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First principles studies of electron tunneling in proteins

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Abstract

A first principles study of electronic tunneling along the chain of seven Fe/S clusters in respiratory complex I, a key enzyme in the respiratory electron transport chain, is described. The broken-symmetry states of the Fe/S metal clusters calculated at both DFT and semi-empirical ZINDO levels were utilized to examine both the extremely weak electronic couplings between Fe/S clusters and the tunneling pathways, which provide a detailed atomistic-level description of the charge transfer process in the protein. One-electron tunneling approximation was found to hold within a reasonable accuracy, with only a moderate induced polarization of the core electrons. The method is demonstrated to be able to calculate accurately the coupling matrix elements as small as 10^{-4} cm^{-1} . A distinct signature of the wave properties of electrons is observed as quantum interferences of multiple tunneling pathways.

Keywords

Electron tunneling; proteins; electron transport chain; cell respiration

I. Introduction

NADH:ubiquinone oxidoreductase (complex I) is a large L-shaped membrane-bound enzyme which serves as an entry point in respiratory electron transport chain of mitochondria and bacteria[1–4]. Complex I transfers electrons over approximately 90 Å along a chain of redox co-factors consisting of flavin mononucleotide (FMN) and seven iron-sulfur (Fe/S) clusters (N3, N1b, N4, N5, N6a, N6b, N2), from NADH to ubiquinone [5], which passes electrons further along the chain, Fig. 1. In turn, the reaction provides the driving force for creating the proton gradient across the membrane necessary for ATP synthesis [6].

Seven Fe/S clusters form a chain with long (edge-to-edge) distances between nearest neighbors of 8.5–14 Å. N1b Fe/S cluster is binuclear (2Fe-2S), and six Fe/S clusters (N3, N4, N5, N6a, N6b, N2) are tetranuclear (4Fe-4S). Under physiological conditions, the 2Fe-2S cluster has two redox states with formal iron valences (+3,+3) and (+3,+2), and 4Fe-4S clusters have two redox states with formal iron valences (+3,+3,+2,+2) and

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(+3,+2,+2,+2) to work as single electron carriers [7]. The formal charges and schematic spin configurations of two redox states of 4Fe-4S clusters are shown in Figure 1 (right). Due to the anti-ferromagnetically coupled high-spin iron atoms, the Fe/S clusters have unique electronic properties [8–11]. The main feature of such clusters is the exceptionally high quasi-degeneracy of the electronic states, which has its origin in the 5-fold degeneracy of the *d*-orbitals of Fe ions. Theoretical studies of such anti-ferromagnetic systems had been performed by combining Heisenberg type Hamiltonian and the so-called broken-symmetry (BS) state [9], which resemble the pure spin ground state with some higher spin state contributions.

The first report on the details of electron tunneling in complex I has been recently published by these authors [12]. This study extends our previous work, emphasizing computational aspects. Here a semi-empirical electronic structure method ZINDO is used to examine the tunneling pathways and the strength of electronic coupling between Fe/S clusters in complex I. The method is based on the analysis of BS states of the donor and acceptor quasi-continuum “bands” to obtain a dynamically averaged picture, which are found in good agreement with broken-symmetry DFT calculations.

ZINDO has been successfully applied to simulate the spin states and the electronic spectra of transition metal complexes with accuracy comparable to that of DFT (time-dependent DFT for spectra) [13–15]. The Heisenberg spin Hamiltonian and the resulting spin states of polynuclear transition metal complexes computed with ZINDO agree well with the experimental data [16]. ZINDO also reproduce the electronic absorption spectra of mononuclear and binuclear Fe/S clusters [17]. ZINDO has also been extensively used for calculations of electron transfer in a variety of inorganic and organic systems [18]; the method has an advantage in describing the through-space interactions of noncovalently bonded protein atoms due to its Slater-type orbitals [19].

In Condon and Born-Oppenheimer approximations, the electron transfer rate is determined by the square of the electronic coupling matrix element (also denoted as tunneling matrix element) between the donor and acceptor electronic states [20–21]. Since the electronic couplings of long-range electron tunneling in biological systems are generally orders of magnitude smaller than the accuracy of the typical electronic structure calculations (~1 kcal/mol), the direct calculation of the energy difference between the two adiabatic states at the transition is not feasible [21]. In this study, the simulation of electron transfer between redox centers is based on the tunneling current theory [20–21], which had been successfully applied to several systems previously. The calculations focus on evaluating the transition flux between donor and acceptor states. Coarse-graining by introducing the atomic basis set gives interatomic currents, which describe the tunneling flux at the atomic level [21]. Based on the tunneling flux theorem, the tunneling matrix element is expressed as the total flux across the dividing surface between the donor and acceptor groups [20–21]. The method treats many-electron wave functions explicitly by incorporating both the tunneling electron orbitals and the induced polarization of core electrons. The method can accurately predict the protein-mediated electronic couplings of the molecular orbitals of the metal clusters separated in the enzyme by a distance of up to 15–20 angstroms. While the tunneling couplings in such systems can be as small as 10^{-2} – 10^{-3} cm⁻¹, the tunneling orbital tails in

the middle of donor and acceptor, which primarily determine the tunneling coupling, can be calculated with sufficient accuracy.

