Biochemistry (Moscow), Vol. 66, No. 5, 2001, pp. 541-547. Translated from Biokhimiya, Vol. 66, No. 5, 2001, pp. 670-677.

Studies of Electron Transport Dynamics in Photosynthetic Reaction Centers Using Fast Temperature Changes

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Received October 10, 2000
Revision received October 31, 2000

Abstract—Rates of thermoinduced conformational transitions of reaction center (RC) complexes providing effective electron transport were studied in chromatophores and isolated RC preparations of various photosynthesizing purple bacteria using methods of fast freezing and laser-induced temperature jump. Reactions of electron transfer from the primary to secondary quinone acceptors and from the multiheme cytochrome *c* subunit to photoactive bacteriochlorophyll dimer were used as probes 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. 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. In contrast to the quinone complex, the thermoinduced transition of the macromolecular RC complex to the state providing effective electron transport from the multiheme cytochrome *c* to the photoactive bacteriochlorophyll dimer within the temperature range 220-280 K accounts for tens of seconds. This transition is thought to be mediated by large-scale conformational dynamics of the macromolecular RC complex.

Key words: photosynthetic reaction center, cytochromes, electron transport, conformational changes, purple bacteria

Studies of the temperature dependence of electron transfer rates in biological systems provide important insight into the 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 photoinduced primary charge separation [1]. In addition to three integral subunits, RC complexes of certain species of photosynthesizing purple bacteria (*Rhodopseudomonas viridis*, *Chromatium vinosum*, *Chromatium minutissimum*, *Ectothiorhodospira shaposhnikovii*, etc.) contain a multiheme cytochrome subunit exposed to the water phase [2]. The quantum efficiency of the primary processes of charge separation in bacterial RC does not decrease on cooling to cryogenic temperature [3, 4]. On the other hand, temperature decrease has a significant effect on the reactions of electron transfer from the primary to the secondary quinone acceptors and from the multiheme cytochrome *c* to the photooxidized dimer of RC bacteriochlorophyll [5-7]. 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 [8] or deceleration of conformational mobility of protein subunits of RC [9] have been suggested. However, experimental data obtained under steady-state temperature conditions were found to be insufficient to distinguish between these models.

The goal of this work was to study the temperature-induced transitions in bacterial RC taking reactions of electron transfer between quinone acceptors and electron transfer from the multiheme cytochrome *c* to photoactive bacteriochlorophyll dimer as examples.

MATERIALS AND METHODS

Chromatophores and isolated pigment–protein RC complexes from the photosynthesizing bacteria *Rhodospirillum rubrum*, *Rhodobacter sphaeroides*, *Chromatium minutissimum*, and *Ectothiorhodospira shaposhnikovii* were used. Bacterial cells were grown and chromatophores and RC preparations were isolated as

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described in [10]. Bacterial cells of *Halobacterium salinarium* S-9 (wild type) were grown and purple membranes were isolated as described in [11]. Chromatophores and isolated RC preparations were suspended in 0.05 M sodum 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 nsec) or an incandescent lamp (wavelength, >700 nm; exposure time, 0.5-10 sec). Redox reactions of the RC bacteriochlorophyll dimer (P870) were monitored as absorption changes at the Soret band (430 nm) or at 870 nm. Redox reactions of cytochrome *c* were monitored at the gamma-band at 425 nm. Spectral lines of monitoring light were isolated using corresponding interference filters.

