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# Cooperative Interaction of High-Potential Hemes in the Cytochrome Subunit of the Photosynthetic Reaction Center of Bacterium *Ectothiorhodospira shaposhnikovii*

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**Abstract**—Cooperative interaction of the high-potential hemes ($C_h$) in the cytochrome subunit of the photosynthesizing bacterium *Ectothiorhodospira shaposhnikovii* was studied by comparing redox titration curves of the hemes under the conditions of pulse photoactivation inducing single turnover of electron-transport chain and steady-state photoactivation, as well as by analysis of the kinetics of laser-induced oxidation of cytochromes by reaction center (RC). A mathematical model of the processes of electron transfer in cytochrome-containing RC was considered. Theoretical analysis revealed that the reduction of one heme $C_h$ facilitated the reduction of the other heme, which was equivalent to a 60 mV positive shift of the midpoint potential. In addition, reduction of the second heme $C_h$ caused a three- to four-fold acceleration of the electron transfer from the cytochrome subunit to RC.

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*Key words*: purple bacteria, reaction center, cytochromes, electron transport, mathematical model, *Ectothiorhodospira shaposhnikovii*

Cytochrome *c* is the secondary electron donor for the photooxidized reaction center (RC) bacteriochlorophyll dimer in the majority of photosynthesizing bacteria. In some photosynthesizing bacteria, cytochrome *c* is represented by four-heme subunit incorporating two high-potential ($C_h$) and two low-potential ($C_l$) hemes. The 3-D structure of the cytochrome subunit in *Blastochloris viridis* has been resolved with atomic resolution using X-ray diffraction analysis [1], whereas, indirect methods (small-angle X-ray scattering, EPR, and certain biochemical methods) were used to probe the structure of the cytochrome subunit in RC of *Chromatium vinosum* [2, 3], *C. minutissimum* [4-6], and some other species [7]. The results of these studies indicate that the cytochrome hemes are densely packed, which implies possible interaction between them. Some kinetic and thermodynamic aspects of cooperative interaction between electron carriers in the photosynthetic electron-transport chain were considered in [8-10]. Significant anti-cooperative interactions of cytochrome C559 with RC bacteriochlorophyll dimer, low-potential heme C552, and the other high-potential heme were demonstrated using the RC of *B. viridis* with the cytochrome complex as a model system [11]. Interaction between neighboring electron carriers contributes substantially to their "working" midpoint redox potentials, thereby implementing dynamic control over operation of the electron-transport chain. It was experimentally shown that such interaction had an effect on the efficiency of electron transfer from the cytochrome complex to RC bacteriochlorophyll dimer [12, 13]. The detailed kinetic model of electron transfer between the cytochrome subunit hemes and special pair of RC bacteriochlorophyll in *B. viridis* was constructed taking into consideration the energy of interaction between neighboring electron carriers [14, 15]. Meanwhile, there is significant variability in the primary sequence of the four-heme cytochromes between species, in the structure of the contact area with RC, heme orien-

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*Abbreviations*: ETP) electron-transport particles; RC) reaction center.
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tation relative to the membrane plane, and midpoint redox potentials of individual hemes [2, 7, 16, 17]. Thus, the functional structure of the cytochrome complex (including inter-heme interaction) may vary from species to species of the photosynthesizing bacteria.

An approach to studies of inter-heme interaction is to compare the cytochrome redox titration curves measured under conditions of laser pulse photoactivation inducing single turnover of the electron-transport chain with those measured under conditions of photoactivation with continuous light inducing steady-state electron flow through the chain.

In this work, redox transitions of the high potential hemes $C_h$ were studied in chromatophores and subchromatophore electron-transport particles (ETP) isolated from the photosynthesizing bacteria *E. shaposhnikovii*, and compared with predictions of the theoretical model considering possible cooperative interactions between the hemes.

## MATERIALS AND METHODS

Growth protocols for the bacterium *E. shaposhnikovii* and isolation of chromatophores and subchromatophore electron-transport particles were described earlier [18]. We failed to isolate *E. shaposhnikovii* RC preparations containing photoactive cytochromes *c*, because none of available detergents proved able to solubilize the photosynthetic membrane of *E. shaposhnikovii* completely. The photoreactions of the cytochromes $C_h$ in chromatophores and subchromatophore ETP were measured in γ-band (424 nm) rather than in α-band (near 550 nm). It should be noted that spectral maximums of individual hemes are resolved only in the α-band, whereas individual hemes are not spectrally resolved in the γ-band, but amplitudes of differential maximums in the γ-band are significantly larger. The photoinduced reactions of the cytochromes $C_h$ were measured using the locally-made differential spectrophotometer and automated pulse laser spectrophotometer described in [19]. The method of redox titration of photoinduced reactions of cytochromes $C_h$ was described elsewhere [20].

