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Superexchange Coupling and Electron Transfer in Globular Proteins via Polaron Excitations

G.N. CHUEV, V.D. LAKHNO and M.N. USTITNIN
Institute of Mathematical Problems of Biology, Russian Academy of Sciences, Pushchino, Moscow Region, 142292

Abstract. The polaron approach is used to treat long-range electron transfers between globular proteins. A rate expression for the polaron transfer model is given along with a description of appropriate conditions for its use. Assuming that electrons transfer via a superexchange coupling due to a polaron excitation, we have estimated the distance dependence of the rate constant for the self-exchange reactions between globular proteins in solutions. The distance dependence of the polaron coupling and solvent reorganization energy are provided as a basis for understanding and interpreting a long-range electron transfer experiment. The difficulties and problems of the polaron treatment of long-range electron transfers are discussed, and suggestions for new experiments are made.

Key words: Cytochrome, electron transfer, excited transition state, globular protein, polaron, superexchange coupling

1. Introduction

Electron transfer (ET) is an elementary and fundamental act of biological processes such as respiration and photosynthesis. In most cases it determines their rate and direction. In essence, proteins are molecular devices created by nature to optimize ET in biosystems. Many efforts including experimental measurements [1–2], computer simulations [3–7] and theoretical treatments [8–11] have been performed to understand ET. The main problem is that ET occurs between oxidation-reduction centers typically spaced at 20–25 Å and embedded in protein medium. Such large distance is unlike that in chemical reactions, where an electron usually transfers via a direct contact of reagents. Besides, the aperiodic and complicated nature of the potential barrier between the donor and acceptor in protein medium does not allow to treat ET by the conventional condensed matter theory.

Most of the recent studies of ET [1, 4–6] propose the ET to occur along certain specific pathways via superexchange coupling when an electron jumps from the donor to an adjacent bound or an atom (bridge) in a more favorable position, then to the next bridge and so on until it comes to the acceptor. In this case, the ET rate is determined by positions of bridges and an electron state on an individual bridge. Various semiempirical or ab initio quantum-chemical methods were used to calculate the electronic structure of intervening medium [4–6], and though some

progress has been achieved, a number of questions concerning the functioning of the protein environment still remain to be clarified. For example, small changes in the bridge positions or account of weak interaction between distant protein sites can substantially change the calculated ET pathway and rate, since the intermediate electron state is a linear combination of a large number of individual electron states at each protein site.

In our opinion, a coupling between an electron state and the protein environment plays a key role in the protein functioning and ET in globular proteins. From this point of view, the transition electron state by which the electron transfers can be considered as a collective electron excitation and can be calculated by the condensed matter methods [12–14]. The formation of this excitation is a nonlinear effect similar to arising of solitons in polymer chains. In principle, polarization and vibrational modes take part in the formation of such an excitation, shifting their equilibrium positions upon the change of the electron state. Here, we will consider only the influence of polarization modes which should be dominant given a large number of polar peptide groups. A similar effect of polarization is well known in condensed matter physics and referred to as 'polaron' effect [15]. The polaron formation was studied for ET in photosynthesis [16, 17].

The aim of our work is to discuss difficulties and problems of the polaron approach [12–14, 18] for describing long-range electron transfer between globular proteins, with regard to the complicated and irregular nature of potential barriers in globular proteins. We apply the method to calculate the distance dependence of the ET rate constant and compare it with experimental data.

2. The ET self-exchange reactions

A sufficient number of polar groups is required for a polaron to form. It takes place for self-exchange reactions between globular proteins in solutions. In this case, the presence of a polar solution is also favorable for the arising polaron. Formally, the self-exchange reaction rate k is written in the nonadiabatic limit as [8]

$$k = k_{\mathrm{A}} S T_{\mathrm{DA}}^2 \sqrt{\frac{\pi}{\lambda k_{\mathrm{B}} T \hbar^2}} \exp\left[-\frac{\lambda}{4 k_{\mathrm{B}} T}\right], \tag{1}$$

where k_{A} is the equilibrium constant for the formation of precursor state from the two globules, S is the steric factor, T_{DA} is the electronic transition matrix element between the donor (D) and the acceptor (A), λ is the standard reorganization energy, T is temperature, while $\hbar$ is Planck's constant divided by 2π and and k_{B} is Boltzmann's constant.

