Simulation of the Effect of Reorientation of Hydrogen Bonds in Proteins on Long-Range Electron Transfer

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Abstract. We present Monte Carlo simulations illustrating the influence of reorientation of hydrogen bonds in proteins on long-range interprotein electron transfer. The lattice protein model employed mimics the electron donor (or acceptor) interacting with an antiparallel β sheet. In addition, we take into account harmonic vibrations of the medium and also the dependence of the coupling matrix element on orientation of hydrogen bonds near the donor and/or acceptor. The results obtained show that the interaction between the tunneling electron and amino-acid residues, which are responsible for the formation of hydrogen bonds, may result in broadening the parabolic dependence of the electron-transfer rate constant on the reaction exothermicity and also in deviations from this dependence especially in the cases when the β sheet is linked with the electron donor.

Key words: electron tunneling, β sheets, lattice protein models, Monte Carlo simulations

1. Introduction

Life on our planet is intimately connected with long-range interprotein electron transfer (ET). This process is fundamental to respiration, photosynthesis, and redox reactions in intermediary metabolism [1]. For this reason, the interprotein ET has long attracted attention. The understanding of this phenomenon is however still limited due to complexity of the protein structure and multitude of factors which may be relevant for its description.

Physically, the long-range ET occurs via electron tunneling from a donor site to an acceptor site. The ET rate is determined by the coupling between the initial and final electronic states, V , and by the nuclear fluctuations necessary to bring these states into resonance. The role of these factors was first scrutinized by Marcus about 40 years ago. His famous general expression for the ET rate constant is

$$k = \frac{\pi^{1/2} V^2}{\hbar (E_r k_B T)^{1/2}} \exp \left[-\frac{(E_r - J)^2}{4 E_r k_B T} \right], \quad (1)$$

where J is the reaction exothermicity, and E_r the reorganization energy of the medium. Derivation of Eq. (1) implies applicability of the linear-response theory (practically this usually means interaction of an electron with classical harmonic nuclear vibrations as in the case of the polaron theory) and the Landau-Zener model for describing nonadiabatic electron jumps. If necessary, it can be complemented by the Franck-Condon factors for quantum nuclear vibrations, dependence of V on the protein structure, protein dynamics, and/or inelastic processes during electron tunneling (see a comprehensive review by Stuchebrukhov [2]).

The main goal of the molecular dynamics (MD) simulations of interprotein ET was to explicitly calculate the free energy needed in order to bring the initial and final states into resonance. In principle, the MD method makes it possible to comprehensively analyze reconfigurations of the protein structure, and accordingly there is no doubt that this method is often superior. At present, this technique does not allow however simulations of proteins on times longer than about 100 ns. Meanwhile, the time scale of protein reconfiguration is often much longer. For this reason, applications of MD to calculations of the ET rate is far from straightforward. Under this state of art, it makes sense to construct generic coarse-grained models of interprotein ET in order to illustrate the physics behind this phenomena.

Following the line above, it is of interest to combine the lattice approximation for describing the protein structure, main ingredients of the Marcus theory related to the medium reorganization of nuclear vibrations, and conventional approximations used to treat electron underbarrier propagation. The adaptation of the lattice approximation is especially instructive, because during the past decade, the lattice models have been very widely used to study various aspects of protein folding [3] and aggregation [4], ligand binding to proteins [5], protein adsorption [6], and enzymatic reactions [7]. Recently, we have used [8] a lattice model in order to analyze non-Markovian effects in fast long-range interprotein ET due to reorientation of hydrogen bonds in proteins. In the present paper, we employ a similar model to systematically illustrate the manifestation of reorientation of hydrogen bonds in the dependence of the interprotein ET rate on the reaction exothermicity.

