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THE PRIMARY PHOTOREACTIONS IN THE COMPLEX CYTOCHROME-*P*-890 · *P*-760 (BACTERIOPHEOPHYTIN$_{760}$) OF *CHROMATIUM MINUTISSIMUM* AT LOW REDOX POTENTIALS

V. A. SHUVALOV and V. V. KLIMOV

Institute of Photosynthesis, U.S.S.R. Academy of Sciences, Pushchino, Moscow Reg. (U.S.S.R.)

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SUMMARY

Experimental evidence for electron transfer, photosensitized by bacteriochlorophyll, from cytochrome *c* to a pigment complex *P*-760 (involving bacteriopheophytin-760 and also bacteriochlorophyll-800) in the reaction centers of *Chromatium minutissimum* has been described. This photoreaction occurs between 77 and 293 °K at a redox potential of the medium between −250 and −530 mV. Photoreduction of *P*-760 is accompanied by development of a wide absorption band at 650 nm and of an EPR signal with $g = 2.0025 \pm 0.0005$ and linewidth of 12.5 ± 0.5 G, which are characteristic of the pigment radical anion.

It is suggested that the photoreduction of *P*-760 occurs under the interaction of reduced cytochrome *c* with the reaction center state P^+-890 · P^--760 which is induced by light. The existence of short-lived state P^+-890 · P^--760 is indicated by the recombination luminescence with activation energy of 0.12 eV and $\tau_{\frac{1}{2}} \leqslant 6$ ns. This luminescence is excited and emitted by bacteriochlorophyll and disappears when *P*-760 is reduced.

At low redox potentials, the flash-induced absorbance changes related to the formation of the carotenoid triplet state with $\tau_{\frac{1}{2}} = 6$ μs at 20 °C are observed. This state is not formed when *P*-760 is reduced at 293 and 160 °K. It is assumed that this state is formed from the reaction center state P^+-890 · P^--760, which appears to be a primary product of light reaction in the bacterial reaction centers and which is probably identical with the state P^F described in recent works.

INTRODUCTION

Recent works [1–3] show that the "primary" electron acceptor of bacterial reaction centers is a complex including both ubiquinone and Fe. However, when the primary electron acceptor is reduced, the transient states of reaction centres are observed, such as the triplet state of *P*-870 [4], short-lived states P^F and P^R [5, 6]. The P^F formation occurs with a high quantum yield both at 15 and 295 °K, and state P^F may be interpreted as an intermediate in the photooxidation of *P*-870 [5–7].

The study of these photoreactions is of great interest for understanding the mechanism of charge separation in the reaction centers.

One of the points related to this problem is the question of the role of bacteriopheophytin in the primary processes. As far back as 1951, the capability of bacteriopheophytin to be photoreduced was demonstrated in vitro [8]. In vivo, the photoreduction of ubiquinone in centers of *Rhodopseudomonas spheroides* [9] and *Rhodospirillum rubrum* [10] is accompanied by the shift of a bacteriopheophytin absorption band at 760 nm, with negligible changes at 540 nm. The bacteriopheophytin absorption bands at 540 and 750 nm bleach in the difference spectrum of P^F [5–7]. Furthermore, in the chromatophores and B890 complex of *Chromatium minutissimum* at low redox potentials, the reversible photoreduction of bacteriopheophytin has been observed [10, 11]. This reaction was found to be related to the activity of reaction centers [10, 11] and accompanied by a decrease in fluorescence yield which has been described earlier [12].

The study of photoreduction of the pigment complex *P*-760, including bacteriopheophytin and bacteriochlorophyll-800, and its relation to the primary photoreaction in the B890 complex of *Chr. minutissimum* is described here.

MATERIALS AND METHODS

The B890 complex of *Chr. minutissimum* was prepared as described in detail elsewhere [13]. Chromatophores were treated with Triton X-100 (2.5–3 %) and the B890 complex was isolated by means of electrophoresis in polyacrylamide gel. For use in experiments, the B890 complex was suspended in 0.05 M phosphate buffer, pH 7.0, or in a mixture of this buffer and glycerol (1 : 4, v/v) for low temperature measurements. The ratio *P*-890/B890 in the B890 complex was 1 : 32. The oxidation of antenna bacteriochlorophyll with negligible loss of *P*-890 activity was achieved by the incubation of the B890 complex in the presence of ferricyanide (1 mM) for 10 h at 20 °C. This preparation was only used for the measurement of the sensibilization by *P*-890 of *P*-760 photoreduction.

The absorbance changes induced by continuous actinic light were measured in a 10-mm cuvette with the phosphoroscopic set-up described earlier [14]. To measure the action spectra of the absorbance changes, a grating monochromator MDR-2 with $f/d = 2.5$ was used.

