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ELECTRON ACCEPTORS IN REACTION CENTER PREPARATIONS FROM PHOTOSYNTHETIC BACTERIA

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SUMMARY

1. Kinetics and difference spectra of light-induced absorbance changes were measured in reaction center preparations from *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum*.

2. The results indicate the photoreduction of an electron acceptor X by P870. X is subsequently reoxidized in the light by a secondary endogenous electron acceptor A. The half times of the back reaction from X^- to $P870^+$ and from A^- to $P870^+$ are 0.12–0.15 s and 20–60 s, respectively. Photoreduced X^- can also be reoxidized by ferricyanide, the net result being a reversible photooxidation of P870 by ferricyanide. Alternatively, photooxidized $P870^+$ can be re-reduced by ascorbate, the net result being photoreduction of X, or of X and A, by ascorbate.

3. On the basis of these results, reduced *minus* oxidized difference spectra were obtained for P870, X and A. There is only one mole of X per mole of P870. The difference spectra indicate that X is ubiquinone and X^- is the semiquinone anion. The absorbance of A does not alter markedly upon oxidation or reduction.

INTRODUCTION

It is generally accepted that, in contrast to higher plants, only one photosystem is working in photosynthetic bacteria. The primary reaction in bacterial photosynthesis is the photooxidation of a bacteriochlorophyll type which in purple bacteria is called P870 (*i.e.* a pigment absorbing at 870 nm). The substance which functions as the primary electron acceptor during this reaction is still unknown, although much work is being done, and has been done, to identify it. The role of primary electron acceptor has often been attributed to ubiquinone. However, a practical difficulty in testing this assumption was that the reduction of ubiquinone to ubiquinol can be monitored only by absorption changes in the ultraviolet region of the spectrum. Light-induced ultraviolet absorption changes do occur in photosynthetic bacteria and in chromatophore preparations, but so far it was not clear which part of these changes was due to ubiquinone photoreduction and which part was due to P870 photooxidation.

Several authors (*e.g.* refs 1–3) assumed that all of the light-induced absorption change at 275 nm was due to ubiquinone reduction. This led to the conclusion[2] that one mole of ubiquinone was reduced per mole of photooxidized P870. However,

this hypothesis was difficult to reconcile with the fact that ubiquinone requires two electrons for reduction to ubiquinol, and P870 is one-electron donor[4]. Loach *et al.*[5], on the other hand, assumed that the light-induced 275-nm absorption change was completely due to P870 photooxidation. Ke and Chaney[6], working with subchromatophore particles from *Chromatium* observed different decay kinetics at 881 and at 280 nm, respectively. This indicated that the change at 280 nm was due in part to a substance other than P870. Since, however, these measurements were not extended to other wavelengths, they provided no information about the nature of this substance.

More promising results were obtained with reaction center preparations. Clayton and Straley[7] and Clayton[8] measured light-induced absorbance changes in reaction center particles from a carotenoidless strain of *Rhodopseudomonas spheroides* in the presence of exogenous oxidants or reductants. They found absorbance changes that could be ascribed to reduction or reoxidation of an electronic acceptor. McElroy *et al.*[9] and Feher[10] working with the same particles, observed light-induced ESR signals at 1.4 °K, which they tentatively ascribed to reduction of iron.

This paper gives the results of experiments with reaction center preparations from *Rps. spheroides* (wild strain) and *Rhodospirillum rubrum* (wild strain). The results can be summarized by the following scheme:

$$PXA \overset{h\nu}{\rightleftharpoons} P^+X^-A \rightleftharpoons P^+XA^-$$

in which P is P870, and X and A are endogenous electron acceptors. The light requirement is indicated by *hν*. Absorbance difference spectra (reduced *minus* oxidized) were obtained for P870, A and X or X + A. It is suggested that X is ubiquinone and X^- is the anion of ubisemiquinone.

MATERIALS AND METHODS

Reaction center fractions were prepared as described earlier: Treatments of chromatophores with sodium dodecylsulphate resulted in the isolation of a reaction center fraction designated as SDS-RC. Treatment of SDS-RC fractions with urea and Triton X-100 at pH 10.0 gave a more purified reaction center fraction[11,12] designated as AUT-RC. The preparations were stored in Tris buffer, containing 0.05 M Tris–HCl, pH 8.0, and 0.01 M $MgCl_2$.