2. Model

In the lattice approximation, a protein is schematically viewed as a linear sequence of N amino acids. The structure of the amino acids is usually not analyzed explicitly. Instead, the protein is simulated by a chain of monomers, which are constrained (along the chain) to be nearest neighbors on the 3D cubic lattice (each lattice site can be occupied at most once). In the bulk of available lattice simulations, proteins are treated as heteropolymers [3], i.e., the chain representing a protein is considered to be perfectly flexible and the monomer-monomer interactions are assumed to be isotropic. The direct consequence of these approximations is that the predicted native protein structures are usually highly compact (e.g., close to cubic). In principle, one can relate long-range ET with reconfiguration of such structures.

In practice, however, this strategy is not constructive, because the heteropolymer model does not describe explicitly local features of real proteins (for this reason, the heteropolymer model is not universal; despite all its merits [3], it can hardly be used to simulate, e.g., such generic protein structures as β sheets). Meanwhile, the ET process is sensitive to such features.

In real proteins, α helices and/or β sheets are often the main motifs [1]. Such low dimensional fragments of proteins are stabilized primarily by hydrogen bonds, i.e., by strongly anisotropic bonds between oxygen and hydrogen atoms belonging to amino-acid residues that are nearest-neighbor (nn) in space but not nn in the polypeptide chain. Hydrogen bonds are formed only if the orientation of amino-acid residues is favorable for their formation (other interactions between amino-acid residues can also depend on their orientation but not so strongly). To scrutinize the role of hydrogen bonds in stabilization of proteins, we designed [9] a relatively simple lattice model, making it possible to simulate the folding kinetics of β sheets. In the present study, we adopt that model for description of long-range interprotein ET.

The full version of our model takes into account isotropic monomer–monomer interactions, corresponding to the heteropolymer model, anisotropic interactions mimicking the formation of hydrogen bonds, and chain rigidity. Conformational changes include conventional end, corner, and crankshaft monomer moves and also monomer reorientation governed by anisotropic interactions. Our present simulations of long-range interprotein ET are focused on reorientation of hydrogen bonds. To articulate the role of these bonds, we consider that the ET process is not accompanied by changes in the global protein structure (the position of the electron donor and/or acceptor is considered to be fixed as well). Practically, this means that we prohibit end, corner, and crankshaft monomer moves and allow only monomer reorientations. In this case, there is no need to explicitly include isotropic interactions and chain rigidity. On the other hand, we should complement the model by the terms describing the change of the energy of hydrogen bonds due to the presence or absence of an electron on the donor and acceptor sites.

Specifically, we assume that the electron donor or acceptor site is located slightly above the center of a three-turn antiparallel β sheet (Figure 1). To describe the hydrogen bonds stabilizing this structure, we introduce the unit vector $\mathbf{s}_i$ characterizing the orientation of monomer i . Physically, this vector shows the orientation of an amino-acid residue (specifically, of the $-\text{C}-\text{O}$ and $-\text{N}-\text{H}$ groups) with respect to the chain. For this reason, the orientations of $\mathbf{s}_i$ along the peptide bonds formed by monomer i with monomers $i - 1$ and $i + 1$ are prohibited (thus, $\mathbf{s}_i$ is oriented in one of the four directions). An electron located on the donor (or acceptor) site is considered to interact with four nn monomers (such situations when the electron donor and/or acceptor is in contact with a few amino-acid residues is typical in real systems). In particular, the electron energy becomes lower if the monomers are oriented toward the donor (or acceptor) site. This orientation is considered to be perpendicular to the β sheet, because the donor (or acceptor) site is located above

