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PHOTOCHEMICAL ACTIVITIES OF REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHAEROIDES* AT LOW TEMPERATURE AND IN THE PRESENCE OF CHAOTROPIC AGENTS

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SUMMARY

Light-induced absorbance changes were measured at low temperatures in reaction center preparations from *Rhodopseudomonas sphaeroides*. Absorbance difference spectra measured at 100 °K show that ubiquinone is photoreduced at this temperature, both by continuous light and by a short actinic flash. The reduction occurred with relatively high efficiency. These results give support to the idea that ubiquinone is involved in the primary photochemical reaction in *Rhodopseudomonas sphaeroides*. Reduction of ubiquinone was accompanied by a shift of the infrared absorption band of bacteriopheophytin.

The rate of decay of the primary photoproducts (P^+870 and ubisemiquinone) appeared to be approximately independent of temperature below 180 °K and above 270 °K; in the region between 180 and 270 °K it increased with decreasing temperature. The rate of decay was not affected by *o*-phenanthroline. Secondary reactions were inhibited by lowering the temperature.

The light-induced absorbance changes were inhibited by chaotropic agents, like thiocyanate and perchlorate. It was concluded that these agents lower the efficiency of the primary photoconversion. The kinetics indicated that the degree of inhibition was not the same for all reaction centers. The absorption spectrum of the photoconverted reaction centers appeared to be somewhat modified by thiocyanate.

INTRODUCTION

The primary photochemical reaction of bacterial photosynthesis consists of the transfer of an electron from bacteriochlorophyll ("*P*-870" in purple bacteria) to an acceptor molecule. Although during the last few years much work has been done to identify the primary electron acceptor, its chemical nature is still in doubt: there is evidence that it may be either ubiquinone or a non-heme iron protein [1].

The assignment to ubiquinone of the function of primary acceptor in *Rhodopseudomonas sphaeroides* was based upon the observation of optical signals at room

temperature [2, 3] and ESR signals both at low and room temperature [4–6]. The contribution of the primary acceptor to the absorbance difference spectrum at room temperature resembled the difference spectrum obtained upon reduction of ubiquinone to ubisemiquinone anion [2, 3]. An ESR-signal with $g = 2.0046$, attributed to ubiquinone, could be observed at low temperature in reaction centers treated with sodium dodecyl sulfate [4, 5]. Further evidence was given recently by extraction and reconstitution experiments [7].

A broad iron-like ESR-signal, observed at low temperature in iron-containing reaction centers of *Rps. sphaeroides* mutant R-26 [8, 9] supported the idea that a non-heme iron protein may function as the primary acceptor. Recently, however, it was found that the photochemical activity at low temperature in these reaction centers was not lowered by replacement of part of the iron by manganese [10], suggesting that the iron atom itself is not involved in the primary reaction. The possibility that an iron-ubiquinone complex performs the function of the primary acceptor has also been proposed [4, 6].

In this paper we present the results of optical studies with reaction centers from *Rps. sphaeroides* at low temperatures and in the presence of chaotropic agents. Kinetic studies and light-induced difference spectra at low temperatures show that ubiquinone is photoreduced at 100 °K with relatively high efficiency. These results indicate that in *Rps. sphaeroides* ubiquinone is involved in the primary photochemical reaction.

Chaotropic agents (ionic compounds that weaken hydrophobic interactions) appeared to inhibit the light-induced absorbance changes. It is concluded that these agents lower the efficiency of the primary photoconversion.

MATERIALS AND METHODS

Reaction center particles were prepared from wild type *Rps. sphaeroides* by the method described by Slooten [11]. Briefly, a reaction center fraction was obtained by treatment of chromatophores with sodium docecylsulfate. This fraction was purified further by gradient centrifugation in the presence of urea and Triton X-100 at pH 10.0, followed by dialysis against a solution of 0.05 M Tris (pH 8.0) containing 0.01 M $MgCl_2$.

