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ARTICLE

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# Electron transport dynamics at the quinone acceptor site of bacterial photosynthetic reaction centers as probed using fast temperature changes

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**Abstract** Methods of laser-induced temperature jumps and fast freezing were used for testing the rates of thermoinduced conformational transitions of reaction center (RC) complexes in chromatophores and isolated RC preparations of various photosynthesizing purple bacteria. An electron transfer reaction from primary to secondary quinone acceptors was used as a probe of electron transport efficiency. The thermoinduced transition of the acceptor complex to the conformational state facilitating electron transfer to the secondary quinone acceptor was studied. To investigate the dynamics of spontaneous decay of the RC state induced by the thermal pulse, the thermal pulse was applied either before or during photoinduced activation of electron transport reactions in the RC acceptor complex. The maximum effect was observed if the thermal pulse was applied against the background of steady-state photoactivation of the RC. It was shown that neither the characteristic time of the thermoinduced transition within the temperature range 233–253 K nor the characteristic time of spontaneous decay of this state at 253 K exceeded several tens of milliseconds. Independent support of the estimates was obtained from experiments with varied cooling rates of the samples tested.

**Keywords** Conformational changes · Cytochromes · Electron transport · Photosynthetic reaction center · Purple bacteria

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**Abbreviations** bR: bacteriorhodopsin · P: photoactive bacteriochlorophyll · $Q_A$: primary quinone acceptor · $Q_B$: secondary quinone acceptor · RC: reaction center

## Introduction

The temperature dependence of electron transfer rates in biological systems provides essential information about physical mechanisms of biological processes. In addition to thermoinduced equilibrium states, the temperature jump method allows dynamic parameters of transitions between the thermoinduced states to be studied.

The bacterial photosynthetic reaction center (RC) includes three integral membrane polypeptide subunits. These subunits are bound to the chromophore groups mediating the processes of the photoinduced primary charge separation (Allen et al. 1987; Deisenhofer et al. 1995). The quantum efficiency of the primary processes of charge separation in the bacterial RC does not decrease upon cooling to cryogenic temperatures (Arnold and Clayton 1960; Clayton 1980). On the other hand, the temperature decrease has a significant effect on the electron transfer reaction from the primary to the secondary quinone acceptors (Dutton and Prince 1978; Rubin et al. 1987). Various mechanisms of low-temperature inhibition of electron transport reactions in bacterial RC are discussed in the literature. For example, models of the temperature-induced shift of redox levels (Case and Parson 1971; Kaminskaya et al. 1990) or deceleration of the conformational mobility of protein subunits of the RC (Rubin et al. 1989) were suggested. However, experimental data obtained under steady-state temperature conditions were found to be insufficient to distinguish between these models.

Variation of the rate of the temperature change provides a promising experimental approach to studies of the role of conformational dynamics of phototransformation proteins and the charge transfer mediated by these proteins. This approach provides an opportunity not only to trap nonequilibrium protein states involved in charge

transfer but also to assess kinetic and energy parameters of the protein transition between different conformational substates affecting the rates of functional processes (Zubov et al. 1983; Parot et al. 1987; Kirmaier and Holten 1990; McMahon et al. 1998; Xu and Gunner 2001, 2002). In one of the first works devoted to this problem, light-induced structural changes of the photosynthetic RC protein were experimentally observed by rapid immersion of RC preparations of *Rhodobacter sphaeroides* in liquid nitrogen in the light and in the dark (Kleinfeld et al. 1984a). The same approach was later applied to RC crystals. In combination with X-ray diffraction analysis, this revealed distinct changes in position and orientation of the secondary quinone acceptor $Q_B$ in the RC interior upon $Q_B$ photoreduction (Stowell et al. 1997). The conformational changes associated with the photoinduced electron transfer to the secondary quinone can be trapped by cooling the RC preparations in the light. The quantum efficiency of electron transfer from bacteriochlorophyll to $Q_B$ is maintained at a high level (quantum yield of up to 100%) even at temperatures below 77 K on cooling the samples in the light (Xu and Gunner 2001). In contrast, the efficiency of this process in RC preparations frozen in the dark rapidly declines upon cooling below 260–270 K. This decline is due to inhibition of electron transfer from the primary quinone acceptor ($Q_A$) to $Q_B$. The rate of light-induced conformational changes of the RC facilitating rapid transfer of an electron from $Q_A$ to $Q_B$ was assessed from the temperature dependence of the quantum yield of this reaction (Xu and Gunner 2001). According to these estimates, the rate constant of this process decreases to less than 1 $s^{-1}$ at $T < 200$ K (Xu and Gunner 2001). The conformation trapped upon cooling in the light provides effective and reproducible (under conditions of repetitive photoactivation) transfer of electrons from $Q_A$ to $Q_B$ at low temperatures. At temperatures above 120 K this conformation is gradually transformed into the conformation typical of RC preparations frozen in the dark (Xu and Gunner 2001).