II. Spin States of Fe/S clusters

In the reduced state $[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^{3-}$, three ferrous Fe^{2+} sites have spin $S=2$ with multiplicity 5, and one ferric Fe^{3+} site has spin $S=5/2$ with multiplicity 6; given the four possibilities of the ferric Fe site (Figure 1), the total number of states is 3000 ($=5^3 \times 6 \times 4$). These states are grouped into 380 overlapping clusters by the total spin. The spin state energy diagram is shown in Figure 2 (left panel) based on the following phenomenological Hamiltonian:

$$H = \sum_{i,j} J_{ij} S_i \cdot S_j \pm B(S_{34} + 1/2) \pm B'(S + 1/2) \quad (1)$$

where S_{34} is the spin of the oxidized layer and S is the total system spin [9]. J_{ij} , B , and B' represent the Heisenberg spin coupling parameter, intra-layer resonance delocalization, and inter-layer resonance delocalization, respectively. Uniform J_{ij} are usually assumed for all Fe pairs. Here small variations of the spin couplings are introduced by assuming $J_{12} = J_0 +$ and $J_{34} = J_0 -$. The solution thus becomes:

$$H = \frac{J_0}{2} S(S+1) + \frac{\Delta}{2} S_{12}(S_{12}+1) - S_{34}(S_{34}+1) \pm B(S_{34} + 1/2) \pm B'(S + 1/2). \quad (2)$$

The energies are calculated by employing calculated J_0 , B , and B' by BS-DFT [22] and are scaled to match the experimentally observed J_0 of 250 cm^{-1} [10]. Δ is set as 5% of J_0 . The total degeneracy of a given S is $a_S \times 4$, where a_S is the number of irreducible representations for a given spin; for example, $a_{1/2} = 9$, $a_{3/2} = 15$. The spin-orbit coupling is also expected to be not too much smaller than $k_B T$ in these systems, and will further lift the $2S+1$ degeneracy of these states to give $a_S \times 4 \times (2S+1)$ different spin states.

Estimated density of spin states and integrated number of states versus energy (in $k_B T$) are plotted in Fig. 2 (right panel). 12 core atoms (4Fe + 8S) are expected to have significant vibronic couplings to the electronic states of the spin Hamiltonian. The fluctuation of the total vibrational energy associated with these atoms is estimated as

$\sqrt{(12 \times 3-6)k_B T} \approx 5.5k_B T$. As many as 30 spin states of spin $1/2$, $3/2$, $5/2$ and possibly $7/2$ are present within the energy interval of $5.5 k_B T$ above the ground state, and can therefore be mixed through the vibronic coupling during the thermal dynamics. The number of states accessible during the thermal fluctuations is not sensitive to the introduced asymmetry of the spin couplings when Δ is varied from 1% to 10% of J_0 , which further suggests that many states are dynamically mixed regardless of the degree of asymmetry. All relevant vibrations (Fe-S stretch, ring breathing, ring deformation, etc) of a Fe/S cluster calculated with BS-DFT have frequencies in $200\text{--}400 \text{ cm}^{-1}$, which corresponds to the time scale of $80\text{--}160 \text{ fs}$ [23–24], and therefore the time scale of the resulting mixing is expected to be 6 to 8 orders of magnitude faster than that of the slowest electron transfer.

Broken-Symmetry (BS) ground state of isolated $[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^{3-}$ are calculated at the ZINDO level with an optimized geometry by BS-DFT calculation [25]. The converged electronic structure reproduces the anti-ferromagnetic spin structure of previous BS-DFT calculations [9] where two iron sites have spin up with positive atomic spin densities and two iron sites have spin down with negative atomic spin densities. The absolute value of the calculated Mulliken atomic spin densities of four iron atoms is about 4.1 in ZINDO and 3.7 in DFT. The calculated BS HOMO, which contributes most to the electron tunneling, is a combination of Fe-Fe σ^* and S-S σ^* , in agreement with one of the two spin states coexisting at the room temperature [9]. Figure 3 shows that both DFT and ZINDO produce similar results [26], suggesting the reasonable accuracy of ZINDO comparable to that of DFT in the electron tunneling calculations.

III. Protein Pruning

Due to the high complexity of the electronic spin structure of the Fe/S clusters, ZINDO method is not feasible to solve the electronic structure of the whole hydrophilic domain of complex I which has more than 18000 atoms including nine Fe/S clusters. Since the electron transfer reaction occurs locally between the neighboring Fe/S clusters, only the peptide close to the electron tunneling pathway significantly contributes to the reaction. The most part of the protein far from the region of electron tunneling only participates through the electrostatic interactions. We therefore perform calculations in two stages: first we identify the most important parts of whole protein with an approximate one-electron extended Hückel method using the procedure of protein pruning [27]; then we proceed with more accurate many-electron treatments using DFT and ZINDO semi-empirical method. In the pruning procedure, the tunneling matrix element is calculated by using the perturbation theory Green's functions, assuming a weak coupling of the donor and acceptor orbitals to the bridge orbitals. Here, we first include all the peptide residues located within a cylinder of radius R_{cut} with its axis connecting the donor and acceptor complexes; the cut-off radius R_{cut} is varied to find the minimum value which gives the tunneling matrix element within 5% error of the converged value. The tunneling matrix element is then calculated excluding residues one by one to identify the most essential ones. The peptide residues which change the tunneling matrix element by more than 1 % are retained. The numerical errors due to the protein pruning are typically less than 10%. The neutral states of all residues are assumed. The neutral hydroquinone state of riboflavin is employed as a model of the reduced FMN. Terminal dangling bonds are eliminated by capping C-terminals with $-\text{NHCH}_3$ groups and N-terminals with acetyl ($-\text{COCH}_3$) groups. The resulting pruned systems contain 200~500 atoms.