In contrast to our previous observation[11,12] this procedure was also successful for *Rsp. rubrum*, provided the cells were frozen at 77 °K and thawed again prior to the preparation of chromatophores. The absorbance spectrum of the reaction center fractions from *Rsp. rubrum* was essentially the same as that of AUT-RC particles from *Rps. spheroides*[11], except for a shoulder at 405 nm (possibly due to oxidized cytochrome) and a more pronounced peak at 530 nm (probably due to bacteriopheophytin) which gave the preparations a purple colour.

Light-induced absorbance changes were measured aerobically in 1-mm cuvettes with an apparatus described earlier[13]. The concentration of P870 was about 3 μM. If necessary the signals obtained at a certain wavelength were fed into a signal averager (Nuclear Chicago, Model 7100 Data Retrieval Computer) to improve the signal-to-noise ratio. Actinic light provided by a 125-W quartz–iodine lamp was

filtered by a Balzer B-40 803-nm interference filter in combination with a Schott RG10-2 and a Kodak 89B far-red cut-off filter. The intensity of the actinic light was 4.5 neinsteins·cm^{-2}·s^{-1} unless otherwise indicated.

RESULTS

Light-induced absorbance changes observed without additions

Fig. 1 shows the light-induced absorbance changes at a few representative wavelengths, observed in AUT-RC particles from *Rps. spheroides*, suspended in Tris buffer without additions (A), with 30 μM ferricyanide (B) and with 10 mM ascorbate (C). Figs 1B and 1C will be discussed later. Fig. 1A shows that without additions the kinetics both in the light and in the dark were biphasic. The fast and the slow phase in the light will be referred to hereafter as Phase a and b, respectively.

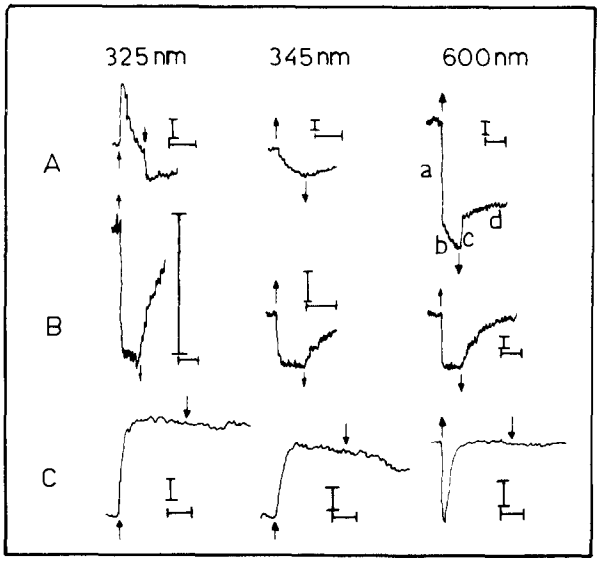

Fig. 1. (A) Light-induced absorbance changes at 325, 345, and 600 nm in AUT-RC particles from *Rps spheroides*, suspended in Tris buffer. No additions. Actinic light was switched on and off at the times indicated by the upward and downward pointing arrows The horizontal bars indicate a time interval of 10 s The vertical bars indicate an absorbance change (ΔA) of 0.001. Further details: see Methods. Each sample was illuminated only once and then allowed to stand in the dark for at least 2 h. (B) Same as (A), except that 30 μM ferricyanide was present. In order to avoid the exhaustion of ferricyanide, a fresh sample was taken after three illumination cycles consisting of 10 s light and 60 s dark Each point is the average of three, six or nine illumination cycles (C) Same as (A), except that 10 mM ascorbate was present. Each sample was illuminated only once

The fast and the slow phase of the decay kinetics will be called Phase c and d, respectively. Phase a was complete within 1 s after the onset of the actinic light. Phase b reached a steady-state level after 2–5 min of actinic illumination. The decay kinetics of Phase c and d were first order when plotted semi-logarithmically, with half times of 0.12–0.15 s and 30–70 s, respectively, depending upon the preparation.