The goal of this work was to assess the rate of temperature-induced transitions relevant to the reaction of electron transfer from $Q_A$ to $Q_B$ in bacterial RCs at the initial segment of the temperature dependence of this reaction (230–250 K). Two independent methodological approaches were used to attain this goal: variation (within the range of two orders of magnitude) of the rate of cooling of RC preparations of purple bacteria and pulsed laser-induced heating of RC preparations cooled in the dark to various temperatures.

## Materials and methods

Chromatophores and isolated pigment-protein RC complexes from wild-type corotenoid-containing strains of the photosynthesizing bacteria *Rhodospirillum rubrum* and *Rhodobacter sphaeroides* were used. The method of chromatophore isolation and the initial stages of isolation of the RC preparations were similar to those described in Clayton and Clayton (1978). After the chromatophores had been incubated for 30 min at 4 °C in 0.01 M Tris-HCl or sodium phosphate buffer (pH 7.0) containing 0.5% lauryldimethylamine oxide (LDAO), they were centrifuged (144,000×*g*, 90 min, 4 °C). The supernatant contained the RC fraction, which was purified by adsorption chromatography on a hydroxyapatite column. The optical density at 870 nm of the suspension of chromatophores used in experiments was about 1 in a 1-mm cuvette. The concentration of the isolated RC was about 10 μM. Bacterial cells of *Halobacterium salinarium* S-9 (wild type) were grown and purple membranes were isolated as described in Becher and Cassim (1975). Chromatophores and isolated RC preparations were suspended in Tris-HCl or phosphate buffer containing 0.2 M sucrose and 5 mM $MgSO_4$ (pH 7.0). Some experiments were performed using air-dry films or water-glycerol suspensions of chromatophores and isolated RC preparations.

Photoreactions in chromatophores and RC preparations of purple bacteria were induced by the second harmonic of a pulsed YAG:Nd laser (wavelength 530 nm; pulse duration 15 ns) or an incandescent lamp (wavelength > 700 nm; exposure time 0.5–1 s). Redox reactions of the RC bacteriochlorophyll dimer (P870) were monitored as absorption changes at the Soret band (425 nm). Spectral lines of the monitoring light were isolated using corresponding interference filters.

Radiation of a YAG:$Er^{2+}$ pulsed laser (wavelength 2.94 μm) or a continuous-mode $CO_2$ laser (wavelength 10.6 μm) was used to induce pulsed heating (temperature jump). Measuring the inverse current of a KD-103 diode attached to the sample tested monitored the initial steady-state temperature.

The maximum magnitude and kinetics of heating induced by the YAG:$Er^{2+}$ laser were calculated from the laser radiation intensity, extinction coefficients of sample and support at the laser radiation wavelength, and heat capacity of sample and support. The kinetics of heating induced by the continuous-mode $CO_2$ laser was mainly determined by the switching rate of the mechanical shutter. The calculated half-time of the temperature increase induced by the YAG:$Er^{2+}$ laser or $CO_2$ laser was 150–400 μs or 1 s, respectively. The amplitude of the laser-induced temperature increase was varied over a broad range by changing the laser radiation intensity (using glass filters).

