Electron tunneling through sensitizer wires bound to proteins

Matthew R. Hartings, Igor V. Kurnikov, Alexander R. Dunn, Jay R. Winkler, Harry B. Gray, Mark A. Ratner

1. Introduction

We have shown that electrons and holes can be delivered rapidly to the active sites of metalloenzymes through substrates (or ligands) linked to redox-active photosensitzers (“sensitizer wires”) [1–12]. Electron transfer (ET) is initiated by optical excitation of the sensitizer at the end of the wire and monitored by spectroscopic methods. Dramatic variations of ET rates through the wires have been observed upon changes in the chemical compositions of both linkers and substrates [1,3,6,10,12].

We would like to know the origin of these large variations in wire ET rates, as detailed understanding will aid the design of improved wires for the study of biological redox reactions.

There have been many investigations of the effects of molecular fluctuations on ET rates in bridge-mediated systems [13–25]. Bridge dynamics can have profound effects on ET kinetics in biological systems, as, for example, in conformationally gated processes.

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Gating occurs when the coupling between two cofactors is altered as a result of large-scale nuclear motions and the rate of charge transfer is greatly increased. Bridge dynamics can also enhance electronic coupling through equilibrium fluctuations of the torsional angles along the length of the bridge.

We have calculated the rates of electron transfer through Ru-diimine sensitizer wires to the heme of cytochrome P450. Using a combination of molecular dynamics and electronic coupling calculations, we have found that subtle rearrangements modulate electron flow from the Ru-diimine sensitizer through a wire to the heme. We have analyzed the effects of these rearrangements on the experimentally observed rates as well as other factors that might lead to more rapid electron transfer in these protein:wire conjugates.

2. Structures

Rapid heme reduction is successfully achieved through two similar photoprocesses in the case of five related Ru(II) sensitizer wires. In the first process, electronically excited Ru(II), or Ru(II)*, directly reduces the heme. The driving force of the reaction ($-\Delta G^\circ$) is dependent on the Ru(II) complex employed in the experiment. Here, we have examined two complexes: $[\text{Ru(2,2'}^-\text{-bipyridine)}_2\text{L}]^{2+}$ (bpyRu) and $[\text{Ru(4,4',5,5'-tetramethylbipyridine)}_2\text{L}]^{2+}$ (tmbpyRu). The (bpyRu)3+/2+* potential is $-0.62$ V vs NHE; (tmbpyRu)3+/2+* is $-0.75$ V vs NHE. In the second process, Ru(II)* is reductively quenched by an external redox partner, followed by Ru(I) to Fe(III) ET to produce an Fe(II) heme. The (bpyRu)2+/+ potential is $-1.24$ V vs NHE.

The sensitizer wires that have been investigated are shown in Fig. 1. Two types of hydrophobic bridges have been examined: aliphatic hydrocarbon chains (C11 and C13) and fluorinated biphenyls (F8bp). Three types of terminal substrate groups have been investigated: imidazole (Im), adamantane (Ad) and ethylbenzene (EB). One difference in the terminal groups that is significant for electron transfer is the ability of imidazole to coordinate directly to the Fe atom in Fe-porphyrins, while interactions of adamantane or ethylbenzene with the Fe-porphyrin are noncovalent in nature. We have performed calculations on $[\text{bpyRu-F8bp-Ad}]^{2+}$, which showed no heme reduction in flash/quench experiments, because it is similar to $[\text{bpyRu-F8bp-Im}]^{2+}$ and $[\text{tmbpyRu-F8bp-Im}]^{2+}$, whose electronic excited states are capable of directly reducing the Fe(III) heme on submicrosecond timescales.

3. Molecular dynamics simulations

Crystal structures are available for P450 conjugates with bpyRu-F8bp-Ad and bpyRu-C9-Ad (Fig. 2) wires. Other wires were constructed using molecular mechanics modeling. Substantial flexibility of the wires in conjugates with P450 is expected (as indicated by the presence of “alternative” structures in the X-ray determinations of P450 with bpyRu-F8bp-Ad and bpyRu-C9-Ad); therefore, molecular dynamics simulations were used to generate a representative set of configurations for the protein:wire conjugates.

Simulations of P450:wire conjugates were performed with the AMBER 7 suite of programs. Parameters for protein amino acids and the Fe-porphyrin were taken from the Amber-94 force field. Parameters for the carbon and hydrogen atoms of Ad and hydrocarbon bridges were taken as in aliphatic amino acid sidechains such as valine and leucine. Atomic charges for atoms of fluorinated biphenyls (F8bp) were set using Hartree–Fock ab initio calculations with a 6-31G* basis set and the Merz–Kollman charge-fitting scheme. The equilibrium geometry of F8bp was taken from an X-ray structure. Force-field parameters for a torsional angle defining the relative orientation of fluorinated-phenyl rings were ones that fit the potential energy profile obtained by constrained energy minimization of F8bp (the torsional angle value was a parameter in quantum chemical calculations) using the MP2 method and a 6-31G* basis set. The initial geometry for bpyRu-F8bp-Ad was taken from X-ray data. Initial geometries for other structures were generated by replacing the wire in the X-ray structure of bpyRu-F8bp-Ad or bpyRu-C9-Ad followed by energy minimization to remove unfavorable atom–atom contacts. 500-ps molecular dynamics trajectories were generated for conditions of constant pressure (1 atm) and constant temperature (300K). The first 100 ps of MD trajectories were discarded as nonequilibrated and snapshots were then collected at regular intervals along remaining portions of the trajectories.
4. Electronic coupling calculations

To compute electronic donor–acceptor interactions we used an energy splitting method where eigenstates of a model electronic Hamiltonian of the system that corresponds to the donor and acceptor electronic states are tuned into quasi-degeneracy by a Hamiltonian perturbation (in this work by applying an electric field in the donor–acceptor direction) [29–31]. The minimal energy splitting of these two eigenstates of the system is equal to twice the donor–acceptor electronic coupling $\Delta E_{\text{min}} = 2H_{DA}$. In Hartree–Fock calculations of donor–acceptor systems, $H_{DA}$ can be found from the energy splitting of eigenstates (molecular orbitals) of the one-electron Hamiltonian (Fockian) of the system. For our calculations we compute the energy splitting for both electron and hole transfer. To perform these calculations we either add an extra electron (for the case of electron transfer) or remove an electron (for the case of hole transfer) from the system in accordance with Koopman’s theorem and literature precedent [32–36].