IV. BS-ZINDO Calculations

BS donor and acceptor states of the pruned system at the ZINDO level are used for electron tunneling calculations. The BS-ZINDO calculations were performed as follows. First, the pruned peptide system, including the electron donor and acceptor, is dissected into several fragments with no covalent bonds between them. An electronic structure calculation is performed for each peptide fragment. The donor fragment has +1/2 and 0 spin and the acceptor fragment has 0 and +1/2 spin in the donor and acceptor electronic states,

respectively. Due to the slight deformation of symmetric Fe/S clusters in the protein environment, several nearly degenerate BS ground states exist. The energy is lowest when each layer has the highest spin state, namely $S_{12}=9/2$ and $S_{34}=9/2$ for oxidized $[\text{Fe}_4\text{S}_4\text{R}_4]^{2-}$ (R denotes ligands), or $S_{12}=4$ and $S_{34}=9/2$ for reduced $[\text{Fe}_4\text{S}_4\text{R}_4]^{3-}$. BS-ZINDO calculations are carried out for such states of the oxidized donor and acceptor Fe/S fragments by employing 12 SCF initial guesses where 6 possible choices of two oxidized Fe^{3+} and two reduced Fe^{2+} sites and 2 possible different spin alignments are considered. 2 to 10 different electronic spin states are obtained as converged SCF solutions, and 1 to 3 BS states with the total energy less than 0.15 eV higher than the lowest one are considered in the subsequent calculations. The electronic spin states of the reduced $[\text{Fe}_4\text{S}_4\text{R}_4]^{3-}$ are obtained by employing initial guesses from the converged electronic states of the oxidized $[\text{Fe}_4\text{S}_4\text{R}_4]^{2-}$ clusters with $N+1$ electrons.

The resulting average $\langle S^2 \rangle$ values of the oxidized and reduced clusters are 8.9 and 8.6 in reasonable agreement with expected 9 and 8 by the Hamiltonian of Eq. 1 [9]. The BS states consist mainly of $S = 1/2$, $3/2$, and $5/2$ pure spin states stabilized by B and B' delocalization, which are in the range of thermal fluctuations (Figure 2).

BS-ZINDO calculations are performed for the total system with a set of different SCF initial guesses from the low-lying BS state pairs of the donor and acceptor fragments. The local electronic spin properties of each Fe/S cluster (namely, atomic spin densities, Mulliken charges, etc) are well preserved through the SCF procedure to the final converged solutions. The electron tunneling pathway and the tunneling matrix elements then calculated for different pairs of BS donor and acceptor states.

V. Electron Tunneling Calculation

Suppose the system is artificially “frozen” at the transition state of electron transfer reaction, where the energy of donor and acceptor electronic states are in resonance; the time-dependent wave function is described as a linear combination of the donor and acceptor diabatic states $|\Psi_D\rangle$ and $|\Psi_A\rangle$:

$$|\Psi(t)\rangle = \cos\left(\frac{T_{DA}t}{\hbar}\right) |\Psi_D\rangle - i\sin\left(\frac{T_{DA}t}{\hbar}\right) |\Psi_A\rangle, \quad (3)$$

where T_{DA} is the tunneling matrix element. By combining Eq. (3) and a general expression of the probability current $\vec{j}(r,t) = \langle \Psi(t) | \hat{j}(r) | \Psi(t) \rangle$ where $\hat{j}(r)$ is the quantum-mechanical multi-electron flux operator, we have

$$\vec{j}(r,t) = -\vec{J}(r) \sin \frac{2T_{DA}t}{\hbar}. \quad (4)$$

The spatial part of the current (also called the transition flux) is described by [20–21, 28]

$$\vec{J}(r) = -i \langle \Psi_A | \hat{j}(r) | \Psi_D \rangle. \quad (5)$$

In the diabatic donor and acceptor states, the tunneling electron is localized on their respective complexes. When the donor and acceptor states are represented by the single Slater determinant many-electron wave functions, a set of biorthogonal (corresponding) orbitals is obtained by appropriate rotations of the canonical MO's of both states:

$$\begin{aligned} |\Psi_D\rangle &= |\varphi_{1\alpha}^D \cdots \varphi_{m\alpha}^D \varphi_{1\beta}^D \cdots \varphi_{n\beta}^D\rangle \\ |\Psi_A\rangle &= |\varphi_{1\alpha}^A \cdots \varphi_{m\alpha}^A \varphi_{1\beta}^A \cdots \varphi_{n\beta}^A\rangle, \end{aligned} \quad (6)$$

where the overlap matrix of $|\Psi_D\rangle$ and $|\Psi_A\rangle$ is diagonal: $\langle \varphi_{i\sigma}^A | \varphi_{j\sigma}^D \rangle = \delta_{ij} s_i^\sigma$ [29–33].