Absorbance spectra were measured on a Cary model 14R spectrophotometer. Light-induced absorbance changes were measured with a split-beam apparatus equipped with two measuring beams, as described in ref. 12. Low temperature measurements were performed with the same apparatus using the attachment described by Visser et al. [13] or with a single-beam apparatus as described in ref. 14. Measurements below 320 nm were not possible with this arrangement due to the high absorbancy of the perspex vessels. Clear samples were obtained upon cooling by mixing the reaction center preparation with glycerol (final concentration 55 %, v/v) after addition of sucrose to a concentration of 1.0 M. The actinic light for kinetic measurements was provided by either a Xenon flash lamp (duration of the flash 8 μs at one-third of the peak) or a quartz-iodine lamp, equipped with suitable interference and absorption filters. In some experiments a signal averager (Nuclear Chicago, Model 7100 Data Retrieval Computer) was used to improve the signal-to-noise ratio.

RESULTS

Light-induced absorbance changes at low temperatures

Fig. 1 shows the kinetics of the light-induced absorbance changes of *P*-870 at different temperatures. At room temperature the kinetics were biphasic, both in the light and in the dark. Slooten [2] has shown that the slow phases of the rise and decay kinetics can be ascribed to a secondary reaction consisting of the reduction of an unknown electron acceptor A by ubisemiquinone. Reaction centers that are in the state $P^+_{870}XA^-$ after illumination have a low rate of back reaction, whereas the reaction between P^+_{870} and X^- is much faster. Therefore, the second, slow phase in the light is caused by accumulation of reaction centers (P^+XA^-) with a slow back reaction, so that the extent of the slow phases in the light and upon darkening reflects the rate of reduction of A during the light period [2]. Recordings A–E of Fig. 1 show that this reduction is slowed down by lowering the temperature: at 260 °K the reaction was at least five times slower than at room temperature and below 220 °K it could not be observed at all. Comparison of the slopes of the slow phase of the dark decay of *P*-870 indicated that the rate of the back reaction of the reduced acceptor A^- with

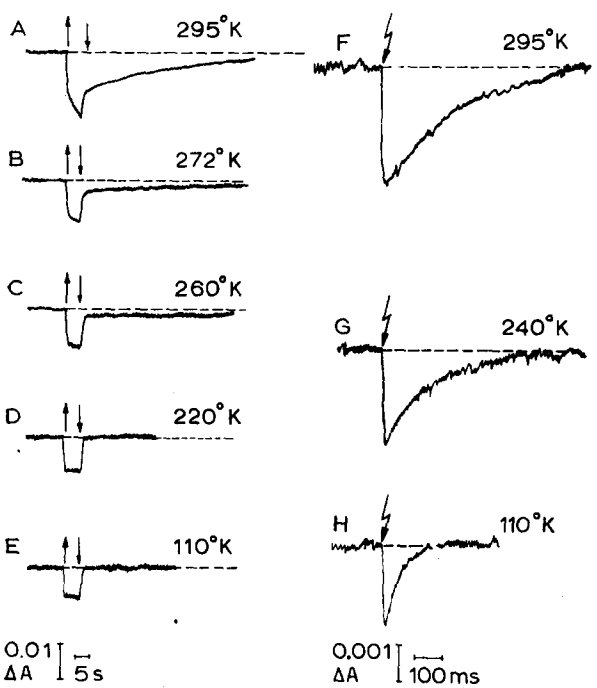

Fig. 1. Light-induced absorbance changes at 600 nm in reaction center particles of *Rps. sphaeroides* at different temperatures. Reaction centers (*P*-870 concentration, 3.0 μM) were suspended in a sucrose-containing water-glycerol mixture. The optical pathlength was 1.0 mm. Illumination at 880 nm (band width 20 nm). Recordings A–E, continuous actinic light (intensity 0.6 mW/cm^2, duration 5 s) was switched on and off at the times indicated by the upward and downward pointing arrows. Recordings F–H, a non-saturating actinic flash was given at the times indicated.

oxidized *P*-870 decreased also upon cooling; it decreased by about four-fold upon lowering the temperature from 295 °K to 260 °K.