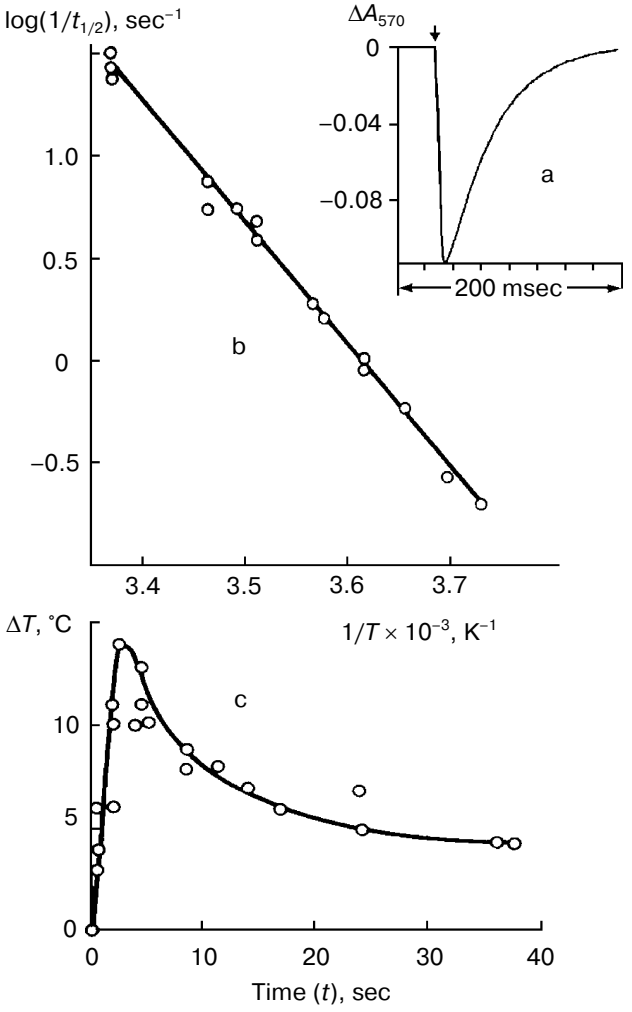

Fig. 1. Kinetic profile of thermal pulse generated by CO_2-laser radiation (wavelength, 10.6 μm; pulse duration, 1 sec). a) Kinetics of absorption changes of the main form of bacteriorhodopsin at 570 nm (bR-570) induced by the second harmonic of pulsed YAG:Nd laser (wavelength, 530 nm; pulse duration, 15 nsec). 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 CO_2-laser with radiation energy of 8.3 J.

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). The initial steady-state temperature was monitored by measuring the inverse current of a KD-103 diode attached to the sample.

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 mechanical shutter. The calculated half time of the temperature increase induced by YAG:Er^{2+} laser or CO_2 laser was 150-400 μsec or 1 sec, 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 uncertain in some cases, because they depend on such uncertain factors as distribution of laser radiation over cross-section of laser beam and 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 fluorescence of rhodamine B. It is well known that the Arrhenius plot of the rate of dark recovery of bR-570 followed 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 CO_2- rather than 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 CO_2-laser.

A kinetic curve of the bR-570 absorption changes at room temperature and Arrhenius plot of the rate constant of this process are shown in Figs. 1a and 1b, respectively. The kinetic profile of the thermal pulse generated by CO_2-laser was calibrated as follows. When a sample of bacteriorhodopsin had been cooled to given temperature, it was exposed to a 1-sec pulse of 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 recovery kinetics of bR-570. An example of the kinetic profile of the thermal pulse gener-

ated at the CO_2-laser pulse energy of 8.3 J is shown in Fig. 1c. The optical settings used in calibration were identical to those used in further experiments with chromatophores or photosynthetic RC preparations.

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

In case of dry films of chromatophores (thickness, 10-20 μm), the rise-time of the thermal pulse generated by YAG:Er^{2+}-laser was approximately the same (150-400 μsec), whereas the half-time of temperature relaxation and time of virtually complete relaxation to the initial level were about 10 msec and several tens of seconds, respectively [12, 13]. Thus, the use of pulsed YAG:Er^{2+}-laser and quasi-pulsed CO_2-laser (continuous generation modulated with a mechanical shutter) provides the opportunity to study the kinetics of both sufficiently fast (milliseconds) and slow (tens of seconds) thermoinduced processes in biological membranes.

Kinetics of 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 [7].

RESULTS AND DISCUSSION

Electron transfer in the system of quinone acceptors of bacterial photosynthetic reaction center. The efficiency of electron transfer from the primary (Q_A) to the secondary (Q_B) quinone acceptor was assessed by the relative amplitude of slow component of charge recombination as described in [7, 14]. At high redox potential of reaction medium (aerobic conditions), photoactivation of chromatophores or isolated RC preparations induces limited pseudocyclic electron transfer between P870 and quinone acceptors (Q_A and Q_B) [7]. Typical kinetic curves of the photoinduced absorption changes of P870 in dry films of the *R. rubrum* chromatophores are shown in Fig. 2. At room temperature, a photomobilized electron is trans-