We will show in the next sections that the Phases a–d are caused by the following reactions.

$$\text{a:} \quad PXA \xrightarrow{h\nu} P^+X^-A \tag{1}$$

$$\text{b:} \quad P^+X^-A \longrightarrow P^+XA^- \tag{2}$$

$$\text{c:} \quad P^+X^-A \longrightarrow PXA \tag{3}$$

$$\text{d:} \quad P^+XA^- \longrightarrow PXA \tag{4}$$

According to this scheme, the spectrum of the absorbance changes obtained in Phase a represents the difference spectrum (P^+X^-—PX), and the spectrum of the absorbance changes observed in Phase d represents the difference spectrum (P^+A^-—PA). These spectra are shown in Fig. 2. The spectra were matched at 600 nm. They were identical in the regions of 580–720 nm and 780–950 nm (see ref. 11, Fig. 1B), so it could be safely assumed that the bleaching at 600 nm was due to P870 oxidation only (*cf.* ref. 11). Consequently, by taking the difference between the two difference spectra shown in Fig. 2, we eliminated the difference spectrum (P^+—P) and obtained the difference spectrum [(X^-—X)—(A^-—A)], *i.e.* the difference spectrum of X *minus* the difference spectrum of A. This spectrum is shown in Fig. 3 (open circles). The same difference spectrum was obtained with SDS-RC particles from *Rps. spheroides* (not shown).

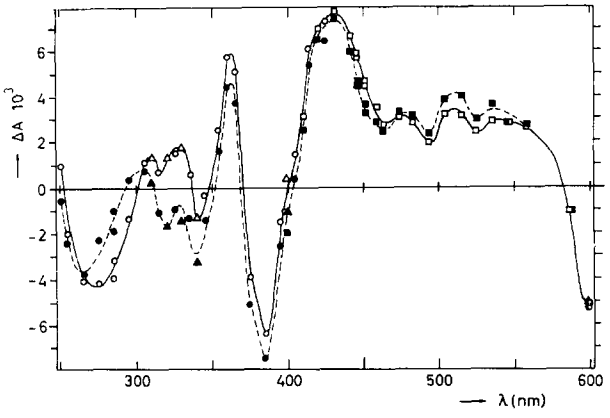

Fig. 2 Light *minus* dark absorbance difference spectrum of AU-TRC particles from *Rps. spheroides*, suspended in Tris buffer. No additions. Illumination time 10 s. Open symbols, solid line: difference spectrum of Phase a, as observed at 0.7 s after switching on the light. Solid symbols, dashed line: difference spectrum of Phase d, as observed at 2 s after darkening. The illumination time was chosen such that at 600 nm Phase d was approximately equally large as Phase a. The circles, triangles and squares refer to experiments with three different batches of particles. The spectra were matched at 600 nm; the ordinate scale refers to the spectrum with the open circles.

According to Eqns 1 and 3 Phase c represents the reversal of Phase a. This was supported by the results shown in Fig. 4. The difference spectrum (dark *minus* light) of Phase c (open circles) was proportional to the difference spectrum of Phase a (solid line). (The dashed line representing Phase d was drawn just for comparison.)

