# LONG-RANGE ELECTRON TRANSFER IN GLOBULAR PROTEINS BY MEANS OF POLARON EXCITATIONS

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A polaron model is used to calculate the electron state localized on the protein haeme. Using the parameters of this state, namely, the electron density distribution and energy, the self-exchange reaction rate for cytochrome *c* (equine heart), the energy of reorganization and the matrix element and the dependence of the rate of this transfer on the distance between haemes are calculated. The results of calculations and experimental data and other model evaluations are compared. A hypothesis regarding the role of polaron excitations in long-range electron transfer in globular proteins is discussed.

Electron transfer in globular proteins is an elementary act of all biochemical reactions: it determines the direction and rate of bioenergetic processes. In essence, proteins are molecular machines created by nature to optimize electron transfer. Because of the lack of experimental evidence on the molecular mechanisms of long-range electron transport, theoretical and computer methods of exploring this problem are of considerable importance.

One of the most intriguing problems of electron transport is long-range transfer [1]. In biological systems the rate of electron transfer varies from picoseconds to milliseconds and essentially depends on the state of the active centre of the protein globule, its environs and also on the temperature. The problem of modelling long-range electron transfer usually reduces to investigating the influence of these factors.

In our view, the interaction of the electron with the protein medium plays a key role in electron transfer. Although the analogy between this interaction and the mechanism of the formation of a polaron was emphasized some time ago [2 — 4], detailed calculations of the polaron state in the protein globule were made only recently [5 — 8].

This paper is concerned with use of the polaron model to explain the possible mechanism of long-range electron transfer in globular proteins.

## STRUCTURE AND PHYSICAL PROPERTIES OF THE PROTEIN GLOBULE

The protein globule has a unique physical structure. Its characteristic size is 30 Å or more in diameter, while the active centre is of the order 4 — 6 Å and is surrounded by non-polar peptide groups. The peripheral part of the globule contains strongly polar peptide groups and molecules of the protein environs (most often water molecules). Such a heterogeneous structure of the globule means that its dielectric properties essentially depend on the distance to the active centre of the globule. In the simplest model of a «dielectric cavity» [9] this effect is taken into account by considering individual regions $R_1 < R_2 < R_3 < \ldots$, with a different permittivity $\varepsilon_1 < \varepsilon_2 < \varepsilon_3$:

$$\varepsilon(r) = \varepsilon_1, \; r < R_1, \; \varepsilon(r) = \varepsilon_2, \; R_1 < r < R_2, \; \varepsilon(r) = \varepsilon_3, \; r < R_3. \tag{1}$$

More complex models [10] allow for the non-local character of the dependence for the Fourier component of the permittivity

$$\varepsilon(k) = \varepsilon_1 + (\varepsilon_2 - \varepsilon_3)/(1 + \varepsilon_2/\varepsilon_1 l^2 k^2), \tag{2}$$

where $l$ is the correlation length. Experiments and computer modelling of the dielectric properties of the protein globule show [11—13] that the dependence of the permittivity on the distance to the active

centre of the globule is approximated quite well by relation (2).

Proteins are multimodal systems with a complex hierarchy of characteristic relaxation times and oscillation frequencies. However, various experiments [14] suggest that the interaction of an electron with vibrations with an effective frequency $\Omega \sim 10^{12}$— $10^{13}$ s $^{-1}$ plays the main role in electron transfer. The role of slower conformational rearrangements was investigated in [16]. Note also that the dielectric characteristics of the peripheral part of the globule are close to the characteristics of strongly polar media such as ionic crystals or their melts. The interaction constant $\alpha = 1/2 C_g e^2 / \hbar\Omega (2m\omega/\hbar)^{1/2}$ (where $m$ is the mass of the electron, $C_g = (1/\varepsilon_0 - 1/\varepsilon_\infty)$ and $\varepsilon_0$, $\varepsilon_\infty$ are the low- and high-frequency permittivities) will be of the order of $\alpha \geq 100 >> 1$. In other words, the electron—globule interaction is strong and according to the polaron theory [15] such interaction enables the polaron state to be formed with a characteristic radius $\langle r \rangle \sim \pi^2 \hbar^2 / C_g m e^2 \sim 5\text{Å}$ and energy $W \sim -2 / \pi^2 e^4 m C_g^2 / \hbar^2 \sim$ -2eV.

