BBA 45616

THE ROLE OF P870 IN BACTERIAL PHOTOSYNTHESIS

WILLIAM W. PARSON*

Department of Biophysics and Physical Biochemistry, Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. (U.S.A.)

(Received July 11th, 1967)

SUMMARY

In *Chromatium* chromatophores, a 30-nsec flash causes an absorbance decrease at 882 nm which is interpreted as an oxidation of P870. The quantum yield is close to one. The reaction is complete in less than 0.5 μsec. It does not occur in the presence of dithionite.

With a half-time of 2 μsec, the 882-nm absorbance increases again. Separate measurements show that cytochrome C422 becomes oxidized at the same rate. The 882-nm absorbance increase is interpreted as a reduction of oxidized P870 ($P870^+$). There appears to be a direct electron transfer between cytochrome C422 and $P870^+$.

The 2-μsec $P870^+$ reduction is generally incomplete at 298 °K. It does not occur at 80 °K, nor does the cytochrome oxidation occur at this temperature. At 298 °K, the addition of *N*-methylphenazonium methosulfate (PMS) causes the 2-μsec reduction to be complete. In the presence of PMS, the cytochrome oxidation occurs with a quantum yield near one, and its rate and extent are similar to those which occur in whole cells.

In *Rhodospirillum rubrum* chromatophores, the $P870^+$ reduction is much slower. In this species, the initial absorbance changes of P800 are complete within 0.2 μsec.

The results support the view that P870 oxidation is the primary chemical reaction of photosynthesis in *Chromatium* chromatophores.

INTRODUCTION

Illumination of photosynthetic bacteria causes absorbance changes which appear to reflect the oxidation of a specialized bacteriochlorophyll[1,2]. The specialized bacteriochlorophyll, called P870 or P890, occurs in a concentration of about 1 % that of the bulk bacteriochlorophyll. DUYSENS, CLAYTON and others have proposed that P870 oxidation is the primary chemical reaction of bacterial photosynthesis, and refs. 1 and 2 review various lines of evidence for this proposal. At present, however, there exists a controversy as to whether the oxidation of P870 has any importance at all in photosynthesis. The disagreement arises because one generally observes P870 oxidation only if the oxidation of cytochromes is saturated or inhibited. Cytochrome *c*

Abbreviation: PMS, *N*-methylphenazonium methosulfate.

* Present address: Department of Biochemistry, University of Washington, Seattle, Wash. 98105 (U.S.A.).

oxidation must be an important step in bacterial photosynthesis, because in undamaged cells it occurs with a quantum yield near one[3,4].

Upon illumination of *Chromatium* cells, cytochrome C422* oxidation commences immediately, but P870 oxidation appears only after a lag[8]. BEUGELING AND DUYSENS[8] account for this by assuming that illumination causes a rapid P870 oxidation, but that ferrous cytochrome C422 reduces the P870 again. The P870 continues to cycle until the supply of ferrous cytochrome is exhausted. There is an alternative explanation, however[9]. P870 may play no role in the cytochrome oxidation, but may only divert energy after cytochrome oxidation becomes impossible. Either of these theories can account for all of the findings which have been reported to date, such as the greater P870 oxidation which occurs after disruption of cells with inhibitors or sonication. Observation[10,11] of high quantum yields of oxidized P870 have always involved preparations in which the cytochrome oxidation was negligible.

An experiment to resolve the uncertainty surrounding P870 is simple in principle. Following a brief flash of light, one should observe very rapid oxidation of P870. Subsequently, oxidized P870 ($P870^+$) should become reduced again as cytochrome oxidation occurs, or faster. A major obstacle to performing this experiment is that fluorescence from bacteriochlorophyll obscures measurement of P870 shortly after an actinic flash. Measurements[12] with *Rhodospirillum rubrum* chromatophores, in which the cytochrome *c* oxidation is slow, demonstrated that the initial oxidation of P870 was faster than that of the cytochrome, and experiments with inhibitors indicated that cytochromes *b* and *c* were somehow involved in the reduction of the $P870^+$. There appeared to be $P870^+$ reduction as cytochrome *c* became oxidized. These results were unconvincing, however, because the amount of cytochrome *c* oxidation was extremely small and the rate was much lower than that which occurs in whole cells.

It seemed important to extend these measurements to *Chromatium*, in which the cytochrome oxidation is dramatic in rate and magnitude. We have done this, and have found that $P870^+$ is reduced as cytochrome *c* becomes oxidized. This appears to settle the controversy in favor of a fundamental role for P870 in photosynthesis.

MATERIALS AND METHODS

Chromatophores of *R. rubrum* and *Chromatium* were prepared and stored as described previously[12]. Dr. HEINZ SCHLEYER generously supplied the *Chromatium* cells, which were collected after 48 h of autotrophic growth. P870 was measured spectrophotometrically at 882 nm. In DISCUSSION, we consider the assumption that a bleaching at this wavelength does reflect P870 oxidation.