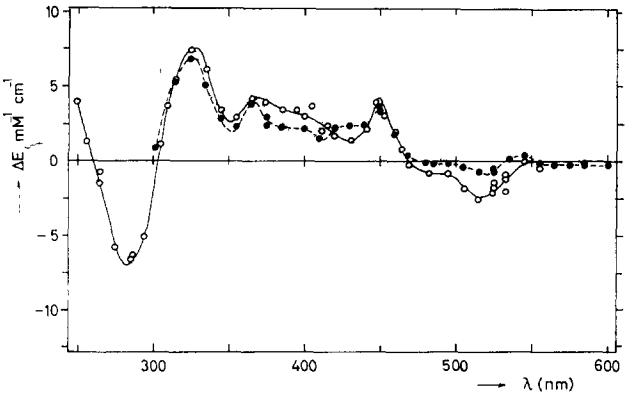

Fig 3 ○—○: AUT-RC particles from *Rps spheroides* Spectrum of [(X⁻—X)—(A⁻—A)], as determined by the difference between the two difference spectra shown in Fig 2. The ordinate is given in molar extinction units To this end, the spectra of Fig. 2 were expressed per mmole of photooxidized P870, and thus, according to our model, per mmole of X and A, before subtraction. The differential extinction coefficient of P870 (reduced *minus* oxidized) was taken to be 16 $mM^{-1} \cdot cm^{-1}$ (*cf* ref 11). ●- - -●: AUT-RC particles from *Rps spheroides* Spectrum of (X⁻—X) or [(X⁻—X) + (A⁻—A)], as determined by the light-induced absorbance changes observed in the presence of 10 mM ascorbate. In order to ensure that P870 was completely reduced, the absorbance changes were measured at 1.3 s after switching off the actinic light The data were expressed as the maximum absorbance changes per mmole of P870 present. The concentration of P870 was determined from the maximum light-induced absorbance change at 600 nm, as observed without additions : reduced *minus* oxidized difference spectrum of ubisemiquinone anion *minus* ubiquinone Data from ref. 15

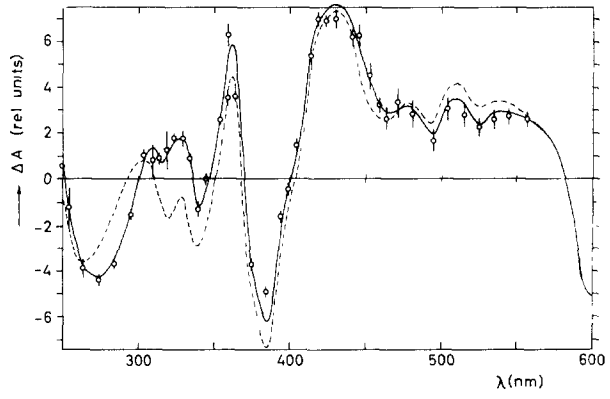

Fig 4. AUT-RC particles from *Rps spheroides* suspended in Tris buffer without additions. Open circles: difference spectrum of Phase c of the light-induced absorbance change (see Fig. 1A), after switching off the light The bars indicate the standard deviation of the mean. Solid and dashed line: difference spectra of Phase a and Phase d, respectively, as copied from Fig 2 The data concerning Phase c were obtained with the same samples as those of Fig 2 The spectra were matched at 600 nm. At this wavelength, the magnitude of Phase c was about one third of the magnitude of Phase a and d

Fig. 5 shows the kinetics of P870, measured at 600 nm as a function of the actinic light intensity and of the illumination time. In Fig. 5 the total amount of photooxidized P870 after a certain period of illumination (left) was analyzed into the fast decaying fraction (middle) and the slowly decaying fraction (right, solid lines) after switching off the actinic light. This was done at four different actinic light intensities. After