Sets of simultaneous differential equations were solved analytically or using the GraFit data analysis and graphics program (Erithacus Software, Ltd).

## RESULTS AND DISCUSSION

The effect of the redox potential on the amplitude and kinetics of high-potential cytochrome $C_h$ oxidation induced by laser pulse or continuous light was studied in chromatophores and ETP of *E. shaposhnikovii*. Redox titration curves of the amplitude of the differential absorption signal associated with cytochrome oxidation induced by single laser pulse or continuous light in chromatophores and ETP of *E. shaposhnikovii* are shown in Fig. 1a.

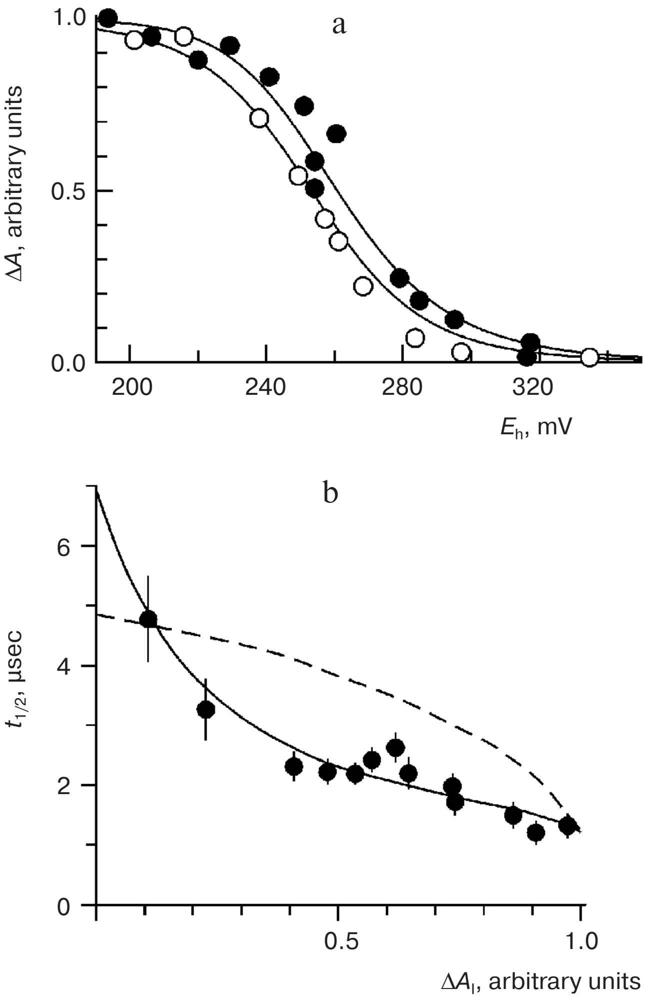

**Fig. 1.** a) Redox titration curves of high-potential heme $C_h$ absorption changes in *E. shaposhnikovii* chromatophores induced by single laser pulse (closed circles) or continuous light (open circles). Experimental data are best approximated by Eqs. (2) and (3), respectively, with a value for parameter *d* (the measure of cooperative interaction) of 0.30 ± 0.08. b) Dependence of half-time of cytochrome $C_h$ oxidation induced by a single laser pulse in *E. shaposhnikovii* chromatophores on the relative amplitude of the absorption change signal. The theoretical curve calculated for parameter $d = 0.30$ and $t_{1/2} = 1.2$ and 6.9 μsec (for the states with two and one reduced heme, respectively) is drawn through the experimental points. The curve plotted for the case of independent hemes ($d = 1.0$) at limiting values of $t_{1/2} = 1.2$ and 4.7 μsec is given for comparison as a dashed line.

The redox titration of the absorption changes associated with cytochrome oxidation induced by continuous light yielded the dependence close to the Nernst two-electron curve. This implies the possibility of positive cooperative interaction between the hemes. In addition, there was a significant (more than four-fold) increase in the rate of laser-induced cytochrome oxidation upon decreasing the medium redox potential (Fig. 1b). It is logical to suggest that this effect is due to an increase in the rate of electron transfer from the cytochrome complex to RC bacteriochlorophyll dimer upon a transition from the complex state with one reduced heme $C_h$ to the state with two reduced hemes $C_h$. Thus, analysis of $C_h$ heme oxidation kinetics at different redox potentials of the reaction medium provides additional information about the possibility of cooperative interaction between $C_h$ hemes.