* *Chromatium* contains at least 3 different *c*-type cytochromes, which can be distinguished by their absorption spectra, reduction potentials, and solubilities, and by their responses to actinic light intensity, aerobiosis, temperature, inhibitors, and starvation of the cells[4–7]. OLSON AND CHANCE[5] designated the cytochromes according to the wavelength of the largest Soret band absorbance difference between the reduced and the oxidized forms. CUSANOVICH[6] used a nomenclature based on the absorbance differences in the α bands. BEUGELING AND DUYSENS[8] studied cells which were suspended in an aerobic mixture of glycerol, water, and potassium glycerophosphate. Under these conditions, illumination causes the oxidation of a single cytochrome[4] which is probably cytochrome C422 (in the nomenclature of OLSON AND CHANCE[5]), also designated cytochrome C555 (in that of CUSANOVICH[6]). Arguments given below indicate that the present experiments concern the same cytochrome.

The apparatus for measuring rapid absorbance changes after a 694.3-nm actinic laser flash was that of CHANCE AND DEVAULT[13], with the following modifications. (A complete description of the apparatus is in preparation[14].)

(i) A faster preamplifier was developed. This was essential, not only to allow resolution of times shorter than 1 μsec, but also to insure rapid recovery from their overload which results from a large pulse of bacteriochlorophyll fluorescence.

(ii) A much brighter measuring light was obtained by the use of a xenon flash lamp. This was essential so that the signal to noise ratio would be adequate for the rapid measurements. (Shot noise is the principal limitation.) It also increased the ratio of measuring light to fluorescence. The strong xenon discharge line at 881.9 nm is ideal for measurements of *Chromatium* P870, providing about 44 times the intensity of a BXJ tungsten lamp. A choke in series with the flash lamp gives enough of a plateau in the discharge to allow measurements with oscilloscope sweep speeds of 2 μsec/cm or faster. The intensity incident on the sample was about 1 nEinstein·cm^{-2}·sec^{-1} and the sample was exposed to the light for about 100 μsec before and during the measurement. This gave a total incident illumination of about 10^{-13} Einstein·cm^{-2}. For most of the measurements, the chromatophore concentration was such that a saturating flash from the Q-switched laser oxidized more than 0.2 nmole of cytochrome *c* per cm^2, so the actinic effect of the xenon measuring light was less than 0.05 % that of the laser.

(iii) A second monochromator between the cuvette and the photomultiplier greatly improved the resolution of transmitted light from fluorescence. A Wratten 88A filter on the photomultiplier rejected second-order light from the monochromator. The use of 2 monochromators is feasible only for optically clear suspensions of chromatophores.

Q-switching the laser with a spinning prism gives a single flash lasting about 30 nsec. Fixing the prism in position gives a burst of flashes. The amplitude of the flashes decreases during the burst, which lasts about 500 μsec. The laser flash energy was measured with a ballistic thermopile (TRG Co.), after partial attenuation with a negative lens. The ballistic thermopile was calibrated by passing a current through a coil of fine wire suspended within the cone and measuring the current, the voltage across the wire, and the ballistic thermopile output. The result agreed with the manufacturer's calibration within 5 %.

The absorption of 694.3-nm light by the sample was measured with continuous light from a monochromator, in an integrating sphere which accepted the experimental cuvettes. Following LATIMER[15], we built an icosahedral apparatus with 20 International Rectifier S1020E4PL silicon solar cells. The samples generally absorbed 20 to 50 % of the 694.3-nm light.

Other materials and methods were the same as those described previously[12]. The chromatophore suspensions were aerobic, in 0.1 M glycylglycine (pH 7.4) except for those of Fig. 2. The temperature was approx. 298 °K, except in the experiment of Fig. 2.

RESULTS

Fig. 1 shows measurements of P870 and cytochrome oxidation in *Chromatium* chromatophores, following actinic flashes of two different lengths. The oscilloscope sweep was relatively slow in these experiments, and the time constant of the apparatus was relatively long, so the records show only absorbance changes that lasted

longer than about 10 μsec. Under these conditions, a 30-nsec flash appeared to cause only 10 to 40 % as much P870 oxidation as a 500-μsec flash did (Figs. 1A and B). A 30-nsec flash caused appreciable cytochrome oxidation (Fig. 1C). These measurements were made with saturating flash energy; with low energy flashes, 30-nsec and 500-μsec flashes appeared to oxidize equal amounts of P870.

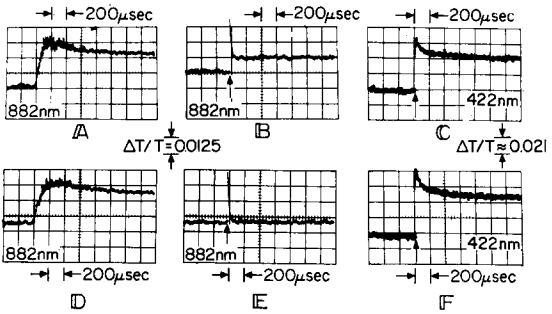


Fig. 1. Absorbance measurements at slow oscilloscope sweep speeds (200 μsec/cm). An RC filter between the preamplifier and the oscilloscope filters out very rapid absorbance changes and most of the fluorescence artifact. An upward deflection is an absorbance decrease. A, 500-μsec flash (note small spikes of fluorescence during the flash), total flash intensity absorbed was 2.8 nEinsteins·cm^{-2}, total bacteriochlorophyll was 30.4 nmoles·cm^{-2} (to calculate the μM concn., divide by the cuvette path length, 0.16 cm), measuring light was 882 nm from a tungsten lamp, filter time constant 10 μsec. B, same as A except 30-nsec flash with total absorbed intensity 1.94 nEinsteins·cm^{-2}. C, 30-nsec flash with total absorbed intensity 1.82 nEinsteins·cm^{-2}, total bacteriochlorophyll was 85.2 nmoles·cm^{-2} (a different chromatophore preparation from that used in A). Measuring light 422 nm from the tungsten lamp, filter time constant 3 μsec. D, same as A, except 45 μM PMS present. E, same as B, except 45 μM PMS. F, same as C, except 83 μM PMS.