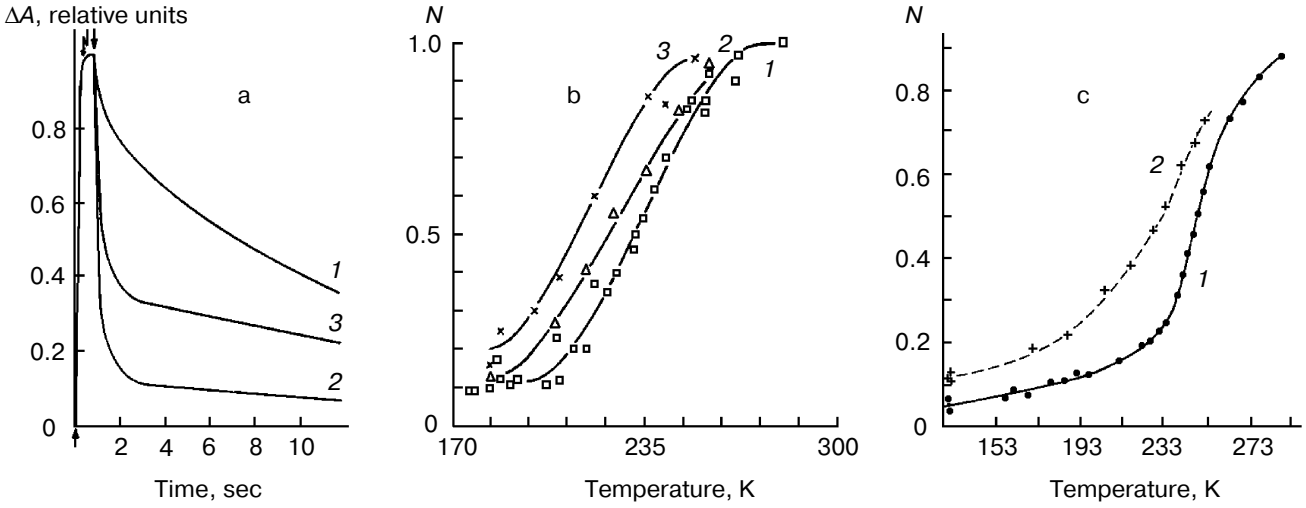

Fig. 2. a) Kinetics of photoinduced absorption changes of the reaction center bacteriochlorophyll P870 as measured at 425 nm in air-dry films of the *R. rubrum* chromatophores. Upward and downward arrows show the moments of actinic light (wavelength, >700 nm; exposure time, 1 sec) on and off, respectively. Temperature: *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 thermal pulse of YAG:Er-laser at 228 K. The maximum heating at the peak of the thermal pulse of YAG:Er-laser corresponded to temperature increase of about 100°. Relative contribution of slow component of $P870^+$ reduction (*N*): *1*) 0.83; *2*) 0.20; *3*) 0.46. b) Temperature dependence of relative contribution of slow component of $P870^+$ reduction (*N*) in isolated RC preparations of *Rb. sphaeroides*: *1*) without thermal pulse; *2*) against the background of application of thermal pulse with amplitude of 12°; *3*) against the background of application of thermal pulse with amplitude of 19°. Experimental samples contained 55 vol. % glycerol. Abscissa in curves *2* and *3* corresponds to the initial temperature of experimental sample before application of thermal pulse. Photoactivation duration, 0.5 sec. c) Temperature dependence of relative contribution of slow component of $P870^+$ reduction (*N*) in air-dry films of the *R. rubrum* chromatophores: *1*) control (without thermal pulse); *2*) against the background of thermal pulse of YAG:Er-laser with temperature pulse amplitude of 100°.

ferred 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 characteristic time of the process in this case is about 5 sec. 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. Relative contribution of slow component of $P870^+$ reduction (*N*) can be regarded 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 RC based on measurements of *N* is described in more detail in [14].

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 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 were exposed to the thermal pulse which profile is shown in Fig. 1c. Changes in the efficiency of the electron transfer reaction were probed 3 and 10 sec 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 laser-induced temperature pulse causes an increase in the electron transfer efficiency. Experimental curves shown in Fig. 2b were measured at the heating temperature (Temperature jump) values of 12 and 19°C (curves *2* and *3*, respectively). It is seen that curves *2* and *3* are approximately 10 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 sec even at low temperature (about 200 K).

Similar results were obtained in 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 YAG:Er^{2+}-laser (tens of milliseconds).

To study the dynamics of spontaneous decay of the RC state induced by thermal pulse, the thermal 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 YAG:Er^{2+}-laser in thin layers of water–glycerol suspension of chromatophores or isolated RC 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 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 sec) or very small (time interval between thermal pulse and photoactivation was 10 msec). It follows from the kinetic curves shown in Fig. 3 that the thermal pulse of YAG:Er^{2+}-laser applied 10 msec before photoactivation caused only a 4% increase in the contribution of 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 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 characteristic times of these transitions can be obtained either from additional experiments or from profound theoretical analysis of thermoinduced processes in this system.