It should be noted that the results of these calculations are approximate in some cases, because they depend on such uncertain factors as the distribution of the laser radiation over the cross-section of the laser beam and the laser radiation distribution between sample and support. Therefore, it was reasonable to measure the laser-induced temperature changes experimentally. Laser-induced temperature changes were calibrated using absorption changes of the initial form of bacteriorhodopsin (bR-570) or the fluorescence of rhodamine *B*. It is well known that the Arrhenius plot of the rate of dark recovery of bR-570 following flash-induced excitation of the photocycle is a linear function. Because the recovery time of bR-570 (milliseconds at room temperature) is much shorter than the relaxation time of the temperature jump induced by the $CO_2$ laser, rather than the YAG:$Er^{2+}$ laser, the absorption change kinetics of bR-570 can be used as a low-inertia sensor of the temperature changes induced by the $CO_2$ laser.

A kinetic curve of the bR-570 absorption changes at room temperature and an Arrhenius plot of the rate constant of this process are shown in Fig. 1a and Fig. 1b, respectively. The kinetic profile of the thermal pulse generated by the $CO_2$ laser was calibrated as follows. When a sample of bacteriorhodopsin (water-glycerol suspension, 55 vol% glycerol, of the *H. salinarium* S-9 purple membranes) had been cooled to a given temperature, it was exposed to a 1-s pulse from the $CO_2$ laser. After a variable time interval *t*, the bacteriorhodopsin photocycle in the sample was activated with the second harmonic of a YAG:Nd laser (530 nm). The sample temperature at the moment *t* was calculated from the calibration curve shown in Fig. 1b and the recovery kinetics of bR570. An example of the kinetic profile of the thermal pulse generated at the $CO_2$ laser pulse energy of 8.3 J is shown in Fig. 1c. The effects of laser-induced thermal pulses on the photocycle of bacteriorhodopsin have been studied in more detail by Zubov et al. (1983).