Figure 1. Two conformations of a model three-turn antiparallel β sheet. Monomers mimicking amino-acid residues are represented by circles. The vectors $\mathbf{s}_i$ describing the orientations of monomers are (i) indicated by bars if they are in the plane of the sheet, (ii) shown by open circles if they are directed perpendicular to the sheet and toward the donor (or acceptor) site (this site, marked by the star sign, is considered to be located above the sheet), and (iii) not shown if they are directed outwards. For the end monomers and monomers forming corners, the orientation vectors are not introduced (the role of these monomers in the formation of hydrogen bonds is assumed to be minor). Panel (a) exhibits a typical conformation in the case when an electron located on the donor (or acceptor) site does not influence the hydrogen bonds ($\epsilon_{es} = 0$) and accordingly the orientations of monomers correspond to a nearly perfect β sheet. Panel (b) shows a typical conformation in the situation when the interaction between the electron and nn monomers is relatively strong, $\epsilon_{es} = 0.15$ eV. In the latter case, all the four nn monomers are oriented towards the electron.

the sheet, and accordingly the monomers contacting the donor (or acceptor) site do not form hydrogen bonds with other monomers.

The Hamiltonian corresponding to the model described is given by

$$H = \sum_{|i-j| \geq 3} \epsilon_{ij}^{ss} \delta(r_{ij} - a) + \sum_i A(\mathbf{s}_i \mathbf{s}_{i+1}) - n\epsilon_{es}, \quad (2)$$

where r_{ij} is the distance between monomers i and j , a the lattice spacing, $\delta(0) = 1$ (zero otherwise), $(\mathbf{s}_i \mathbf{s}_{i+1})$ is a scalar product of the orientation vectors, and n ($n \leq 4$) is the number of monomers located near the electron and reducing its energy, i.e., the number of nn monomers oriented toward the donor (or acceptor) site (e.g., $n = 0$ and 4 in the cases shown in Figures 1 (a) and (b)).

The first term in the right-hand part of Eq. (2) corresponds to the hydrogen bonds in topological contacts. These bonds are considered to be formed only provided that (i) one of the monomers is directed toward the other monomer (e.g., $\mathbf{r}_i + \mathbf{s}_i = \mathbf{r}_j$), and (ii) simultaneously the vectors $\mathbf{s}_i$ and $\mathbf{s}_j$ are parallel. If both these conditions

are fulfilled, the energy parameter ϵ_{ij}^{ss} (the superscript ss is for the s - s contacts) is negative ($\epsilon_{ij}^{ss} = \epsilon_{ss} < 0$), i.e., the monomers attract each other (in all the other cases, $\epsilon_{ij}^{ss} = 0$). The second term (with $A > 0$), constructed in analogy with the Ising antiferromagnetic spin-spin interaction, describes the orientational dependence of the interaction between nn monomers linked via the peptide bonds. Its goal is to reproduce an antiparallel orientation of nn monomers. The third term (with $\epsilon_{es} > 0$) takes into account decrease of the electron energy due to interaction with the properly oriented nn monomers.

The lattice model introduced does not describe explicitly the medium and accordingly the model parameters are effective. To employ the model for simulations of the long-range interprotein ET, we assume that the effect of the reorientation of hydrogen bonds in a protein on harmonic vibrations of the medium is relatively weak and accordingly the ET rate for each configuration of hydrogen bonds can be calculated by using the Marcus theory (Eq. (1)) with the reorganization energy, E_r , corresponding to harmonic vibrations of the medium. Specifically, E_r is assumed to be independent of the configurations of hydrogen bonds in a protein. This assumption is physically reasonable, because reorganization of harmonic vibrations of the medium *due to* reconfiguration of the hydrogen bonds in a protein is a secondary process compared to the reconfiguration.* Applying the Marcus formalism, we take into account that the interaction of an electron with nn monomers slightly changes the reaction exothermicity and also modifies the matrix element V (note that the latter effect is beyond the conventional Marcus theory).