Traces F, G and H of Fig. 1 show the kinetics of *P*-870 upon illumination with short actinic flashes. Even at room temperature the rate of reduction of A was too slow for a measurable accumulation of A^- during or after a flash. In Fig. 2 the decay kinetics are plotted on a semilogarithmic scale; in all cases investigated the decay turned out to be first-order and monophasic. The temperature dependence of the half-time of the decay of the primary reaction is shown in Fig. 3. At low temperatures up to 200 °K the half-time of decay remained approximately constant at a value of 20–40 ms, depending upon the preparation. Between 200 °K and 270 °K there was

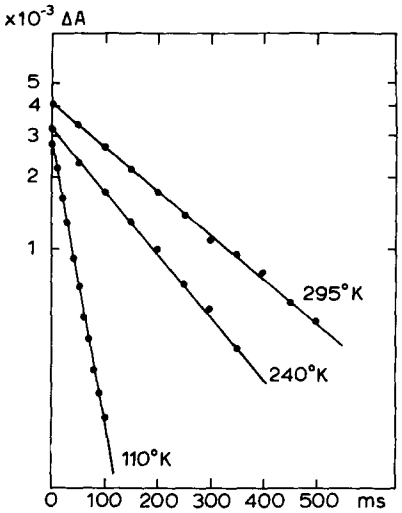

Fig. 2. Semilogarithmic plot of the decay following a non-saturating actinic flash. Same conditions as in Fig. 1, recordings F–H.

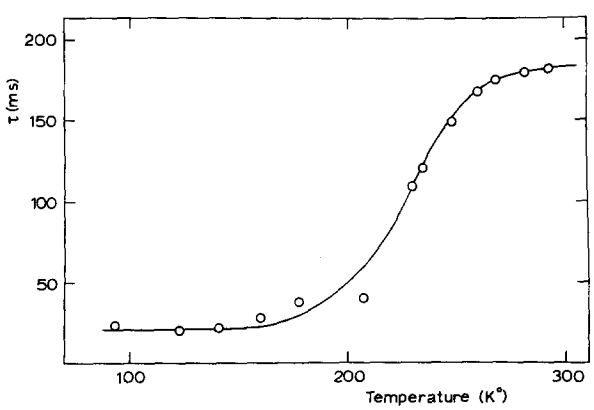

Fig. 3. Temperature dependence of the half-time of decay (τ) at 360 nm following a non-saturating actinic flash. Conditions as for Fig. 1, recordings F–H.

an increase to a value of 180–220 ms. At temperatures between about 270 °K and 295 °K the half-time of the decay was almost independent of temperature again. At 295 °K the rate of decay was the same in the absence of glycerol and sucrose; however, it is apparently dependent upon the type of reaction center preparation. Higher rates were observed with reaction centers prepared from wild type *Rps. sphaeroides* with lauryl dimethyl amine oxide instead of sodium dodecyl sulfate; with this preparation we measured a half-time of decay of 100 ms. After illumination with continuous actinic light the fast phase of the decay showed the same rate constant as after a short flash both at room and at low temperature.

Figs. 4 and 5 show the absorbance spectrum and the light-induced absorbance

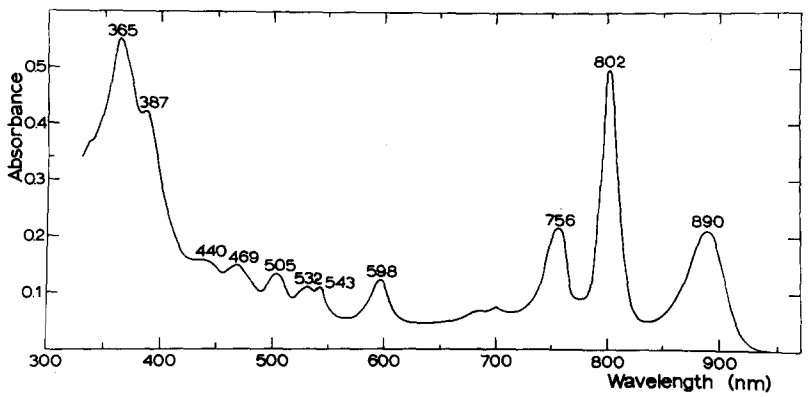

Fig. 4. Absorption spectrum of reaction center particles of *Rps. sphaeroides* at 100 °K. The particles were suspended in a sucrose containing water-glycerol mixture. Optical pathlength 1 mm.