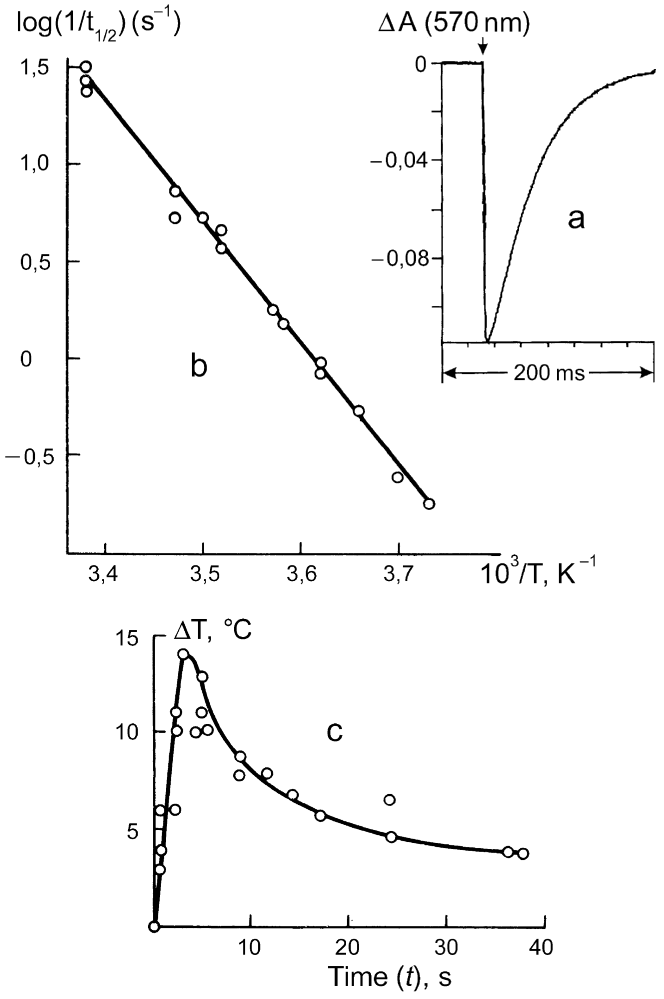


**Fig. 1a–c** Kinetic profile of the thermal pulse generated by $CO_2$ laser radiation (wavelength 10.6 μm; pulse duration 1 s). **a** Kinetics of absorption changes of the main form of bacteriorhodopsin at 570 nm (bR-570) induced by the second harmonic of a pulsed YAG:Nd laser (wavelength 530 nm; pulse duration 15 ns). An *arrow* shows the moment of photoactivation. **b** Arrhenius plot of the rate constant of the absorption changes of the main form of bacteriorhodopsin bR-570 at 570 nm. **c** Kinetic profile of thermal pulse generated by a $CO_2$ laser with radiation energy of 8.3 J

The optical settings used in the calibration (geometry, volume and optical density of sample, etc.) were identical to those used in further experiments with chromatophores or photosynthetic RC preparations, so that the temperature-jump conditions used for the bacteriorhodopsin calibration were assumed to be reproduced sufficiently accurately for both RCs and chromatophores.

The kinetic profiles of the thermal pulses generated by the YAG:$Er^{2+}$ laser in thin films (10–20 μm) of water-glycerol suspensions were calibrated similarly using either faster intermediates of the bacteriorhodopsin photocycle or fluorescence of rhodamine *B*. The results of these measurements, as well as the theoretical simulation, showed that both the leading and trailing edges of the thermal pulse in this case are much faster than in the case of the $CO_2$ laser. According to the pulse energy, the rise-time of the thermal pulse generated by the YAG:$Er^{2+}$ laser ranged from 150 μs to 400 μs, whereas the temperature relaxation kinetics contained two stages. The half-time of the temperature decay (temperature relaxation to the level of 50%) was about 1 ms, whereas the time of complete relaxation to the initial level was several tens of milliseconds.

In the case of dry films of the chromatophores (thickness 10–20 μm), the rise-time of the thermal pulse generated by the YAG:$Er^{2+}$ laser was approximately the same (150–400 μs), whereas the half-time of the temperature relaxation and the time of virtually complete relaxation to the initial level were about 10 ms and several tens of seconds, respectively. Thus, the use of the pulsed YAG:$Er^{2+}$ laser and quasipulsed $CO_2$ laser (continuous generation modulated with a mechanical shutter) provides an opportunity to study the kinetics of both sufficiently fast (milliseconds) and slow (seconds) thermoinduced processes in biological membranes.

The kinetics of the photoinduced electron transport reactions induced in chromatophores and isolated RC preparations by steady-state light were measured using a differential single-beam spectrophotometer as described in Rubin et al. (1987).

## Results and discussion

The efficiency of electron transfer from the primary ($Q_A$) to the secondary ($Q_B$) quinone acceptor was assessed by the relative amplitude of the slow component of charge recombination as described by Chamorovsky et al. (1976) and Rubin et al. (1987). Because neither of these works is available in electronic form, it is worthwhile resuming briefly the most important points of the method. Absorption of a light quantum by a RC gives rise to the $P^+Q_A^-Q_B$ state formation. The electron from $Q_A$ can either recombine with $P^+$ ($\tau_{AP} \approx 0.1$ s) or be transferred to $Q_B$, giving rise to formation of the state $P^+Q_AQ_B^-$ ($\tau_{BP} \approx 1$ s or more). At room temperature, $K_{AB} \gg K_{AP}$, $K_{BP}$ and the photomobilized electron is effectively transferred to $Q_B$. The relative contribution of the slow component ($1/K_{BP} = \tau_{BP}$) to the kinetics of the dark reduction of $P^+$ after photoactivation of the RC represents the efficiency of electron transfer from $Q_A$ to $Q_B$ ($N$). At room temperature, $N = 1$. As the temperature decreases, the rate of electron transfer from $Q_A$ to $Q_B$ declines, and the fast component of $P^+$ reduction, representing the dark recombination between $P^+$ and $Q_A^-$ ($1/K_{AP} = \tau_{AP}$), appears in addition to the slow component. The ratio of the slow ($N$) to the fast ($1-N$) components can be regarded as a quantitative characteristic of the efficiency of electron transfer from $Q_A$ to $Q_B$. A similar approach was also used by Kleinfeld et al. (1984a, 1984b).