To estimate reorganization energy we should calculate the total change in the equilibrium positions of all peptide groups. Electrostatic forces as well as charge screening yield the main contribution to the reorganization energy. For the simplest estimate we can use different approximations of dielectric constants which are a direct measure of protein polarizability and determine the reorganization energy.

The method of these approximations and data extracted from molecular dynamic simulations are presented in [20] for several proteins. Previously, we have estimated the reorganization energy for the polaron formation, and found it to be about 1–1.2 eV [12–14] for self-exchange reactions between cytochromes. Recent estimations using continuum dielectric approach as well as free energy perturbation calculations and linear response approximation [19] yield the similar value about 20–23 kcal mol^{-1} for the reorganization energy of cytochrome c in solution. Here, we will estimate the electronic transition element and calculate the distance dependence of the ET rate constant.

The electronic transition element T_{DA} is given by the sum of the direct interaction between the donor and the acceptor and the superexchange coupling which contains information about electronic excitations of the intervening medium. Simple estimates show that the last term is dominant for the long-range ET and T_{DA} is written as

$$T_{\mathrm{DA}} = V_{\mathrm{DE}} V_{\mathrm{EA}}^{-1}, \tag{2}$$

where E_{A}, E_{D}, and E_{exc} are the electronic energies for acceptor, donor and excitation, $G(E_{exc})$ is the Green's function of the medium, V_{DE}, V_{AE} are matrix elements between donor, acceptor and excited states, they are proportional the overlap integrals between the corresponding wave functions, i.e.

$$V_{\mathrm{DE}}(R) \propto \int \varphi_{\mathrm{D}}(\mathbf{r})\varphi_{exc}(\mathbf{r}-\mathbf{R})d\mathbf{r}, \;\; V_{\mathrm{DA}}(R) \propto \int \varphi_{\mathrm{A}}(\mathbf{r})\varphi_{exc}(\mathbf{r}-\mathbf{R})d\mathbf{r}\,. \tag{3}$$

Here R is the transfer distance. In the case of self-exchange reactions, the initial and final electron states on the donor and acceptor are the same.

We suggest the following ET scheme (see Figure 1). The electron weakly bounded to the donor transfers to the ETS separated by the distance R from the donor, and then to the acceptor. Thus, the problem is reduced to the calculation of the electronic coupling between the ground and excited electron states. In our opinion, the ground electron state determines energetic parameters controlling the ET reaction, while the excited state strongly affects the distance dependence of the electron transfer. Here, we discuss only the latter effect. To find the distance dependence, we should calculate electron density distributions in the ground and excited electron states.

3. Calculation of the ground and excited electron states

The protein globule has a unique physical structure (see Figure 2). Its characteristic size is about 30 Å, while the active center with hemeporphyrin surrounded by non-polar peptide groups is of the order of 4–6 Å and surrounded by non-polar peptide groups. The peripheral part of the globule contains strongly polar peptide groups and environmental molecules (most often water). Such a heterogeneous structure of a protein globule leads to that the ground and excited electron states have different

