KINETICS OF LASER-INDUCED OXIDOREDUCTIONS IN THE PHOTOSYNTHETIC REACTION CENTRE OF *ECTOTHIORHODOSPIRA SHAPOSHNIKO VII*

A. A. KONONENKO, S. M. REMENNIKOV, A. B. RUBIN, L. B. RUBIN, P. S. VENEDIKTOV and E. P. LUKASHEV

Biology Department, State University, Moscow (U.S.S.R.)

(Received June 20, 1973)

SUMMARY

Light-induced absorbance changes at 400–450 nm associated with oxidoreductions of bacteriochlorophyll P890, high (C_h) and low (C_l) potential cytochromes were studied in cells and chromatophores of the *Ectothiorhodospira shaposhnikovii* bacterium. The half-time of the pigment reduction after a single laser flash (694.3 nm, 30 ns, 3 mJ cm^{-2}) coincides with the half-time of oxidation of cytochrome C_l (1.6 μs) under anaerobic conditions and with that of cytochrome C_h (2.2 μs) under aerobic conditions. We inferred that cytochrome C_h becomes available for oxidation only after cytochrome C_l has been already oxidized, which is a result of specific conformation changes in the photochemical reaction centre. At 80 K, under anaerobic conditions, reversible photo-oxidation of P890 is induced only by the first laser pulse; under aerobic conditions this low-temperature reaction can be demonstrated repeatedly. Data obtained strongly support the suggestion made in our laboratory earlier that only one type of cytochrome oxidizing photochemical system exists in purple sulphobacteria.

INTRODUCTION

In previous papers[1-4] we presented several lines of evidence that in *Ectothiorhodospira shaposhnikovii* cells a single photochemical system with the same bacteriochlorophyll P890, and with the same primary electron acceptor, oxidizes both high- and low-potential cytochromes associated with cyclic and non-cyclic electron paths respectively.

This work is aimed to study kinetic parameters of direct electron transfer between these components in the photosynthetic reaction centre. Methods applied include laser flash spectroscopy, which proved valuable in such studies[5-7].

EXPERIMENTAL

E. shaposhnikovii were grown as described elsewhere[1]. 4–7 day old cells in culture medium were used. For experiments under aerobic (oxidizing) conditions, cell suspensions were bubbled for 15 min with air, whereas to create anaerobic (reducing) conditions, the addition of Na_2S (up to 0.5 mM final concentration) was followed by continuous bubbling with argon. Sample absorbances were 0.4 at 590 nm.

Bacterial chromatophores were prepared as described in ref. 8. The reaction mixtures in this case contained chromatophores equivalent to 30 μg/ml bacterio-chlorophyll in 50 mM Tris–HCl buffer (pH 8.0) with 250 mM sucrose and 5 mM $MgCl_2$.

The single beam kinetic spectrophotometer for measuring rapid absorbance changes after a short laser flash has been described previously[9,10]. The measuring light beam from the 1000 W high pressure Xe arc lamp passed through the grating monochromator set to give a band width of 6 nm. The intensity of the measuring light at the sample cuvette position was 1 mJ cm^{-2} s^{-1}. Dark adapted cell suspensions were exposed to the measuring light beam for 1–4 ms prior to the actinic laser pulse. It was determined in special experiments that the measuring light oxidized not more than 6% of the total amount of cytochromes that could be oxidized by a single laser flash of saturating intensity. To measure absorbance changes in the region 400–450 nm, the photodetector was covered by a cut-off filter which rejected stray actinic light and sample luminescence. The response time of our laser-spectrophotometer was 0.3 μs. The energy of a single laser flash of 30 ns duration at 694.3 nm was 3 mJ cm^{-2}.

Optical absorbance changes caused by continuous light (700–1100 nm, 1 mJ cm^{-2} s^{-1}) were measured in a single beam difference spectrophotometer with a 5 ms response time[11].

RESULTS AND DISCUSSION

Figure 1 shows transient curves of absorbance changes at 400–450 nm caused by a single laser pulse in *E. shaposhnikovii*. Changes of two different kinetic types can be distinguished. The first type is characterized by a fast recovery, and the second type by a slow one. Measurements at 440 nm and 410 nm allow separate observation of the two types of laser induced absorbance change respectively.

