absorbance changes at 270 nm (left) and at 865 nm (right) of $K_3Fe(CN)_6$-treated *R. rubrum* chromatophores. The intensities of the actinic light, $\lambda = 593$ nm, were 1.9 nEinstein·cm^{-2}·sec^{-1} (a) and 3.9 nEinstein·cm^{-2}·sec^{-1} (b). The switching on and off of the actinic light is indicated by upward and downward pointing arrows, respectively.

induced absorbance changes at 270 and 865 nm in the bleached chromatophores were similar.

Quantum requirements for the photoreduction of one molecule of ubiquinone and for the photooxidation of one P_{890} molecule were determined in the same $K_3Fe(CN)_6$-bleached sample; these were 0.9 and 0.8, respectively. The extinction coefficient for the difference in absorption of ubiquinone and ubiquinol at 270 nm was taken to be $1.53 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ (see ref. 22).

The results mentioned above are consistent with the hypothesis of CLAYTON[7] and others[23] that ubiquinone is intimately connected with the photochemistry of chromatophores and cells of purple bacteria. CLAYTON[7] suggested that ubiquinone itself is the primary electron acceptor of bacterial photosynthesis. So far no direct experimental support for his statement is available. LOACH[9] concluded that the primary acceptor is a pigment (denoted as $P_{-0.02}$) with a redox potential of −0.02 V. He supposed that $P_{-0.02}$ is situated between P_{890} and ubiquinone. However, if ubiquinone or $P_{-0.02}$ would act as the primary acceptor the additional assumption should be made that the reduction of the low-potential compound NAD^+, which was shown to occur in intact cells and chromatophores[13,25–27], needs a high-energy compound such as ATP.

The reduction of one molecule of ubiquinone requires two electrons, but according to titration experiments, the bleaching of one P_{890} molecule involves only one electron[17]. Thus our results would imply that the efficiency for ubiquinone reduction and the total amount of ubiquinone that is reduced when calculated per equivalent are twice as high as the corresponding numbers for P_{890}. It is possible, as was already pointed out by LOACH[9], that a second electron is supplied by the environment of P_{890}. For example, this second electron could be supplied by P_{800}, or one quantum of light might liberate two electrons from one double molecule:

$$A\text{–}P_{890} \xrightarrow{h\nu} A^+ + P^+_{890} + 2e$$

If we assume that the molecular amounts of photooxidized P_{890} and of reduced ubiquinone are equal, then it can be calculated from the sizes of the light-induced absorbance changes at 865 and 270 nm and the extinction coefficient for ubiquinone reduction mentioned above, that the extinction coefficient for the absorption maximum of P_{890} in the bleached chromatophores is somewhat lower than the value of 10^5 $M^{-1} \cdot cm^{-1}$ used for the estimation of the quantum requirement of P_{890} oxidation. The calculated values ranged from $8.3 \cdot 10^4$ to $9.3 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$. These values are lower than the value of $11.3 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ estimated in another way by CLAYTON[11] for P_{890} in *Rps. spheroides*.

As mentioned, we could not observe a photooxidation of a cytochrome in the bleached chromatophores. When, however, horse-heart cytochrome *c* (15 % reduced) was added to the preparation, a light-induced oxidation of the reduced cytochrome took place. Sometimes addition of small amounts of 2,6-dichlorophenolindophenol (DCIP) or DCIP + ascorbate were necessary to achieve cytochrome oxidation.

In Fig. 7 light-induced absorbance changes are shown of the preparation in the blue spectral region with and without cytochrome. From the light-induced difference spectrum (Fig. 8) with a minimum at 420 nm and the large absorbance changes that can take place at this wavelength when enough cytochrome is present (Fig. 7) it can be concluded that the added cytochrome is indeed oxidized. *R. rubrum* cytochrome c_2

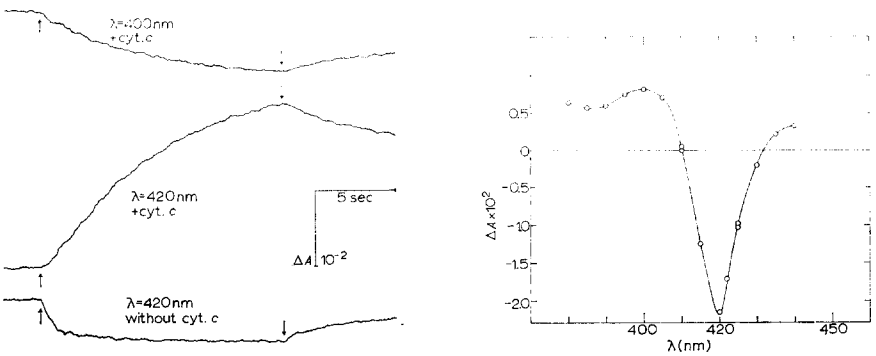

Fig. 7. Kinetics of the light-induced absorbance changes of $K_3Fe(CN)_6$-treated *R. rubrum* chromatophores with and without horse-heart cytochrome *c*. Lower trace: without cytochrome *c*, upper traces in the presence of about 0.11 mM cytochrome *c*. The intensity of the actinic light, $\lambda = 800$ nm was 3.5 nEinstein·cm^{-2}·sec^{-1}. The switching on and off of the actinic light is indicated by upward and downward pointing arrows, respectively.

Fig. 8. Light-induced difference spectrum of $K_3Fe(CN)_6$-treated *R. rubrum* chromatophores in the presence of about 0.11 mM horse-heart chtochrome *c*. The intensity of the actinic light, $\lambda = 800$ nm, was 3.5 nEinstein·cm^{-2}·sec^{-1}. Total absorbance changes were measured after an illumination period of 15 sec.

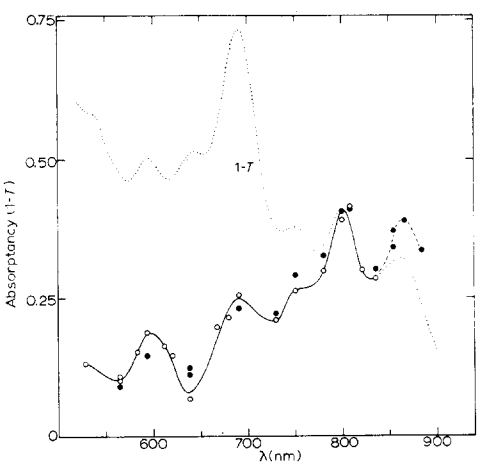

Fig. 9. A comparison of the action spectra of the light-induced bleaching of P_{890} in $K_3Fe(CN)_6$-treated *R. rubrum* chromatophores (O—O) and of the light-induced cytochrome oxidation in this preparation in the presence of about 0.11 mM horse-heart cytochrome *c* (●). The absorbance decreases of P_{890} and of cytochrome were measured at 865 and 420 nm, respectively. ....., absorption spectrum corrected for scattering. The spectra are normalized at about 800 nm.

and reduced DCIP were also oxidized by the bleached chromatophores (H. DE KLERK, T. BEUGELING, W. J. VREDENBERG, unpublished results).

Action spectra of the $K_3Fe(CN)_6$-treated chromatophores were measured for the photooxidation of P_{890} and the horse-heart cytochrome *c*. Fig. 9 indicates that the oxidation of both compounds is brought about by the same pigment system. This suggests that the cytochrome is directly or indirectly oxidized by P_{890}. The relatively low activity of actinic light of 690 nm shows that light absorbed by the oxidation product of bacteriochlorophyll is much less active than light of 800 nm absorbed by P_{800}.

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