At high redox potentials of the reaction medium (aerobic conditions), photoactivation of chromatophores or isolated RC preparations (air-dried film or water-glycerol suspension alike) induces limited pseudocyclic electron transfer between P870 and the quinone acceptors ($Q_A$ and $Q_B$) (Rubin et al. 1987). Typical kinetic curves for the photoinduced absorption changes of P870 in dry films of the *R. rubrum* chromatophores are shown in Fig. 2. Although the redox potential ($E_h$) was not controlled in these samples, it was shown in special experiments with the ferri/ferrocyanide redox couple that the same kinetic pattern is observed within the $E_h$ range 390–410 mV. At room temperature a photomobilized electron is transferred from P870 through $Q_A$ to $Q_B$. The rate of the electron return from $Q_B^-$ back to $P870^+$ is rather slow (Fig. 2a, curve 1). The

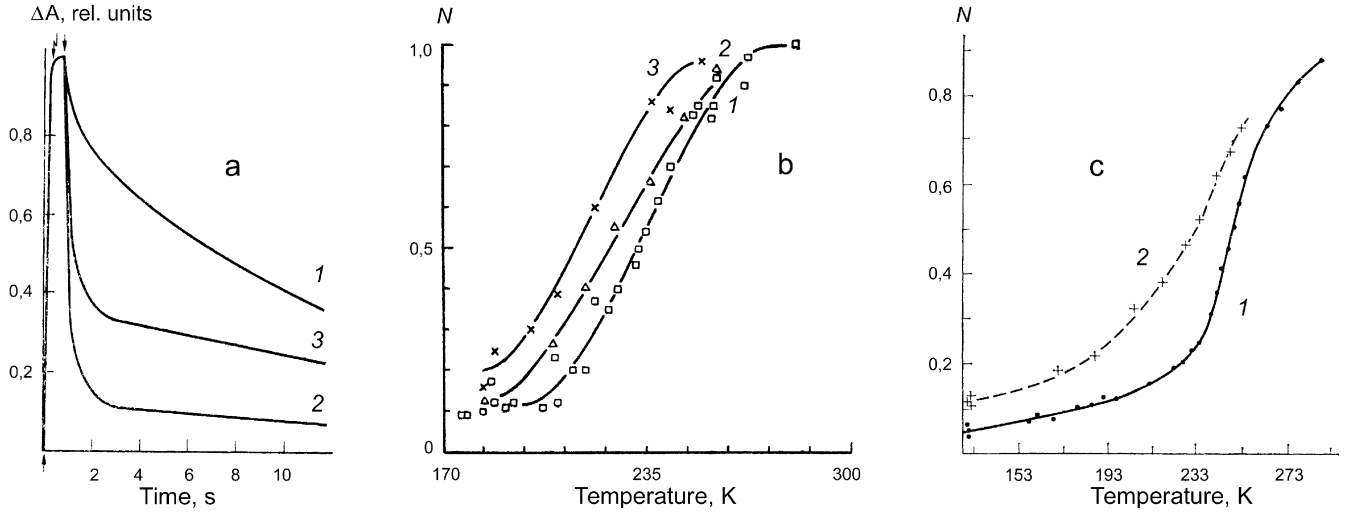


**Fig. 2** **a** Kinetics of photoinduced absorption changes of the reaction center bacteriochlorophyll P870 as measured at 425 nm in air-dry films of the *R. rubrum* chromatophores. *Upward* and *downward arrows* show the moments of actinic light (wavelength, $\lambda > 700$ nm; exposure time 1 s) on and off, respectively. Temperatures: *1*, 283 K; *2*, 228 K; *3*, 228 K + pulse heating against the background of photoactivation. A *zigzag arrow* indicates the moment of application of the thermal pulse of the YAG:Er laser at 228 K. The maximum heating at the peak of the thermal pulse of the YAG:Er laser corresponded to a temperature increase of about 100 °C. Relative contribution of the slow component of $P870^+$ reduction (*N*): *1*, 0.83; *2*, 0.20; *3*, 0.46. **b** Temperature dependence of the relative contribution of the slow component of $P870^+$ reduction (*N*) in isolated RC preparations of *Rb. sphaeroides* (water-glycerol suspension): *1*, control (without a thermal pulse); *2*, against the background of application of a thermal pulse generated by a $CO_2$ laser with an amplitude of 12 °C; *3*, against the background of the application of a thermal pulse generated by a $CO_2$ laser with an amplitude of 19 °C. The experimental samples contained 45 vol% glycerol. The *abscissa* in curves *2* and *3* corresponds to the initial temperature of the experimental sample before application of the thermal pulse. Photoactivation duration, 0.5 s. **c** Temperature dependence of the relative contribution of the slow component of $P870^+$ reduction (*N*) in air-dry films of the *R. rubrum* chromatophores: *1*, control (without a thermal pulse); *2*, against the background of a thermal pulse of a YAG:Er laser with a temperature pulse amplitude of 100 °C. No reductants or redox mediators were added

characteristic time of the process in this case is about 5 