As usual for the underbarrier tunneling [10], the squared coupling matrix element is represented as $V^2 = B \exp(-\gamma R/a_0)$, where R is the donor-acceptor distance, a_0 is the atomic unit length, and B and γ are the parameters dependent on the electron energy and properties of the protein and solution. The very presence of the protein can dramatically change V [2]. We are however interested only in modification of V due to reorientation of hydrogen bonds. The change of the electron energy due to the reorientation of any single hydrogen bond is relatively small. For this reason, the dependence of B on the monomer orientations can be neglected in our model. The effect of fluctuations of orientations of non-nn amino-acid residues on γ can be neglected as well because the corresponding change of the electron energy is small (our model does not contain these terms). The influence of reorientation of nn amino-acid residues on γ is also not large but nevertheless may appreciably change the tunneling rate. To describe this effect, we expand the parameter γ with respect to the interaction $\epsilon_{es}n$ and keep only the zero- and first-order terms. This yields $V^2 = V_0^2 \exp(-\eta \epsilon_{es} n R/a_0 E_0)$, where η is the dimensionless parameter, $E_0 \simeq 10$ eV is the scale of the electron energy (this energy is chosen to be about two times larger than typical energy of tunneling electrons, because the expansion coefficient is expected to be about 1/2), and V_0 is the coupling corresponding to the case when

*To relax this assumption, one can introduce the dependence of E_r on n . The corresponding terms are however expected to be small compared to $n\epsilon_{es}$ and accordingly their role seems to be inferior.

the electron energy is independent of the orientation of hydrogen bonds. From the experiment and theory [10], one has $\gamma \simeq 0.5$ and accordingly the absolute value of η is expected to be about 0.5 as well (at present, accurate calculation of these parameters is hardly possible). The sign of η is usually positive (because, as a rule, γ increases with increasing electron binding energy), but in principle it can be negative (because sometimes γ decreases with increasing the binding energy in a narrow range of energies).

In addition to the assumptions above, we consider that the reorientation of hydrogen bonds occurs much faster than the ET process. This means that the ET kinetics is Markovian and accordingly the apparent ET rate constant, k , can be calculated in our model as an average of the rate constants corresponding to different orientations of monomers (the non-Markovian effects related to slow reorientation of hydrogen bonds are discussed in [8]). The explicit expression for k depends on the location of the donor and acceptor.

If the donor site is located near the β sheet, the electron-monomer interactions reduce the reaction exothermicity and accordingly the ET rate constant is represented as (cf. Eq. (1))

$$k = k_0 \sum_{n=0}^4 P_n \exp \left[-\frac{\eta \epsilon_{es} n R}{a_0 E_0} - \frac{(E_r - J + n \epsilon_{es})^2}{4 E_r k_B T} \right], \quad (3)$$

where $k_0 = \pi^{1/2} V_0^2 \hbar^{-1} / (E_r k_B T)^{1/2}$, and P_n is the probability that the electron has n properly oriented nn monomers.

If the acceptor site is located near the β sheet, the electron-monomer interactions increase the reaction exothermicity and one gets

$$k = k_0 \sum_{n=0}^4 P_n \exp \left[-\frac{\eta \epsilon_{es} n R}{a_0 E_0} - \frac{(E_r - J - n \epsilon_{es})^2}{4 E_r k_B T} \right], \quad (4)$$

where P_n is the probability that the vacant acceptor site has n nn monomers oriented towards this site.

To calculate P_n in Eq. (3), one should use the full Hamiltonian (2) because in this case the electron located on the donor site interacts with the β sheet. In contrast, the probability P_n in Eq. (4) should be calculated by employing Hamiltonian (2) without the last term (i.e., with $\epsilon_{es} = 0$), because in the latter case the electron initially (before the jump) does not interact with the β sheet.

3. Results of Simulations

In the model formulated above, the positions of monomers are fixed and the ordering of hydrogen bonds is mimicked via change of the monomer orientations. Specifically, the probabilities of the orientations depend on the effective parameters

Figure 2. Probability P_n for $\epsilon_{es} = 0, 0.1$ and 0.15 eV.