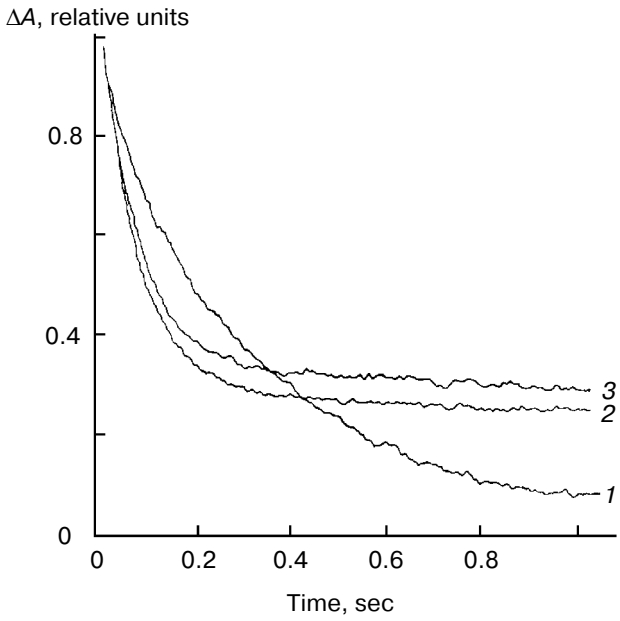

Fig. 3. Kinetics of dark reduction of bacteriochlorophyll $P870^+$ in water–glycerol suspension (60 vol. % glycerol) of the *R. rubrum* chromatophores photoactivated with the second harmonic of pulsed YAG:Nd^{3+} laser (wavelength, 530 nm; pulse duration, 15 nsec): *1*) at 293 K; *2*) at 255 K; *3*)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

Independent support of these estimates was obtained from independent experiments with rapid cooling of 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 slow component to the recombination kinetics of separated charges would be higher than the equilibrium value inherent in given temperature.

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

The temperature dependencies of relative contribution of slow component of $P870^+$ reduction (*N*) as measured at average (0.1°/sec) and the maximal (15°/sec) rates of cooling are shown in Fig. 4 (curves *1* and *2*, respectively). It follows from Fig. 4 that the contribution of 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°/sec). 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 true, 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 temperatures higher than 250 K. This is indeed observed in experiments (Fig. 4). Perhaps, more profound theoretical analysis of mechanisms of thermoinduced processes in RC 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 with each other.

Electron transfer from the multiheme cytochrome *c* to photoactive bacteriochlorophyll dimer of reaction center. The temperature dependence of the amplitude of the photoinduced absorption decrease at 425 nm caused by cytochrome *c* oxidation in water–glycerol suspension of the *C. minutissimum* chromatophores is shown in Fig. 5, curve *1*. After the chromatophore preparations had been cooled down to required temperature, they were exposed to the laser thermal pulse which kinetic profile is shown in Fig. 1c. Two series of experiments were performed with laser pulse energy of 11.5 and 23 J (Temperature jump amplitude of 17 and 33°, respectively). In the two experimental series, the photoinduced electron transfer reactions of cytochrome *c* oxidation were probed 7 sec after

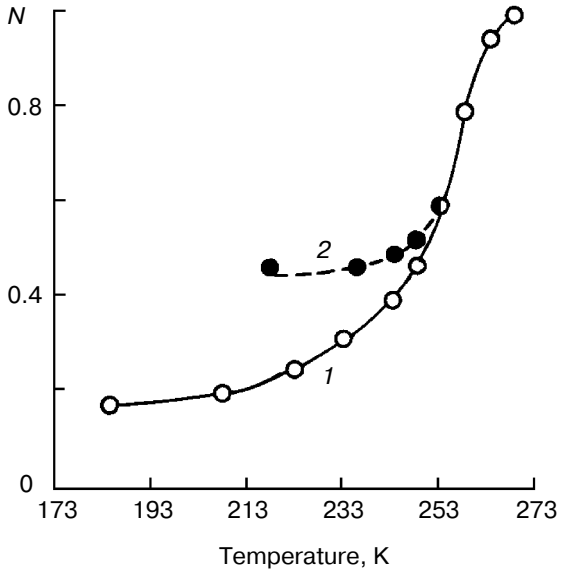

Fig. 4. Temperature dependence of relative contribution of slow component of $P870^+$ reduction (*N*) in water–glycerol suspension of the *R. rubrum* chromatophores at different rates of cooling from room temperature: *1*) 0.1°/sec; *2*) 15°/sec. Kinetics of photoinduced absorption changes of $P870^+$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