At 80 °K no cytochrome oxidation occurred, as CHANCE AND NISHIMURA found with continuous actinic light[16], and the short and long flashes appeared to cause the same amount of P870 oxidation. The amount of P870 oxidation which occurs at 80 °K with either flash is evidently the same as that which occurs with the longer flash at 298 °K. This conclusion comes from the experiment of Fig. 2. In this experiment, the chromatophores were suspended in 75 % glycerol. At 298 °K, the effects of short and long flashes in oxidizing cytochrome and P870 were the same for chromatophores in 75 % glycerol as for those in aqueous buffer. Fig. 2 shows the difference spectra caused by 500-μsec flashes at 298 °K, and at 80 °K after slow cooling to give a clear glass. The areas under the two curves are approximately the same.

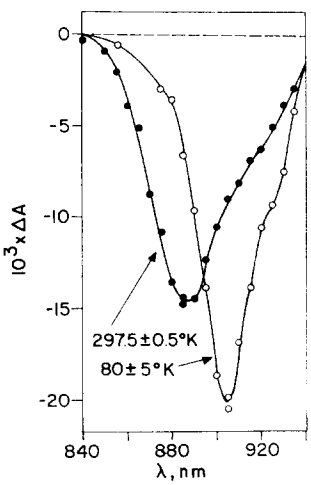
In chromatophores of *R. rubrum*, the short and long flashes oxidized the same amount of P870, both at 80 and at 298 °K. Again, the areas under the difference spectra were similar at 80 and 298 °K.

Addition of oxidized or reduced *N*-methylphenazonium methosulfate (PMS) to *Chromatium* chromatophores has a dramatic effect (Fig. 1). A 30-nsec flash at 298 °K now oxidizes more cytochrome *c* and no P870 at all, again judging from measurements with relatively low oscilloscope sweep rates. The 500-μsec flash still oxidizes the same amount of P870, indicating that PMS does not destroy the ability of the chromatophores to oxidize P870.

Chromatium whole cells behaved like chromatophores which had been treated with PMS. No P870 oxidation was apparent one msec after a 30-nsec flash, whereas

a 500-μsec flash oxidized about the same amount of P870 (per bacteriochlorophyll) as it did in chromatophores.

Fig. 3 shows the spectrum of the absorbance changes which accompany cytochrome oxidation in *Chromatium* chromatophores. These measurements were made with 30-nsec flashes, in the presence of PMS. The spectrum is similar to that which DEVAULT AND CHANCE[17] found with whole cells. DEVAULT and co-workers[18,19] have identified the cytochrome which one measures in this way as cytochrome C422 (OLSON AND CHANCE[5]) also designated C555 (CUSANOVICH[6]). In addition to the spectrum, the evidence for the assignment is that this cytochrome is not autoxidizable; the fast photooxidation occurs in aerobic as well as anaerobic samples. Further, the photooxidation does not occur at 80 °K in chromatophores, as VREDENBERG AND DUYSENS[4] found for C422 in whole cells.



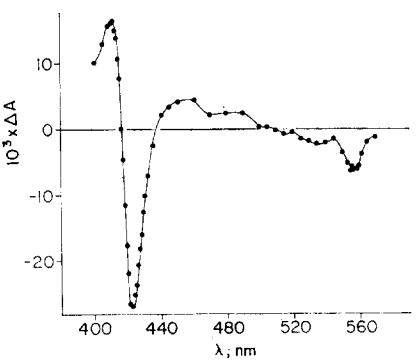


Fig. 2. Spectrum of P870 absorbance changes. *Chromatium* chromatophores, bacteriochlorophyll 30.4 nmoles·cm^{-2}, suspended in 75% glycerol, saturating 500-μsec flashes, tungsten measuring lamp, approx. 5.3-nm half band width. ●—●, 297.5 ± 0.5 °K. ○—○, 80 ± 5 °K.

Fig. 3. Spectrum of cytochrome absorbance changes. *Chromatium* chromatophores, bacteriochlorophyll 54.5 nmoles·cm^{-2}, 91 μM PMS, saturating 30-nsec flashes (approx. 1.8 nEinsteins·cm^{-2} absorbed), tungsten measuring lamp, 1.65-nm half band width. The α- and γ-minima are at 556 ± 1 nm and 422 ± 1 nm.

