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On the interaction of photoactive bacteriochlorophyll with the primary electron acceptor in the reaction centre of *Ectothiorhodospira shaposhnikovii*

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SUMMARY

In *Ectothiorhodospira shaposhnikovii* cells, the half-time of photooxidized bacteriochlorophyll P890$^+$ dark reduction is 60 ms and does not change with increasing actinic illumination period; whereas the half-time of high-potential cytochrome c_H^+ reduction varies from 80 ms up to 300 ms. Interaction between P890$^+$ and the primary electron acceptor A_1^- provides for fast reduction of pigment when cytochrome c_H is oxidized or reduced slowly. It is suggested that P890 and A_1 form a complex such that A_1^- is not able to donate its electron to secondary acceptors until P890$^+$ has accepted an electron from cytochrome.

Characteristic time values of electron transfer in the photosynthetic reaction centre of purple bacteria have been determined in dark-adapted organisms by the method of pulse laser spectroscopy[1-3]. Since prolonged steady illumination brings about complex redistribution of electrons between electron pools in the chain and turns on energy control mechanisms it can also change actual time values needed for electrons to transfer from one carrier to another.

In this paper, data are presented on dark reduction rates of photooxidized bacteriochlorophyll and high-potential cytochrome in the reaction centres of *Ectothiorhodospira shaposhnikovii*, measured after different periods of steady actinic illumination. The experiments were conducted under anaerobic and aerobic conditions at different sulphide concentrations on intact and washed bacterial cells cultivated as described elsewhere[4].

A single beam difference spectrophotometer with response time of the recording system of 1 ms was assembled. The samples had an absorbance of 0.6 at 590 nm. The intensity of the monochromatic measuring light beam did not exceed 5 erg·cm^{-2}·s^{-1}. The actinic light (480–620 nm, 10^4 erg·cm^{-2}·s^{-1}) was shut off by a mechanical shutter with closing time of approximately 5 ms. To minimize errors resulting from scattered actinic light, fluorescence and delayed light emission, a monochromator was placed between the sample and the photomultiplier. Half-times of dark reduction of photooxidized bacteriochlorophyll and of high-potential cytochrome in the reaction centre were estimated from the absorbance increase at 810 nm[5] and the fast component of the absorbance increase at 424 nm[4], both measured after cessation of actinic illumination.

It has been shown in our laboratory[4] that two *c*-type cytochromes functioning in the photosynthetic electron transport system of *E. shaposhnikovii* cells are similar to c_{555} (c_{422}) and c_{553} ($c_{423.5}$) cytochromes of *Chromatium* D which have been thoroughly studied in such respects as kinetics of dark reduction, dependence of oxidation on light intensity, reactions at low temperatures and response to the intrinsic redox potential of the reaction media. However, in *E. shaposhnikovii* these cytochromes do not demonstrate valid spectral differences and therefore we denote them as cytochrome c_H (high potential, cyclic) and cytochrome c_L (low potential, non-cyclic). We have recently reported[6] that both cytochrome c_H and c_L in *E. shaposhnikovii* are components of a single photosynthetic reaction centre with an active bacteriochlorophyll denoted by P890 as accepted generally.

Half-times ($\tau_{1/2}$) of P890$^+$ and cytochrome c_H^+ reduction processes were found to be 60 ms and 80 ms correspondingly, provided that the steady actinic illumination period did not exceed 5 s (Figs 1A and 1C). As the illumination period increases, the dark reduction rate of P890$^+$ does not change (Figs 1B and 1D), whereas that of