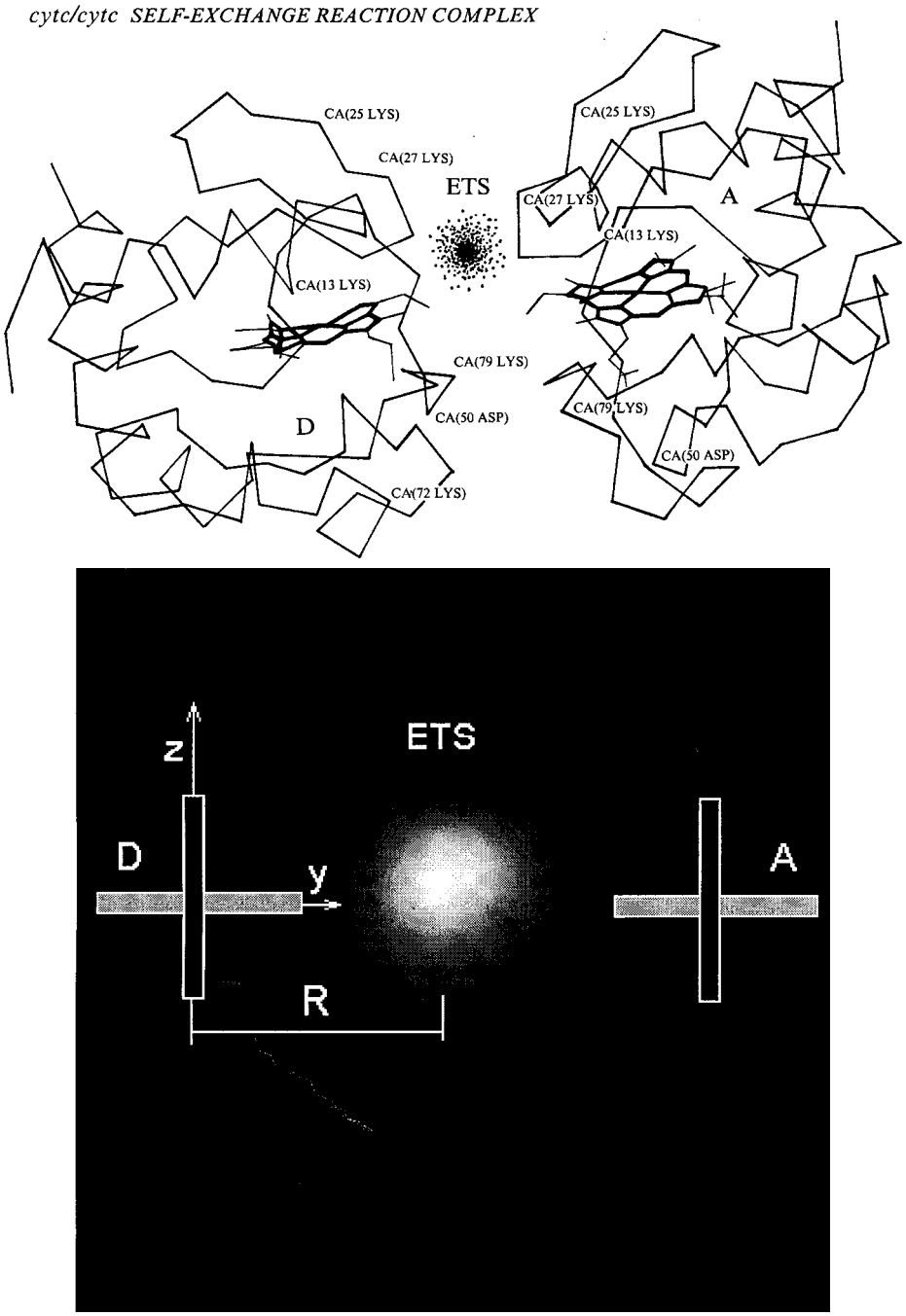

Figure 1. The scheme of self-exchange ET reaction between cytochromes (a) and mutual positions (b) of hemes and excited transition state (ETS), donor (D), acceptor (A). The hemes can be oriented along the axis y or z.

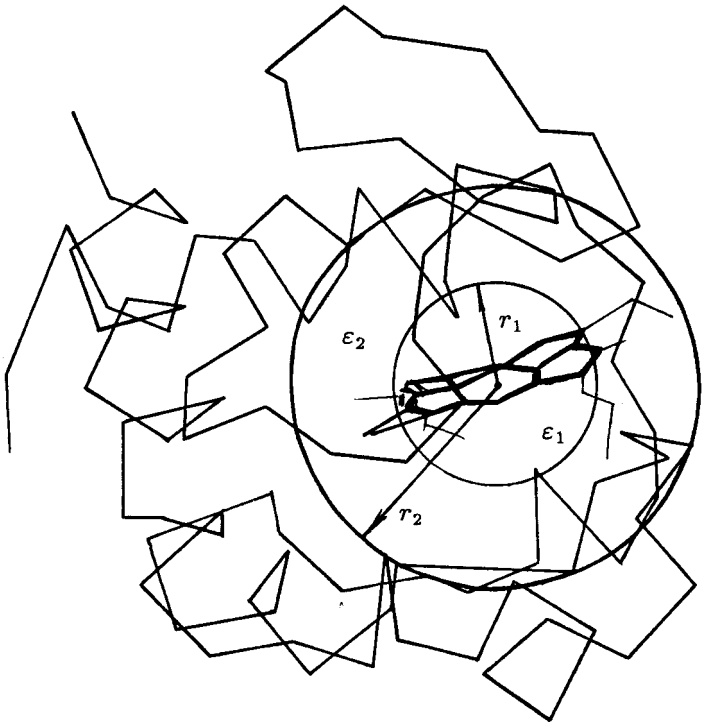

Figure 2. The model of dielectric properties of protein globule.