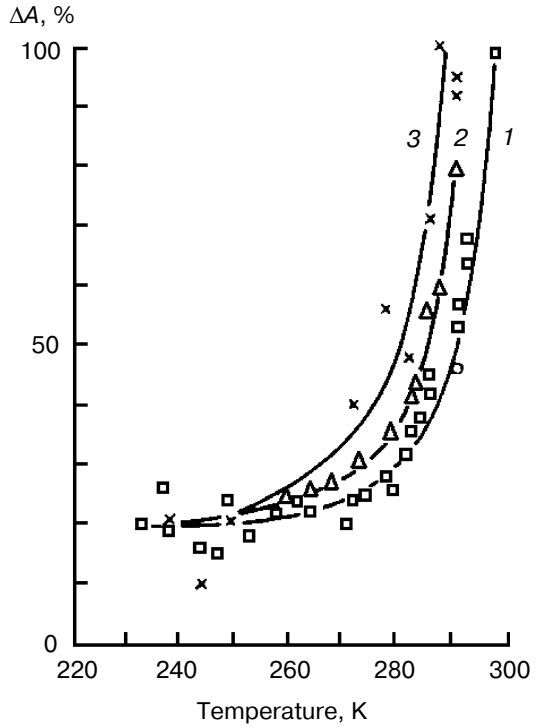

Fig. 5. Temperature dependence of the amplitude of the photoinduced absorption changes at 425 nm associated with cytochrome *c* oxidation in the *C. minutissimum* chromatophores: *1*) control (without thermal pulse); *2*) against the background of thermal pulse of CO_2-laser with amplitude of 17°; *3*) against the background of thermal pulse of CO_2-laser with amplitude of 33°. Abscissa in curves *2* and *3* corresponds to the initial temperature of experimental sample before application of thermal pulse. Experimental samples contained 70 vol. % glycerol. Photoactivation duration, 0.5 sec.