## MODELLING OF THE POLARON IN THE PROTEIN GLOBULE

The electron bound at the active centre plays the main role in the process of electron transfer. Calculations of the ground and excited states of such an electron in the protein globule have been made based on the polaron model [5-8]. In general, the effective Hamiltonian for such an electron may be written as follows:

$$H_{pol} = -\frac{\hbar^2}{2m}\Delta + V(r) + P(r). \tag{3}$$

Here, the first term is the kinetic energy of the electron, the second is the potential of the active centre of the globule and the last term represents the interaction of the electron with the protein medium.

The potential $V(r)$ includes both the short-range interactions, determined by the microstructure of the active centre and the Coulomb interaction between the electron and the charge of the active centre. Again, in general, it is necessary to make quantum-chemical calculations of this potential. However, in considering electron transfer, we will be primarily interested in the energy of the electron and the asymptotic form of the decrease in its wave function, which will be first and foremost determined by the long-range forces. Therefore, in modelling the polaron state, like [17], we approximated the short-range part of the potential $V(r)$ by a potential well, the depth of which is determined from experiment (for example, from the redox potential or from the absorption spectrum). As a result the potential $V(r)$ is represented in the form [8]:

$$V(r) = V_0, r < R, \ V(r) = \frac{Ze^2}{\varepsilon(r)|r|}, r > R, \tag{4}$$

where the function $\varepsilon(r)$ was approximated in different ways. For models of bilayer and trilayer cavities we used relation (1) with two and three different constants. Accordingly, for the model of non-local permittivity we used relation (2) for the Fourier component.

The interaction potential of the electron with the protein medium $P(r)$ also includes the short-range and long-range interactions, although taking into account the above ideas on the predominant influence of polarization interactions on the asymptotic form of the wave function and the energy of the electron, we approximated this potential in the following form:

$$P(r) = -e^2 \int_{r>R} [1/\varepsilon_\infty - 1/(|r'|)] \frac{\varphi^2(r')}{|r - r'|} dr' \tag{5}$$

where $\varphi(r)$ is the wave function of the electron and $R$ is the size of the active centre. Expression (5) follows from the polaron theory of a strong bond [18] and its meaning resides in the fact that the electron considered so induces shifts of the atoms of the medium that the resulting density of the

induced charge is proportional to $[1/\varepsilon - 1/\varepsilon(|r'|)]\varphi^2(r')$. To calculate the state of the polaron in the protein globule, using the COLCON software package we numerically solved Schrödinger's non-linear equation with the potential determined from (3). To approximate $V_0$, we used two types of model: continual models [5-7], where it is assumed that $V_0 = 0$, and semicontinual models [8], where this parameter is determined from the redox potential.

According to our calculations, for continual models over an acceptable range of permittivities for the globule $\varepsilon \approx 4\text{-}20$, the energy of the polaron state varies in the range -1.5 to -2.5 eV and its radius varies from 2.5 Å to 4 Å, the heterogeneity of the dielectric properties of the globule having only a slight effect on the characteristics of the polaron state. Although, as shown below, the continual polaron models give a correct assessment of the rate of electron transfer, they do not match the experimental data on the absorption spectra and also the data on the redox potential, according to which, it should be $W \leq$ -5 eV.

To remove this drawback, in [8] a semicontinual polaron model was developed which takes into account the contribution both of the polar and non-polar interactions to $V(r)$ through self-consistent calculations. As a result, for cytochrome $c$ the value $V_0 = $ - 6.18 eV was obtained, which corresponds to $W = $ - 4.76 eV and a value of the redox potential of $E_{1/2} = 0.26$ eV. The results of calculations of the energy and the mean radius are given in Table I for the ground and first excited spherically symmetrical states of the polaron for different parameters of the model. According to these calculations, the ground state of the polaron is very localized $\langle r \rangle \approx 2.7$ Å, which is less than the radius of the active centre (in our calculations it was 3.2 A and depended only slightly on the surroundings of the centre). On the other hand, the excited state is very diffuse, $\langle r \rangle \approx$ 5-6 Å and its energy and radius depend very much on the dielectric properties of the globule.

## Table 1. Energy $W$ (eV) and mean radius $\langle r \rangle$ (Å) of polaron states in the protein globule.

| Parameters of the model | | Ground state | | First excited state | |
|---|---|---|---|---|---|
| $\varepsilon$ | $V_0$, эB | | | | |
| 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 |