For proteins the one-electron tunneling approximation holds [28], which means that a single pair of the corresponding orbitals with the smallest overlap s_1^σ gives the largest and dominant contribution to the tunneling current, whereas the rest (core orbitals) undergo induced polarization and participate as an electronic Franck–Condon factor [34]. For example, for N6a→N6b pair, the overlap of the tunneling orbitals is calculated to be $s_1^\alpha = 5.0 \times 10^{-5}$. The next smallest overlap is 0.6 and all other overlaps are larger than 0.9, suggesting that core orbitals undergo only minor induced polarizations. The electronic Franck–Condon factors were typically in the range between 0.44 and 0.75 for all process studied. The one tunneling electron approximation thus holds in the electron tunneling of complex I system where the donor and acceptor are multinuclear Fe/S clusters.

Comparing the canonical and corresponding MO's, the atomic population of the corresponding donor and acceptor tunneling orbitals are significantly more localized on a single atom within the redox complex, suggesting that the electron tunneling occurs primarily between a pair of donor and acceptor atoms in the corresponding orbital picture.

In ZINDO approximation, there is a band gap of ~8 eV between the occupied (valence) and unoccupied (“conduction”) orbitals. Both donor and acceptor orbitals are in resonance (by nature of the transition state of electron transfer reaction) and are off-resonant from all molecular orbitals of the peptide bridge, which supports the notion of electron tunneling between the donor and acceptor Fe/S clusters without any intermediate state involved where the electron is localized on some peptide residue.

By introducing the atomic basis set and the Mulliken type coarse-graining of the tunneling current, the tunneling transition flux is expressed in terms of the interatomic currents, which has the following form [28, 35]:

$$J_{ab} = \prod_{i \neq 0} s_i^\alpha \prod_j s_j^\beta \sum_{\nu \in a} \sum_{\mu \in b} h_{\nu\mu} - E_0 S_{\nu\mu} \quad A_\mu D_\nu - D_\mu A_\nu. \quad (7)$$

Here ν and μ are the atomic orbitals of atom a and b ; D_μ and A_ν are the expansion coefficients of the donor and acceptor tunneling orbitals, respectively; h_ν and $S_{\nu\mu}$ are core Hamiltonian and overlap matrix, and E_0 is a tunneling orbital energy defined by

$$E_0 = \sum_{\lambda,\rho} D_\lambda \bar{F}_{\lambda\rho} D_\rho = \sum_{\lambda,\rho} A_\lambda \bar{F}_{\lambda\rho} A_\rho, \quad (8)$$

where $\bar{F}_{\lambda\rho}$ is the Fock matrix. The second equality in Eq. (8) corresponds to the resonance of the donor and acceptor redox potentials at the transition state of electron transfer reaction. The resonance is achieved by applying a static electric field mimicking the electrostatic environment due to the surrounding protein region, solvation and protonation of the protein.

Eq. (7) shows that the interatomic current is primarily determined by the overlap of the tails

of the two tunneling orbitals. The electronic Franck-Condon factor ($\prod_{i \neq 0} s_i^\alpha \prod_j s_j^\beta$) contributes as a uniform scaling factor for all inter-atomic currents, namely, larger induced polarization of the core electrons gives smaller tunneling currents. In case the electronic Franck-Condon factor is significantly smaller than 1 (for example, <0.1), the rearrangement of the core electrons is not negligible and the one-electron approximation no longer holds.

The total atomic current through a given atom a is expressed as

$$J_a^{tot} \equiv \frac{1}{2} \sum_b |J_{ab}|, \quad (9)$$

which is proportional to the probability that a tunneling electron is passing through this atom in the tunneling transition. The tunneling matrix element, according to the total flux theorem [20–21], is given by the surface integral of the tunneling flux across the dividing surface Ω_D separating donor and acceptor complexes,

$$T_{DA} = -\hbar \int_{\partial\Omega_D} (d\vec{s} \cdot \vec{J}). \quad (10)$$

In a correct calculation of donor and acceptor orbitals, in particular their “tails” which are exponentially small in the region between metal complexes (i.e. under the tunneling barrier) and which determine the magnitude of electronic coupling, the total flux should be independent of the position of the separating surface, as long as it runs in the tunneling barrier region. In order to check that the total flux does not indeed depend on the position of the dividing surface, the total flux is plotted as a function of the position of the dividing plane perpendicular to the line connecting donor and acceptor complexes in Fig. 4. The flux is found conserved in sufficiently large range of the position of the dividing plane around the midpoint (0.5) between the donor and acceptor.

VI. Electron Tunneling Pathways

For different pairs of donor and acceptor BS states, the tunneling pathways are mainly similar, except for some details at the Fe/S clusters themselves. Most typical tunneling pathways are shown in Figure 5. For shortest electron tunneling distances, the pathway runs either along the two cysteine wires of the donor and acceptor in direct van der Waals contact ($N1b \rightarrow N4$ and $N6a \rightarrow N6b$), or along a single mediator residue located in the middle of the two Fe/S complexes with two through-space jumps ($N4 \rightarrow N5$). For intermediate distances, e.g. $N6b \rightarrow N2$, one cysteine ligand and one mediator residue participate. For longest distance tunneling ($N3 \rightarrow N1b$ and $N5 \rightarrow N6a$), one neighboring residue of either exit or entrance cysteine also participates as an extended through-bond wire in addition to the two cysteine residues. One to three through-space jumps are involved, all over distances below 3.2 Å, which indicates that the tunneling electron avoids pathways with longer through-space jumps due to higher energy costs