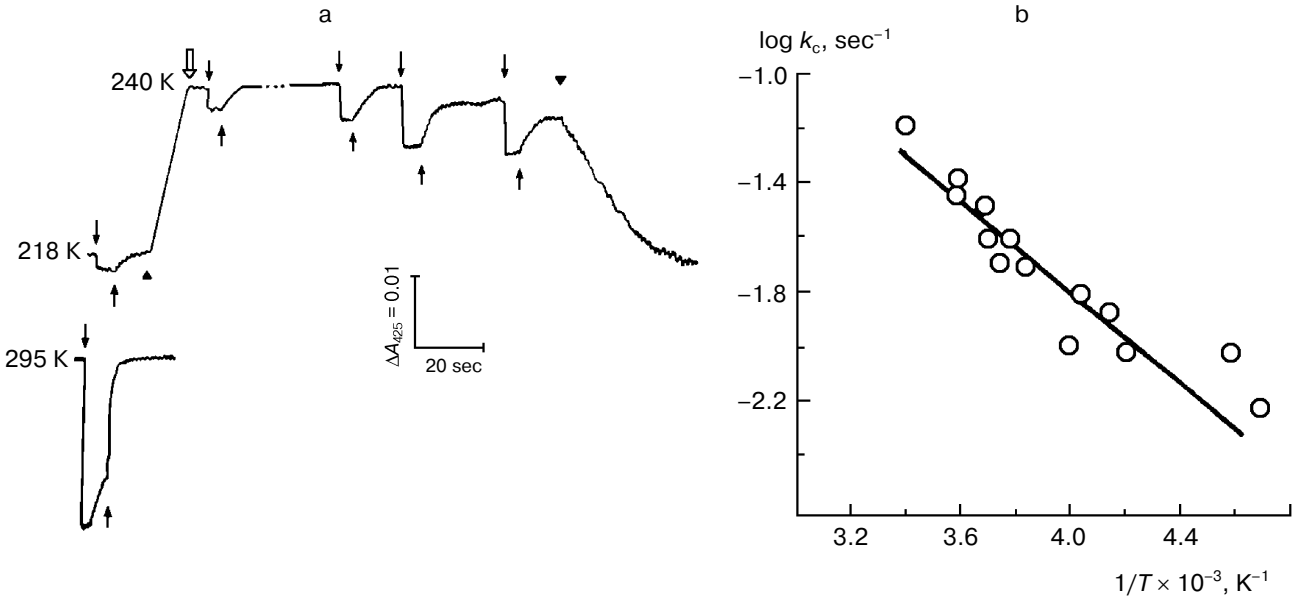

Fig. 6. a) Kinetics of photoinduced absorption changes at 425 nm associated with cytochrome *c* oxidation in air-dry films of the *E. shaposhnikovii* chromatophores. Thin downward and upward arrows indicate the moments of actinic light on and off, respectively. Triangles with the vertex up and down indicate the moments of the thermal radiation of YAG:Er-laser on and off, respectively. A thick open arrow indicates the moment of attenuation of the YAG:Er-laser radiation with KS glass filters (explanation in text). b) Arrhenius plot of the temperature dependence of the rate constant (k_c) of the thermoinduced conformational transition of RC complex to the state with photoactivated electron transfer from cytochrome *c* to bacteriochlorophyll dimer.

the laser pulse application. The results of the first and second experimental series are shown in Fig. 5 (curves *2* and *3*, respectively). It follows from Fig. 5 that although thermal pulses indeed increased the amplitude of the photoinduced signals, the increment of the thermoinduced increase was less than expected (17 and 33° in the first and second experimental series, respectively). In addition, the increment of the thermoinduced increase of the photoinduced signal was temperature-dependent. Indeed, the increment decreased upon decreasing the initial temperature of the sample, and within the temperature range from 253 to 243 K thermal pulse induced only an insignificant increase in the photoinduced signal amplitude. Thus, it can be suggested that the lifetime of the thermal pulse (7 sec) is too short to attain the conformational equilibrium between the multiheme cytochrome *c* subunit and photoactive bacteriochlorophyll dimer providing high-efficiency electron transfer in this system.

Other experimental settings were used for testing the kinetics of the thermoinduced implementation of the conformational equilibrium between the multiheme cytochrome *c* subunit and photoactive bacteriochlorophyll dimer. The YAG:Er^{2+}-laser was set at the spontaneous generation mode with pulse repetition frequency of 3 Hz, pulse duration of 150 μsec, and pulse energy of 0.5 J. When the required sample temperature had been reached, it was stabilized by attenuating laser radiation energy with KS glass filters. The temperature stability was judged by invariability of sample absorption. After a steady-state temperature of sample had been attained and maintained stable for a time interval *t*, the sample was exposed to actinic light, and amplitude of photoinduced oxidation of cytochrome *c* was recorded. An example of such record is shown in Fig. 6a. The time interval required to attain the maximum amplitude of the signal of photoinduced oxidation of cytochrome *c* at 240 K was 60 sec. The Arrhenius plot of the temperature dependence of the characteristic time interval required to attain the maximum amplitude of the signal of photoinduced oxidation of cytochrome *c* within the temperature range from 220 to 280 K is shown in Fig. 6b. The slope of the curve corresponds to the activation energy of corresponding conformational transition of 16 kJ/mol. Both kinetic and thermodynamic activation characteristics of this transition are typical of either displacement of large fragments of macromolecules or conformational changes of macromolecules as a whole. It should be noted that studies of the temperature dependence of the photoinduced electron transport from the multiheme cytochrome *c* to the photoactive bacteriochlorophyll dimer in seven mutant strains of *Rps. viridis* with the amino acid residue Tyr-62 substitution (Tyr-62 is located in RC between the multiheme cytochrome *c* and photoactive bacteriochlorophyll dimer) also suggested that the low-temperature inhibition of this reaction depends on the conformational transition of the protein moiety of RC and is mediated by a network of water molecules [15].

It should also be noted that rates of many biochemical reactions are changed without accompanying conformational translations. However, the magnitude and kinet-

ic characteristics of the processes studied in this work imply involvement of temperature-induced conformational transitions of proteins

Thus, to sum up the results of the study, it should be noted that the rate of the thermoinduced conformational transition providing effective electron transfer between redox centers embedded into the integral RC protein structure (Q_A and Q_B) is significantly higher than the rate of similar thermoinduced conformational providing electron transfer between redox centers located at peripheral and integral subunits (multiheme cytochrome *c* and bacteriochlorophyll dimer, respectively). The kinetic and thermodynamic characteristics of the latter transition imply that the molecular model of corresponding process should be based on large-scale conformational dynamics (e.g., association/dissociation of the cytochrome subunit of RC).

This study was supported by the Russian Foundation for Basic Research, project No. 00-04-48106.

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