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Effect of protein relaxation on electron transfer from the cytochrome subunit to the bacteriochlorophyll dimer in *Rps. sulfoviridis* reaction centers within mixed adiabatic/nonadiabatic model

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Abstract

The broad set of nonexponential electron transfer (ET) kinetics in reaction centers (RC) from *Rhodopseudomonas sulfoviridis* in temperature range 297–40 K are described within a mixed adiabatic/nonadiabatic model. The key point of the model is the combination of Sumi–Marcus and Rips–Jortner approaches which can be represented by the separate contributions of temperature-independent vibrational (v) and temperature-dependent diffusive (d) coordinates to the preexponential factor, to the free energy of reaction $\Delta G = \Delta G_v + \Delta G_d(T)$ and to the reorganization energy $\lambda = \lambda_v + \lambda_d(T)$. The broad distribution of protein dielectric relaxation times along the diffusive coordinate is considered within the Davidson–Cole formalism.

Keywords: Marcus theory; Free energy; Energy of reorganization; Protein dynamics; Dielectric relaxation

1. Introduction

In the last two decades, a considerable progress has been achieved in the quantitative description of long-range electron transfer (ET) between redox centers in native and in various chemically and genetically modified proteins. Special interest attract the reactions in the proteins under the lowered mobility: in membranes, in great protein complexes, in films, on the electrode surface or at lowered temperatures. The rates of electron transfer in such conditions reveal a great variety and they required special approaches for corrected description and analysis. For example, the course of kinetics of ET reaction between proximal heme of cytochrome subunit and photooxidized primary donor P^+ in protein complex of reaction centers (RC) from *Rhodopseudomonas viridis* changes greatly in the broad temperature range from 300 to 8 K [1,2]. Quite different temperature dependencies of ET rate one can observe in a set of heme and Cu-containing proteins [3–9]. If the changes in ET rate at temperatures below 150 K can be related mainly to vibrational degrees of freedom [1–3], the substantial increase in ET rates around 170–180 K for myoglobin and hemoglobin [4–6], or even at 220 K for cytochrome *c*–cytochrome *c* peroxidase complex [7,8], can be associated with the appearance of diffusive motion due to a glass-like transition in the solvent–protein matrix. In this paper, we propose the new model for description of ET kinetics in proteins, taking into account the vibrational and diffusive channels of energy dissipation, based on the combination of Sumi–Marcus and Rips–Jortner approaches [10,11]. This model is used for quantitative analysis of kinetics for electron transfer reactions from the heme c-559 of tetraheme cytochrome *c* to the special pair of bacteriochlorophylls in reaction centers from the photosynthetic purple bacterium *Rps. sulfoviridis*. The electron transfer kinetics were measured and analysed for free state of cytochrome reduction in temperature range 295–40 K.

2. Description of the model

In nearly all publications, ET reactions in proteins are analysed in terms of the nonadiabatic approach of the

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Marcus theory. According to the classical Marcus and Sutin's theory [12], under conditions of fast matrix reorganization ($V_{ab}^2 \ll \{\lambda h\nu/4\pi\}$), in the nonadiabatic approximation:

$$K_{et}^{NA} = K_0 \exp\left(-\frac{E_a}{RT}\right) \quad (1)$$

where

$$K_0 = \frac{2\pi V_{ab}^2(R)}{\eta\sqrt{4\pi\lambda kT}} \quad (2)$$

$$E_a = \frac{(\Delta G + \lambda)^2}{4\lambda}. \quad (3)$$

In these equations, K_{et}^{NA}, E_a, V_{ab}, λ, ΔG and ν, are the nonadiabatic electron transfer constant, the activation energy, the tunneling matrix element, the nuclear reorganization energy, the free energy of reaction and the nuclear reorganization characteristic frequency, respectively.

The application of the nonadiabatic approximation depends on the magnitudes of ν and V_{ab}, which can broadly vary in proteins. As was estimated in publications [13–15], $V_{ab} = 10^{-1} - 10^{-3}$ cm^{-1} for electron transfer in reaction centers (RC), in heme proteins myoglobin, cytocrome c and azurin at distances 8–26 Å. If the nuclear reorganization is governed by fast vibrations or by the motion of free water molecules with $\nu = 2 \times 10^{12}$ s^{-1} and $\lambda = 1$ eV, the nonadiabatic limit is fulfilled for all ET reactions in these proteins. However, the relaxation frequency of the myoglobin heme pocket is as low as $10^8 - 10^4$ s^{-1} even at room temperature [16–18]. Such slow relaxations may take place due to conformational transitions or local protonation and reveal diffusive character. If $\nu = 2 \times 10^4$ s^{-1}, the nonadiabatic limit is fulfilled at $V_{ab} \ll 10^{-2}$ cm^{-1}, which is not valid for some reactions in metal-containing proteins.