s. Upon decreasing the temperature to 228 K (Fig. 2a, curve 2), the process of slow reduction of $P870^+$ by electrons from the secondary quinone $Q_B$ is partially replaced by faster (millisecond) reduction from the primary quinone $Q_A$. This replacement is due to partial inhibition of electron transfer from $Q_A$ to $Q_B$ at low temperature. The relative contribution of the slow component of $P870^+$ reduction (*N*) can be regarded as a quantitative characteristic of the efficiency of electron transfer from $Q_A$ to $Q_B$. The method of calculation of the efficiency of electron transfer between the quinone acceptors of the RC based on measurements of *N* is described in more detail in Chamorovsky et al. (1976).

The kinetics of the thermoinduced transitions in photosynthetic RC was tested using the following procedure. After a sample under study had been cooled down to the initial steady-state temperature ($T_0$), it was exposed to a thermal laser pulse. The $T_0$ value was chosen to correspond to a given degree of inhibition of the reaction of interest, whereas the laser pulse amplitude was chosen to unblock the reaction.

The *Rb. sphaeroides* RC preparations (water-glycerol suspension, 55 vol% glycerol) were exposed to the thermal pulse, whose profile is shown in Fig. 1c. The temperature profile was determined as described above using a water-glycerol suspension (55 vol% glycerol) of the *H. salinarium* S-9 purple membranes as a suitable test object. Changes in the efficiency of the electron transfer reaction were probed 3 s and 10 s after the laser pulse application (i.e., at the moments of maximum temperature increase and 30–50% of the maximum temperature increase, respectively).

It follows from Fig. 2b that exposure to the laser-induced temperature pulse causes an increase in the electron transfer efficiency. The experimental curves shown in Fig. 2b were measured at the heating temperature (temperature jump) values of 12 °C and 19 °C (curves 2 and 3, respectively). It is seen that curves 2 and 3 are approximately 10 °C and 20 °C shifted toward a higher temperature relative to the initial curve 1, respectively. Within the limits of experimental error, these values correspond to the laser-induced pulse temperatures. Therefore, the equilibrium configuration of the acceptor complex that provides electron transfer from $Q_A$ to $Q_B$ is attained faster than 3 s even at low temperature (about 200 K).

Similar results were obtained for the *R. rubrum* chromatophores (Fig. 2c). Moreover, it was shown that in this case the equilibrium configuration of the acceptor complex providing high-efficiency electron transfer from $Q_A$ to $Q_B$ is attained during the relaxation time of the thermal pulse generated by the YAG:$Er^{2+}$ laser (tens of milliseconds).