One can conclude that changes of slow recovery reflect cytochrome oxido-reductions since their spectra are quite similar to "oxidized minus reduced" spectra of c-type cytochromes.

Absorbance changes at 440 nm show a rapid increase ($t_{1/2} \sim 0.5 \mu$s) and somewhat longer recovery. Within experimental error, the half-time of the recovery is the same as that of cytochrome oxidation (Fig. 1). Spectra of laser induced fast
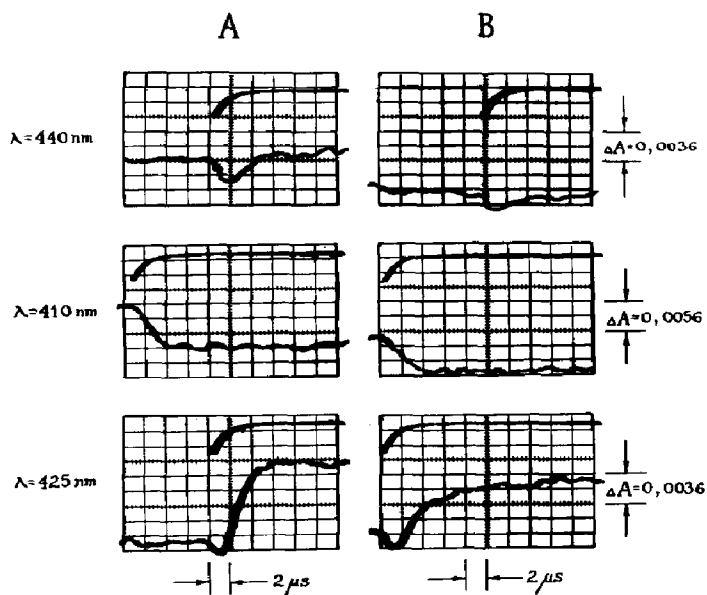
A   B

Fig. 1. Kinetic traces of laser-induced absorbance changes at 440, 410 and 425 nm in the bacterium cells. A, reducing; B, oxidizing conditions. Start of the upper trace in oscillograms indicates the time of a laser flash.
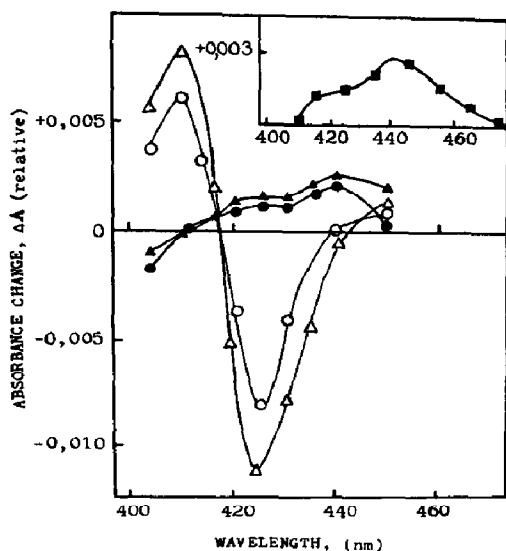

Fig. 2. Spectra of laser-induced fast recovering (closed symbols) and slow recovering (open symbols) absorbance changes in the bacterium cells. △, reducing; ○, oxidizing conditions. The insert shows the spectrum of absorbance changes induced by continuous actinic light in the bacterium chromatophores. $K_3Fe(CN)_6$ was added in the chromatophore suspension to 50 μM final concentration.

absorbance changes are shown in Fig. 2. We also found such absorption increases with a similar broad band centred at 440 nm in bacterial chromatophores illuminated with continuous actinic light in the presence of $K_3Fe(CN)_6$ added to oxidized cytochrome components (the insert in Fig. 2). Except for spectral changes in the 400–450 nm region, no other absorbance changes are observed under these conditions, but there is bleaching at 890 nm and blue-shift at 800 nm referring to the oxidation of bacteriochlorophyll in the reaction centres of photosynthetic units[12]. The observed changes in the visible and near infra-red spectral range both saturate at approximately the same continuous light intensity of 0.10–0.15 mJ $cm^{-2} s^{-1}$, and have the same half-times for their dark recovery of 5–6 μs; they can be demonstrated at 80 K.