Fig. 4 shows measurements of the amount of cytochrome oxidation which occurs in the presence of PMS, as a function of the absorbed flash energy. Because the extinction coefficient of cytochrome C422 is uncertain, the ordinate gives the observed absorbance change. The units are such that dividing by $\Delta\varepsilon$ (mM^{-1}·cm^{-1}) gives the cytochrome oxidation in nmoles·cm^{-2}. The quantum yield is $90/\Delta\varepsilon$. This is essentially the same as the value ($94/\Delta\varepsilon$) which VREDENBERG AND DUYSENS[4] measured with continuous illumination of whole cells. It seems likely that $\Delta\varepsilon$ is close to 90 mM^{-1}·cm^{-1} and the quantum yield is approx. 1 (see ref. 4).

Assuming that $\Delta\varepsilon$ is 90 mM^{-1}·cm^{-1}, the asymptote at high flash energy gives a maximum cytochrome oxidation of 1 cytochrome per 160 bacteriochlorophylls. In Fig. 3, which involved a different chromatophore preparation, the ratio was 1 cytochrome per 180 bacteriochlorophylls; other preparations gave values which averaged

about 1 per 170 bacteriochlorophylls. As the flash duration is very short compared to the cytochrome oxidation time, these measurements give the photosynthetic unit size, if preparation of the chromatophores does not inactivate any units. A measurement in *Chromatium* whole cells gave 1 cytochrome per 300 bacteriochlorophylls; DEVAULT's[18] measurements gave 1 per 500 bacteriochlorophylls, after recalculation for a $\Delta\varepsilon$ of 90 $mM^{-1}\cdot cm^{-1}$. The amount of cytochrome oxidation (per bacteriochlorophyll) in chromatophores thus appears to be no less than it is in whole cells. In fact, it may be greater. Some cytochrome *c* is lost in preparation of *Chromatium* chromatophores (M. NISHIMURA, personal communication), but this must be a different cytochrome from the one which the 30-nsec flash oxidizes.

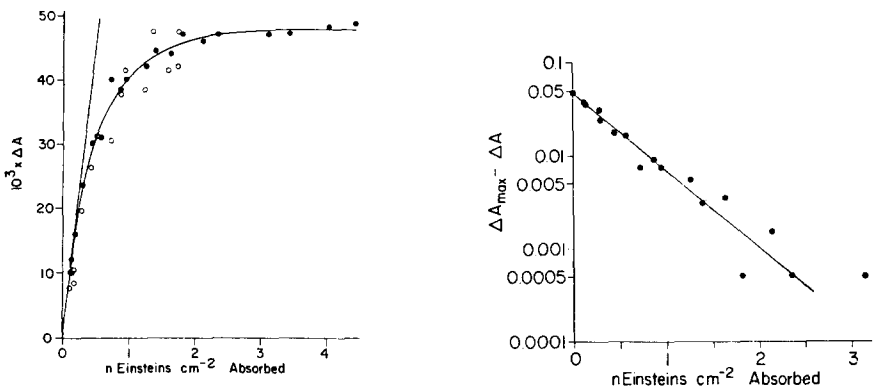


Fig. 4. Dependence of P870 and cytochrome oxidation on the flash energy. *Chromatium* chromatophores, bacteriochlorophyll 85.2 nmoles·cm^{-2}, 83 μM PMS, 30-nsec flashes. The ordinate gives the absolute magnitude of the observed absorbance decrease. ●—●, cytochrome measurements at 422 nm with the tungsten lamp. ○—○, P870 measurements at 882 nm with xenon flash lamp. The P870 measurements were made from photographs like those of Figs. 7A, B, and C by extrapolation to the time of the flash. The fluorescence artifact was measured by the first method described in the text, from photographs like those of Figs. 8A, B, and C, again by extrapolation to the time of the flash. All of the P870 data were corrected by subtracting the deflection due to the artifact.

Fig. 5. Cytochrome data of Fig. 4 replotted on a semilog grid. The abscissa is the same as in Fig. 4; to convert it to quanta absorbed per photosynthetic unit, divide by the concentration of units (47.5/90 = 0.53 nmoles cm^{-2} from Fig. 4 on the assumptions of one cytochrome C422 per unit and of $\Delta\varepsilon$ = 90 $mM^{-1}\cdot cm^{-1}$). The line is given by the equation $\Delta A_{max} - \Delta A = \Delta A_{max}\, e^{-Q}$, with Q = the quanta absorbed per unit, calculated in this way.

The data of Fig. 4 fit an exponential equation, $\Delta A_{max} - \Delta A = \Delta A_{max} e^{-Q}$, where Q is the number of quanta absorbed per photosynthetic unit (Fig. 5). One predicts such an equation if there is rapid energy migration to the trap within a photosynthetic unit, but no energy migration between 2 different units[20]. DUYSENS[20] found with whole cells that a plot of cytochrome C422 oxidation *versus* time under constant illumination did not fit this equation. He interpreted his results in terms of interunit energy migration. Our results suggest that interunit energy migration is negligible in the chromatophores.

Following the flash, part of the cytochrome recovers with a half-time of about 50 μsec (Figs. 1C and 2F). A similar recovery occurs in whole cells[18]. With chromatophores, the rate and extent of this step varies considerably from preparation to preparation. The rate generally decreases as the chromatophores age.