Significant quantum interferences are observed between some Fe/S cluster pairs ($N3 \rightarrow N1b$, $N4 \rightarrow N5$ and $N5 \rightarrow N6a$). In $N5 \rightarrow N6a$ process, clusters N5 has two cysteine (${}^3\text{Cys}^{119}$ and ${}^3\text{Cys}^{122}$) wires connected by neighboring ${}^3\text{Pro}^{120}$ and ${}^3\text{Thr}^{121}$ residues making the backbone loop ($N5\text{-}{}^3\text{Cys}^{119}\text{-}{}^3\text{Pro}^{120}\text{-}{}^3\text{Thr}^{121}\text{-}{}^3\text{Cys}^{122}\text{-}N5$) stabilized by a hydrogen bond and the ${}^3\text{Pro}^{120}$ rigidity. The main electron tunneling flux from N5 is through ${}^3\text{Cys}^{119}$ and ${}^3\text{Pro}^{120}$, part of which is reflected back to N5 through ${}^3\text{Thr}^{121}$ and ${}^3\text{Cys}^{122}$, the rest is transmitted to ${}^9\text{Cys}^{56}$ with a 2.7 Å or 2.6 Å through-space jump across the subunit boundary of Nqo3 and Nqo9. The electron then tunnels through ${}^9\text{Cys}^{56}$ ligand to reach N6a. The resulting quantum interference is destructive where the tunneling flux through the two pathways is in the donor-to-acceptor (${}^3\text{Cys}^{119} \rightarrow {}^3\text{Cys}^{122}$) and acceptor-to-donor (${}^3\text{Cys}^{122} \leftarrow {}^3\text{Thr}^{121}$) directions. Destructive interference is also prominent in $N3 \rightarrow N1b$ process with flux through two pathways in opposite directions (${}^1\text{Cys}^{356} \rightarrow {}^1\text{Thr}^{357}$ and ${}^1\text{Cys}^{359} \leftarrow {}^1\text{Pro}^{358}$). The peptide bridge connecting N3 and N1b clusters has the same topology as that of $N5 \rightarrow N6a$ since the protein backbone loop ($N3\text{-}{}^1\text{Cys}^{356}\text{-}{}^1\text{Thr}^{357}\text{-}{}^1\text{Pro}^{358}\text{-}{}^1\text{Cys}^{359}\text{-}N3$) formed with the donor complex N3 is facing a cysteine ligand of the acceptor complex N4. In process $N6a \rightarrow N6b$, the electron tunnels along two different pathways depending on the involved BS states, suggesting the sensitive nature of the pathways to the local protein environment.

The whole electronic wiring of complex I is obtained by combining tunneling pathways of individual processes, as shown in Figure 6. It is clear that specific peptide residues serve as electronic wires connecting neighboring Fe/S clusters; individual electron tunneling paths involve up to three protein residues, including two cysteine ligands and one additional key residue. Notably, the clusters in the protein are oriented in a specific way – corner to corner – with Cys ligands mostly pointing toward each other, which is clearly the most efficient way to transfer electrons from one cluster to another. The protein subunit boundaries are clearly seen as significant gaps in the electronic wiring of complex I where internal water should exist.

VII. Electron Transfer Rates

The electron transfer rates between the clusters are calculated by using Marcus' theory [36]

$$k_{ET} = \frac{2\pi}{\hbar} \frac{\langle T_{DA}^2 \rangle}{\sqrt{4\pi\lambda k_B T}} \exp\left[-\frac{(\Delta G^0 + \lambda)^2}{4\lambda k_B T}\right], \quad (11)$$

with a generic reorganization energy $\lambda = 0.5$ eV [37–38] and the driving force $G = 0$ eV for all processes with an exception of $G = -0.15$ eV for N6b→N2 [1, 39]. The dynamical

average of the electronic coupling matrix elements squared $\langle T_{DA}^2 \rangle$ is evaluated in the following procedure. For a given pair of donor and acceptor tunneling orbitals the tunneling

matrix element is renormalized as: $T_{DA}^* = T_{DA} / \sqrt{P_D P_A}$, where P_D and P_A are the atomic populations on the respective gateway atoms defined as core atoms of Fe/S clusters which give the largest contribution to the transition. For different pairs of donor and acceptor orbitals the renormalized matrix element T_{DA}^* is about the same, and reflects the maximum possible coupling between redox centers, which occurs when donor and acceptor orbitals are localized on the respective gateway atoms. The dynamical and statistical average of the electronic coupling that determines the electron transfer rate is $\langle T_{DA}^2 \rangle = \gamma_D \gamma_A (T_{DA}^*)^2$, where $\gamma = 1/8$ for 4Fe-4S clusters and $\gamma = 1/4$ for 2Fe-2S clusters. Here we assume a statistically equal distribution of the tunneling orbitals over eight core atoms of 4Fe-4S cluster, and over four core atoms of 2Fe-2S cluster.