The fact that half-times for cytochrome oxidation following a single laser pulse are quite close to the half-times for the recovery of laser-induced absorbance changes centred at 440 nm suggest that the latter may be attributed to the oxidation of bacteriochlorophyll P890, as was done for 883 nm[5], 610 nm[13] and 785 nm[14] absorbance changes. Data obtained in our experiments with continuous actinic light illumination give additional support for such a conclusion. Seibert and DeVault[15] have resently observed laser-induced absorbance changes at 400–450 nm in *Chromatium D* chromatophores which they also showed to be a spectral component of the photosynthetic reaction centre.

It is generally accepted[6,7] that a laser pulse induces the oxidation of either low-potential (C_l) or high-potential (C_h) cytochromes depending on whether the cells are anaerobic or aerobic respectively. Our studies on the kinetics of cytochrome dark reduction after a single laser pulse (Fig. 3) confirm this observation. In fact, under anaerobic conditions, with both cytochromes C_l and C_h being initially reduced, relaxation half-times were 5 s thus indicating a cytochrome C_l reaction[2]. On the other hand, half-times of reduction under aerobic conditions were found to be 50 ms, the same as for cytochrome C_h[2,4].
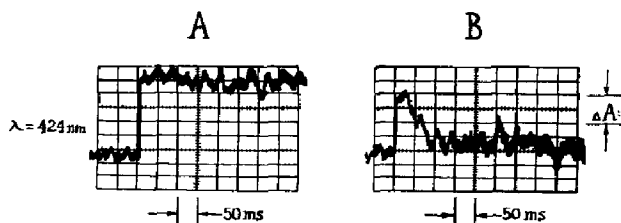

Fig. 3. Kinetics of cytochromes dark reduction after a single laser pulse in the bacterium cells. A, reducing; B, oxidizing conditions, $\Delta A = 0.003$.

Laser-induced oxidation half-times for C_l and C_h cytochromes are also different. Kinetic records on Fig. 1 show cytochrome oxidation to occur with a half-time of 1.6 ± 0.3 μs under anaerobic and 2.2 ± 0.3 μs under aerobic condi-

tions. Each time value is the average of 20 separate determinations. The rate of C_l cytochrome oxidation exceeds that of C_h cytochrome by not more than a factor of two. Some explanation is therefore required for the selective response of only C_l to a laser flash when both cytochromes are in reduced form in the dark. Seibert and DeVault[7] accounted for this fact in the case of *Chromatium D* by assuming that two cytochromes have separate primary electron acceptors, their switching being under the control of the redox potential of the environment. There is a possibility that certain control mechanisms may well operate on the cytochrome level. According to this suggestion, reduced cytochrome C_h occupies such a position in relation to the photochemically active bacteriochlorophyll that it cannot be oxidized until cytochrome C_l has given its electron to the reaction centre. After that, owing to some conformational changes in the cytochrome–pigment complex, the position of C_h shifts so that the latter becomes available for the oxidation with half-times of 2.2 μs. Providing this suggestion is true, the proposed cytochrome switching, irrespective of its precise molecular mechanism, which at present is of course rather unclear may nevertheless explain the way close contact is brought about between bacteriochlorophyll and each of two cytochrome macromolecules which is necessary to obtain the high rate of laser-induced oxidation reactions.

The half-time of the oxidized bacteriochlorophyll P890 reduction after a single laser flash (as estimated from the absorbance increase at 440 nm) coincides with the oxidation half-time of the participating cytochrome (Fig. 1). This correlation, along with the obvious similarity between absorption difference spectra of the pigment with either C_h or C_l being oxidized, suggests that both cytochromes are oxidized by a single photosynthetic reaction centre bacteriochlorophyll.