ϵ_{ss} , A and ϵ_{es} . Numerous simulations of protein folding indicate that the values of the effective parameters should be comparable to or slightly larger than $k_B T$. In our Monte Carlo (MC) simulations performed for $T = 300$ K, we use $\epsilon_{ss} = -0.06$ eV and $A = 0.02$ eV. [Our conclusions drawn on the basis of the calculations with these parameters hold for other physically reasonable parameters (e.g., with decrease of ϵ_{ss} by about 0.01 eV or changing of A by ± 0.01 eV) as well.]

To calculate the probabilities P_n with the parameters above, the vectors characterizing the monomer orientation were rotated (the angle change was $\pm\pi/2$) by employing the standard Metropolis algorithm. Initially, the monomer orientations corresponded to the ideal β sheet. The first 10^5 rotation trials (per monomer) were performed to reach equilibrium. Then, the next 10^8 trials were used to get P_n . The results obtained are shown in Figure 2 for $\epsilon_{es} = 0, 0.1$ and 0.15 eV (as physically expected, these value are taken to be of the same scale as ϵ_{ss}). If there is no electron–monomer interaction ($\epsilon_{es} = 0$), the orientations of monomers near the donor (or acceptor) site usually correspond to the ideal β sheet (Figure 1(a)). This means that $P_0 \simeq 1$ and the other probabilities (with $n \neq 0$) are exponentially small. If the electron–monomer interaction is appreciable, $\epsilon_{es} = 0.1$ eV, the perturbation of the orientations of nn monomers is significant and all the orientations can be found with comparable probabilities. If the electron–monomer interaction is strong, $\epsilon_{es} = 0.15$ eV, the nn monomers are as a rule oriented toward the electron (Figure 1(b)).

The dependence of the ET rate constant on the reaction exothermicity can be calculated by substituting probabilities P_n (Figure 2) into Eqs. (3) and (4). To use these equations, we need the value of the reorganization energy, E_r , corresponding to harmonic vibrations of the medium. In experiments, the ET rate constant is usually fitted by employing Eq. (1) with $E_r = 1$ – 2 eV [10]. These *apparent* values taking

into account the solution and protein are large on the scale of the protein stability. This means that the contribution of the solution to the reorganization energy is appreciable. What we need is the reorganization energy corresponding to harmonic vibrations of the medium. The latter value is expected to be somewhat lower than 1–2 eV. In our calculations (Figures 3–6), we use $E_r = 1$ and 0.5 eV. With these values of E_r , we have found that the effect of reorientation of monomers on ET is significant if the β sheet is associated with the donor (Figures 3–5).

In particular, Figure 3(a) constructed for $\eta = 0$, $E_r = 1$ eV, and $\epsilon_{es} = 0, 0.1$ and 0.15 eV shows that in this case the monomer reorientations result in a shift of the maximum of $\ln k$ to the right and, what is most essential, in broadening the curve. Both these effects are related to reducing the reaction exothermicity due to the electron–monomer interaction. In the ‘normal’ regime (where k increases with increasing J), the ET process is controlled primarily by the conformations with $n = 0$ or 1 (Figure 3(b)), because for these conformations the exothermicity is larger and accordingly the electron tunneling is faster. In the ‘inverted’ regime (where k decreases with increasing J), the role of the conformations with $n = 3$

Figure 3. (a) ET rate constant as a function of the reaction exothermicity for $\eta = 0$, $E_r = 1$ eV, and $\epsilon_{es} = 0, 0.1$ and 0.15 eV (thick, thin, and thick solid lines) in the case when the β sheet is linked with the donor. (b) The same curve (thick solid line) for $\epsilon_{es} = 0.1$ together with the contributions of the conformations with $n = 0$ and 4 (thin and dashed lines).

Figure 4. As Figure 3(a) for $E_r = 0.5$ eV.