The absorbance changes following a flash excitation were measured using an apparatus which consisted of a flash lamp of 4 μs, stabilized light source, monochromator, photomultiplier and oscilloscope. To measure the absorbance changes in the region of 380–600 nm, a blue filter (CZC-22) was placed between the sample and photomultiplier. Flash light was passed through a red filter (KC-15).

To excite the luminescence ("fluorescence") with $\tau_{\frac{1}{2}} < 10^{-7}$ s, the beam from a helium-neon laser with $\lambda = 632.8$ nm was used. The beam was modulated with a frequency of 8.4 MHz by means of an ultrasonic liquid chamber. The photomultiplier signal was measured both at the modulation frequency of 8.4 MHz and in direct current. The comparison of the degree of modulation in the beam of exciting light (m_s) with that of luminescence (m_l) allowed the estimation of the lifetime ($\tau_{\frac{1}{2}}$) of luminescence to be made by the following equation [15]:

$$\tau_{\ddagger} = \frac{m_s\sqrt{1-(m_l/m_s)^2}}{2\pi f \cdot m_l}$$

To measure the excitation spectrum of the luminescence, the monochromatic exciting light (from an incandescent lamp) was polarized perpendicular to the light emission.

Light-induced changes of the luminescence excited by laser, were measured with a disc phosphoroscopic set-up which is similar to that described earlier [16]. The dark interval between an actinic light ($\lambda \geqslant 720$ nm, intensity $9 \cdot 10^4$ ergs $\cdot$ s$^{-1} \cdot$ cm^{-2}) and the measurement of the luminescence was 0.4 ms.

An EPR signal was measured in a 3-cm EPR spectrometer using a cuvette of $30 \times 10 \times 0.1$ mm.

The value of the redox potential (E_h) of the medium was measured in a hermetically sealed cuvette under anaerobic conditions using platinum and calomel electrodes [11].

RESULTS

Photoreduction of pigment complex P-760. Fig. 1A shows the absorption spectra of the B890 complex of *Chr. minutissimum*, before and after the oxidation of antenna bacteriochlorophyll. Figs. 1B and 1C illustrate the difference absorption spectra (light minus dark) of the B890 complex measured at different redox potentials of medium. At the redox potential of $+300 \pm 10$ mV, the difference spectrum of *P*-890 oxidation is observed. At the redox potential of -450 ± 20 mV, the difference spectrum is characterized by bleaching in bacteriopheophytin absorption bands near 543 and 760 nm, and the bacteriochlorophyll ones near 595 and 805 nm with the development of band at 790 nm and of broad bands at 430 and 650 nm (Fig. 1C). No bleaching in absorption at 890 nm is observed. At the redox potential of -450 mV, the intensity of the absorbance changes at 805 nm is similar to that at the redox potential of $+300$ mV.

The photoprocess at low redox potentials (Fig. 1C) occurs between -250 and -530 mV. The lowering of the redox potential does not influence the photoprocess rate, but it decreases the recovery rate of the absorbance changes in the dark. This indicates the reductive nature of the photoprocess. The complex of light-induced spectral changes, obtained at low redox potential and described earlier [10, 11], are designated here as the photoreduction of the pigment complex *P*-760.

Quantum yields were estimated from the comparison of *P*-760 photoreduction rate at the redox potential of -450 mV and that of *P*-890 photooxidation at the redox potential of $+300$ mV under weak, continuous illumination. Fig. 2 shows that the rates of both *P*-760 photoreduction and *P*-890 photooxidation, as measured by the absorbance changes at 805 nm, are just the same at the 600 : 1 ratio of actinic light intensities. It means that the quantum yield of *P*-760 photoreduction is approx. 0.002 of that of *P*-890 photooxidation.

Fig. 3 shows the action spectra of *P*-890 photooxidation and *P*-760 photoreduction. One can see that both photoprocesses are sensitized by light absorbed by bacteriochlorophyll-890. It should be noted that the *P*-760 photoreduction was also observed after the oxidation of antenna bacteriochlorophyll. In this case the process was sensitized by *P*-890 itself.