## MODELLING OF ELECTRON TRANSFER OVER LONG DISTANCES

To model the process of electron transfer over long distances, let us consider the self-exchange reaction for cytochrome $c$ (equine heart) in aqueous solution. This reaction has been quite well studied experimentally. At room temperature and an ionic strength $\mu = 0.1$, the reaction rate is determined [19] as follows:

$$K = SK_a k_{max}\exp(-E_r/4T) = K_a H_{et}^2(\pi/E_r T)^{1/2}\exp(-E_r/4T), \qquad (6)$$

where $S$ is the steric factor, $K_a$ is the equilibrium constant, $H_{et}^2$ is the matrix element of interaction, $E_r$ is the energy of reorganization of the medium, and $T$ is the temperature. Taking into account the fact that for the self-exchange reaction the states on the donor and acceptor are identical, we obtain the following relations for the energy of reorganization and the matrix element as a function of the distance of electron transfer $d$

$$H_{et}(d) \approx WL(d) = W\int\varphi(\mathbf{r})\varphi(\mathbf{r} - d)d\mathbf{r} \quad H_{et}(d) \approx WL(d) = W\int\varphi(\mathbf{r})\varphi(\mathbf{r} - d)d\mathbf{r}, \qquad (7)$$

$$E_r = e^2 \int (1/\varepsilon_0 - 1/\varepsilon(\mathbf{r})) \frac{\varphi^2(\mathbf{r})\varphi^2(\mathbf{r}') - \varphi^2(\mathbf{r})\varphi^2(\mathbf{r}' - d)}{|\mathbf{r} - \mathbf{r}'|} d\mathbf{r}d\mathbf{r}'. \qquad (8)$$

The results of our calculations and also a comparison of these with different experimental and model calculations are given in Table 2, where we present the parameters of the self-exchange reaction in the following units: maximum transfer rate $k_{max}$ — in $10^9$ s$^{-1}$, energy of reorganization $E_r$ — in eV, the reaction rate $K$ — in $10^3$ s$^{-1} \times$M$^{-1}$, transfer distance — in Å and also the parameter associated with the equilibrium constant, $SK_a$ — in $10^3$ s$^{-1} \times$M$^{-1}$. In [19] $SK_a$ is estimated as 0.001 and in [22, 23] as 0.0001. In our calculations we assumed $SKg = 0.01$ — 1. The self- exchange reaction rate $K$ which we calculated is close to the experimental estimates [20, 22] and theoretical estimates [19, 23]. This is obviously not a sufficient argument in favour of our model, since the order of magnitude of this transfer rate is essentially determined by the parameter $SK_a$ and estimates of this have a spread of several orders of magnitude. Another parameter of the transfer rate — the energy of reorganization $E_r$ — has much the same value for all the calculations considered, a consequence of the assumption of the predominant influence of the electrostatic interactions.

Table 2 also presents the parameter $\beta$(Å$^{-1}$), which determines the exponential dependence of the matrix element on the transfer distance

$$H_{et}^2(d) = C_0 \exp(-\beta d). \qquad (9)$$

### Table 2. Experimental and model parameters of the cyt $c \to$ cyt $c$   transfer rate.

| Source | $SK_a$ | $k_{max}$ | $E_r$ | $K$ | $\beta$ | d |
|---|---|---|---|---|---|---|
| [20] | | | 1,2 | 1-10 | | |
| [21] | | | 0,26 | | | |
| [19] | 0,01 | 5 | 1,04 | 2 | 1,2 | 6,4 |
| [22] | 0,001 | 49 | 0,72 | 5,1 | 0,9 | 5,9 |
| [23] | 0,0001 | | 0,45÷0,81 | 4,3 ± 1 | 0,8÷1,1 | 7,7 |
| [6] ε =4,0 | 1 | 0,7 | 1,24 | 3 | 0,94 | 17,7 |
| [6] ε =5,1 | 1 | 3,2 | 1,32 | 6 | 0,84 | 17,7 |
| [8] | 0,1 | 0,3 | 0,74 | 1,4 | 0,94 | 17,7 |

In our calculations the function $H_{et}(d)$ differs somewhat from exponential because of the weak power dependence of the pre-exponential factor, and the estimate of the parameter $\beta$ will depend on the choice of $C_0$. Note that the transfer distance $d$ is defined differently in different models: either as the minimum distance between the edges of the haernes or (in our case) as the distance between the centres of the haernes, since the wave functions are centred on the haeme. In [19, 22, 23] to evaluate the parameter $\beta$, extrapolation of the estimates of this parameter for intraprotein transfer was used. However, according to the latest estimates [24], this parameter for modified cytochromes $c$ amounts to $\beta = 0.73$. Estimates of this parameter for photosynthetic reactions [14] give $\beta \approx 0.9$, but for the reactions of interglobular transfer $\beta = 0.72$ [25]. In our research this parameter was calculated directly and was close to the values indicated. Relation (9) and the value of the parameter $\beta$ may obviously serve as arguments for or against the model used. We discuss this problem below.