To study the dynamics of spontaneous decay of the RC state induced by a thermal pulse, the pulse was applied either before or during photoinduced activation of electron transport reactions in the RC acceptor complex.

Because the time of complete relaxation of the thermal pulse generated by the YAG:$Er^{2+}$ laser in thin layers of a water-glycerol suspension of chromatophores or isolated RCs is significantly shorter than in dry films of these preparations, these experiments were performed in thin layers of water-glycerol suspensions. The maximum effect was observed if the thermal pulse was applied against the background of steady-state photoactivation of the RC. If the thermal pulse was applied before photoactivation, the effect was either zero (time interval between thermal pulse and photoactivation was longer than 0.5 s) or very small (time interval between thermal pulse and photoactivation was 10 ms). It follows from the kinetic curves shown in Fig. 3 that the thermal pulse of the YAG:$Er^{2+}$ laser applied 10 ms before photoactivation caused only a 4% increase in the contribution of the slow component $N$ (from 23% to 27%). It should be noted that the same thermal pulse applied against the background of steady-state photoactivation caused a significantly larger increase in the signal amplitude.

Thus, it may be concluded that both the time of the thermoinduced transition to the conformational state of the acceptor complex providing high-efficiency electron transfer from $Q_A$ to $Q_B$ within the temperature range from 233 to 253 K and the time of spontaneous decay of this state at 253 K are about tens of milliseconds. More accurate estimates of the characteristic times of these transitions can be obtained either from additional experiments or from profound theoretical analysis of thermoinduced processes in this system.

Independent support for these estimates was obtained from experiments with rapid cooling of the samples tested. Indeed, if the cooling rate were comparable or faster than the rate of spontaneous decay of the conformational state of the acceptor complex providing high-efficiency electron transfer from $Q_A$ to $Q_B$, the contribution of the slow component to the recombination kinetics of the separated charges would be higher than the equilibrium value inherent in the given temperature.

These experiments were performed in a water-glycerol (1:3, vol/vol) suspension of the *R. rubrum* chromatophores. The cooling rate was varied from 0.1 to 15 °C/s. The maximum rate of cooling was achieved by dipping a special low-inertia thin-layer cuvette with the experimental sample into liquid nitrogen for several seconds and its further transfer into a transparent Dewar flask containing cold ethanol of the required temperature. The rate of cooling was monitored with a thermocouple. This procedure provided a virtually linear temperature decrease with time.

The temperature dependencies of the relative contribution of the slow component of $P870^+$ reduction ($N$) as measured at average (0.1 °C/s) and the maximal (15 °C/s) rates of cooling are shown in Fig. 4 (curves 1 and 2, respectively). It follows from Fig. 4 that the contribution of the slow component of $P870^+$ reduction in samples cooled below 250 K with the maximal rate is significantly larger than in the same samples cooled to the same temperature with an average cooling rate (0.1 °C/s). If the estimates of the lifetime of the configuration of the acceptor complex that provides effective electron transfer from $Q_A$ to $Q_B$ obtained by the temperature-jump method are correct, during the time interval of rapid cooling from room temperature to 250 K a fraction of the complex should be immobilized in the conformational state typical of