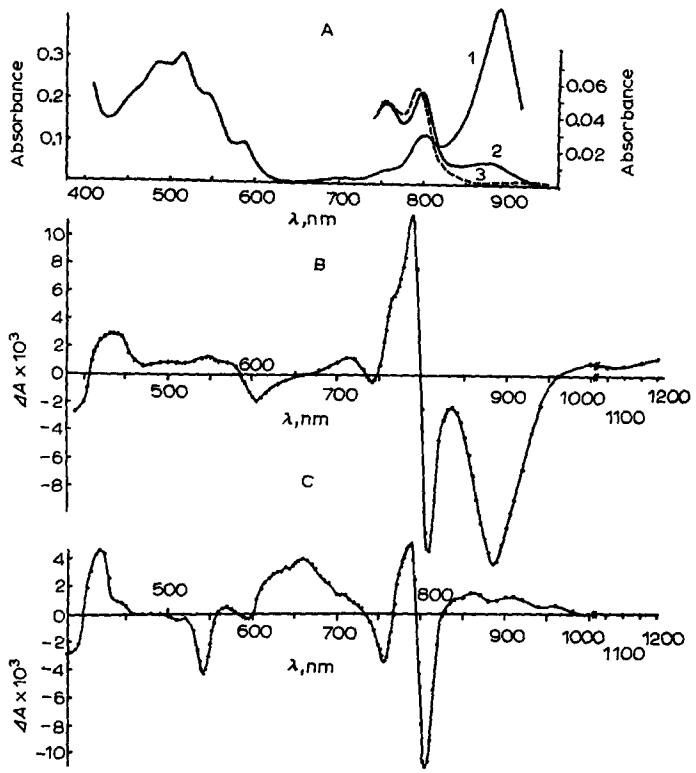


Fig. 1. Some spectral properties of B890 complex of *Chr. minutissimum* under various conditions. A absorption spectrum of control (curve 1) and absorption spectra of control after the oxidation of B890 pool with ferricyanide (1 mM for 10 h at 20 °C) which were measured without (curve 2) or with (curve 3) continuous actinic illumination at $E_h = +300$ mV; the left scale of absorbance is for curve 1, the right one is for curves 2 and 3. B and C, difference absorption spectra ("light minus dark") of complex B890 at $E_h = +300 \pm 10$ mV (B) and in the presence of 0.5 mg/ml dithionite and 3 μM methyl viologen under anaerobic conditions ($E_h = -450 \pm 20$ mV) (C). Experiments were carried out in phosphate buffer, pH 7.0, at 20 °C. Intensities of measuring and actinic light were 0.5 ergs · cm^{-2} · s^{-1} and 9 · 10^4 ergs · cm^{-2} · s^{-1} ($\lambda \geqq 720$ nm), respectively.

In a film obtained by drying the B890 complexes in the presence of dithionite and *o*-phenantroline, there is in addition to absorbance changes related to *P*-760 photoreduction, a light-induced oxidation of cytochrome *c* (Cyt. *c*): the development of band at 410 nm and the bleaching of bands at 423 and 552 nm (Fig. 4A). Kinetics of the light-induced oxidation of cytochrome are similar to that of *P*-760 photoreduction, and the dark kinetics of cytochrome$^+$ reduction ($\tau_{\frac{1}{2}} \simeq 6$ s) resembles that of P^--760 oxidation (Fig. 4C).

Electron transfer from cytochrome to pigment *P*-760, photosensitized by bacteriochlorophyll, is also observed at 77 °K in the film (Fig. 4B). 1 mol of oxidized cytochrome is generated approximately per mol of bacteriopheophytin if $\varepsilon_{423\,nm}^{ox\text{-}red} = 70\ mM^{-1} \cdot cm^{-1}$ for cytochrome [17] and $\varepsilon_{543\,nm} = 28\ mM^{-1} \cdot cm^{-1}$ for bacteriopheophytin [18]. The photoprocess at 77 °K occurs with approximately the same

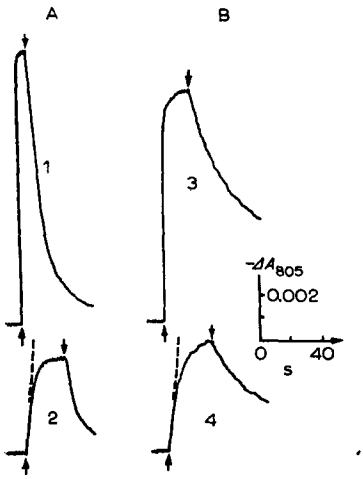


Fig. 2. Kinetics of light-induced absorbance changes at 805 nm related to photooxidation of *P*-890 at $E_h = +300$ mV (A) and to photoreduction of *P*-760 at $E_h = -450$ mV (B) on illumination of B890 complex by actinic light with $\lambda \geqq 720$ nm of various intensities: 100 % ($9 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}) saturating both photoreactions (curves 1 and 3); 0.03 % (curve 2) and 18 % (curve 4); at 20 °C. ↑, actinic light on; ↓, actinic light off. The absorbance of the sample was 0.3 at 890 nm.

quantum yield as at 293 °K. Only 10 % of the light-induced absorbance changes are reversible in the dark at 77 °K (Fig. 4C).

The illumination of B890 complex at the redox potential of −430 mV gives an EPR signal with $g = 2.0025 \pm 0.0005$ and linewidth of 12.5 ± 0.5 G (Fig. 5A). Kinetics of light-induced EPR signal correspond to that of *P*-760 photoconversion (Figs. 5B and 5C).