If the conditions of the nonadiabatic approach are not fulfilled, the adiabatic limit must be used. According to the simplest version of the theory [19,20], if $V_{ab}^2 \gg \{\lambda h\nu/4\pi\}$, the adiabatic electron transfer rate constant is expressed by the equation:

$$K_{et}^{AD} = \nu A \exp\left(-\frac{E_a}{RT}\right). \quad (4)$$

The intermediate case between nonadiabatic and adiabatic limits was considered for a model with a single diffusive mode [11,19,20]. In this case, the electron transfer constant K_{et} is expressed by the equation:

$$K_{et} = \frac{K_0}{1 + \frac{K_0}{A}\tau} \exp\left(-\frac{E_a}{RT}\right) \quad (5)$$

where $\tau = 1/\nu$ is the thermally activated relaxation time for the diffusive motion.

In more complicated cases, if the times of the electron transfer and of the matrix reorganization are comparable and there is a Davidson–Cole distribution of the relaxation times described by the function:

$$g(\tau) = \frac{\sin(\pi\beta)}{\pi\tau}\left(\frac{\tau}{\tau_0 - \tau}\right)^{\beta} \quad 0 < \tau \le \tau_0 \quad (6)$$

$$g(\tau) = 0 \quad \tau > \tau_0$$

then, according to Rips and Jortner [11], the average ET constant at short times is expressed as:

$$K_{et} \sim (1/\tau_0)^{\beta} V_{ab}^{2(1-\beta)} \exp\left(-\frac{E_a}{RT}\right) \quad (7)$$

where $0 \le \beta \le 1$ is the time distribution parameter of the Davidson–Cole dielectric relaxation spectrum and τ_0 is the characteristic relaxation time, which corresponds to the upper limit of the τ distribution.

In order to describe the kinetics of the ET reactions in RC from *Rps. sulfoviridis*, we propose in this paper a model that combines Sumi–Marcus [10] and the Rips–Jortner approximations. Sumi–Marcus model takes into account the possibility of energy dissipation simultaneously by two modes: by fast vibrational and by a diffusive degrees of freedom.

Obviously, along with the diffusive motion, proteins display vibrational (nondiffusive) motions including intramolecular vibrations, internal rotations, etc. Such motions have very short relaxation times and never freeze out even at low temperatures. If the motion along both coordinates, nondiffusive q and diffusive X, is fast, the reaction goes through the optimum, fastest nonadiabatic pathway from state of reactant to state of product with the lowest activation energy E_a^{min} at point S representing the transition state for the reaction (see Fig. 1 in Ref. [10]). However, if the motion along the diffusive coordinate X freezes out at low temperature due to a change of τ, the ET process still persists due to the possibility of reaction along the nondiffusive coordinate via a different transition state with a higher activation energy E_a, $E_a^{min} \le E_a$. In this case, the reaction proceeds in a mixed adiabatic/nonadiabatic regime.

Such a shift of the transition state can be described phenomenologically by introducing a τ-dependent activation energy and a preexponential factor, as described by Kotelnikov et al. [21]:

$$K_{et}(\tau) = \sigma(\tau)\exp\left[-\frac{E_a(\tau)}{RT}\right] \quad (8)$$

$$E_a(\tau) = \frac{[\Delta G(\tau) + \lambda]^2}{4\lambda(\tau)}. \quad (9)$$

The dependence on τ is given by the function:

$$\phi(\tau) = \frac{1}{1 + \frac{K_0}{A}\tau} \quad (10)$$

which serves as a switch between the high-temperature (nonadiabatic) and low-temperature (adiabatic along diffu-

sive coordinate) limits since $\varphi(\tau) \sim 1$ at room temperature and $\varphi(\tau) \ll 1$ at low temperature, like in Eq. (5). The pre-exponential factor is represented as a sum of vibrational and diffusive contributions:

$$\sigma \equiv \sigma_v + \sigma_d = K_0[P_\sigma + (1 - P_\sigma)\varphi(\tau)] \quad (11)$$