Figure 5. ET rate constant versus the reaction exothermicity for $E_r = 1$ eV, $\epsilon_{es} = 0.1$ eV, $E_0 = 10$ eV, $R = 30$ at. un., and $\eta = 0$ (dashed line) and 0.5 (thick solid line). For the latter case, the thin lines show the contributions of the conformations with $n = 0$ and 4. The β sheet is linked with the donor.

or 4 increases, because for these conformations the exothermicity is smaller. If the electron–monomer interaction is appreciable and accordingly the probabilities P_n with $n = 3$ or 4 are not exponentially small, the latter conformations play the main role in the electron jumps (Figure 3(b)). This change in the type of the rate-determining conformations gives rise to broadening the $\ln k$ - J curve maximum.

The shape of the ET rate constants calculated with $E_r = 1$ eV and $\epsilon_{es} = 0.1$ and 0.15 eV (Figure 3) is nearly parabolic, because in this case the reorganization energy is relatively large and accordingly the harmonic vibrations of the medium dominate. With decreasing E_r down to 0.5 eV (Figure 4), the dependence of k on J remains close to parabolic for $\epsilon_{es} = 0.1$ eV. For $\epsilon_{es} = 0.15$ eV, there are however deviations from the parabolic shape. It is of interest also to note that in the latter case

Figure 6. ET rate constant as a function of the reaction exothermicity for $\eta = 0$, $E_r = 1$ eV, and $\epsilon_{es} = 0$ (thick solid line) and 0.1 eV (open circles) in the case when the β sheet is linked with the acceptor.

due to the reorientation of hydrogen bonds the apparent value of the reorganization energy is appreciably larger than 0.5 eV.

The role of the R -dependent factors, $\exp(-\eta\epsilon_{es}nR/a_0E_0)$, is illustrated in Figure 5. For typical parameters $\eta = 0.5$, $R = 30$ at. un., and $\epsilon_{es} = 0.1$ eV [10]), these factors are nearly negligible in the normal regime, because this regime is controlled primarily by the conformation with $n = 0$. In the inverted case, the conformation with $n = 4$ dominates and accordingly the ET rate constant is reduced compared to that calculated for $\eta = 0$. This effect is however not strong.

If the β sheet is linked with the acceptor, the electron tunneling rates corresponding to different monomer orientations are also different, but the whole ET process is completely controlled by the conformation with $n = 0$, because the probabilities of the other conformations are negligibly small (see the curve for $\epsilon_{es} = 0$ in Figure 1). For this reason, the effect of reorientation of monomers on the ET rate constant is nearly not visible (Figure 6).

4. Conclusion

Using realistic values of the model parameters, we have explicitly demonstrated that the reorientation of hydrogen bonds in proteins may significantly influence the rate of long-range interprotein ET especially in the case when the reorientation occurs in the protein linked with the electron donor. Specifically, the interaction between the tunneling electron and the amino-acid residues, which are responsible for the formation of hydrogen bonds, may result in (i) broadening the dependence of the ET rate constant on the reaction exothermicity and (ii) deviations from the parabolic law especially if the reorganization energy of the solution is relatively small (≤ 0.5 eV). The former effect may be significant. The latter effect is usually

relatively weak, because near maximum the ET rate constant can formally always be approximated by a parabola even if the maximum results from the interplay of the Gaussian fluctuations of the medium and the non-Gaussian statistics of hydrogen bonds. These findings extend the conceptual basis of the general theory of interprotein ET. Concerning experiment, we may note that the identification of the role of reorientation of hydrogen bonds in real ET processes is rather difficult, because apparently the effects predicted are similar to those related to quantum nuclear vibrations and/or inelastic processes during electron tunneling [2].

Finally, it is appropriate to note that our simulations of interprotein ET have been performed for the simplest case when this process is slow compared to reorientation of hydrogen bonds, and there are no global changes in the protein structure. The lattice approximation can however be employed in more complex cases as well. In particular, kinetic MC simulations, based on the lattice models, can be used to analyze nontrivial global reconfiguration of the protein structure and also other interesting effects, e.g., non-Markovian kinetics of fast interprotein ET [8].

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