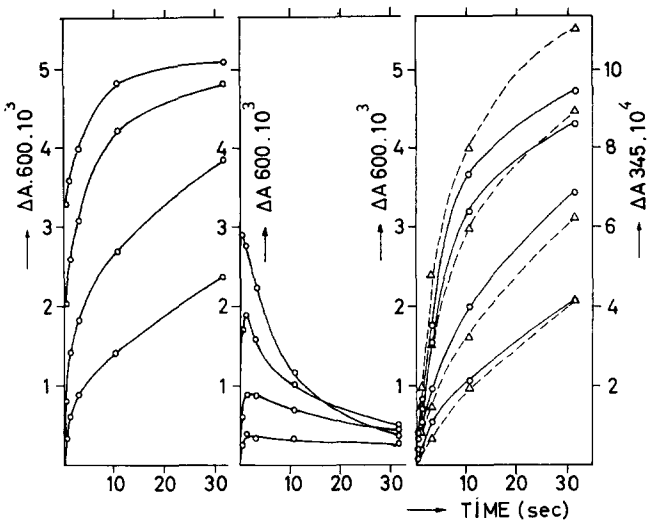

Fig 5 AUT-RC particles from *Rps. spheroides* in Tris buffer without additions Kinetics of the light-induced absorbance changes at 600 nm (○—○) and at 345 nm (△---△). Actinic light, 803 nm Intensities from upper to lower curves, 4 5, 1.5, 0 45 and 0.15 neinsteins·cm^{-2}·s^{-1}. Left: total absorbance change at 600 nm, obtained in the light, as a function of the time of illumination Middle: magnitude of Phase c of the decay of the absorbance change at 600 nm, as a function of the time of illumination. Right: magnitude of Phase d of the decay of the absorbance change at 600 nm (○—○), and magnitude of the absorbance change at 345 nm (△---△) as a function of the illumination time The values shown in the open circles were obtained by plotting the observed decay curves on a semilog scale and extrapolating to zero time after switching off the light

a short illumination time most of the photooxidized P870 decayed as Phase c. After 30 s of illumination most of the photooxidized P870 decayed as Phase d. The data fitted with a model on the form in which the k's are rate constants, I is

$$PXA \underset{k_3}{\overset{k_1 \cdot I \cdot \sigma}{\rightleftharpoons}} P^+X^-A \underset{k_4}{\overset{k_2}{\rightleftharpoons}} P^+XA^- \qquad (5)$$

the incident light intensity and σ is the absorption cross section of a reaction center particle. In fact we do not know whether A^- donated its electron to $P870^+$ (Eqn 4) directly or *via* X, but this was of no consequence for our model because of the widely different values of k_3 and k_4. For the experiment shown in Fig. 5 we found by computer simulation approximate values of 0.22, 6.7 and 0.015 s^{-1} for k_2, k_3 and k_4, respectively, and the value of $k_1 \cdot I \cdot \sigma$ ranged from 0.33 to 10 M· einstein^{-1}. In this model the Phases a, b, c and d of the absorbance changes shown in Fig. 1A represent reactions indicated by $k_1 \cdot I \cdot \sigma$, k_2, k_3 and k_4, respectively. Fig. 5 also shows that the absorbance change at 345 nm (which is an isosbestic point for Phases a and c as shown in Fig. 1A) behaved kinetically as $P^+X\ A^-$ in Eqn 5.

Light-induced absorbance changes observed in the presence of ferricyanide

Fig. 1B shows the kinetics of the light-induced absorbance changes observed in the presence of 30 μM ferricyanide. These absorbance changes were reversible within 50 s after switching off the light. The observed kinetics could be explained

with the scheme shown in Eqn 5. The reoxidation of X^- in the light proceeded in the presence of ferricyanide at a much higher rate than without additions. For instance, in the presence of 20–25 μM ferricyanide, an initial absorbance increase at 325 nm, due to the photooxidation of P870 by X was still visible, but the rate of the subsequent absorbance decrease was strongly enhanced. In the presence of 30 μM ferricyanide, however, the rate of reoxidation of X^- was so high that X^- did not accumulate and only a negative absorbance change was observed.

Fig. 6 shows the light-induced difference spectrum observed in the presence of 30 μM ferricyanide (open circles). As expected, this spectrum was different from the difference spectrum of (P^+X^-—PX) (solid line, copied from Fig. 2). It was, however, also different from the difference spectrum of (P^+A^-—PA) (dashed line), notably at wavelengths below 280 nm and above 460 nm. This indicated that in this case ferricyanide, instead of A, had reoxidized X^- in the light, the net result being a light-driven oxidation of P870 by ferricyanide. After subtraction of the absorbance changes due to ferricyanide reduction there remained a reduced *minus* oxidized difference spectrum which was attributable to P870 alone (see Discussion). This spectrum was not significantly different from the difference spectrum of (P^+A^-—PA), except at wavelengths above 460 nm. This indicates that the absorbance difference spectrum of (A^-—A) was insignificantly small except at wavelengths above 460 nm, and that the difference spectrum [(X^-—X)—(A^-—A)] (Fig. 3) is mainly the difference spectrum of (X^-—X) at wavelengths below 460 nm.