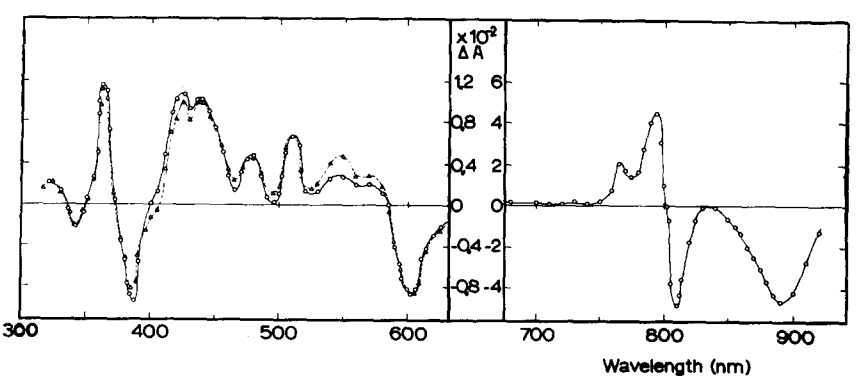

Fig. 5. ○−○, Light minus dark absorption difference spectrum of reaction centers of *Rps. sphaeroides* at 100 °K. Illumination with non-saturating actinic light of 880 nm (intensity 2.3 mW/cm²) for the region 320–630 nm and actinic light of 426 nm (intensity 3.7 mW/cm²) for the region 600–930 nm. Both regions of the spectrum were matched at 602 nm. The concentration of reaction centers was the same as used at the measurement of the absorption sprectum of Fig. 4. $\Delta-\Delta$, Light minus dark difference spectrum under the same conditions, but in the presence of 0.1 M thiocyanate. The spectra were matched at 602 nm.

difference spectrum at 100 °K. Most bands due to bacteriochlorophyll and carotenoid were sharper than in the corresponding spectra [11] of similar preparations obtained at room temperature. In the infra-red region the shift of the *P*-870 band in the absorbance spectrum from 870 nm at room temperature to 890 nm at 100 °K is reflected by a shift of the corresponding peak in the difference spectrum. The band near 765 nm is probably identical to the one observed by Clayton and Straley [3] at room temperature accompanying the reduction of ubiquinone in the presence of *N*-methylphenazonium methosulfate and ascorbate, which was attributed to a shift of the bacteriopheophytin band. The band in the room temperature difference spectrum near 430 nm was resolved into two bands near 420 and 440 nm. The last one, together with the peaks at 480 and 510 nm, may be partly due to a shift of the carotenoid absorbing at 440, 469 and 505 nm.

The difference spectrum in the ultraviolet region is of special interest since absorption changes due to ubiquinone reduction are most easily observed in this region [2, 3, 15]. Comparison of the shape of the spectrum at 320–350 nm with room temperature difference spectra reported by Slooten [2] and Ke et al. [15] indicates that there is a contribution of ubiquinone photoreduction to the spectrum shown in Fig. 5. The same spectrum was obtained after short actinic flashes. This indicates that ubiquinone is reduced with high efficiency at 100 °K. The decay after a flash (not shown) was the same at 330 nm (where the contribution due to ubiquinone was relatively high) and at 360 nm, both at 100 °K and 295 °K.

The effect of o-phenanthroline and chaotropic agents

The addition of low concentrations of *o*-phenanthroline, which is assumed to inhibit electron transfer between the primary and secondary acceptor [15–19] had little or no effect upon the kinetics of the light-induced absorbance changes at room temperature. In contrast to the findings of Clayton et al. [16] no inhibiting effect was found with concentrations up to $5 \cdot 10^{-4}$ M *o*-phenanthroline. However, the electron transfer to externally added ubiquinone appeared to be blocked in the same way as reported by Clayton and coworkers. We confirmed the observation [16] that a higher concentration *o*-phenanthroline (2 mM) inhibited the primary photochemical reaction. It did not affect the rate of the re-reduction of oxidized *P*-870 however.