nature. Our earlier studies show that the polaron effect on the ground electron state is negligible. The ground state is mainly determined by the rigid structure of the hemeporphyrin molecule, and the influence of polarization of the protein environment is small. The excited electron state is more extended, and a large number of

Table I. Energy W (eV) and mean radius $\langle r \rangle$ (Å) of polaron states for the simplest model of protein globule

Parameters of the model		Ground state		First excited state	
ϵ	V_0 (eV)	W	$\langle r \rangle$	W	$\langle r \rangle$
3	–6.18	–4.76	2.69	–1.71	5.77
5	–6.18	–4.76	2.69	–1.67	5.89
5	–7.00	–5.56	2.59	–1.94	4.84
5	–8.00	–6.41	2.49	–2.42	3.79
10	–6.18	–4.76	2.69	–1.66	5.92
20	–6.18	–4.76	2.69	–1.63	6.04

peripheral polar groups take part in the formation of the excited state, therefore the influence of protein polarization is strong. The total potential includes the short range term V_0, the coulomb part V_C caused by the charge distribution on the donor, and the self-consistent polarization potential induced by the electron density distribution. At the first step, we will ignore the detailed molecular structure of the protein globule and use the parameters averaged over protein globule, considering the active center as a well whose averaged depth is V_0, while the peripheral part is characterized by dielectric constant ϵ. We write the self-consistent polarization potential $P(\mathbf{r}, \varphi(\mathbf{r}))$ similar to that for polaron

$$P(\mathbf{r}, \varphi(\mathbf{r})) = -e^2 \int \left[\frac{1}{\epsilon_{op}} - \frac{1}{\epsilon(|r'|)} \right] \frac{\varphi^2(\mathbf{r}')}{|\mathbf{r} - \mathbf{r}'|} \, d\mathbf{r}' \,, \tag{4}$$

where ϵ_{op} and ϵ are the high-frequency and static dielectric constants. Various models of dielectric properties of the protein globule were used in such calculations [12–14]. We divided the globule into sublayers with different dielectric constants

$$\epsilon(r) = \epsilon_1, \qquad r < r_1, \qquad \epsilon(r) = \epsilon_2, \qquad r_1 < r < r_2 \cdots$$

or used nonlocal approximation of the distance dependence of the dielectric constant (see for example [21]). In this case we should use nonlocal value $\epsilon(|r' - r|)$ in (4). We calculated numerically the nonlinear Schrödinger equation, which includes V_0 as well as the self-consistent polarization potential for the chosen parameters of the number and size of the sublayers in the globule and their static dielectric constants. Details and the method of the calculations are described in [22]. Table 1 presents the calculated electron energy W and the mean radius $\langle r \rangle = \int r^2 \varphi^2(\mathbf{r}) d\mathbf{r}$ for the excited and ground states found for the simplest model with $\epsilon_1 = \epsilon_2$. It is seen that the ground electron state depends strongly on the model parameters and quantum-chemical methods are required to calculate the state carefully. Such calculations will be performed elsewhere [23].

However our model is suitable for the excited state, the mean radius of ETS is about 3–6 Å, which is comparable with the globule radius equal 15 Å. The energy and the mean radius weakly depend on the heme potential V_0 and on the parameters of dielectric layers.

We also investigate the effect of the asymmetric shift of the heme from the globule center. In this case the ground electron state is not spherically but cylindrically symmetrical. Figure 3 shows the dependence of the electron energy W (curve *1*) and the mean radius $\langle r \rangle$ (curve *2*) on the heme shift z with respect to the globule center at $V_0 = 0$ eV. Although the asymmetric shift changes quantitatively the parameters of the electron state, the local electron density distribution changes weakly. Figure 4 shows the electron density distributions at different asymmetric shifts.