Turning now to more rapid measurements, Fig. 6 shows the kinetics of the cytochrome oxidation. The half-time is 2 ± 0.5 μsec, the same as the half-time in whole cells[17]. The rate is approximately the same in the presence and absence of PMS (Fig. 6C) or PMS *plus* ascorbate (Fig. 6D). It is the same for chromatophores in 75 % glycerol as for chromatophores in aqueous buffer.

In some measurements (*e.g.* Fig. 6B) there appeared to be a lag of 0.5 to 2 μsec after the flash before cytochrome oxidation began. The apparent lag probably was an artifact which resulted from the superposition of several effects. The 430-nm pigment (see below) generally caused a small, rapid absorbance increase, and the laser flash caused a small spike in the opposite direction. The sum of these effects and the

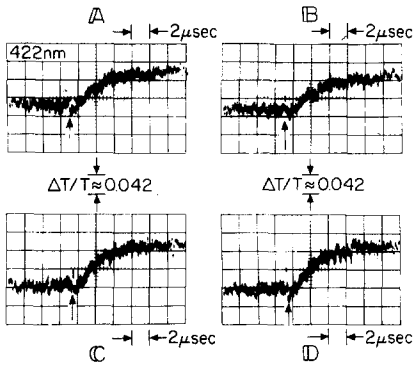


Fig. 6. Kinetics of the cytochrome oxidation. *Chromatium* chromatophores, bacteriochlorophyll 85.2 nmoles·cm^{-2}, tungsten measuring light, 422 nm. Small upward spikes indicate the time of the 30-nsec flash, 1.82 nEinsteins·cm^{-2} absorbed. A and B, no additions. C, 83 μM PMS. D, 83 μM PMS *plus* 830 μM ascorbate. An upward deflection is an absorbance decrease.

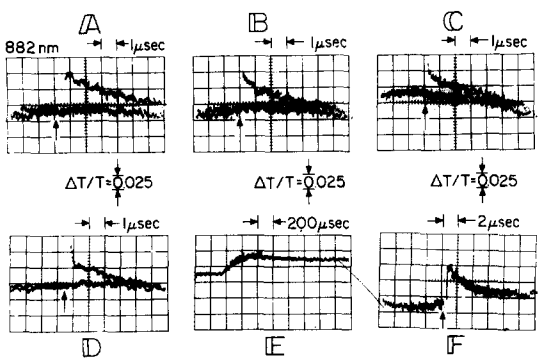


Fig. 7. Kinetics of P870 oxidation and reduction in *Chromatium* chromatophores. All measurements at 882 nm. Except in E the measuring light was from the xenon flash lamp. A, bacteriochlorophyll 46.4 nmoles·cm^{-2}, 0.91 nEinsteins·cm^{-2} absorbed from the 30-nsec actinic flash. B, same as A, except 0.47 nEinsteins·cm^{-2} absorbed. C, same as A except 0.19 nEinsteins·cm^{-2} absorbed. D, bacteriochlorophyll 30.4 nmoles·cm^{-2} (a different preparation from that of A, B, C and F), 0.80 nEinsteins·cm^{-2} absorbed, 45 μM PMS. E, same as D, but a 500-μsec flash with 1.94 nEinsteins·cm^{-2} absorbed, boosted tungsten measuring light, oscilloscope sweep 200-fold slower. F, same as A except sweep 2-fold slower, 1.17 nEinsteins·cm^{-2} absorbed. From measurements of the laser flash, the response (1/e) time of the apparatus was estimated as 0.07 μsec in A, B, C, and F; in D it was increased by an RC filter to about 0.1 μsec; in E it was 10 μsec. An upward deflection is an absorbance decrease.

absorbance decrease due to cytochrome oxidation could give the appearance of a lag in the oxidation.

Fig. 7 shows rapid measurements of P870 under several conditions, and one slower measurement for comparison. The xenon flash lamp discharge was fairly regular from flash to flash, but generally was not flat at these measurement speeds. Accordingly, most of the figures include a baseline measurement with no laser flash. The measurement with a laser flash was made 1 min later.

These measurements show that the 30-nsec laser flash does cause a fast P870 oxidation even in the presence of PMS, but that a recovery occurs with a half-time of about 2 μsec. The fast recovery is complete in the presence of PMS (Fig. 7D), but incomplete in its absence. (Complete recovery does occur within several minutes in the absence of PMS.) Within experimental error, the half-time of the fast recovery is the same as that of the cytochrome oxidation. A saturating 30-nsec flash appears to cause the same initial P870 oxidation (Fig. 7D) as does a saturating 500-μsec burst of flashes (Fig. 7E). The recovery after the 500-μsec flash is thousands of times slower, presumably because all of the cytochrome C422 becomes oxidized during the flash.

In evaluating these measurements, the critical question is the extent to which they are distorted by artifacts. A fluorescence artifact would exaggerate the appearance of a fast absorbance decrease and recovery. We have 2 ways of measuring the artifact due to bacteriochlorophyll fluorescence. Figs. 8A, B, and C show measurements in which there was a laser flash, but no measuring light. These measurements were identical with those in Figs. 7A, B, and C, respectively, except that the path of the measuring light was blocked, and the d.c. input to the preamplifier was removed. (The d.c. ordinarily balanced the photomultiplier anode current resulting from the measuring light.) Before the laser flash, the net input to the preamplifier was thus the same, but the photomultiplier was in darkness. Figs. 8A, B, and C will overestimate the artifact if the photomultiplier response to the fluorescence spike is greater in darkness than during illumination by the measuring light.