In the presence of chaotropic agents the photochemical activity of the reaction center particles was decreased considerably. Traces a to d of Fig. 6 show the effect of 0.5 M potassium thiocyanate upon the kinetics of the light-induced absorbance changes at 600 nm at room temperature. Apparently the efficiency of the *P*-870 photo-oxidation was decreased by the addition of thiocyanate. This effect was also shown by other chaotropic agents. Table I gives the extent of the fast phase of the rise kinetics at a high, but non-saturating intensity of illumination for some chaotropic agents. At low temperatures the absorbance changes in the presence of thiocyanate were smaller also, as can be seen from traces e and f of Fig. 6. The effect was dependent on the concentration: at 0.05 M the absorbance change was 50 %, at 2.0 M only 5 % of the control.

At 100 °K, the rate of the decay of the signal upon darkening, measured at 360 nm, was the same with and without thiocyanate. At 0.1 M the half-time was found to be 40 ± 5 ms, both after a flash and after continuous illumination, compared to 35 ± 5 ms for the control. At room temperature the results were somewhat more

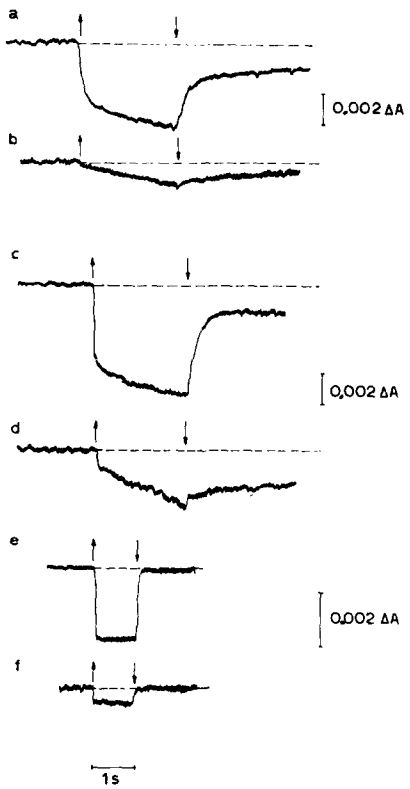

Fig. 6. Light-induced absorbance changes at 600 nm with and without thiocyanate present at different intensities of actinic light (880 nm) at room and at low temperature. Concentration of *P*-870, 1.0 μM. Conditions: a–d, room temperature; e, f, 100 °K; a, b, light intensity 1.1 mW/cm^2; c, d, 4.5 mW/cm^2; e, f, 2.3 mW/cm^2; a, c, e, no additions; b, d, f, 0.5 M thiocyanate.

TABLE I

EFFECT OF SOME CHAOTROPIC AGENTS UPON THE ACTIVITY OF THE PRIMARY PHOTOCHEMICAL REACTION

Photochemical activities are expressed as the extent of the fast phase of the light-on kinetics (see Fig. 6) at 600 nm, relative to the control. Light intensity: 4.5 mW/cm^2. The concentration of the chaotropic agents added was 0.5 M with all measurements. Incubation 15 min at room temperature. Further conditions as for Fig. 6c.

Addition	Photochemical activity (% of control)
No additions	100
Perchlorate	18
Thiocyanate	26
Iodide	26
Chloroacetate	34
Fluoroacetate	35
Urea	47
Guanidine hydrochloride	78

complicated: thiocyanate had no effect upon the decay after continuous illumination, but after a flash the decay appeared to be faster with than without thiocyanate (half times 80–100 ms and 180 ms, respectively). The low temperature difference spectrum was somewhat modified by thiocyanate (Fig. 5, dotted line), especially near 550 and 400 nm. As the absorption spectrum of the unbleached preparation was not significantly affected, these differences must be due to an effect of thiocyanate upon the absorption spectrum of the photoconverted reaction centers. The difference spectrum in the region 320–350 nm was not affected, indicating that photoreduction of ubiquinone occurred in the presence of thiocyanate also.

DISCUSSION

The results reported in this paper support the hypothesis that ubiquinone is the primary electron acceptor in *Rps. sphaeroides*. Comparison of the light-induced difference spectra at room and low temperature shows that ubiquinone is reduced at 100 °K, both in a short flash and by continuous light. Both the amount of *P*-870 oxidized and the amount of ubiquinone reduced at 100 °K by a non-saturating flash were 45 % lower than at room temperature, which indicates that the quantum efficiency of the reaction is somewhat decreased by lowering the temperature. Our results do not exclude that some other substance accepts electrons before ubiquinone; if so, however, they show that this substance reduces ubiquinone within a few ms at 100 °K.