where P_σ is a phenomenological parameter, $\sigma_v = K_0 P_\sigma$ is a temperature-independent vibrational contribution and $\sigma_d = K_0(1 - P_\sigma)\varphi(\tau)$ is a temperature-dependent (via τ) contribution of the diffusive motion. The activation energy in Eq. (9) is expressed in terms of τ-dependent reorganization energy and driving force as:

$$\lambda(\tau) \equiv \lambda_v + \lambda_d = \lambda[P_\lambda + (1 - P_\lambda)\varphi(\tau)] \quad (12)$$

$$\Delta G(\tau) \equiv \Delta G_v + \Delta G_d = \Delta G[P_G + (1 - P_G)\varphi(\tau)] \quad (13)$$

where P_λ and P_G are phenomenological parameters and the same $\varphi(\tau)$ is used as a switch between the low- and high-temperature limits. Since we suppose that τ characterizes the temperature-activated diffusive motion, the first terms in the square brackets in Eqs. (11)–(13) represent constant, temperature-independent contributions from the vibrational mode q, $\sigma_v = \sigma P_\sigma$, $\lambda_v = \lambda P_\lambda$ and $\Delta G_v = \Delta G P_G$, whereas the second terms are temperature-dependent parts of $\sigma(\tau)$, $\lambda(\tau)$ and $\Delta G(\tau)$ corresponding to the diffusive coordinate X_1, $\sigma_d = \sigma(1 - P_\sigma)$, $\lambda_d = \lambda(1 - P_\lambda)$ and $\Delta G_d = \Delta G(1 - P_G)$. Note, however, that the numerator of Eq. (9) contains the constant reorganization energy λ corresponding to the room temperature limit of Eq. (12) with $\varphi(\tau) = 1$, in the low-temperature limit:

$$E_a(\tau) = \frac{[\Delta G_v + \lambda]^2}{4\lambda_v}, \quad (14)$$

which is easy to verify. As a result, $E_a(\tau)$ must increase as temperature decreases.

We have performed the fitting of ET kinetics in RC from *Rps. sulfoviridis* using Eqs. (8)–(13) with parameters $P_\lambda = 0.7$ and $P_G = 0.1$, as was determined in Ref. [21]. A computer analysis showed that these parameters give optimum results for the present system, too. Concerning the thermodynamic parameters, we accept the values from Ref. [22]: the ΔG values for electron transfer in the first, second and third states of reduction of cytochrome are $\Delta G_1 = -0.14$ eV, $\Delta G_2 = -0.18$ eV and $\Delta G_3 = -0.29$ eV, respectively; for all the reactions, $\lambda = 0.29$ eV.

3. Experimental

Rps. sulfoviridis, a species closely related to *Rps. viridis*, was grown and reaction centers were prepared as described in Ref. [23]. Purified reaction centers were handled as described in Ref. [1] for *Rps. viridis*.

Flash-induced absorption changes under several redox conditions and at various temperatures were measured in time domain 0.1–4500 μs as in Ref. [1] using a ruby laser as