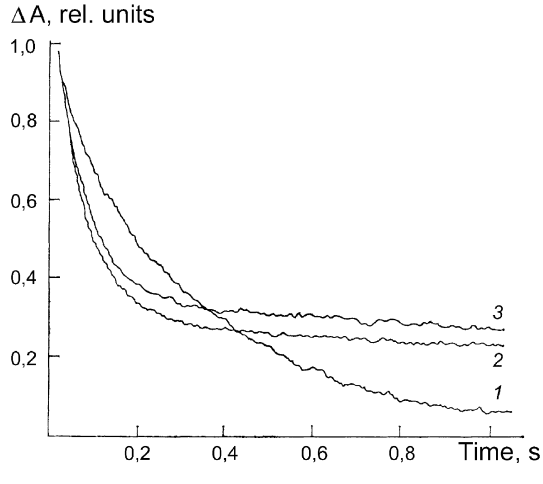


**Fig. 3** Kinetics of dark reduction of bacteriochlorophyll $P870^+$ in a water-glycerol suspension (60 vol% glycerol) of the *R. rubrum* chromatophores photoactivated with the second harmonic of a pulsed YAG:Nd laser (wavelength 530 nm; pulse duration 15 ns): *1*, at 293 K; *2*, at 255 K; *3*, at 255 K + a thermal pulse of a YAG:Er laser with a temperature pulse amplitude of 45 °C and a delay time (after light pulse) of 10 ms. Explanation in text

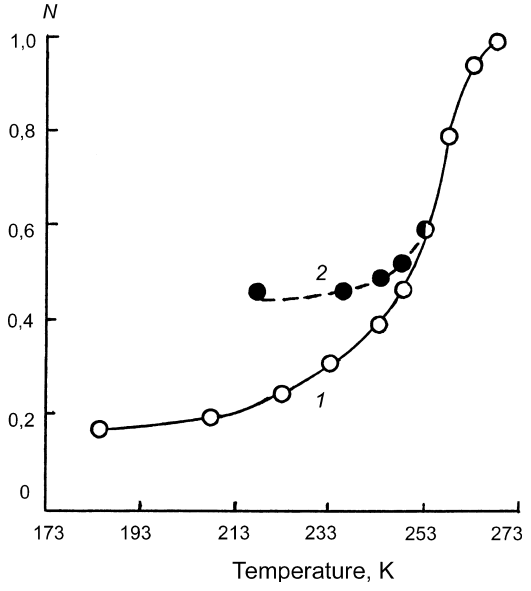


**Fig. 4** Temperature dependence of the relative contribution of the slow component of $P870^+$ reduction ($N$) in a water-glycerol suspension of the *R. rubrum* chromatophores at different rates of cooling from room temperature: *1*, 0.1 °C/s; *2*, 15 °C/s. The kinetics of the photoinduced absorption changes of P870 (870 nm) were measured at a photoactivation duration of 10 s

temperatures higher than 250 K. This was indeed observed in the experiments (Fig. 4). Perhaps more profound theoretical analysis of the mechanisms of thermoinduced processes in RCs is required for quantitative comparison between the results obtained by the methods of rapid cooling and temperature jump. However, it is quite obvious that the results obtained by these methods are qualitatively consistent.

Effective transfer of an electron to the secondary quinone acceptor in RCs of purple bacteria prevents the electron from being rapidly returned back to the oxidized bacteriochlorophyll. The efficiency of direct transfer is determined by the processes of electrostatic stabilization of the $Q_B$ negative charge. This stabilization is thought to be associated with displacement of protons and surrounding groups in the RC interior. This is accompanied by changes in the p*K* values of protonated amino acids within a distance of up to 15–17 Å (Okamura and Feher 1992; Lancaster et al. 1996; Miksovska et al. 1996). Charge transfer to $Q_B$ modifies the location and orientation of the quinone itself (Stowell et al. 1997). Presumably, these processes of electron stabilization in quinone acceptors should depend on the mobility of the protein moiety of RCs in the vicinity of the electron transfer cofactors. The role of protein dynamics in the functional activity of the RCs is presently a subject of intense research (Mäntele 1995; Maroti and Wraight 1997; McMahon et al. 1998; Balabin and Onuchic 2000; Labahn and Schmid 2000). The results of our studies of the effects of rapid temperature changes on the efficiency of electron transfer to $Q_B$ at the initial segment of the temperature dependence of this reaction (230–250 K) provide at least a qualitative basis for further research into the role of RC dynamics in the functional activity of the pigment-protein complex. This is particularly true in the case of estimates obtained by the method of laser-induced temperature jumps, because this method has not yet been applied to studies of electron transfer processes in the RCs of purple bacteria.

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