Figs. 8D, E, and F show a second way of measuring the artifact. This is to add $Na_2S_2O_4$ to the chromatophores, blocking the P870 oxidation[21]. The measurements of Figs. 8D, E, and F are identical with those of Figs. 7A, B, and C respectively, except

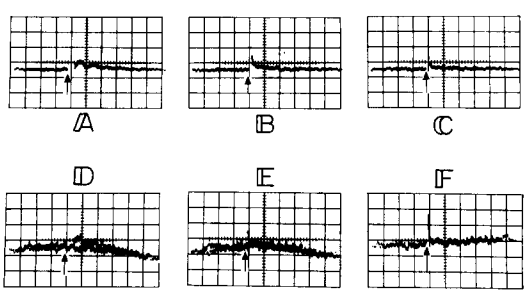


Fig. 8. Measurements of artifacts. In A, B, and C, there was no measuring light and no d.c. offset was applied to the preamplifier. In D, E, and F, the measuring light and d.c. offset were present but the chromatophores had been treated with a few mg of solid $Na_2S_2O_4$. Other conditions (dynode voltage on the photomultiplier, chromatophore concentration, actinic flash strength, oscilloscope settings, *etc.*) were the same in A and D as in Fig. 7A; in B and E as in Fig. 7B; and in C and F as in Fig. 7C. Strongly damped oscillations occur in the recovery from a large fluorescence pulse (A and D).

for the presence of $Na_2S_2O_4$. They overestimate the artifact, because $Na_2S_2O_4$ increases the yield of bacteriochlorophyll fluoresence[22].

The measurements of Fig. 8 show that the artifact is negligible at low laser flash energies. At higher energies there is an appreciable artifact (Figs. 8A and D), but one is still able to distinguish the artifact from a true absorbance change.

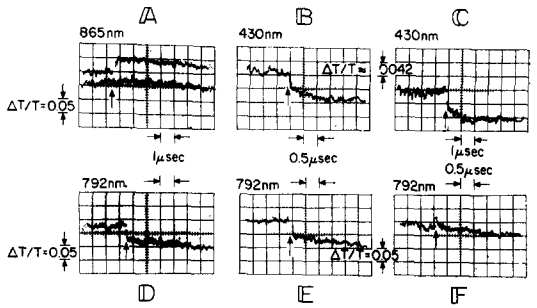


Fig. 9. Measurements with *R. rubrum* chromatophores. All measurements used the xenon flash lamp. A, 865 nm, bacteriochlorophyll 17.3 nmoles·cm^{-2}, 0.29 nEinsteins·cm^{-2} absorbed. B, 430 nm, bacteriochlorophyll 54.4 nmoles·cm^{-2}, 1.43 nEinsteins·cm^{-2} absorbed. C, same as B except slower sweep, and 3.46 nEinsteins·cm^{-2} absorbed. D, 792 nm, bacteriochlorophyll 31.7 nmoles·cm^{-2}, 0.48 nEinsteins·cm^{-2} absorbed. E, same as D, except faster sweep. F, same as E, after addition of solid $Na_2S_2O_4$. An upward deflection is an absorbance decrease.

A valuable control would be one in which the cytochrome oxidation is slow or inoperative. The only satisfactory control of this type which is presently available is the use of *R. rubrum* chromatophores. Fig. 9A shows that there is no fast reduction of $P870^+$ in these. This agrees with earlier measurements[12] of the half-time for cytochrome oxidation in *R. rubrum* chromatophores (10 msec), and with the experiments with short and long flashes. Low temperatures offer another such control for *Chromatium* chromatophores, and the experiments with short and long flashes indicate that there is no fast reduction of $P870^+$ at 80 °K. Unfortunately, even glasses of 80% glycerol scatter the measuring light enough to interfere with very fast measurements at low temperatures. Inhibitors which appear to damage cytochrome oxidation under continuous actinic light[7] (0.5 mM phenylmercuric acetate, 10 mM sodium mersalyl, 0.4 mM *o*-phenanthroline, and mild heating) had no obvious effect on the cytochrome C422 oxidation.

A second question which is critical for evaluating the P870 oxidation is whether it occurs with low-energy actinic flashes. Fig. 7 shows several measurements with different flash energies, and Fig. 4 shows the results of a more detailed investigation. Clearly, the P870 oxidation does occur with weak flashes. Further, the quantum yield is probably close to one. As the quantum yield of cytochrome oxidation is near one under the same conditions, quanta which cause P870 oxidation must not go to waste. Again, the exact quantum yield depends on an assumption of $\Delta\varepsilon$ for P870 oxidation. With a 500-μsec flash, the maximal absorbance change at 882 nm was approximately the same as that at 422 due to cytochrome C422 oxidation (perhaps slightly smaller). Assuming that P870 and cytochrome C422 are oxidized in equimolar concentration, this suggests a $\Delta\varepsilon$ of about 90 mM^{-1}·cm^{-1} for P870 oxidation.