The calculated electronic couplings and the rates are shown in Table 1. Four electron transfer processes of N1b→N4, N4→N5, N6a→N6b, and N6b→N2 are faster than both the reported estimates of the total transfer rate: 170 s^{-1} and 10^4 s^{-1} . However, the transfer rate of N3→N1b ($\sim 10^3 \text{ s}^{-1}$) is slower than the highest estimate, and N5→N6a ($\sim 10 \text{ s}^{-1}$) is drastically slower than the reported rates. Both are the electron tunneling across the protein subunit boundaries, where internal water molecules should be present [40], although the intervening water is not seen in the crystal structure due to its possible significant mobility.

We have therefore performed the calculations of electron tunneling in N3→N1b, N5→N6a, and N6b→N2 pairs, across the protein subunit boundaries, with the additional simulated internal water. With the included water between the subunits, the tunneling rates are dramatically increased by two to three orders of magnitude (Table 1). With this increase, the overall transfer rate is now in a typical physiological range [37], and is roughly equal to a typical electron transfer rate for such distance [41]. With the water included the negative slope of the distance dependence or ET rates in natural logarithm becomes 1.3, which agrees well with a typical 0.6 in base-10 log [41], Fig. 7. The internal water at the subunit boundaries is therefore an essential mediator for the efficient electron transfer along the redox chain of complex I. The calculated enhancement of the ET rate due to the internal water agrees well with a recent study on the bovine liver cytochrome b₅ [42].

VIII. Summary and conclusions

Theory and the atomistic simulations of the electron tunneling along Fe/S chain in respiratory complex I have been presented. The calculations involved the broken-symmetry (BS) states of the Fe/S clusters imbedded in the protein matrix. To simplify the system, the protein pruning was performed; the pruned protein system consists of anti-ferromagnetically coupled binuclear or tetranuclear Fe/S donor and acceptor clusters and a peptide bridge region connecting them.

While the tunneling tails of the donor and acceptor molecular orbitals determine the tunneling pathways, the induced polarization of the core electrons contributes as a scaling factor (the electronic Franck-Condon (FC) factor). The calculated FC factors are in the range of 0.44~0.75, and the small overlaps of the tunneling orbitals ($<10^{-4}$) suggests that even in the case of the multinuclear (2Fe-2S or 4Fe-4S) anti-ferromagnetic donor and acceptor systems, the one tunneling electron approximation still holds. Only one electron/hole primarily participates in tunneling while other electrons undergo induced polarization without any significant orbital and spin rearrangement. However, the induced polarization effect is still significant, and should be considered in the electron transfer rate calculations since the rates are scaled by the square of the FC factor (0.19~0.56).

Population analysis of the tunneling orbitals shows that the tunneling donor and acceptor orbitals are localized on donor and acceptor clusters, respectively, with exponentially decaying tunneling tails extending toward each other through the peptide bridge region. The atomic populations of the donor orbital around the acceptor complex and the atomic population of the acceptor orbital around the donor complex are as small as 10^{-20} ~ 10^{-15} depending on the tunneling distance, and therefore cannot be calculated accurately. However, both donor and acceptor orbitals have much larger atomic populations of 10^{-10} ~ 10^{-7} in the vicinity of the midpoint between the donor and acceptor clusters, where the tunneling matrix element is evaluated by the flux theorem. The tunneling current theory thus has an advantage compared to the direct evaluation of the tunneling matrix element, which requires the overlap and integration over the whole space of two tunneling orbitals, and more susceptible to numerical errors.

By analyzing the donor, acceptor and bridge contributions to the corresponding donor and acceptor orbitals of N6a→N6b pair, it is found that the tunneling energy is ~3 eV higher than Fermi level in the middle of the energy gap between valence and conduction bands of the bridging peptide. This is reasonable since the tunneling orbitals are mainly from the *d*-orbitals of Fe atoms, which are off-resonant in energy with any orbitals of the protein bridge, which mainly consist of *s* and *p* orbitals of carbon, oxygen, nitrogen and hydrogen. This supports the notion that the electron transport occurs via quantum tunneling.

A signature of the wave properties of the tunneling electrons is observed when multiple electron tunneling pathways exist. Quantum interferences are pronounced in a characteristic peptide configuration where the peptide backbone loop connected to the donor is facing the cysteine ligand wire attached to acceptor in a few Å distance (N3→N1b and N5→N6a).

In N6a-N6b, electron tunnels along different pathways depending on the BS states involved. Considering that the spin states of the Fe/S clusters constantly change, driven by the thermal fluctuations of the protein local environment, the result suggests that the electrons tunneling pathways also fluctuate accordingly.

The averaging procedure of the coupling matrix element T_{DA} reflects the fact that many tunneling orbitals are statistically mixed in the course of protein dynamics. Different nearly degenerate electronic states are typically localized differently in the Fe/S cluster due to the anti-ferromagnetism; this means that the tunneling orbitals in the core regions are constantly changing in time, driven by the thermal dynamics of the protein environment. On the other hand, different tunneling orbitals, while having different structure at the cluster regions, are similar in the intervening protein space, where they extend their tunneling tails. It is the tail region of the wave functions that determines the efficiency of electron coupling between metal sites and the character of electron propagation in the protein matrix. Since the tunneling tails are more or less invariant in shape but depend drastically, in absolute value, on the orbital types, and since different orbitals with close energies are expected to be mixed in the course of thermal dynamics of the protein, a statistical renormalization of the tunneling orbitals and the corresponding tunneling matrix element is reasonable to evaluate the dynamical average of the coupling matrix element.