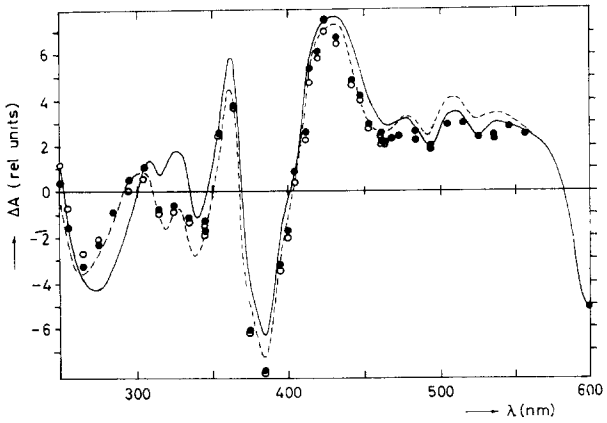

Fig. 6 AUT-RC particles from *Rps. spheroides* in Tris buffer with 30 μM ferricyanide. The same batches as shown in Fig. 2 were used Open circles: absorbance changes as observed after 10 sec of illumination. Closed circles: absorbance changes after subtraction of the difference spectrum of ferrocyanide *minus* ferricyanide, assuming that one mole of ferricyanide was reduced per mole of photooxidized P870 Solid and dashed line: absorbance changes of Phase a and d, respectively, as copied from Fig. 2. The spectra were matched at 600 nm The light-induced absorbance changes observed with ferricyanide were about half the maximum absorbance changes observed without additions See further Fig. 1B

Effect of ascorbate

Fig. 1C shows the kinetics of the light-induced absorbance changes observed in the presence of 10 mM ascorbate. The 600-nm absorbance change indicates that under these circumstances the photooxidation of P870 by X was followed by

reduction of $P870^+$ by ascorbate. Consequently, the absorbance differences observed in the steady state at other wavelengths reflect the reduction of X, or possibly of X and A.

Fig. 3 (solid circles, dashed line) shows the spectrum of the light-induced absorbance changes observed in the presence of 10 mM ascorbate, at 1.3 s after switching off the actinic light. The spectrum was similar to the one of $[(X^-—X)—(A^-—A)]$ (Fig. 3, open circles). A comparison of the magnitude of these two difference spectra indicates that, in the presence of ascorbate, only 1 mole of X was reduced per mole of P870 present. Apparently the photoreactive pool of X is identical in size to that of P870. This was also found by Clayton *et al.*[14].

The difference spectrum given by the solid circles was similar in the blue region to those obtained by Clayton and Straley[7]. Without knowing further details it is difficult to compare our data to those mentioned by Clayton[8] in a more recent paper.

As discussed above, the solid circles represent either the spectrum $(X^-—X)$ or $[(X^-—X) + (A^-—A)]$. Consequently, the difference between the open and solid circles should give either the spectrum $(A^-—A)$ or $(2A^-—2A)$. This spectrum had maxima at 515 nm and 430 nm and a negative band below 415 nm. The accuracy was low as can be judged from Fig. 3. In principle the spectrum $(A^-—A)$ could also be calculated from the $(P^+A^-—PA)$ and $(P^+—P)$ spectra (Fig. 6, solid circles, and Fig. 2, solid symbols, respectively) but the accuracy of measurement would make such a calculation practically meaningless.

Reaction center particles from Rsp. rubrum

Measurements of light-induced absorbance changes in SDS-RC particles from *Rsp. rubrum* gave essentially the same results as in AUT-RC particles from *Rps.*