Some additional discussion of the $Na_2S_2O_4$ control is necessary. We reported

earlier[12] that the P870 absorbance changes still occurred in the presence of $Na_2S_2O_4$. This conclusion came from measurements at 430 nm, at a time when we were unable to measure infrared absorbance changes sooner than several μsec after the flash. Under other conditions, we had not found significant differences between the absorbance changes of P870 and those at 430 nm. Other workers[21,23,24] had found kinetic differences, but we felt that these resulted from different measuring light intensities.

Evidently, $Na_2S_2O_4$ does block the P870 and P800* absorbance changes in both *Chromatium* (Figs. 8D, E, and F) and *R. rubrum* (Fig. 9F), but not the 430-nm absorbance change. In fact, at high flash energy the latter is larger in the presence of $Na_2S_2O_4$ than in its absence. (It can be at least twice as large with *R. rubrum* chromatophores and a strong flash. With low-energy flashes, the 430-nm absorbance changes have the same magnitude in the presence and absence of $Na_2S_2O_4$.)

There may be a further difference between the infrared absorbance changes and those of the 430-nm pigment. The absorbance changes at 792 nm in *R. rubrum* (P800) appear to be complete in less than 0.2 μsec (Figs. 9D and E), whereas those at 430 nm appear biphasic. About half of the 430-nm absorbance change is complete within 0.2 μsec, but the remainder occurs with a half-time of about 0.5 μsec (Figs. 9B and C). The nature of the pigment which causes the 430-nm absorbance change remains obscure.

DISCUSSION

These experiments concentrate on chromatophores because of the technical difficulties of making very fast infrared absorbance measurements in turbid cell suspensions. Chromatophores are satisfactory, however, only if they preserve an undamaged interaction between the cytochrome *c* and bacteriochlorophyll. The experiments with short and long flashes indicate that a portion of the cytochrome C422 may be uncoupled from the bacteriochlorophyll in untreated *Chromatium* chromatophores. A saturating flash fails to oxidize all of the cytochrome C422, and part of the $P870^+$ is inert. PMS somehow restores complete coupling.

Possibly, the defect in untreated chromatophores is not in the oxidation of cytochrome C422, but in reduction of the ferric cytochrome. Part of the cytochrome C422 may already be oxidized in the dark before the actinic flash, and PMS may restore the reduction.

In the presence of PMS, the cytochrome C422 oxidation in *Chromatium* chromatophores is similar to that which occurs in whole cells. The quantum yield is near 1; the ratio of oxidizable cytochrome to bacteriochlorophyll is at least as great as it is in whole cells; the rate of the initial oxidation is the same as it is in cells; and all of the $P870^+$ appears to turn over actively. Although we do not know the mechanism of action of PMS, *Chromatium* chromatophores under these conditions seem suitable for detailed study.

Even with a low-energy actinic flash, a bleaching of P870 precedes the oxidation of cytochrome C422. The following evidence indicates that the rapid absorbance

* In addition to bleaching P870, illumination causes a blue shift of an absorption band at 800 nm. CLAYTON[2,25] attributes the 800-nm band to 2 bacteriochlorophyll molecules, P800, which are close neighbors of P870. The oxidation of P870 apparently has a strong, indirect effect on the absorption spectrum of P800.

decrease at 882 nm reflects an oxidation of P870. First[21,26,27], chemical oxidants cause similar absorbance changes in the dark; reductants do not. Second[12,21], chemical reductants hasten the reversal of the absorbance changes in the dark following illumination; oxidants retard the reversal. Third, the reversal of the P870 absorbance change correlates kinetically with the loss of an electron from cytochrome C422. Fourth[12], under some conditions the reversal of the P870 absorbance change is slow, requiring several seconds for completion. This suggests that bleached P870 is relatively stable, as one might expect for $P870^+$, but not for a metastable excited species. A protective environment might prolong the life of a metastable species, but disruptive treatments such as heating make the reversal of the absorbance change even slower.

The following evidence supports the proposal that P870 oxidation is a fundamental reaction of photosynthesis in chromatophores. First, it occurs with a quantum yield close to one, under conditions which also give cytochrome C422 oxidation with a high quantum yield. Second[28], it occurs at temperatures as low as 1 °K. Third, it occurs extremely rapidly. Present measurements indicate that the P870 oxidation is complete within 0.5 μsec, and probably within 0.2 μsec.

$P870^+$ reduction occurs as cytochrome C422 becomes oxidized. As the rate of $P870^+$ reduction is equal to the rate of cytochrome oxidation, and as there probably is no significant lag before cytochrome oxidation begins, there is no evidence that any other electron carrier intervenes between $P870^+$ and cytochrome C422.