Thus, our calculations show that the ETS formed due to the total polarization of the whole globule is very extended and weakly depends on the detailed molecular

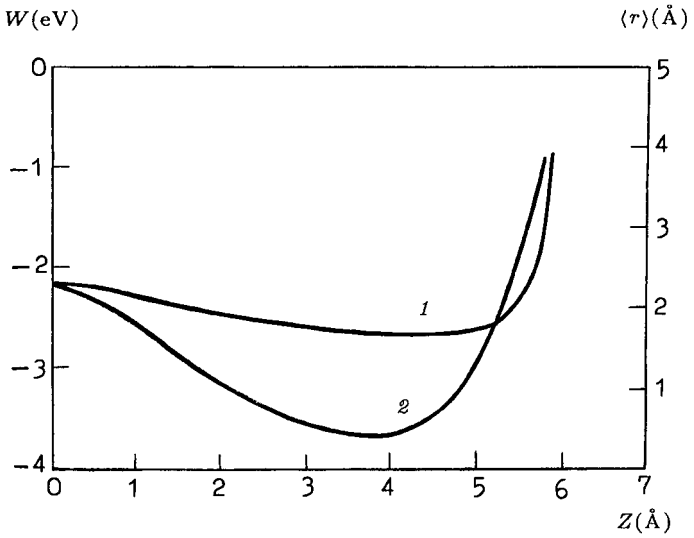

Figure 3. The electron energy W (curve *1*) and the mean radius $\langle r \rangle$ (curve *2*) depending on the heme shift z with respect to the globule center.

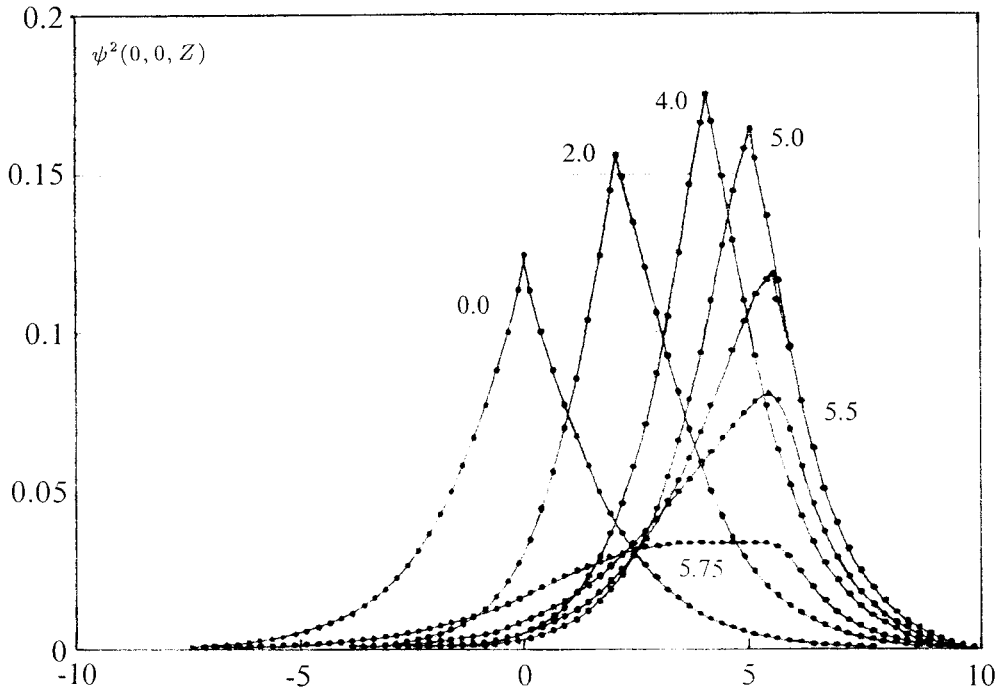

Figure 4. Electron density distribution $\varphi^2(0, 0, z)$ at different asymmetric shift z indicated at each curve.

structure of the protein environment. It is similar to the formation of the solvated electron in polar liquids.

4. The distance and orientation dependence of the ET rate

In principle, the ET can occur via different ETS localized at different distance from the acceptor. To estimate this effect, we investigated the probability of ET via various excited polaron-like states [14] with different symmetry and localized at different distance from the donor. We found that the ET in globular proteins at large distances can efficiently proceed via the lowest polaron excitations. The transfer probability has the maximum when the ETS is localized in the midpoint between the donor and the acceptor. The polaron excitation depends on details of molecular structure of the peripheral part of the protein globule. At the initial step we use a crude approximation and consider the protein environment as a dielectric continuum characterized by the $\epsilon_{op} = 2$ and $\epsilon = 10$. Earlier we numerically calculated the spherically symmetrical polaron state in the similar homogeneous medium [21]. The polaron wave function is approximated as [18]

$$\varphi(r) = C_{norm} \exp[-\alpha r](1.0248 + 0.4922r + 0.1168r^2)\,, \tag{5}$$

where $\alpha = 0.4994$ Å$_1$, $_{norm}$ is the normalized constant, and r is measured in angstroms. The accuracy of the approximation is 0.3% for the calculated wave function and 0.03% for the polaron energy. This type of wave function is used for the polaron model of solvated electron.