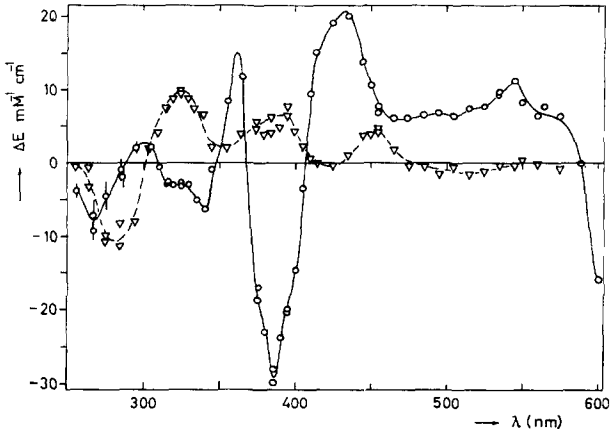

Fig. 7. SDS-RC particles from *Rsp. rubrum*. ○—○ · difference spectrum $(P^+A^-—PA)$ of the slow part of the dark decay after 3 s of actinic illumination. ▽- - -▽ : spectrum of $[(X^-—X) — (A^-—A)]$, obtained in a similar way as the spectrum of Fig. 3 (open circles), *viz.* by subtracting, after normalization at 600 nm, the $(P^+A^-—PA)$ spectrum from the $(P^+X^-— PX)$ spectrum that was obtained by measuring the fast part of the dark decay after 0.20 s of actinic illumination

spheroides, except that the conversion of fast decaying (half time about 0.2 s) P^+X^-A into slowly decaying (half time about 30 s) $P^+X\,A^-$ proceeded at a 5–10 times higher rate. The difference spectrum $[(X^-\!-\!X)^-(A^-\!-\!A)]$ was very similar to the corresponding spectrum of AUT-RC particles from *Rps. spheroides* (see Fig. 7), indicating that for these two organisms X is identical. Fig. 7 also shows the difference spectrum $(P^+A^-\!-\!PA)$, which approximates the difference spectrum $(P^+\!-\!P)$ fairly well, as discussed above.

DISCUSSION

The chemical nature of X

Fig. 3 (dotted line) shows the difference spectrum (reduced *minus* oxidized) of the ubisemiquinone anion *minus* ubiquinone, as measured in methanol. The difference spectrum was calculated from data given by Land *et al.*[15]. This spectrum is remarkably similar to the difference spectra of $[(X^-\!-\!X)\!-\!(A^-\!-\!A)]$ and of $(X^-\!-\!X)$ or $[(X^-\!-\!X) + (A^-\!-\!A)]$, obtained with AUT-RC particles from *Rps. spheroides* (Fig. 3, open and solid symbols). One difference is, however, that the latter spectra seem to be shifted to longer wavelengths by about 10 nm, as compared to the ubiquinone difference spectrum. In addition, the absorbance changes around 285 nm and in the region of 420–450 nm were smaller in the *in vivo* difference spectra than in the ubiquinone difference spectrum. Apart from these discrepancies the shape and the magnitude of the absorbance difference spectra shown in Figs 3 and 7 are so similar that we want to suggest that X is ubiquinone and X^- is the anion of ubisemiquinone in reaction center particles from *Rps. spheroides* and *Rsp. rubrum*. The discrepancies observed in the regions of 280 nm and 420–450 nm might be due to a very tight coupling between ubiquinone and the reaction center bacteriochlorophyll. Such a tight coupling may also be suggested by the observation that the $(X^-\!-\!X)$ or $[(X^-\!-\!X) + (A^-\!-\!A)]$ difference spectrum, as obtained by illumination in the presence of ascorbate, showed small absorbance changes in the region of 730–900 nm (maximum ΔE about 4 $mM^{-1}\cdot cm^{-1}$, as expressed on a P870 basis). Without ascorbate, these changes occurred in the opposite direction during Phase b of the light-on kinetics, although under these circumstances they were only visible in the region of 730–750 nm, where the difference spectrum $(P^+X^-\!-\!PX)$ is small. Clayton[8] interpreted these absorbance changes as small shifts in the absorption bands of bacteriopheophytin, P800 and P870, perhaps due to electric field interactions between these molecules on the one hand and X^- on the other hand.

The idea of a one-electron reduction of ubiquinone in the light is in accordance with results of titration experiments[16] which indicated that the primary oxidant is a one-electron acceptor with a pH-independent midpoint potential. It is not necessarily at variance with the observation by Bolton *et al.*[17] that there is only one unpaired electron per mole of photooxidized P870, as indicated by ESR-measurements at room temperature, because the latter measurements seem to have been done under conditions where X^- had been reoxidized by A already which perhaps cannot be observed by ESR spectroscopy. This is indicated by the rate of the dark decay of the ESR signal which is due to photooxidized P870 in those experiments. The direct return of electrons from X^- to P^+ has a half time of 0.06 s in reaction center preparations, according to Clayton and Yau[18]. Our value of

0.12–0.15 s is not much different. The dark decay of the light-induced ESR signal, however, had a half time of 4 s as shown in Fig. 4 of ref. 17.