The *P*-760 photoreduction occurs in cells, chromatophores and B890 complex of *Chr. minutissimum*, chromatophores and B890 complex of *Thiocapsa roseopersicina* and it is not observed in chromatophores and reaction centers of *Rhs. rubrum*, B870

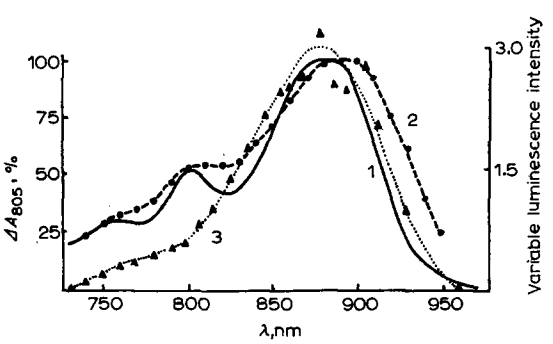


Fig. 3. Action spectra of *P*-890 oxidation at $E_h = +300$ mV (curve 1) and *P*-760 reduction at $E_h = -430$ mV (curve 2) in B890 complex at 20 °C. The spectral width of the slit was 8 nm for curve 1 and 12 nm for curve 2. Absorbance of the sample was 0.3 at 890 nm. Curve 3, the excitation spectrum of variable luminescence (λ of emission $\geqq 900$ nm, actinic light with $\lambda \geqq 720$ nm and intensity of $3 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}), monochromatic exciting light with an intensity of 0.5 erg $\cdot$ cm$^{-2} \cdot$ s^{-1}).

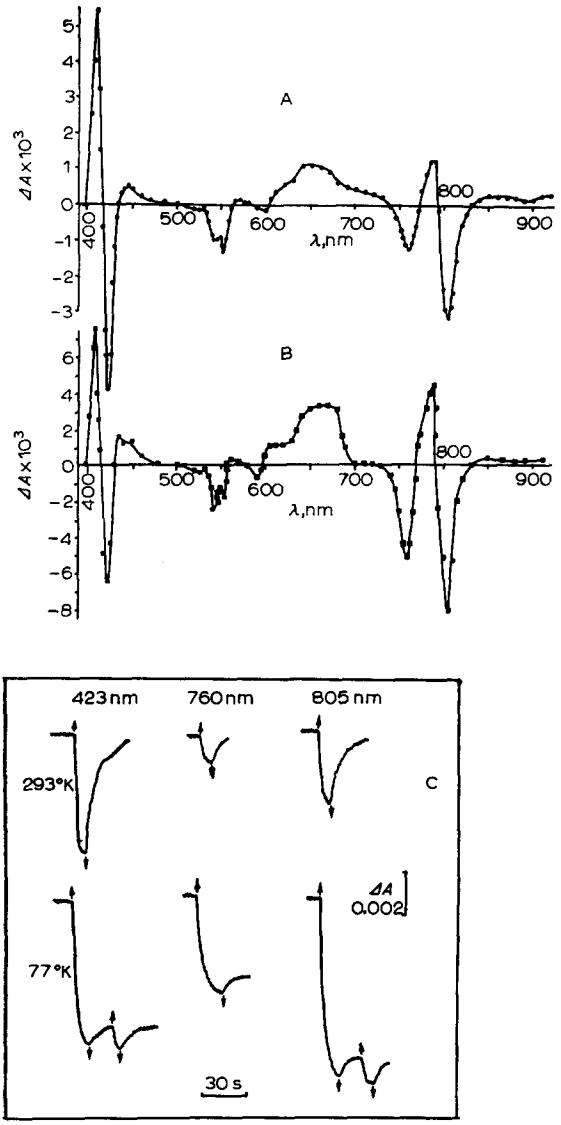


Fig. 4. A and B, difference absorption spectra ("light minus dark") of the film, obtained by drying suspension of B890 complex in the presence of dithionite (0.5 mg/ml) and *o*-phenantroline (0.1 mM), as measured at 293 °K (A) and at 77 °K (B). C, kinetics of light-induced absorbance changes of the sample at 293 and 77 °K. Absorbance of the sample at 890 nm was 0.25. The film was kept under vacuum conditions in the presence of water vapour.

complex of *Rhodopseudomonas palustris* and chromatophores of *Rps. spheroides* (Fig. 6, see also refs. 10, 11).

Variable luminescence ($\tau_{\frac{1}{2}} \leqslant 6$ *ns and activation energy of 0.12 eV*). At the redox potential of +300 mV, a light-induced increase in fluorescence quantum yield is observed with the B890 complex, which is related to *P*-890 photooxidation [11]. On lowering of the redox potential to −450 mV, the "dark" fluorescence (lumines-