A kinetic model of redox transitions in the *E. shaposhnikovii* RC complex with cytochromes was suggested. This model is shown in the scheme below. By analogy with the structure-functional model of cytochrome complex interaction with RC in *B. viridis* [1], it was assumed that the *E. shaposhnikovii* RC bacteriochlorophyll dimer directly interacted with only one heme $C_h$, whereas the other heme $C_h$ was the electron donor for the first heme $C_h$. To make the analysis simpler, it was suggested that the rate of electron exchange between hemes $C_h$ was at least one order of magnitude higher than the rate of electron transfer from the $C_h$ cytochrome complex to RC bacteriochlorophyll dimer, and midpoint redox potentials of the two hemes $C_h$ coincided with one another. To describe the cooperative interaction between the hemes $C_h$ during transition from the state with one reduced heme to the state with two reduced hemes, it was suggested that the midpoint redox potential of each heme $C_h$ was changed from $E'_m$ to $E''_m$. In addition, this scheme takes into account acceleration of electron transfer from $C_h$ to RC during transition from the state with one reduced heme to the state with two reduced hemes (rate constants $k'$ or $k''$, respectively; $k'' > k'$).

The cytochrome complex can attain three states: with one $C_h$ heme reduced, with two $C_h$ hemes reduced, and with both $C_h$ hemes oxidized (states 1, 2, and 0, respectively). The probabilities of the states are $P_1$, $P_2$, and $P_0$ ($P_0 + P_1 + P_2 = 1$).

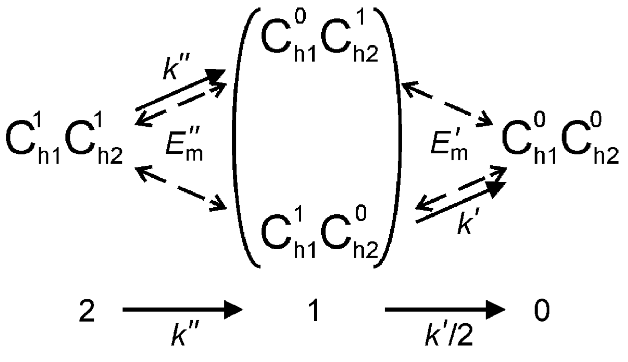

Diagram of functional organization of the high-potential part of the cytochrome complex in *E. shaposhnikovii*. Only one heme ($C_{h1}$) is assumed to be the immediate electron donor to RC bacteriochlorophyll dimer. Dashed arrows show the redox transitions of the complex in the dark; solid arrows, reactions of photoinduced oxidation of cytochrome $C_h$ by RC bacteriochlorophyll dimer

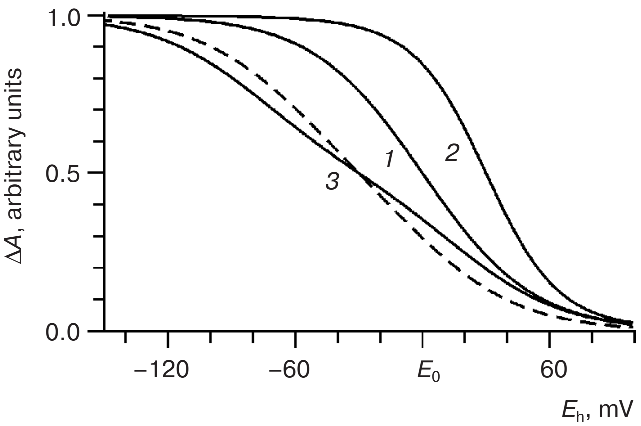

**Fig. 2.** Theoretical curves of redox titration of two-heme cytochrome. $E_0$ is the is the midpoint redox potential of the titration curve for cytochrome with two equivalent hemes $C_h$ in the absence of cooperative effect ($E'_m = E''_m$, curve *1*), corresponds to the first heme $C_h$. Curve *2* corresponds to positive cooperative effect, i.e. reduction of the first heme facilitates reduction of the second heme ($E''_m - E'_m = +60$ mV); curve *3* corresponds to negative cooperative effect, i.e. reduction of the first heme hampers reduction of the second heme ($E''_m - E'_m = -60$ mV). The dashed line corresponds to two initially non-equivalent (the midpoint redox potentials of the hemes differing by 60 mV) and independent (absence of cooperative effect) hemes $C_h$.

The expression for the probabilities of different states of the cytochrome complex at different medium redox potentials poised in the dark was derived on the basis of the Nernst equation:

$$P_1 = 2\alpha d/(1 + 2\alpha d + \alpha^2),$$
$$P_2 = 1/(1 + 2\alpha d + \alpha^2), \qquad (1)$$

where at $T = 298$ K, $\alpha = 10^{(E_h - (E'_m + E''_m)/2)/59}$, $d = 10^{(E'_m - E''_m)/118}$ (values $E'_m$ and $E''_m$ and medium redox potential $E_h$ are in mV). Parameter $d$ describes cooperative interaction (positive cooperative effect at $d < 1$; negative cooperative effect at $d > 1$; $d = 1$ corresponds to the absence of cooperative effect, i.e. lack of interaction or independence of redox reactions of two hemes $C_h$).