Laser-induced changes in the bacteriochlorophyll and cytochrome absorbance were also studied at 80 K (Table 1). Under oxidizing conditions, the reversible reaction of the bacteriochlorophyll photo-oxidation can be carried out repeatedly.

TABLE 1

PHOTOSYNTHETIC REACTION CENTRE BACTERIOCHLOROPHYLL AND CYTOCHROME ABSORBANCE CHANGES INDUCED BY LASER FLASHES GIVEN IN SERIES AT 80 K

Number of	Absorbance changes (relative)	
the flash	at 440 nm	at 410 nm
	Reducing conditions:	
1	2.70	2.20[a]
2	0	0
3	0	0
	Oxidizing conditions:	
1	2.85	0
2	2.60	0
3	2.50	0

[a] Non-reversible absorbance changes.

This reaction seems to be the electron transfer within the complex between the bacteriochlorophyll and the primary electron acceptor (PA_1), since, at 80 K, cytochrome C_h cannot supply an electron to the oxidized pigment (Table 1). Contrary to observations under reducing conditions, reversible photo-oxidation of the reaction centre bacteriochlorophyll is observed only after the first laser pulse, whereas the subsequent pulses do not give any effects. Evidently the same complex PA_1 while in the light-induced state $P^+A_1^-$, can accept an electron from cytochrome C_l at 80 K under reducing conditions (Table 1) and is converted then to the PA_1^- state. Hence, subsequent light-induced electron transfer reaction becomes impossible. These data allow us to conclude that, in the *E. shaposhnikovii* bacterium, not only the bacteriochlorophyll but also the primary electron acceptor in the photosynthetic reaction centre are common both to the C_h and C_l cytochromes. This conclusion is consistent with the suggested mechanism of the alternative oxidation which may operate at the cytochrome level.

REFERENCES

 1 L. B. Rubin, A. B. Rubin, V. N. Dubrovin and Yu. E. Shvinka, *Mol. Biol.* (*U.S.S.R.*), 3 (1969) 700.
 2 A. A. Kononenko in A. B. Rubin (ed.), *Biophotochemistry Problems,* Nauka, Moscow, 1973, p. 180.
 3 A. A. Kononenko, P. S. Venediktov, E. P. Lukashev, D. N. Matorin and A. B. Rubin, *Studia Biophys.,* 28 (1971) 15.
 4 A. A. Kononenko, E. P. Lukashev, A. B. Rubin and P. S. Veneditkov, *Biochim. Biophys. Acta,* 275 (1972) 130.
 5 W. W. Parson, *Biochim. Biophys. Acta,* 153 (1968) 248.
 6 W. W. Parson and G. D. Case, *Biochim. Biophys. Acta,* 205 (1970) 232.
 7 M. Seibert and D. DeVault, *Biochim. Biophys. Acta,* 205 (1970) 220.
 8 V. D. Samuilov and E. N. Kondratyeva, *Biol. Sci.* (*U.S.S.R*), 5 (1969) 97.
 9 A. P. Andreytsev, A. E. Voronkov, V. A. Gavanin, L. N. Grigorov, S. M. Remennikov, A. B. Rubin and L. B. Rubin, *J. Appl. Spectros.* (*U.S.S.R.*), 16 (1972) 938.
10 L. N. Grigorov, V. D. Zhivotchenko, S. M. Remennikov, L. B. Rubin and A. B. Rubin, *Mol. Biol.* (*U.S.S.R.*), 5 (1971) 744.
11 A. A. Kononenko, A. P. Andreytsev and A. B. Rubin, *Biol. Sci.* (*U.S.S.R.*), 8 (1967) 138.
12 A. A. Kononenko. P. S. Venediktov, E. P. Lukashev and A. B. Rubin, *Studia Biophys.,* 28 (1971) 9.
13 P. L. Dutton, T. Kihara, J. A. McCray and J. Ph. Thornber, *Biochim. Biophys. Acta,* 226 (1971) 81.
14 W. W. Parson, *Biochim. Biophys. Acta,* 189 (1969) 397.
15 M. Seibert and D. DeVault, *Biochim. Biophys. Acta,* 253 (1971) 396.