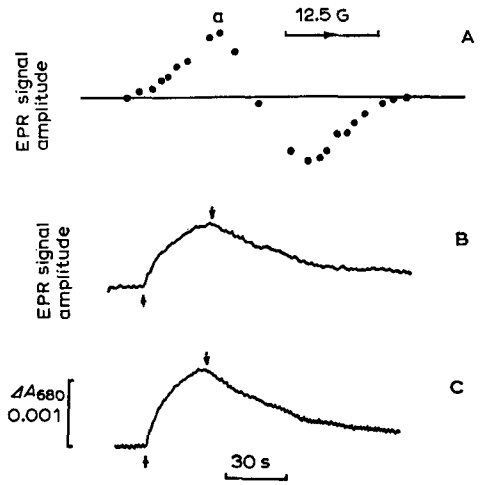


Fig. 5. (A), Difference spectrum ("light minus dark") of EPR. (B), kinetics of light-induced EPR signal at the point "a" of spectrum, and (C) kinetics of absorbance changes at 680 nm of B890 complex at $E_h = -430$ mV (293 °K). EPR spectrometer was set at a modulation amplitude 3 G and microwave power 1 mW. The *P*-890 concentration was 35 μM.

cence) is enhanced. Under these conditions, the reversible light-induced decrease in luminescence is observed. Kinetics of this process correspond to that of *P*-760 photoconversion (Fig. 7).

The estimation of excitation and emission spectra of the variable luminescence at the redox potential of −450 mV showed that this luminescence is excited by light with $\lambda > 860$ nm (absorbed by bacteriochlorophyll) and it is emitted by bacteriochlorophyll (λ of emission $\geqslant 900$ nm) (Fig. 3).

The negative changes of luminescence are decreased when the temperature is lowered (in agreement with data on luminescence of chromatophores [11]). They approach to zero at $T \leqslant 220$ °K, in contrast with the value of *P*-760 photoreduction, which is independent of temperature in the region of 293 to 160 °K in the B890 complex suspended in 80 % glycerol (Fig. 7). The activation energy of the variable luminescence, decreasing under illumination, is 0.12 eV between 220 and 290 °K (Fig. 7D). These results are in contrast with the data on fluorescence increase following the *P*-890 photooxidation: both processes occur over a whole temperature range [11, 19]. This difference suggests that the luminescence decrease following *P*-760 photoreduction reflects the drop of quantum yield of recombination luminescence, rather than that of fluorescence.

The luminescence decrease related to *P*-760 photoreduction, is observed when a photomultiplier signal is registered at the modulation frequency of 8.4 MHz and in direct current (Figs. 7A and 7B). The m_1/m_s ratio is 0.98 ± 0.03, i.e. a lifetime of variable luminescence is less than 6 ns (see Methods).

Flash-induced absorbance changes. Fig. 8 shows that at the redox potential of −450 mV, a light flash ($\lambda > 650$ nm) induces absorbance changes in the B890 complex with $\tau_{\frac{1}{2}} \simeq 6$ μs at 293 °K and 20 μs at 160° K. A difference spectrum of this signal in the 380–450 nm region (Fig. 8A) and its lifetime at 293 °K (Fig. 8B) resemble those of the state P^R described earlier for reaction centres of *Rps. spheroides* [5]. In the 470–

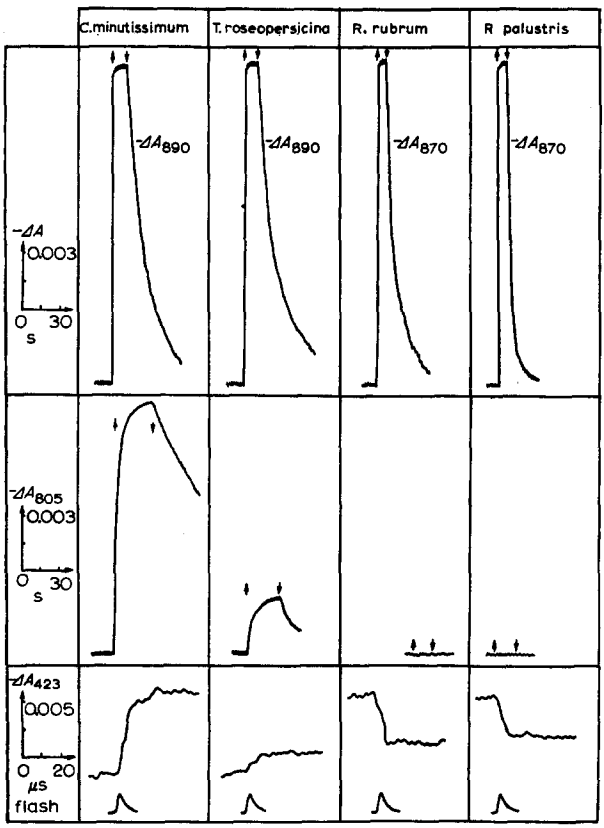


Fig. 6. Comparison of some optical properties of various bacterial preparations: B890 complex of *Chr. minutissimum*, B890 complex of *T. roseopersicina*, B870 complex of *Rps. palustris* and *Rhs. rubrum* chromatophores prepared as described earlier [10, 13]. Light-induced absorbance changes at 870–890 nm indicate photooxidation of *P*-870 (890) at $E_h = +300$ mV. Absorbance changes at 805 nm are related to the *P*-760 photoreduction at $E_h = -450$ mV. Flash-induced absorbance decrease at 423 nm in *Chr. minutissimum* and *T. roseopersicina* in the presence of 1 mM ascorbate and 1 μM 2,6-dichlorophenolindophenol indicates the oxidation of cytochrome *c*; and absorbance increase in the region of 410–440 nm in *Rps. palustris* and *Rhs. rubrum* under the same conditions is related to the *P*-870 photooxidation.