The electron density distribution in the ground state is modeled by the uniform distribution in the box of size of $11.35 \times 11.35 \times 5.35$Å^3. We study the distance dependence of the overlap integral $I(R) \propto V_{\mathrm{DE}}(R)$ for various mutual orientations of hemes and the ETS ($I_y(R)$ corresponds to the case when the hemes are parallel and reside on the same plane, while $I_z(R)$ – in different planes).

The problem is reduced to the one-dimensional integration. This integral is calculated numerically. Figure 5 depicts the calculated data. The results are summarized as:

1. $I(R)$ tends asymptotically to the exponential dependence, $I(R) \sim \exp[-\beta R/2]$ with $\beta \simeq 0.8$ Å^{-1}. The decay coefficient β is mainly determined by decay parameter α for the polaron wave function at large distances.
2. At distances less than $d = 2R = 10 \div 20$ Å the above dependence differs from the exponential one, β varies from 0.3 to 0.8 Å^{-1}.
3. There is a weak orientation dependence of the ET matrix element. The ET rate can increase or decrease by factor of 2, depending on mutual orientations of the heme and ETS.

We also investigate the influence of the asymmetry of the ETS localization with respect to the donor and acceptor. We consider the donor and acceptor located

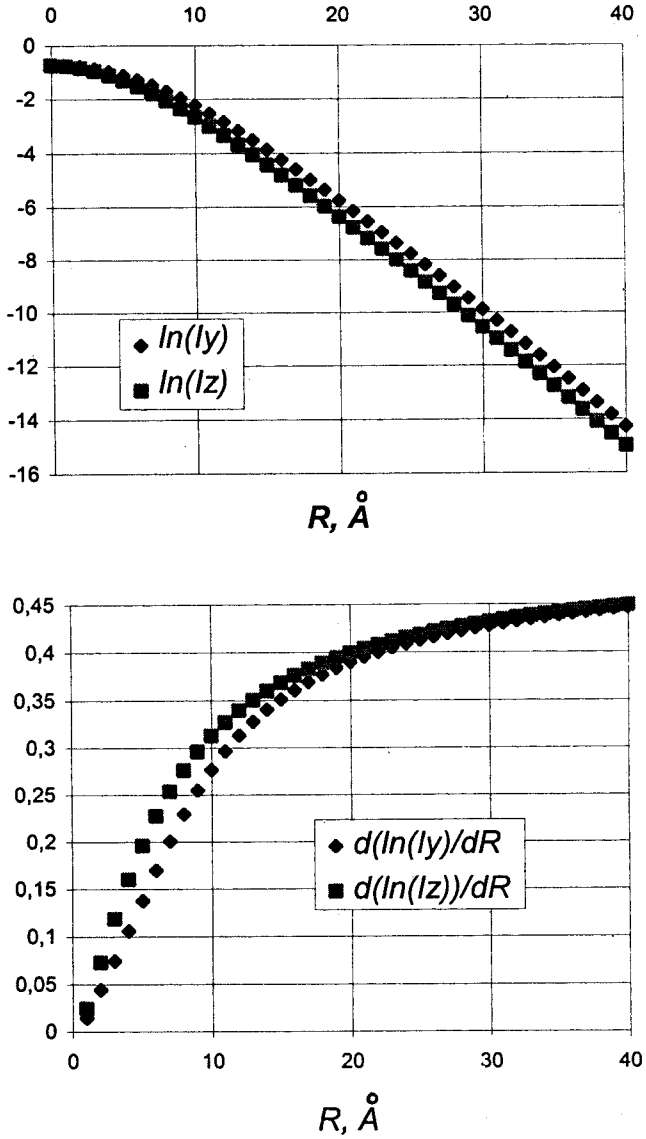

Figure 5. Logarithms (a) and their derivatives (b) of overlap integrals $ln[I_y(R)]$ and $ln[I_z(R)]$ depending on the ET distance R.

at distance 18 Å, which is typical for self-exchange reactions [2, 3]. The ETS is localized at distance R from the donor. Then, the distance dependence of the matrix element is $T_{DA} \sim V_{DE}(R)V_{EA}(18 - R) = P(R)$. Figure 6 depicts this dependence for different heme orientations. According to these data, the asymmetry of ETS localization substantially changes the probability of the transfer, decreasing the ET rate constant.