## DISCUSSION OF THE RESULTS

Our calculations of the polaron state in the protein globule show that, to agree with the experimental data on the absorption spectra and redox potential for the ground state of the electron, it is

necessary to take into account the detailed molecular structure of the active centre. Here the polaron effect for the ground state is low; this state is practically completely determined by the chemical structure of the active centre. However, calculations of the self-exchange reaction of an electron show that direct electron transfer between the ground states $S_a \rightarrow S_d$ (where $S_a$ and $S_d$ signify the ground state of the electron on the acceptor and donor, respectively, gives an estimate for transfer that is too low by a factor of more than $10^6$).

On the other hand, calculation of the polaron excited states shows that they depend slightly on the molecular structure of the active centre. The characteristic size of these states is comparable with that of the globule itself. In essence, such a state is collective electron excitation of the protein medium, formed as a result of the self-consistent and cooperative interaction of the electron with the whole protein medium. Calculations of the self-exchange rate through these states give good agreement with experiment.

In our view the following scheme nay be realized as one of the electron transfer channels: $S_a \rightarrow P_a \rightarrow P_d \rightarrow S_d$, where $P_a$ and $P_d$ are the excited polaron states on the acceptor and donor. In this case the polaron contribution $P_a \rightarrow P_d$ will determine the dependence of the transfer rate on the transfer distance, and the reactions $S_a \rightarrow P_a$ and $P_d \rightarrow S_d$ will determine the influence of the structure of acceptor and donor primarily on the temperature dependence and energy characteristics of the transition. In these terms our model of transfer may be regarded as an extension of the superexchange theory. According to this theory, the interaction of donor and acceptor with the ambient medium results in the formation of a perturbed mixed state (see Fig. 1). Usually, such a state is calculated using electron wave functions (orbitals) for individual atoms [26] or individual chemical bonds [27] as a basis. On the other hand, the polaron state is a smoothed characteristic of the distribution of these orbitals (see Fig. 1) and in essence the problem of the nature of the excited state adds up to the problem of a reasonable choice of the basis of the electron wave functions. When the formation of the collective state involves a small number of isolated types of orbitals of rigidly fixed atoms (as, for example, for bridge compounds), it is obviously best to use a basis resting on chemical bonds. When perturbation includes a large number of orbitals of different types, the most adequate measure is to describe them in terms of collective electron excitations. In proteins, according to [24, 26, 27], 10 — 20 peptide groups take part in transfer.
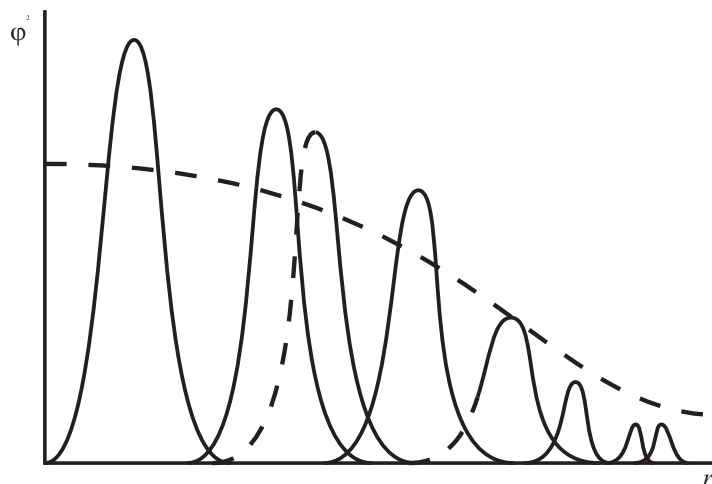


Fig. I. Schematic distribution of the electron density for the excited state:
the broken line is the polaron model and the continuous line is the superexchange model.

In examining such collective excitation, we included in the calculation only the dielectric (long-wave) properties of the protein globule and ignored the anisotropy of these properties for different directions. Our model of the polaron may also be extended to the case of an anisotropic medium and the real spectrum of vibrations of the protein globule. In considering the excited polaron spherically symmetrical state, we obtained the exponential dependence on the transfer distance (9). Although these generalizations of the model may somewhat change this dependence, they do not affect the asymptotic

nature of the exponential behaviour. The asymptotic behaviour of relation (9) is now being actively discussed. Although the experiment and computer calculations do not give a clear-cut answer to this question, we may invoke the reasoning in [28], which argues in favour of our model. In the case of transfer through excited collective states of the polaron type, the whole protein globule takes part in the formation of the state and, therefore, such transfer is more «biologically stable», i.e. the transfer rate will depend only slightly on the change in the individual regions of the globule and on different point mutations.

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