Continuous actinic light induces photooxidation of all hemes $C_h$ pre-reduced in the dark. State (2) contributes more significantly to the measured signal amplitude, because two hemes $C_h$ can be photooxidized in this state. The following expression was derived for the curve of the redox titration normalized to the maximum amplitude of the $C_h$ photooxidation signal induced by continuous actinic light:

$$\Delta A_{con} = (2P_2 + P_1)/2 = (1 + \alpha d)/(1 + 2\alpha d + \alpha^2). \qquad (2)$$

The effect of cooperative interaction between hemes on the shape of the redox titration curve is illustrated in Fig. 2. Cooperative interaction increases the slope of the redox titration curve near its midpoint, whereas anti-cooperative effect (reduction of the first $C_h$ heme hampers reduction of the second $C_h$ heme) decreases the slope of the redox titration curve relative to the one-electron curve; it is difficult to distinguish experimentally the case of anti-cooperative interaction from the case of two independent $C_h$ hemes with different midpoint redox potentials.

Single RC turnover induced by the first light pulse gives rise to photooxidation of one $C_h$ heme in states 1 and 2. The expression for the redox titration curve of $C_h$ photooxidation induced by single laser pulse is:

$$\Delta A_I = P_2 + P_1 = (1 + 2\alpha d)/(1 + 2\alpha d + \alpha^2). \qquad (3)$$

The amplitude of the $C_h$ photooxidation induced by the second light pulse separated from the first pulse by a short time interval (photooxidized cytochrome should not be reduced from external donors during the time interval between the light pulses, but this interval should be long enough to provide electron transfer from the primary acceptor to the secondary acceptor) represents the concentration of the states with two reduced hemes $C_h$:

$$\Delta A_{II} = P_2 = 1/(1 + 2\alpha d + \alpha^2), \qquad (4)$$

where $\Delta A_I$, $\Delta A_{II}$ are cytochrome absorption changes induced by the first and the second laser pulses normalized to the cytochrome absorption maximum, respectively.

Equation (2) describes the symmetric redox titration curve with midpoint $E_m = (E'_m + E''_m)/2$ equally distant from the midpoints of the curves corresponding to the first and the second laser pulses. Combination of Eqs. (2)-(4) gives rise to the expression for the redox potential shift (mV) between the midpoint redox potentials of the titration curves for $C_h$ absorption changes induced by continuous light, the first light pulse ($\delta_I$), and the second light pulse ($\delta_{II}$):

$$\delta_I = -\delta_{II} = 59\log\,(d + [d^2 + 1]^{1/2}). \qquad (5)$$

This shift in chromatophores and ETP of *E. shaposhnikovii* was approximately 8 mV (Figs. 1a and 3a). A similar shift in *Chromatium* chromatophores was 20 mV [2]. According to Eq. (5), the shift is 22 mV in case of two independent and equivalent hemes $C_h$ ($d = 1$). In case of