The internal water has been shown to accelerate the electron transfer kinetics so as to achieve the physiologically meaningful rate; the log of the electron transfer rate (with internal water included), as a function of distance between donor and acceptor has, the slope close to that of a typical protein known from experiments. Both results strongly suggest that the internal water play an essential role as a mediator of electron transfer in proteins in natural conditions.

The current calculations are performed on the frozen protein structure based on the X-ray crystal data [5]. The dynamical mixing of the donor and acceptor electronic states induced by the thermal fluctuations of the protein are effectively taken into account through the renormalization procedure described above. The agreement between the calculated and experimental turnover of the enzyme and the reproduced phenomenological relation between the electron transfer rates and the tunneling distances support that it is reasonable to employ the X-ray structure of the protein with internal water molecules for calculations. More details on the dynamical aspects of the electron transfer, including the thermal nuclear fluctuations of the peptide bridge regions, will be addressed in future studies.

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References

1. Hirst J. *Biochem J.* 2010; 425:325.
2. Zickermann, V.; Kerscher, S.; Zwicker, K.; Tocilescu, MA.; Radermacher, M.; Brandt, U. *BBA - Bioenergetics.* 2009.
3. Sazanov L. *Biochemistry.* 2007; 49:2275. [PubMed: 17274631]

4. Hirst J. *Biochem Soc T*. 2005; 33:525.
5. Sazanov LA, Hinchliffe P. *Science*. 2006; 311:1430. [PubMed: 16469879]
6. Saraste M. *Science*. 1999; 283:1488. [PubMed: 10066163]
7. Ohnishi T. *Biochim Biophys Acta, Bioenerg*. 1998; 1364:186.
8. Blondin G, Girerd JJ. *Chem Rev (Washington, DC, U S)*. 1990; 90:1359.
9. Noodleman L, Peng CY, Case DA, Mouesca JM. *Coord Chem Rev*. 1995; 144:199.
10. Mouesca JM, Lamotte B. *Coord Chem Rev*. 1998; 178:1573.
11. Noodleman L, Han WG. *J Biol Inorg Chem*. 2006; 11:674. [PubMed: 16830148]
12. Hayashi T, Stuchebrukhov AA. *Proc Natl Acad Sci USA*. 2010 (in print).
13. Gorelsky SI, Lever ABP. *J Organomet Chem*. 2002; 659:202.
14. Lalia-Kantouri M, Papadopoulos CD, Quiros M, Hatzidimitriou AG. *Polyhedron*. 2007; 26:1292.
15. Ball DM, Buda C, Gillespie AM, White DP, Cundari TR. *Inorg Chem*. 2002; 41:152. [PubMed: 11782157]
16. O'Brien TA, Davidson ER. *Int J Quantum Chem*. 2003; 92:294.
17. Karacan MS. *Can J Anal Sci Spectrosc*. 2007; 52:25.
18. Newton MD. *Theor Chem Acc*. 2003; 110:307.
19. Zheng XH, Stuchebrukhov AA. *J Phys Chem B*. 2003; 107:6621.
20. Stuchebrukhov AA. *Advances in Chemical Physics*. 2001; 118:1.
21. Stuchebrukhov AA. *Theor Chem Acc*. 2003; 110:291.
22. Mouesca JM, Chen JL, Noodleman L, Bashford D, Case DA. *J Am Chem Soc*. 1994; 116:11898.
23. Shoji M, Koizumi K, Taniguchi T, Kitagawa Y, Yamanaka S, Okumura M, Yamaguchi K. *Int J Quantum Chem*. 2007; 107:116.
24. Shoji M, Koizumi K, Kitagawa Y, Yamanaka S, Okumura M, Yamaguchi K. *Int J Quantum Chem*. 2007; 107:609.
25. Torres RA, Lovell T, Noodleman L, Case DA. *J Am Chem Soc*. 2003; 125:1923. [PubMed: 12580620]
26. All BS-ZINDO and BS-DFT calculations were performed using Gaussian 03 program.
27. Gehlen JN, Daizadeh I, Stuchebrukhov AA, Marcus RA. *Inorg Chim Acta*. 1996; 243:271.
28. Stuchebrukhov AA. *J Chem Phys*. 2003; 118:7898.
29. Cory MG, Zerner M. *J Phys Chem A*. 1999; 103:7287.
30. Amos AT, Hall GG. *Proceedings of the Royal Society of London Series a-Mathematical and Physical Sciences*. 1961; 263:483.
31. Newton MD. *Chem Rev (Washington, DC, U S)*. 1991; 91:767.
32. Goddard WA, Ladner RC. *J Am Chem Soc*. 1971; 93:6750.
33. Zhang, LY.; Murphy, R.; Friesner, R. A. *Ab Initio Quantum Chemical Calculation of Electron Transfer Matrix Element for Large Molecules*. Schrodinger, Inc; Portland, OR: 1997.
34. Newton MD, Ohta K, Zhong E. *J Phys Chem*. 1991; 95:2317.
35. Stuchebrukhov AA. *J Chem Phys*. 1998; 108:8510.
36. Marcus RA, Sutin N. *Biochim Biophys Acta*. 1985; 811:265.
37. Page CC, Moser CC, Chen XX, Dutton PL. *Nature*. 1999; 402:47. [PubMed: 10573417]
38. Gray, HB.; Winkler, JR. *Biological Inorganic Chemistry, Structure and Reactivity*. Bertini, I.; Gray, HB.; Stiefel, EI.; Valentine, JS., editors. University Science Books; Sausalito: 2007. p. 261
39. Dutton PL, Moser CC, Sled VD, Daldal F, Ohnishi T. *Biochim Biophys Acta, Bioenerg*. 1998; 1364:245.
40. Miyashita O, Okamura MY, Onuchic JN. *Proc Natl Acad Sci U S A*. 2005; 102:3558. [PubMed: 15738426]
41. Moser CC, Page CC, Dutton PL. *Philosophical Transactions of the Royal Society B-Biological Sciences*. 2006; 361:1295.
42. Lin JP, Balabin IA, Beratan DN. *Science*. 2005; 310:1311. [PubMed: 16311331]
43. Sazanov LA, Hinchliffe P. *Science*. 2006; 311:1430. [PubMed: 16469879]