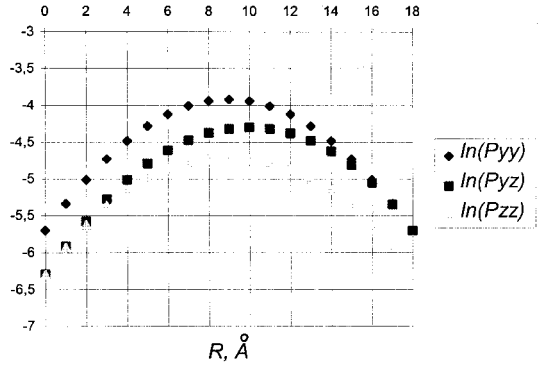

Figure 6. The probability of ET depending on the position of the ETS localization: P_{yy} – the hemes lie in the same plane and parallel, P_{yz} – the hemes are perpendicular P_{yy} the hemes lie in different planes and parallel. The ET distance is equal to 18Å.

5. Discussion

The calculated data are in agreement with the experimental ones [24], where $\beta \sim 0.7 \div 1.4$ Å^{-1}. The indicated scatter of the data can result from the nonexponential behavior of $I(R)$ at distances less than 20 Å as well as from steric and orientation effects. Knowing the reorganization energy, and the electron transition element, we estimate the ET rate for $cytc/cytc$ and $cytb_5/cytb_5$ self-exchange reactions. We extract from the experimental data [2] the factor $k_{max} = ST_{\mathrm{DA}}^2(\pi/\lambda k_{\mathrm{B}} T\hbar^2)^{1/2}$ including the steric factor and electron transition element, the value of k_{max} is equal for $cytb_5$ reaction 6.4×10^9 s^{-1} and 6.1×10^7 s^{-1} for $cytc$ self-exchange reactions, while the heme-heme distances are estimated as 17.5Å and 18.9 Å respectively [2]. We calculated $k_{max} = k_0 I^2(R)$ ($k_0 = 2 \times 10^{11}$s^{-1}) with no separating steric factor ($S = 1$), but for different the ETS localization in favorable and unfavorable positions. Our calculations yield 14×10^7 (favorable position) and 4.5×10^9 s^{-1} (unfavorable position) for the corresponding values of cyt c and $cytb_5$ self-exchange reactions. The calculated rate constants are mainly determined by two factors: reorganization energy and tunneling matrix element. Using polaron approach we found that the reorganization energy is about 1–1.2 eV, while the decay parameter for the tunneling matrix element is about 0.9 Å^{-1}. It yields the experimentally observed rate constants for the self-exchange reactions. The result does not depend on details of the polaron model and is mainly determined by two parameters, the used distance between donor and acceptor and decay parameter α for the polaron wave function in (5), the microscopic nature of the excited state is not essential. The decay parameter is a measure of localization for the excited electron state, if the excited state is extended with $\alpha \sim 0.4 \div 0.6$ Å^{-1}, it yields the experimentally observed values for the rate constants.

In conclusion we consider two questions which are typical in current literature.

First of the them is the exponential distance-dependence of the rate constant. In the general case, the ET rate depends exponentially on distance only at distances exceeding greatly the typical size of changes of tunneling barrier (for our model at $R > 20$ Å). Typical ET distances are less than or equal to this distance. In addition, as we indicated above, the effective ET distance can differ from the minimal donor-acceptor distance, since polar peptide groups required for the ETS to form can be absent in this region. As a result, the distance-dependence of the ET rate can differ from the exponential one.

Another question is that the electron transfers by a specific pathway or not. According to our calculations, there is not a certain ET pathway for self-exchange ET reactions. In these cases the electron transfers via the ETS which are formed due to the polarization of the whole protein globule. It contradicts the usual superexchange theory. At present there are no experimental data in favor of one of the hypotheses, but experiments on ET in proteins modified by point mutations changing the ET pathway can clarify the problem.

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