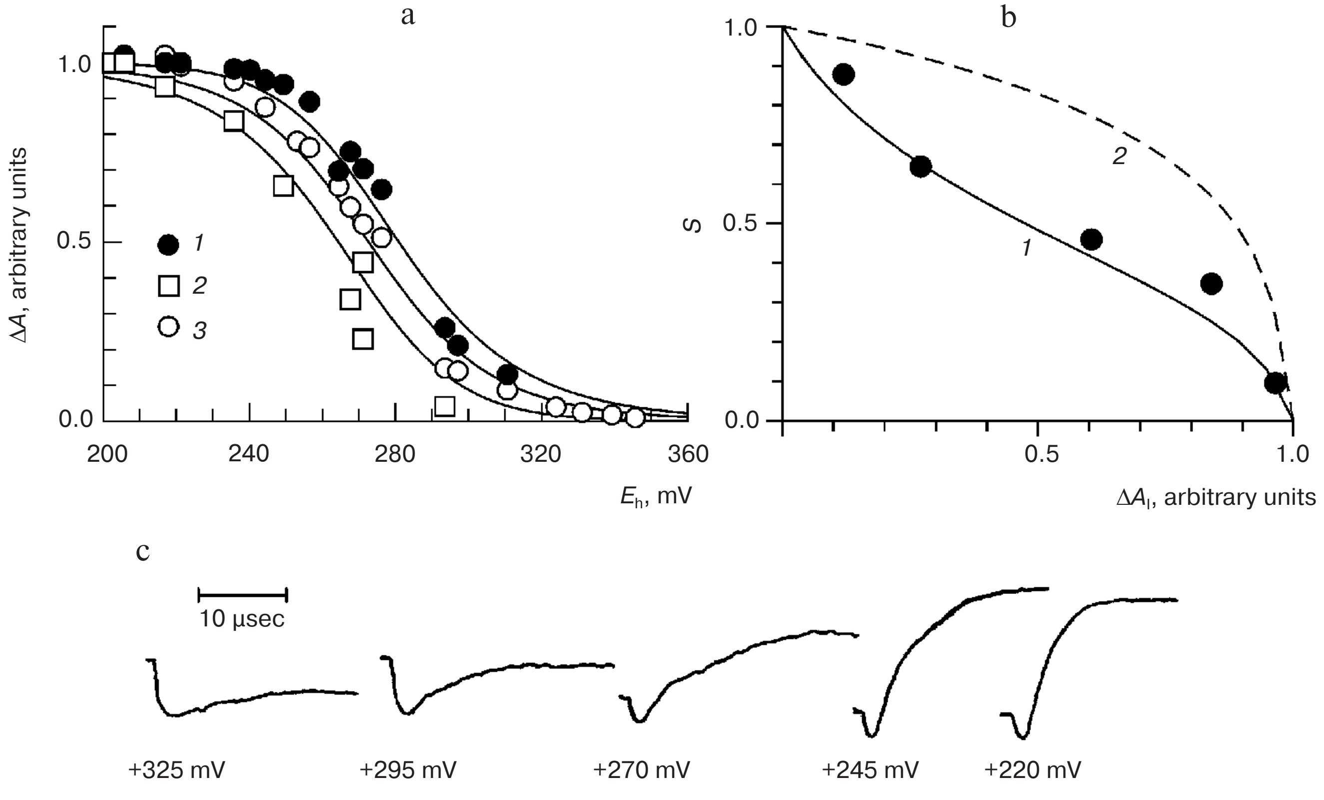

**Fig. 3.** a) Redox titration curves of oxidation of cytochrome $C_h$ induced by the first (*1*) and second (*2*) 50-μsec light flashes as well as continuous light (*3*) in ETP of *E. shaposhnikovii*. Simultaneous approximation with Eqs. (2)-(4) is the best fit to experimental data at $d = 0.33 \pm 0.04$. b) Dependence of contribution of slow component ($t_{1/2} = 6.6$ μsec) to the kinetics of laser induced $C_h$ oxidation on the amplitude of the absorption changes corresponding to this reaction. Experimental records of laser-induced absorption change kinetics corresponding to $C_h$ oxidation are shown at the bottom of the figure. Curves: *1*) a theoretical dependence at cooperativity parameter $d = 0.33$; *2*) a theoretical dependence for two independent hemes $C_h$ ($d = 1.0$) with equal midpoint redox potentials. Experimental conditions: $\lambda_{meas} = 424$ nm. Redox mediators: 4 μM variamine blue, 7 μM dichlorophenol indophenol, 6 μM methylene blue. Buffer solution: 50 mM Tris-HCl (pH 8.0).

two-electron carrier, when states with completely oxidized or completely reduced hemes $C_h$ are only possible ($d = 0$), the shift $\delta_I = 0$. Thus, the cytochrome complex of *E. shaposhnikovii* represents an intermediate case. The theoretical curves (Eqs. (2)-(4)) of the redox titration of $C_h$ photoreactions induced by continuous light and laser pulses are the best fit of experimental points at parameter $d = 0.30$ and 0.33 in chromatophores (Fig. 1a) and ETP (Fig. 3a) of *E. shaposhnikovii*, respectively. This corresponds to a ~+60 mV high-potential shift $E_m'' - E_m'$ during transition from the state with one reduced heme $C_h$ to the state with two reduced hemes $C_h$.

Positive cooperative effect (reduction of the first $C_h$ heme facilitates reduction of the second $C_h$ heme) means that transition from the state with one reduced heme to the state with two reduced hemes occurs within narrower medium redox potential range than in case of independent hemes. The amplitude contributions of the states with one or two reduced hemes in response to saturated laser pulse inducing single RC turnover are equal, because only one heme is subjected to photooxidation in either of the two cases. However, if the rates of the photooxidation of the states with one or two reduced hemes differed from one another, the contributions of the states to total signal amplitude could be determined using kinetic analysis of laser-induced cytochrome oxidation. The kinetics of photooxidation of cytochrome $C_h$ in ETP of *E. shaposhnikovii* induced by a saturated laser pulse contained two components: fast component with $t_{1/2} = 2.2 \pm 0.2$ µsec and slow component with $t_{1/2} = 6.5 \pm 0.5$ µsec. The slow component was substituted by the fast component upon decreasing the medium redox potential (Fig. 3b). It follows from the model described above (scheme), that contributions of fast and slow kinetic components of laser-induced $C_h$ oxidation correspond to contributions of the states with one or two hemes reduced in the dark and kinetics of laser-induced oxidation can be approximated by the following function:

$$c(t) = P_1 e^{-(k'/2)t} + P_2 e^{-k''t}. \qquad (6)$$

The contribution of the slow component ($S$) is:

$$S = P_1/(P_1 + P_2) = 2\alpha d/(1 + 2\alpha d). \qquad (7)$$

It can be shown that the expression for $S$ as a function of the total amplitude of signal $\Delta A_I$ (Eq. (3)) contains the only component, $d$, determining the sign and magnitude of the cooperative effect. Using the value of the parameter $d$ (0.33) obtained during approximation of the titration curve of the cytochrome $C_h$ in *E. shaposhnikovii* ETP, the changes in the contribution of slow component $S$ can be quantitatively described as function of relative amplitude of signal of $C_h$ laser-induced oxidation. It follows from Fig. 3b that theoretically calculated curve fairly describes experimental points, whereas the theoretical curve corresponding to the absence of cooperative effect ($d = 1$) is inconsistent with the experimental data. In *E. shaposhnikovii* chromatophores the signal/noise ratio was rather low, thereby making it difficult to implement detailed kinetic analysis of cytochrome $C_h$ laser-induced oxidation. Therefore, the kinetics of the laser-induced oxidation in chromatophores *E. shaposhnikovii* was quantitatively characterized only by the half-time $t_{1/2}$ of the cytochrome $C_h$ oxidation induced by a single laser pulse (Fig. 1b). Using the value of the parameter $d$ (0.30) obtained during approximation of the titration curve of the cytochrome $C_h$ in chromatophores *E. shaposhnikovii*, a satisfactory correlation between the theory and experiment was obtained at $t_{1/2} = 1.2$ µsec and $t_{1/2} = 6.9$ µsec for electron donation from the states with two electrons and one electron, respectively. It was impossible to approximate experimental data using the theoretical curve calculated assuming that the two $C_h$ hemes ($d = 1$) were independent. The fact that the slow kinetic component was substituted by fast kinetic component at relatively low signal amplitude can be regarded as qualitative evidence in favor of the existence of a positive cooperative effect.

We also verified the suggestion that two $C_h$ hemes in the cytochrome complex of *E. shaposhnikovii* were equivalent. To verify the suggestion, it was assumed hypothetically that initially two hemes $C_h$ in *E. shaposhnikovii* had different midpoint redox potentials $E_1'$ and $E_2'$; during dark reduction of one heme, the midpoint redox potential of the other heme is shifted by a fixed value determined by cooperative interaction. The resulting midpoint redox potential of two hemes are $E_1''$ and $E_2''$, respectively. A new parameter, $n = 10^{(E_2' - E_1')/59}$, is added to the model. This parameter describes the initial difference between the midpoint redox potentials of the hemes $C_h$ (the physical sense of parameter $n$ is the equilibrium constant for electron distribution between the two hemes). Necessary modification of expressions for probabilities of equilibrium states of the cytochrome complex with 0, 1, and 2 hemes reduced in the dark gave rise to new equations for approximation of the redox titration curves shown in Figs. 1a and 3a. Parallel approximation of the redox titration curves measured using laser flashes and continuous actinic light using modified equations allowed the value of the parameter $n$ to be determined ($n = 1.00 \pm 0.04$ and $n = 1.04 \pm 0.02$ for chromatophores and ETP, respectively). Thus, the values of the midpoint redox potentials of individual hemes $C_h$ in *E. shaposhnikovii* proved to be equal (1.5 mV difference fell within the range of experimental error).

This analysis accurately gave the values of the midpoint redox potentials of individual hemes $C_h$ during transition from fully oxidized cytochrome complex to the complex states with one reduced heme. This is also true for the transition between the complex states with one and two reduced hemes (table). It should be noted that both

Midpoint redox potentials of individual hemes $C_h$ in the cytochrome subunit of *E. shaposhnikovii* for the states with one or two reduced hemes

| Object | $E_1'$, mV | $E_2'$, mV | $E_1''$, mV | $E_2''$, mV |
|---|---|---|---|---|
| Chromatophores | +222 ± 6 | +222 ± 6 | +284 ± 15 | +284 ± 15 |
| ETP | +242 ± 4 | +243 ± 4 | +301 ± 7 | +302 ± 7 |

$E_m$ values and kinetic parameters of the reaction of electron transfer from high-potential heme $C_h$ to RC bacteriochlorophyll dimer in chromatophores differ slightly from those in subchromatophore ETP. Perhaps this difference is due to a certain difference in local molecular environment of electron transport cofactors in the preparations. Because it is believed that the photosynthetic membrane structure in chromatophores is nearly native, it is safe to suggest that parameters of electron transfer obtained for chromatophores are close to those *in vivo*.