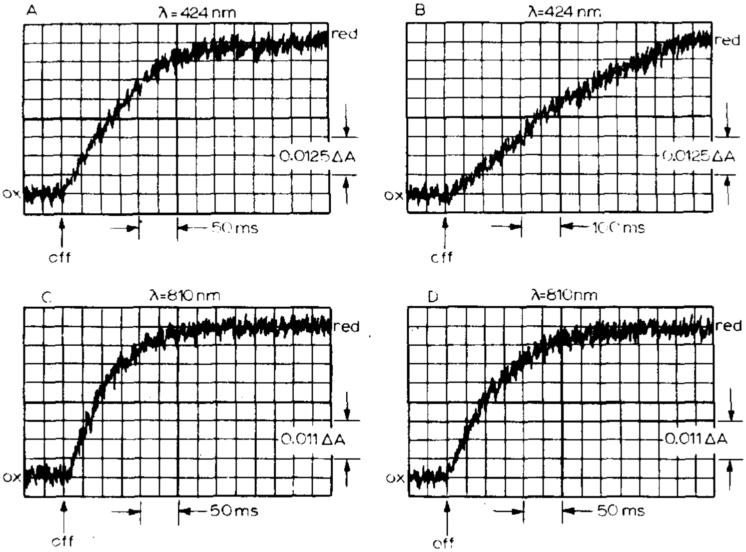

Fig. 1. Kinetic curves of dark reduction of photooxidized high-potential cytochrome (A and B) and bacteriochlorophyll P890 (C and D) measured as the absorbance increase at 424 nm and 810 nm correspondingly, following illumination periods of 5 s (traces A and C) and 120 s (traces B and D) (redrawn from oscilloscope traces). Anaerobic suspension of bacterial cells in culture medium containing Na_2S, 10 mM. Absorbance 0.6 at 590 nm. Actinic light intensity 10^4 erg·cm^{-2}·s^{-1} (480–620 nm).

cytochrome c_H^+ decreases so that its $\tau_{1/2}$ goes up to 300 ms. The slowing down of cytochrome c_H^+ reduction may be due both to the decrease of electron donor concentrations near the reaction centre after prolonged illumination resulting in the lack of photoreducing products and/or to the decrease of the electron transport rate caused by the electrochemical potential generated across photosynthetic membranes. In fact we have found that the observed decrease of the cytochrome c_H^+ dark reduction rate cannot be demonstrated after addition of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone or the exogenous electron donor Na_2S at concentrations of 5 µM and 0.1 mM, respectively.

On the other hand the fact that half-time reduction of P890$^+$, contrary to that of cytochrome c_H^+, did not change after prolonged actinic illumination, even at low exogenous electron donor concentrations, indicates that electrons needed for P890$^+$ reduction are transported from photoreduced products through some pathway which evidently does not include high-potential cytochrome. We suggest that under these conditions the reduction of P890$^+$ occurs as the result of reversed primary reactions.

To test the suggestion, experiments were performed with washed bacterial cells in aerobic donor-depleted media where both cytochromes (c_L and c_H) were already oxidized in the dark due to interaction with molecular oxygen. No light-induced cytochrome reactions were observed in such cells. The time needed for complete P890$^+$ dark reduction under these conditions was found to be practically unchanged ($\tau_{1/2}$ = 70 ms) and independent of the duration of steady actinic illumination. Since it is impossible to accumulate electrons in the pool of secondary accepting compounds in the absence of exogenous electron donors, fast P890$^+$ reduction can be caused only by the primary electron acceptor A_1. In fact such a reaction was shown to occur at high rates even at liquid-nitrogen temperatures.

It is noteworthy that under oxidative conditions P890$^+$ reduction by A_I^- is also complete. Hence all reaction centres during the illumination period were in the state $c_H^+ c_L^+$ P890$^+$ A_I^-, but the probability for an electron localised on A_I^- to be involved in the subsequent direct reactions was far less than a return to P890$^+$. It suggests that P890 and A_1 form a complex where A_I^- is not able to give its electron further to secondary acceptors until P890$^+$ has accepted an electron from cytochrome. Under normal physiological conditions the fast electron transfer from cytochromes to photoactive pigment interferes successfully with the recombination process between A_I^- and P890$^+$ thus preventing energy losses in primary reactions. However at pronounced electron donor deficiency or excess illumination, the suggested mechanism of the P890$^+$ and A_I^- interaction provides for fast reduction of the oxidized pigment, thus eliminating unspecific reactions of the latter with other surrounding compounds.

The primary electron acceptor is still unknown. Numerous attempts to identify it were no more successfull that investigations aimed at chemical isolation of high-energy intermediates of phosphorylation. One can assume that in the primary photochemical act, which is accompanied by bacteriochlorophyll absorbance changes arising at times less then 0.5 µs[5], an electron is not necessarily expelled from the pigment molecule of the reaction centre but may rather be transfered from the system of conjugated π-bonds of the porphyrin ring to the central Mg-atom[1]. Provided this assumption is true, our results on the pigment–acceptor interaction give some evidence that the first actual intermolecular redox reaction is the electron transfer from ferrocytochrome to the $P890^+ A_I^-$ complex.

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