The rates of re-reduction of P^{+}_{870} and reoxidation of ubisemiquinone were the same at 100 °K, as reflected by the half-time of the decay at 360 and 330 nm, respectively. This indicates that a back reaction between these compounds is the only "dark" reaction that occurs at this temperature.

In contrast to these results, Ke et al. [15] obtained a low temperature difference spectrum with subchromatophore particles from *Chromatium vinosum* which showed no contribution of ubiquinone photoreduction. We obtained essentially the same difference spectrum as reported by Ke et al. with a partially purified reaction center preparation from *Chromatium* (unpublished results). This may indicate that the primary electron acceptors in *Rps. sphaeroides* and in *Chromatium vinosum* are not identical. An ESR-signal, observed with *Chromatium* chromatophores and probably arising from the reduction of the primary acceptor, was attributed to an iron-sulphur protein [20]. It should be mentioned that chemical analysis of our reaction centers of *Rps. sphaeroides* indicated that several atoms of non-heme iron were present per reaction center.

The band near 765 nm in the low temperature difference spectrum is probably identical to the small shoulder on the *P*-800 band, which can be observed at room temperature. This band, accompanying ubiquinone photoreduction [3] may be due to a shift of the bacteriopheophytin band. In this way the behavior of bacteriopheophytin appears to be comparable to that of *C*-550 in algae and higher plants [21].

The temperature dependence of the rate of the decay of photo-oxidized *P*-870 was shown in Fig. 3. Our data indicate that this rate is independent of temperature below 180 °K and decreases above this temperature. A temperature independence below 180 °K was also observed by McElroy et al. [22, 23] and by Hsi and Bolton [18] and interpreted in terms of a quantum mechanical tunnelling process. A decrease

of the rate of decay with rising temperature was explained by an increase of the distance between *P*-870 and the primary acceptor [18].

Above about 250 °K the temperature curve levels off again and reaches a value of 180–220 ms at 295 °K. This was not observed by Hsi and Bolton who did not report measurements between 230 and 295 °K. The measurements of Hsi and Bolton were performed in the presence of *o*-phenanthroline; it should be noted that this substance with our preparation did not affect the rate of the back reaction. Clayton and Yau reported a temperature independent decay between 230 and 295 °K with a half-time of 90 ms for a reaction center preparation prepared with lauryl dimethyl amine oxide and suspended in 85 % glycerol [24]. They also noted several components in the decay curve. It is not clear if these reflect secondary and tertiary electron acceptors, or that these are due to the high concentration of glycerol used with these measurements. The shape of our temperature curve may indicate that above 250 °K the Boltzmann-energy becomes sufficient to induce a "normal" back reaction in addition to the tunnelling process. The difference in the decay rates at room and at low temperature was obviously not caused by the action of two different electron acceptors, since the difference spectra at these temperatures were very similar.

The main effect of the chaotropic agents studied appears to be a lowering of the efficiency of photoconversion. This is especially clear from the results obtained at low temperature, where the rate of back reaction between ubisemiquinone and $P^{+}{}_{870}$ was not affected, whereas the extent of the absorbance changes was decreased. The lower efficiency might be due to a shortening of the lifetime of an intermediate state in the photochemical reaction, like e.g. the recently proposed intermediate P_F [25]. Comparison of the kinetics at room temperature with and without added chaotropic agent indicates that some reaction centers are more strongly affected than others. The kinetics at a given intensity in the presence of a chaotropic agent were not the same as those without addition at a lower intensity of illumination, as would be the case when all reaction centers would be affected in the same way. In addition, the kinetics in the presence of thiocyanate suggest that this agent stimulates the reduction of the secondary acceptor A by ubisemiquinone. Such a stimulation was not observed with the other agents used. The observation that the rate of the back reaction between $P^{+}{}_{870}$ and ubisemiquinone was not, or relatively little affected indicates that the chaotropic agents did not alter the structure of the reaction center to such an extent as to change the distance between these compounds.

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