520 nm region (Fig. 8A), the spectrum is characterized by bleaching of absorbance bands which probably belong to carotenoids. The identity of absorbance change kinetics in the region of 380–590 nm and the comparison with data of a recent work [20] can indicate that these absorbance changes are due mainly to formation of a carotenoid triplet (car^T), rather than of the state P^R.

The light of the flash lamp does not saturate the absorbance changes, but the light of Q-switched ruby laser (duration 30 ns, $\lambda = 694$ nm, intensity $3 \cdot 10^6$ ergs $\cdot$ cm^{-2}) does.

The flash-induced absorbance changes of the B890 complex are not observed when *P*-760 is reduced by continuous light at both 293 and 160 °K (Fig. 8C). The P^--760 oxidation in the dark is accompanied by the appearance of the flash-induced absorbance changes (Fig. 8C).

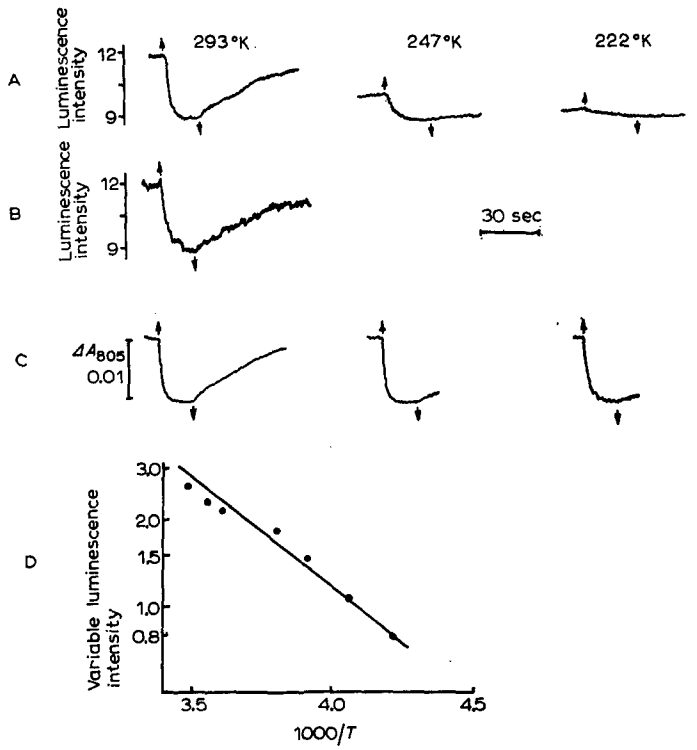


Fig. 7. Dependence on temperature of "fluorescence" (luminescence) changes and absorbance changes at 805 nm of B890 complex at $E_h = -450$ mV in the presence of 1 μM methyl viologen. A and B, kinetics of light-induced changes of luminescence, which is registered both in direct current (A) and at the modulation frequency of 8.4 MHz of exciting beam (B). Helium-neon laser light, $\lambda = 632.8$ nm, was used for excitation; luminescence emission with $\lambda \geqq 900$ nm was registered. ↑, light on; ↓, light off with $\lambda \geqq 720$ nm and intensity of $3 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}. C, light-induced absorbance changes at 805 nm. D, dependence on temperature of light-induced decrease in luminescence. Absorbance of the sample was 0.3 at 890 nm.

DISCUSSION

The data show that in the reaction centers of the B890 complex of *Chr. minutissimum*, electron transfer from cytochrome *c* to the pigment complex *P*-760, sensitized by bacteriochlorophyll-890 or *P*-890, takes place between 77 and 293 °K at the redox potential between −250 and −530 mV, when the primary acceptor is in the reduced form.

The comparison with data obtained in vitro [18, 21] shows that both the development of a broad absorption band at 650 nm in the *P*-760 spectrum and the appearance of the EPR signal (following the *P*-760 photoreduction) with $g = 2.0025 \pm 0.0005$ and the linewidth of approx. 12.5 G are due to the formation of the radical anion of a bacterial pigment. The bleaching of absorption bands at 543, 595, 760 and 805 nm indicates that the complex *P*-760 includes the bacteriopheophytin and the bacteriochlorophyll-800, but not the bacteriochlorophyll-890.