The difference spectrum of (P^+—P)

The difference spectra (P^+—P) of *Rps. spheroides* and (P^+A^-—PA) of *Rsp. rubrum* (Fig. 6, solid circles, and Fig. 7, open circles, respectively) correspond fairly well with the reduced *minus* oxidized difference spectrum of bacteriochlorophyll *in vitro*, as obtained by Loach *et al.*[5]. The main discrepancy is a large blue shift at about 370 nm which is present *in vivo*, but not *in vitro*. This shift is probably due to P800, which also shows a blue shift at about 800 nm upon oxidation of P870. In addition, the (P^+—P) and the (P^+A^-—PA) difference spectra of *Rps. spheroides* exhibit a few humps at 475 nm and at 510 nm which are lacking in the (P^+A^-—PA) difference spectrum of *Rsp. rubrum.* and which are thought to be due to carotenoid shifts[11]. Our data indicate that in the ultraviolet region the difference spectrum of (P^+—P) *in vivo* has a minimum at 265 nm, a maximum at 305 nm and another, more or less clearly separated maximum at 325 nm, with differential extinction coefficients of —8 to —11, +2 and —3 $mM^{-1} \cdot cm^{-1}$, respectively.

The significance of X

Assuming that X is ubiquinone, it does not seem very likely that it is identical with the primary electron acceptor, in view of recent experiments with reaction center[9,10] and subchromatophore[19] particles, which indicated that at very low temperature only one strong ESR signal was observed upon illumination, namely the one arising from $P870^+$. In addition, some very weak light-induced ESR signals were observed which were attributed to the reduction of the primary acceptor, but this has not yet resulted in conclusive evidence as to the nature of the primary electron acceptor (*cf.* refs 9, 10, 19 and the Note added in proof in ref. 8).

It thus appears that X may be a secondary electron acceptor which is reduced within the response time of our apparatus. X may play a key role in the photosynthesis of purple bacteria. It is likely that many of the light-induced absorbance difference spectra observed earlier in chromatophores reflect the photoreduction both of X and of loosely bound (*i.e.* extractable) ubiquinone. For instance, in difference spectra obtained with chromatophores from *Rsp. rubrum*[20], *Rps. spheroides*[5] and *Chromatium*[21], photoreduction of loosely bound ubiquinone was indicated by a minimum at 275 nm, and photoreduction of X is indicated by a negative change at 283 nm (apparent as a shoulder) and by positive changes between 300 and 340 nm. Chromatophores from *Chromatium* extracted with isooctane[21] lacked the minimum at 275 nm so that the absorbance change at 283 nm showed up as a minimum. The minimum at 275 nm appeared again upon readdition of the isooctane extract or of ubiquinone. These experiments may be taken together with our results to indicate that X is a ubiquinone molecule in a special environment which is very strongly bound to the reaction center. In accordance with this observation we found that heptane extraction of SDS-RC particles from *Rps. spheroides*, which removed 3.5 moles of ubiquinone per mole of P870 (*cf.* ref. 11, Table I) did not affect the magnitude of the rate constants k_1 and k_3 (Eqn. 5), but only of k_2 and k_4, as appeared from the kinetics of the light-induced 600-nm absorbance change.

ADDENDUM

In recent experiments with iron-depleted reaction center[22] or subchromatophore[23] particles an easily measurable ESR signal was observed which was ascribed to the photoreduced primary electron acceptor. The signal had a g-value of 2.0050 $\pm$ 0.003 and a peak-to-peak width of 7.0 $\pm$ 0.3 gauss. The very weak ESR signals observed earlier in iron-containing reaction center preparations at very low temperatures[9,10] were recently ascribed[22] to ferrous iron which obtained its electron either from P870 directly or from the primary acceptor observed in ESR experiments with iron-depleted reaction center particles. It was considered possible that the reduced primary acceptor was a semiquinone radical[23]. Thus it is possible that it is identical to what is called X^- in this report, and that X is likewise the primary acceptor in our particles.

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