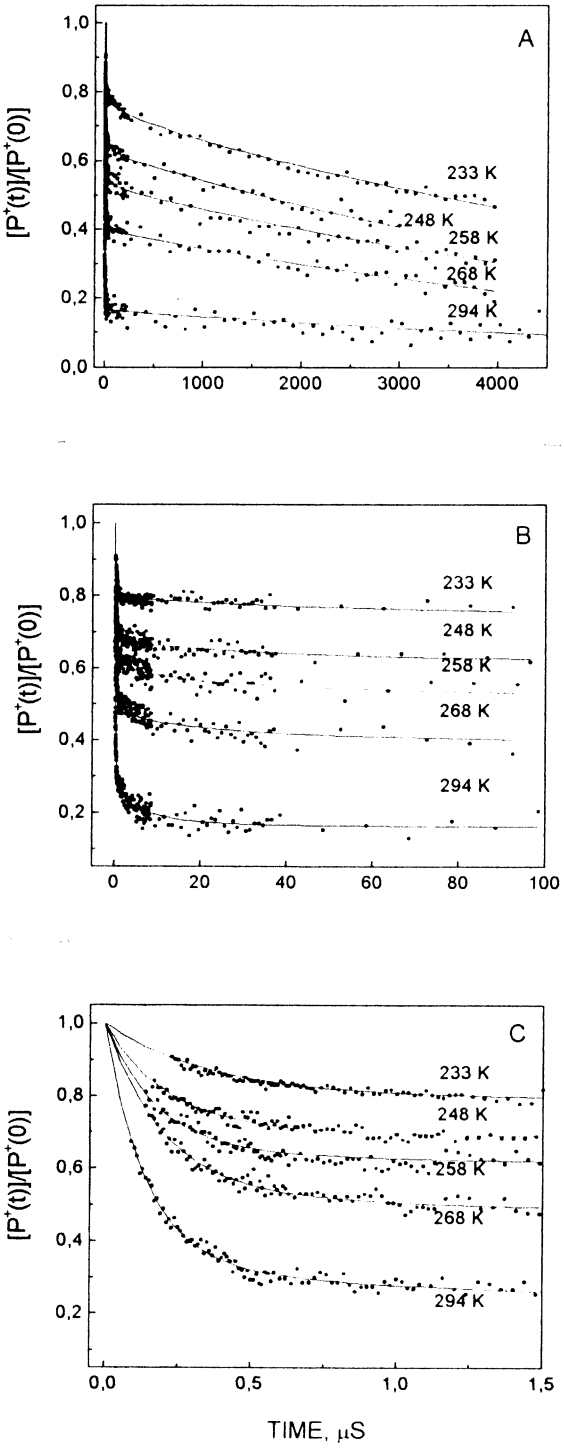

Fig. 1. Kinetics of normalised flash-induced absorption changes at 1283 nm of the cation-radical P^+ in RC from *Rps. sulfoviridis* for the first state of cytochrome reduction ($E_h = +360$ mV) at different temperatures on different time scales. Solid circles: experimental measurements; line graphs: fitting according to the model described in the paper. (A) Full time scale; (B) 100 μs time scale; (C) 1.5 μs time scale.

a source of excitation light. The oxidation of the primary donor P and its subsequent reduction were measured by following at 1283 nm the absorption of P^+. The cuvette contained 60% by volume of glycerol.

Data were acquired as in Ref. [1], except that the decay kinetics were not analyzed in terms of exponential components. Instead, they were analyzed according to the method proposed in this work.

4. Results

Kinetic curves of flash-induced absorption changes of P^+ describing the ET reactions have a strong nonexponential character in a wide temperature range from 295 to 40 K and they depend on the state of cytochrome reduction, as shown previously from similar experiments in RC from *Rps. viridis* [1]. Examples of such kinetic curves at different temperatures and in different states of cytochrome reduction are presented in Figs. 1 and 2.

ET reactions following a flash in the cytochrome–RC complex are as follows:

$$c-559^- \xrightarrow{K_{et}} P^+ \xleftarrow{K_r} Q_A^-.$$

A preliminary analysis of the time behaviour of the function $F(t)=[P^+(t)]/[P^+(0)]$, has shown that in a broad time domain from 0.1 to 4500 μs for various temperatures, all experimental kinetics can be considered as a sum of two curves, which differ significantly (Fig. 1). The initial part of the kinetic curves in the time interval 0.1–40 μs is fast, temperature-dependent and nonexponential, but the next part from 40 to 4500 μs decays slowly and is described by a one-exponential function with a nearly temperature-independent characteristic rate around $(1-4)\times 10^2$ s^{-1}. Since it is known from Ref. [24] that the $P^+ \leftarrow Q_A^-$ reaction have the rate constants $(1-4)\times 10^2$ s^{-1} in temperature range 100–300 K, it seems reasonable to relate the fast nonexponential part of kinetics to the $c\text{-}559^- \rightarrow P^+$ reaction and the slow exponential part to the recombination process $P^+ \leftarrow Q_A^-$.