Our first experiments suggested that a reduction of $P870^+$ did not occur simultaneously with cytochrome oxidation, and we made several preliminary statements to this effect[29,30]. The earlier measurements were made between 780 and 790 nm, where illumination causes an absorbance increase due to P800. A fast absorbance increase and recovery probably were masked by a fluorescence artifact which caused an oscilloscope deflection in the opposite direction. With the use of the xenon flash measuring lamp and the fast preamplifier, the fluorescence artifact now is much smaller and direct measurement of P870 is possible. Another error in the earlier measurements was to anticipate P870 absorbance changes about twice as large as those which were later found. This resulted from assuming a smaller $\Delta\varepsilon$ for cytochrome C422 oxidation and a larger one for P870 oxidation. The values we assumed previously were 60 $mM^{-1} \cdot cm^{-1}$ for cytochrome C422 (the value[31] for *Chromatium* cytochrome C423.5) and 113 $mM^{-1} \cdot cm^{-1}$ for P870 (the value[25] for P870 in *Rhodopseudomonas spheroides*). The present work, and that of VREDENBERG AND DUYSENS[4], suggests that $\Delta\varepsilon$ is approx. 90 $mM^{-1} \cdot cm^{-1}$ for both cytochrome C422 and *Chromatium* P870.

ACKNOWLEDGEMENTS

I am greatly indebted to Dr. H. SCHLEYER for providing the *Chromatium* cells, and to Drs. B. CHANCE, D. DEVAULT, W. HILDRETH, M. NISHIMURA, and H. SCHLEYER for stimulating discussions and many helpful suggestions. U. S. Public Health Service Grants CM 12205 and 5 TI GM-277 supported this work.

REFERENCES

1 R. K. CLAYTON, *Brookhaven Symp. Biol.*, 19 (1966) 62.
2 L. N. M. DUYSENS, *Arch. Biol. Liège*, 76 (1965) 251.
3 J. M. OLSON, *Science*, 135 (1962) 101.

4 W. J. VREDENBERG AND L. N. M. DUYSENS, *Biochim. Biophys. Acta*, 79 (1964) 456.
5 J. M. OLSON AND B. CHANCE, *Arch. Biochem. Biophys.*, 88 (1960) 26.
6 M. A. CUSANOVICH, Ph.D. Thesis, University of California, San Diego, 1967.
7 S. MORITA, M. EDWARDS AND J. GIBSON, *Biochim. Biophys. Acta*, 109 (1965) 45.
8 T. BEUGELING AND L. N. M. DUYSENS, in J. B. THOMAS AND J. C. GOEDHEER, *Currents in Photosynthesis*, Donker, Rotterdam, 1966, p. 49.
9 M. NISHIMURA AND B. CHANCE, *Biochim. Biophys. Acta*, 66 (1963) 1.
10 W. J. VREDENBERG AND L. N. M. DUYSENS, *Nature*, 197 (1963) 355.
11 R. K. CLAYTON, *Photochem. Photobiol.*, 1 (1962) 305.
12 W. W. PARSON, *Biochim. Biophys. Acta*, 131 (1967) 154.
13 D. DEVAULT, in B. CHANCE, R. H. EISENHARDT, Q. H. GIBSON AND K. K. LONBERGHOLM, *Rapid Mixing and Sampling Techniques in Biochemistry*, Academic, New York, 1964, p. 165.
14 B. CHANCE, D. DEVAULT, W. W. PARSON AND A. WEISS, to be published.
15 P. H. LATIMER, Ph.D. Thesis, University of Illinois, 1965; cited in C. N. CEDERSTRAND, E. RABINOWITCH AND GOVINDJEE, *Biochim. Biophys. Acta*, 126 (1966) 1.
16 B. CHANCE AND M. NISHIMURA, *Proc. 5th Intern. Congr. Biochem., Moscow, 1961*, 6 (1963) 267.
17 D. DEVAULT AND B. CHANCE, *Biophys. J.*, 6 (1966) 825.
18 D. DEVAULT, in K. OKUNUKI AND M. D. KAMEN, *Symp. Cytochromes, Osaka, Japan, 1967*, to be published.
19 B. CHANCE, D. DEVAULT, W. W. HILDRETH, W. W. PARSON AND M. NISHIMURA, *Brookhaven Symp. Biol.*, 19 (1966) 115.
20 L. N. M. DUYSENS, in J. B. THOMAS AND J. C. GOEDHEER, *Currents in Photosynthesis*, Donker, Rotterdam, 1966, p. 263.
21 I. KUNTZ, P. A. LOACH AND M. CALVIN, *Biophys. J.*, 4 (1964) 227.
22 R. K. CLAYTON, *Photochem. Photobiol.*, 5 (1966) 679.
23 W. J. VREDENBERG, Thesis, The State University, Leiden, 1965, p. 28.
24 J. M. OLSON AND B. KOK, *Biochim. Biophys. Acta*, 32 (1959) 278.
25 R. K. CLAYTON, *Photochem. Photobiol.*, 5 (1966) 669.
26 J. C. GOEDHEER, *Brookhaven Symp. Biol.*, 11 (1958) 10.
27 R. K. CLAYTON, *Photochem. Photobiol.*, 1 (1962) 201.
28 W. ARNOLD AND R. K. CLAYTON, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 769.
29 W. W. PARSON, *Brookhaven Symp. Biol.*, 19 (1966) 80.
30 W. W. PARSON, *Federation Proc.*, 26 (1967) 860.
31 R. G. BARTSCH, in H. GEST, A. SAN PIETRO AND L. P. VERNON, *Bacterial Photosynthesis*, Antioch, Yellow Springs, 1963, p. 475.