In contrast to *E. shaposhnikovii*, hemes $C_h$ of the cytochrome subunit in *B. viridis* have different midpoint redox potentials: +380 mV in C559 and +310 mV in C556 (C559 is the immediate electron donor for oxidized RC bacteriochlorophyll dimer, whereas C556 reduces oxidized C559) ([14, 21-23] and references therein). There is a between-species difference in the details of the interaction of the cytochrome subunit hemes with each other and with RC bacteriochlorophyll dimer in photosynthetic bacteria. For example, in *C. vinosum* and *R. gelatinosus* the immediate electron donor for RC bacteriochlorophyll dimer has lower midpoint redox potential than the other high-potential heme ($E_m$ = +330 and +360 mV in *C. vinosum*; $E_m$ = +300 and +320 mV in *R. gelatinosus*) [3, 17]. Presently, *B. viridis* is the only species of photosynthetic bacteria in which the structure-functional organization of the cytochrome subunit (orientation of hemes, inter-heme distances, electron transfer sequence, and elementary rate constants at individual electron transport stages) has been accurately determined. There is the following sequence of hemes (according to distance from RC bacteriochlorophyll dimer): $C_h$–$C_l$–$C_h$–$C_l$. Because high-potential and low-potential hemes in *B. viridis* are not immediate neighbors, the inter-heme interaction in pairs $C_h$–$C_h$ and $C_l$–$C_l$ is insignificant (less than 20 mV by absolute value), whereas the interaction between neighboring carriers (heme $C_h$ with RC bacteriochlorophyll dimer and low-potential heme $C_l$ with the two high-potential hemes) is characterized by 50-80 mV $E_m$ shifts [11, 12, 14, 22]. According to the sign, the interactions are anti-cooperative: the oxidized state of the carrier stabilizes the reduced state of neighboring carrier and vice versa. Such effects in heme-containing proteins are mainly electrostatic in nature. For example, theoretical calculations [24] and results of electrochemical redox titration [25] showed that in cytochrome oxidase there was a significant anti-cooperative interaction between hemes *a* and $a_3$ (shift in the $E_m$ value was >100 mV). In the four-heme flavocytochrome from *Shewanella frigidimarina* the anti-cooperative interactions between pairs of hemes were described using a simple electrostatic model (screening of point charge in homogeneous dielectric); the magnitude of the interaction decreased from 56 mV (heme-to-heme distance, ~1 nm) to <10 mV (heme-to-heme distance, >2 nm) [26].

Yet, more interesting are positive cooperative effects observed in certain four-heme and multi-heme cytochromes from bacteria of the genus *Desulfovibrio*. The redox titration of the cytochromes revealed significant deviations from one-electron curve for individual hemes. These deviations were due to two causes: the Bohr effect or static coupling of electron–proton binding (protonation universally stabilizes the reduced heme state) and structural rearrangement of protein environment associated with heme reduction [27-30]. In the case of structural rearrangement of the protein environment, either positive or negative mutual influence of hemes is possible; positive cooperative effect in some cases was fairly significant ($E_m$ shifts were ~50 mV [29, 31]), which was close to $E_m$ shift ~60 mV obtained in our estimates for the high-potential cytochrome complex of *E. shaposhnikovii*. As far as we know, this work describes for the first time positive cooperative effect in the four-heme cytochrome subunit of photosynthesizing bacteria. The deviation in the inter-heme interactions from *B. viridis* argues for possible qualitative difference between the bacterial species in the structure-functional organization of the RC-associated cytochrome subunit.

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## REFERENCES

1. Deisenhofer, J., Epp, O., Sinning, I., and Michel, H. (1995) *J. Mol. Biol.*, **246**, 429-457.
2. Case, G. D., and Parson, W. W. (1971) *Biochim. Biophys. Acta*, **253**, 187-202.