In contrast with the shift of bacteriopheophytin absorption spectrum, wherein

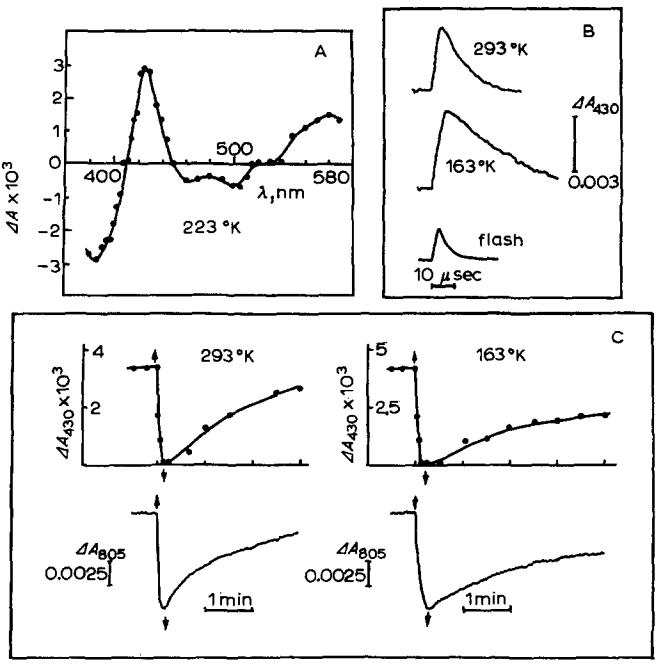


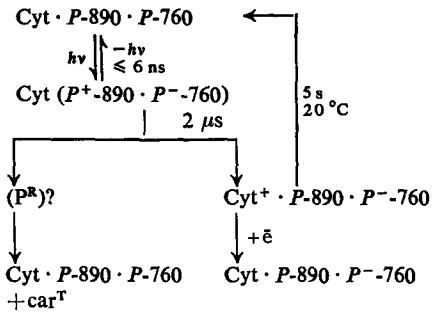
Fig. 8. Some spectral and kinetic properties of flash-induced absorbance changes of B890 complex at $E_h = -450$ mV and at various temperatures. A, difference absorption spectrum. B, kinetics of flash-induced absorbance changes at 430 nm. C, influence of continuous illumination ($\lambda \geqq 720$ nm, intensity of $9 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}) on the flash-induced absorbance changes at 430 nm (for comparison the kinetics of *P*-760 photoreduction, as measured by the absorbance changes at 805 nm, is given). Absorbance of the sample was 0.3 at 890 nm.

the absorbance decrease at 745 nm is accompanied by the absorbance increase at 770 nm with negligible changes of the band at 540 nm [9, 10], in the *P*-760 spectrum, the bands at 543 and 760 nm bleach. The latter bands are similar to the bacteriopheophytin absorption bands by their position and relative intensities. The comparison of spectra presented in Figs. 1B and 1C shows that approximately 1 mol of bacteriopheophytin bleaches, under the illumination at low redox potentials, per 1 mol of *P*-890, if $\varepsilon_{540\,nm}$ for bacteriopheophytin is 28 mM$^{-1} \cdot$ cm^{-1} [18] and $\varepsilon_{890\,nm}$ for *P*-890 is 115 mM$^{-1} \cdot$ cm^{-1} [22].

The absorbance increase at 790 nm and the decrease at 805 nm in the *P*-760 spectrum are characteristic of the shift of the bacteriochlorophyll absorption band at 800 nm. However, the asymmetry of bands at 790 and 805 nm and also the bleaching of band at 595 nm can equally indicate the participation of bacteriochlorophyll-800 and bacteriopheophytin in electron acceptance. The width of the EPR signal (approx. 12.5 G) of reduced *P*-760 shows that the electron localization is shifted to a monomeric pigment, probably bacteriopheophytin.

Equimolar cytochrome oxidation and *P*-760 reduction, sensitized by bacteriochlorophyll at 77 °K, show the electron transfer in a complex including cytochrome, photoactive bacteriochlorophyll *P*-890 and *P*-760. In these complexes, at the redox potential of less than −300 mV, the electron transfer scheme which is consistent with

both our data and recent observations [5–7] is probably as follows:



The assumption of a fast reversible electron transfer in the complex *P*-890 · *P*-760 is confirmed by data on the recombination luminescence with $\tau_{\frac{1}{2}} \leqslant 6$ ns and activation energy of 0.12 eV, as this luminescence is excited and emitted by bacteriochlorophyll and disappears when *P*-760 is reduced. The luminescence activation energy, which is not characteristic of fluorescence, can indicate that the free energy of state P^+-890 · P^--760 is less than the energy of emitted quantum (approx. 1.4 eV) by 0.12 eV. As the midpoint potential of *P*-890 is near +500 mV [23], that of *P*-760 can be in the region of −800 mV. This value is reasonable for a bacteriochlorophyll anion radical [24], rather than for a bacteriopheophytin anion radical, which is −550 mV in solution [18]. However, an interaction between the pigment and protein molecules in the reaction center can shift the midpoint potential of pigments. In fact, the midpoint potential of *P*-760 is less than −620 mV [11].