Figure 1.

Crystal structure of the hydrophilic domain of the respiratory complex I from *Thermus thermophilus* [43] (left), formal charges and schematic anti-ferromagnetic spin configurations of the reduced (right top) and oxidized (right bottom) 4Fe-4S clusters. In each electron transfer process between neighboring Fe/S clusters, the electron is passed from the reduced donor to the oxidized acceptor. Only core (4Fe and 4S) atoms are shown without cysteine ligands for clarity in the right panels.

Figure 2.

Spin state diagram (left panel), density of spin states and integrated number of spin states (right panel) of $[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^{3-}$ cluster. The total 3000 states are grouped by total spin S to 380 clusters overlapping in energy [9]. The experimental value of the Heisenberg constant J is 250 cm^{-1} ; a few tens of low-lying states contribute to the electron tunneling.

Figure 3. HOMO of the $[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^{3-}$ BS ground electronic states calculated at the DFT (B3LYP/6-31G) (left) and ZINDO (right) levels. Both are in reasonable agreement.

Figure 4.

Calculated total flux of N6a→N6b across the dividing plane perpendicular to the line connecting the donor (N6a) and acceptor (N6b) complexes. The coordinate is normalized as 0 at the center of N6a and 1 at the center of N6b. The total flux is conserved in the vicinity of the transition (0.5), which gives the tunneling matrix element T_{DA} by the flux theorem (Eq. (10)).

Figure 5. Electron tunneling pathways of (A) N3→N1b, (B) N1b→N4, (C) N4→N5a, (D) N5→N6a, (E) N6a→N6b, (F) N6b→N2. Total atomic currents proportional to probability that the tunneling electron will pass through a given atom (Eq. (9)) is indicated by red color intensity. Through-space jumps are indicated by green arrows with distances in Å. When multiple paths are present, directions of the electron flux in the primary and secondary paths are marked by solid and dashed arrows, respectively. The arrows and numbers in Å are center-to-center distances between the donor and acceptor clusters. Mediator residues are highlighted with violet circles.

Figure 6.

Calculated complete electron tunneling pathways from FMN to N2 of complex I.

The atoms which have large electron tunneling probability (Eq. (9)) are shown in ball and stick representation with red color intensity corresponding to their total current densities.

The dashed lines indicate the subunit boundaries. Mediator residues of electron tunneling are marked with labels.

Figure 7. Natural logarithms of the simulated electron transfer rates (in s^{-1}) versus the tunneling distance between gateway atoms (in $\text{\AA}$). The negative slope of the least-squares line (solid line) is 1.3. The number is equivalent to 0.56 in base 10 logarithm, reproducing a typical value 0.6 in proteins [41].

Table 1

Distances, mediator residues, electronic Franck-Condon factors (FC), couplings $\langle T_{DA}^2 \rangle$, and calculated rates k_{ET} of ET in Complex I.

Pair	Distance [$\text{\AA}$]		Mediator	FC	$\langle T_{DA}^2 \rangle [\text{cm}^{-2}]$	$k_{ET} [\text{s}^{-1}]$
	C-to-C	G-to-G				
N3→N1b	14.0	11.0	$_3\text{Ala}^{63}$	0.50	4.6×10^{-4} (1.1)	1.3×10^3 (2.9×10^6)
N1b→N4	13.5	10.6	-	0.69	2.3×10^{-2}	6.4×10^4
N4→N5	12.2	8.7	$_3\text{Val}^{232}$	0.67	9.8	2.8×10^7
N5→N6a	16.8	14.0	$_3\text{Pro}^{120}$	0.75	3.2×10^{-6} (2.3×10^{-3})	9.1 (7.3×10^3)
N6a→N6b	12.1	9.3	-	0.60	0.98	2.8×10^6
N6b→N2	13.7	10.5	$_9\text{Ile}^{99}$	0.44	5.8×10^{-4} (5.3×10^{-2})	1.9×10^4 (1.8×10^6)

(C-to-C) and (G-to-G) are center-to-center and gateway-to-gateway atom distances. The gateway atoms are defined as core atoms of Fe/S clusters which give the largest contribution to the transition. When multiple G-to-G distances exist depending on the BS state pairs, the smallest one, which contributes most to ET, are shown. The mediator residues are the key residue, where majority of the flux passes along the main tunneling path, not including cysteine ligands. For couplings and rates, the numbers without parentheses are for the “dry” protein and the numbers in parentheses are for the protein with structural water included.