3. Nitschke, W., Jubault-Bregler, M., and Rutherford, A. W. (1993) *Biochemistry*, **32**, 8871-8879.
4. Chamorovsky, S. K., Zakharova, N. I., Remennikov, S. M., Sabo, Ya., and Rubin, A. B. (1998) *FEBS Lett.*, **422**, 231-234.
5. Erokhin, V. V., Kayushina, R. L., Dembo, A. T., Sabo, Ya., Knox, P. P., and Kononenko, A. A. (1992) *Mol. Cryst. Liq. Cryst.*, **221**, 1-6.
6. Erokhin, V. V., Feigin, L. A., Kayushina, R. L., Lvov, Yu. M., Kononenko, A. A., Knox, P. P., and Zakharova, N. I. (1987) *Stud. Biophys.*, **122**, 231-236.
7. Menin, L., Schoepp, B., Garcia, D., Parot, P., and Vermeglio, A. (1997) *Biochemistry*, **36**, 12175-12182.
8. Rubin, A. B., and Shinkarev, V. P. (1984) *Electron Transport in Biological Systems* [in Russian], Nauka, Moscow.
9. Rubin, A. B., Kononenko, A. A., Pashchenko, V. Z., Gulyaev, B. A., and Chamorovsky, S. K. (1987) *Advances in Science and Technology. Biophysics*, Vol. 20, VINITI, Moscow.
10. Nitschke, W., and Rutherford, A. W. (1994) *Biochem. Soc. Trans.*, **22**, 694-699.
11. Voigt, P., and Knapp, E.-W. (2003) *J. Biol. Chem.*, **278**, 51993-52001.
12. Baymann, F., and Rappaport, F. (1998) *Biochemistry*, **37**, 15320-15326.
13. Alric, J., Cuni, A., Maki, H., Nagashima, K. V. P., Vermeglio, A., and Rappaport, F. (2004) *J. Biol. Chem.*, **279**, 47849-47855.
14. Alric, J., Lavergne, J., Vermeglio, A., Rappaport, F., Matsuura K., Shimada, K., and Nagashima, K. V. P. (2006) *J. Am. Chem. Soc.*, **128**, 4136-4145.
15. Becker, T., Ullmann, T. R., and Ullmann, G. M. (2007) *J. Phys. Chem. B*, **111**, 2957-2968.
16. Dracheva, S., Williams, C. J., van Driessche, G., van Beeumen, J. J., and Blankenship, R. E. (1991) *Biochemistry*, **30**, 11451-11458.
17. Agalidis, I., Othman, S., Boussac, A., Reiss-Husson, F., and Desbois, A. (1999) *Eur. J. Biochem.*, **261**, 325-336.
18. Chamorovsky, S. K., Kononenko, A. A., Petrov, E. G., Pottosin, I. I., and Rubin, A. B. (1986) *Biochim. Biophys. Acta*, **848**, 402-410.
19. Chamorovsky, S. K., Sabo, Ya., and Brown, L. S. (1989) *Biol. Nauki*, No. 6, 98-100.
20. Remennikov, S. M., Chamorovsky, S. K., Kononenko, A. A., Venediktov, P. S., and Rubin, A. B. (1975) *Stud. Biophys.*, **51**, 1-13.
21. Dracheva, S. M., Drachev, L. A., Konstantinov, A. A., Semenov, A. Yu., Skulachev, V. P., Arutyunian, A. M., Shuvalov, V. A., and Zaberezhnaya, S. M. (1988) *Eur. J. Biochem.*, **171**, 253-264.
22. Fritz, F., Moss, D. A., and Mantele, W. (1992) *FEBS Lett.*, **297**, 165-170.
23. Chen, I. P., Mathis, P., Koepke, J., and Michel, H. (2000) *Biochemistry*, **39**, 3592-3602.
24. Krishtalik, L. I. (1985) *Arch. Biochem. Biophys.*, **243**, 701-702.
25. Gorbikova, E. A., Vuorilehto, K., Wikstrom, M., and Verkhovsky, M. I. (2006) *Biochemistry*, **45**, 5641-5649.
26. Pessanha, M., Louro, R. O., Correia, I. J., Rothery, E. L., Pankhurst, K. L., Reid, G. A., Chapman, S. K., Turner, D. L., and Salguiero, C. A. (2003) *Biochem. J.*, **370**, 489-495.
27. Soares, C. M., Martel, P. J., Mendes, J., and Carrondo, M. A. (1998) *Biophys. J.*, **74**, 1708-1721.
28. Teixeira, V. H., Soares, C. M., and Baptista, A. M. (2002) *J. Biol. Inorg. Chem.*, **7**, 200-216.
29. Bento, I., Teixeira, V. H., Baptista, A. M., Soares, C. M., Matias, P. M., and Carrondo, M. A. (2003) *J. Biol. Chem.*, **278**, 36455-36469.
30. Oliveira, A. S. F., Teixeira, V. H., Baptista, A. M., and Soares, C. M. (2005) *Biophys. J.*, **89**, 3919-3930.
31. Turner, D. L., Salguiero, C. A., Catarino, T., Legall, J., and Xavier, A. V. (1996) *Eur. J. Biochem.*, **241**, 723-731.