At low redox potentials, the state P^F has a lifetime of 10±2 ns [20] which corresponds to that of recombination luminescence described here. The P^F difference spectrum has been interpreted as an indicator of the formation of a pigment anion-cation baridical, in which the anion is either bacteriochlorophyll$^-$ or bacteriopheophytin$^-$ [7]. Our data support this conclusion and show that the P^F spectrum corresponds to the sum of the P^+-870 and P^--760 difference spectra, i.e. that the state P^F is probably the biradical P^+-870 · P^--760.

The reduced cytochrome apparently interacts with the state P^+-890 · P^--760 and reduces P^+-890. The low quantum yield of this reaction (approx. 0.002) appears to be determined by a difference between the rate of Cyt $\xrightarrow{\bar{e}}$ P^+-890 reaction (2–4 μs, Fig. 6) and lifetime of state P^+-890 · P^--760 ($\leqslant$ 6 ns). The high rate of reaction between cytochrome and P^+-890 is required for the P^--760 photoaccumulation as the *P*-760 photoreduction at low redox potentials is observed only in the bacterial preparations, which have (in agreement with [19]) a "fast" electron transfer from cytochrome to P^+-890: cells, chromatophores and B890 complex of *Chr. minutissimum*, chromatophores and B890 complex of *Thiocapsa roseopersicina* (Fig. 6). The *P*-760 photoreduction is not observed in chromatophores and reaction centers of *Rhs. rubrum*, B870 complex of *Rps. palustris* and chromatophores of *Rps. spheroides* (Fig. 6), which do not have the "fast" reaction between cytochrome and P^+-870.

It is important that the carotenoid triplet state, observed in B890 complex, is not formed when *P*-760 is photoreduced at 293 and 160 °K. These data can be explained if the carotenoid triplet state is formed from the biradical P^+-890 · P^--760, either directly or through P^R state, by the analogy with assumed reaction: $P^F \rightarrow car^T$

[20]. If this reaction is direct, the high quantum yield of car^T formation [20] can indicate that the biradical P^+-890 · P^--760 has a triplet state.

As the absorbance changes near 800 nm under *P*-760 photoreduction are similar to those under *P*-890 photooxidation, one can assume that both *P*-890 and *P*-760 are involved in a single pigment complex of the reaction center. The ubiquinone is apparently located near bacteriopheophytin, as the ubiquinone photoreduction is only accompanied by the shift of bacteriopheophytin absorption band at 760 nm [9, 10]. Such a localization of the reaction center components can promote the electron transfer from *P*-890 through *P*-760 to ubiquinone and the stabilization of separated charges.

Thus, when the "primary" electron acceptor (ubiquinone) is in the reduced form, in the reaction centers of *Chr. minutissimum* the primary photoreactions are observed, which can be intermediate in the electron transfer from *P*-890 to ubiquinone. In other words, the primary photoact of bacterial photosynthesis, probably, consists in the charge separation in the pigment complex *P*-890 · *P*-760 with the formation of the biradical P^+-890 · P^--760. This suggestion is supported by recent picosecond kinetic studies [7, 25, 26]. When the primary electron acceptor, X, is in the oxidized form, the P^F decay (halftime of approx. 200 ps) is accompanied by the transfer of an electron to X [7, 25]. The P^F state is evidently the pigment biradical in which the cation is P^+-870, as the rise halftime of P^+-870 formation ($\leqslant$ 10 ps) corresponds to that of the state P^F [26].

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Recent work (Shuvalov, V. A., Krakhmaleva, I. V. and Klimov, V. V., unpublished) has shown that the photoreduction of a pigment complex *P*-800 (bacteriopheophytin *b*-800 and bacteriochlorophyll *b*-830) is observed at low redox potentials in a reaction center preparation from *Rhs. viridis*, in which a photoactive cytochrome is present. The circular dichroism spectrum of these centers has been studied in the region of a "exciton splitting" of the 830-nm band. Photooxidation of *P*-960 results in the decrease of a negative band at 848 nm in agreement with the data in ref. 27. Photoreduction of *P*-800 is accompanied by complete disappearance of a positive band at 828 nm without the change of the 848 nm band. Thus, the interaction between bacteriopheophytin *b*-800 and bacteriochlorophyll *b*-830 and between bacteriochlorophyll *b*-830 and bacteriochlorophyll *b*-960 gives the positive 828 nm band and the negative 848 nm band in the circular dichroism spectrum, respectively. These data are interpreted in terms of an exciton interaction of the pigments in the reaction centers which results in the charge separation between *P*-960 and *P*-800.

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