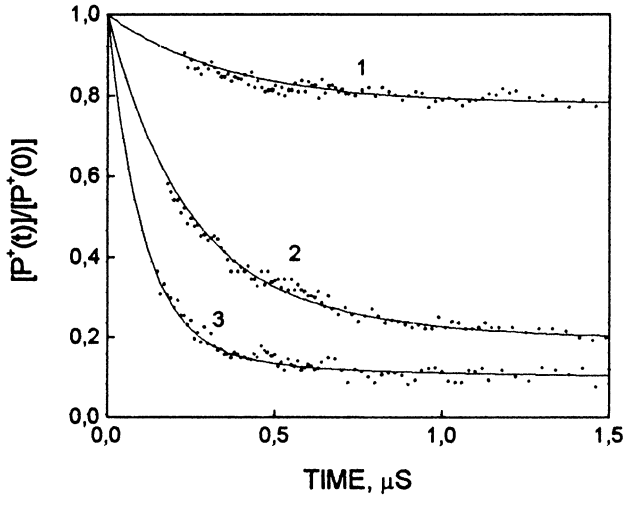

Fig. 2. Kinetics of normalised flash-induced absorption changes at 1283 nm of the cation-radical P^+ in RC from *Rps. sulfoviridis* at temperature 220 K under three different redox conditions on 1.5 μs time scale. (1) First state of cytochrome reduction ($E_h=+360$ mV); (2) second state of cytochrome reduction ($E_h=+250$ mV); (3) third state of cytochrome reduction ($E_h=-20$ mV). Solid circles: experimental measurements; lines: fitting according to the model described in the paper.

Then, the function $F(t)=[P^+(t)]/[P^+(0)]$ can be written as:

$$F(t) = A_1 \int_0^\infty g(\tau)\exp[-K_{et}(\tau)t]d\tau + A_2\exp(-K_r t)]. \quad (15)$$

In this equation, where $A_1+A_2=1$, the first integral term describes the direct fast $c\text{-}559^- \rightarrow P^+$ reaction and the second term describes the back reaction $P^+ \leftarrow Q_A^-$.

5. Discussion

The aim of this work was the description of a whole ensemble of experimental kinetic curves (about 50) in the temperature range 40–298 K for the three states of cytochrome reduction in the framework of a unique model for the determination of unknown parameters: K_0, β, τ_0, A_2 and K_r.

The procedure of computer fitting was divided into several steps. First, A_2 and K_r were determined for each curve from a semilogarithmic plot of the slow kinetics in the time interval 100–4500 μs. Subsequently, each curve in the time interval 0.1–100 μs was fitted for determination of the parameter β, value τ_0 and parameter K_0 by minimizing the standard Φ with respect to the experimental curves.

Finally, parameters λ, ΔG, P_σ, P_λ and P_G were varied around their initial values with monitoring of variations of Φ. As a rule, Φ was minimized for the parameters taken from Ref. [21], only for parameter P_σ was taken $P_\sigma=0$.

5.1. K_0 value

This parameter describes the maximum ET rate in the protein under nonadiabatic conditions. From an analysis of the experimental curves, we can see that $K_0=(1.0-1.6)\times 10^7$ s^{-1} for all states of cytochrome reduction in the temperature range 298–230 K, then it slowly decreases to 10^6 s^{-1} at 40 K (Fig. 3). The average value at room temperature, $K_0=1.4\times 10^7$ s^{-1}, can be compared with $K_0=10^8$ s^{-1}, which is calculated from the empirical equation of the one-dimensional tunnelling barrier model [25,26]:

$$K_0(s^{-1}) = 10^{16}\exp(-\alpha R). \quad (16)$$

Here we used $\alpha=1.4$ Å^{-1} and $R=12.3$ Å, the edge-to-edge distance between the proximal heme c-559 of the cyto-

chrome and P^+. Given $K_0 = 1.4 \times 10^7\ s^{-1}$, V_{ab} can be calculated from Eq. (2). If $\lambda = 0.29$ eV and $T = 295$ K, then $V_{ab} = 0.17\ cm^{-1}$; this value can be compared to $V_{ab} = 0.13\ cm^{-1}$ obtained for the same reaction between cytochrome c_2 and P^+ in reaction centers from *Rhodobacter sphaeroides* [13].

5.2. Parameter β

The parameter β characterizes the time distribution of protein relaxation motions participating in ET reactions. As shown in Fig. 4, the β values are close to 0.03–0.06 for all states of protein reduction in the temperature range 298–220 K. At lower temperatures, such as 200–130 K, β increases from 0.08 to 0.2, and it is equal to 0.57 at 40 K.

It is necessary to note that the magnitude of β is a characteristic of the dielectric spectrum of the medium and therefore, must reflect peculiarities of the object under investigation and of the method of measurements. For pure glycerol, direct dielectric measurements give $\beta = 0.55$–0.6 in the temperature range 198–233 K [27]. The very low β values in the temperature range 298–130 K for RC may be explained by a large contribution of very fast vibrational and librational motions to the dielectric relaxation of the protein matrix with substantial contribution of slow protein relaxation in the course of ET reaction.

5.3. Factor of adiabaticity and characteristic relaxation time τ_0

The parameter $\frac{K_0}{A}\tau_0$, which can determined directly in our calculations, represents, in accord to Eqs. (5) and (10), the degree of adiabaticity of ET reactions for the population of proteins with a maximum relaxation time τ_0 in the Davidson–Cole distribution. The ET reaction is adiabatic if $\frac{K_0}{A}\tau_0 \gg 1$. In our analysis, we have found that for the RC with cytochrome in the first state of reduction, this parameter is equal to 10 at room temperature and increases up to 178 as the temperature decreases to 220 K. This means that even at room temperature, the ET reaction is substantially adiabatic for a fraction of proteins with the longest relaxation times $\tau_0 = 0.35$ μs, and the degree of adiabaticity increases when temperature decreases.

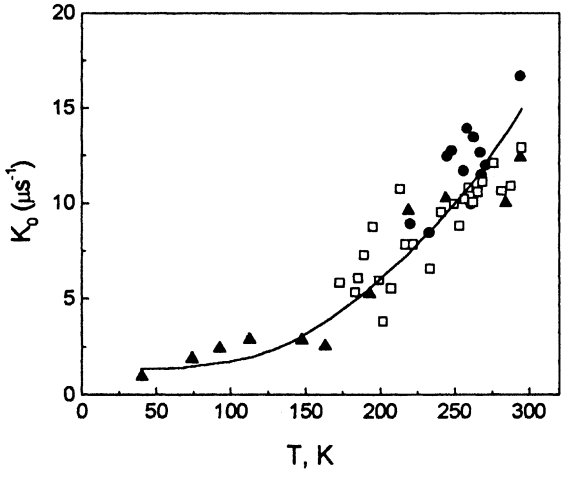

Fig. 3. Temperature dependence of the parameter K_0 for three different states of cytochrome reduction. Solid circles: first state of cytochrome reduction ($E_h = +360$ mV); open squares: second state of cytochrome reduction ($E_h = +250$ mV); solid triangles: third state of cytochrome reduction ($E_h = -20$ mV).

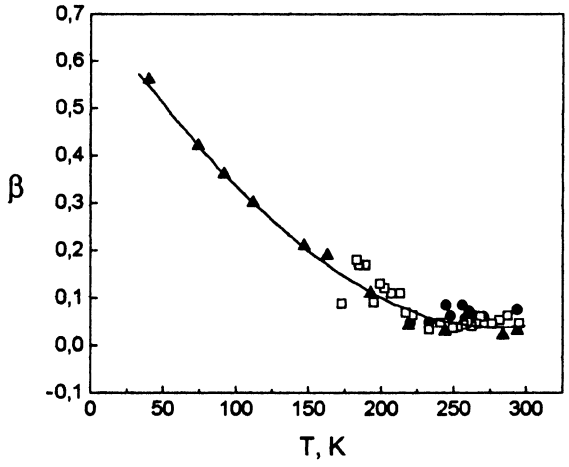

Fig. 4. Temperature dependence of the parameter β for the three different states of cytochrome reduction. Designation as in Fig. 3.

6. Conclusion

In this work, we used a new approach for the description of electron transfer reactions in proteins, taking into consideration the influence of slow molecular dynamics on the dielectric relaxation of the protein matrix during charge separation. A mixed adiabatic/nonadiabatic model is developed for a description of the kinetics of the c-559$^-$ → P^+ ET reaction in the cytochrome–RC complex at different temperatures and states of cytochrome reduction, taking into account a possibility of protein relaxation along vibrational and diffusive coordinates according to the Sumi–Marcus approximation and the Davidson–Cole distribution of diffusive relaxation times (the Rips–Jortner approximation). The parameters of this model, having a simple physical meaning, are determined from a fitting procedure. This model allows to propose new mechanisms of ET rates regulation or gating, connected with changing the parameters τ_0, β, λ_d and ΔG_d under conditions where the protein mobility is restricted. Such conditions are realised in large protein complexes with metal sites deeply embedded into the protein globule. The λ_d parameter is one of the most important of these parameters: it reflects the contribution of the diffusive protein reorganization to the full reorganization energy and can be changed in a discrete manner due to local protonation or selective reduction of some groups in the vicinity of metal sites. This opens the possibility for large

variations of ET rates under conditions of lowered protein mobility. Further investigations are required for a quantitative application of this model to other experiments on long-range electron transfer in proteins.

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