In the ET experiments, the heme at the active site of P450 cycles between Fe(III) and Fe(II) redox states [1–12]. In this redox transition the electronic density changes mainly on the iron atom; therefore, it is likely that both the highest occupied molecular orbital of the Fe(II)-porphyrin and the lowest unoccupied molecular orbital of Fe(III)-porphyrin will be Fe-localized. We assume in our calculations that the occupied Fe-localized orbitals ($d_{xy}$, $d_{xz}$, $d_{yz}$) are equally involved in ET; and, the average ET coupling will be the root mean square (RMS) of ET couplings computed for individual Fe-porphyrin d orbitals.

For the case of computing $H_{DA}$ for electron transfer, the Ru-diimine donor was represented by a single dihydropyrazine ring. The HOMO of dihydropyrazine has the same symmetry and nodal structure as the LUMO of pyridine; thus, the dihydropyrazine HOMO is a reasonable model for the donor one-electron state.

In the second approach for calculating $H_{DA}$ for hole transfer, electrons were removed (to a formal Fe(VI) state). A large compensatory point charge ($-5e$) was placed on Fe; the resulting energies of Fe-localized unoccupied molecular orbitals are roughly $-4$ eV, in agreement with the expected electron binding energies of the acceptor with a reduction potential near $-0.3$ V vs NHE [37] ($0.0$ V vs NHE approximately corresponds to a $-4.5$ eV electron binding energy) [38]. The Ru-diimine donor electronic state was modeled in these calculations by the LUMO of the pyridinium cation. The computed value of the pyridinium cation LUMO energy was $-3.5$ eV in the Hartree–Fock calculations, in agreement with the reduction potential of the donor (approximately $-0.62$ V for the excited state and $-1.2$ V for the anion donor (vs NHE)) [39].

5. Bridge conformational dynamics

The molecular bridges we have investigated are quite flexible, and multiple conformations are observed in MD calculations. The importance of taking into account molecular bridge fluctuations while computing ET rates has been emphasized several times in the literature [13–23]. Fig. 3 (top) shows snapshots of MD trajectories of the P450 complex with a bpyRu-C13-Im wire. We found that the computed electronic coupling varies substantially for the different conformations of the P450:wire conjugate. Fig. 3 (bottom) shows the Fe to Ru ET-coupling ($H_{DA}$) values computed along the MD trajectory. Typically, the computed ET couplings vary by about an order of magnitude from configuration to configuration (corresponding to two orders of magnitude difference in the computed nonadiabatic ET rates). There are geometries with even smaller ET-coupling values, some smaller than the maximal coupling by a factor of 30. The observed rates can be accounted for by the contri-

![Fig. 2. Structure of bpyRu-C9-Ad bound to P450cam (pdb code: 1QMQ) [2].](image)

![Fig. 3. (Top) bpyRu-C13-Im conformations taken from snapshots along the MD simulation. This figure illustrates the large variety of structures available to a sensitizer wire. (Bottom) Average donor–acceptor (pyrazine–Fe) electronic couplings ($H_{DA}$) for different P450:bpyRu-C13-Im MD snapshots.](image)
Table 1
Donor–acceptor couplings (HDA) for electron and hole transfer were computed as described in Section 4. Electron transfer rates are computed from Eq. (1) using the maximum calculated HDA value (HDA = maximum(HDA(electron transfer)), HDA(hole transfer)) divided by the square root of six to account for all of the bipyridine ligands) and assuming $\lambda = 0.9$ eV. Driving forces and experimental rates are taken from references [1,6].

<table>
<thead>
<tr>
<th>Wire</th>
<th>MD RMS HDA (electron transfer) (eV)</th>
<th>MD RMS HDA (hole transfer) (eV)</th>
<th>Driving force, $\Delta G$ (eV)</th>
<th>ET rate computed (s$^{-1}$)</th>
<th>ET rate experimental (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[bpyRu-C11-Im]$^{2+}$</td>
<td>$1.5 \times 10^{-6}$</td>
<td>$5.1 \times 10^{-7}$</td>
<td>0.9</td>
<td>$1.6 \times 10^4$</td>
<td>$2 \times 10^7$ [1]</td>
</tr>
<tr>
<td>[bpyRu-C11-Ad]$^{2+}$</td>
<td>$1.7 \times 10^{-6}$</td>
<td>$8.6 \times 10^{-7}$</td>
<td>0.9</td>
<td>$2.1 \times 10^4$</td>
<td>$2 \times 10^7$ [1]</td>
</tr>
<tr>
<td>[bpyRu-C11-EB]$^{2+}$</td>
<td>$6.7 \times 10^{-7}$</td>
<td>$3.5 \times 10^{-7}$</td>
<td>0.9</td>
<td>$3.3 \times 10^3$</td>
<td>$2 \times 10^7$ [1]</td>
</tr>
<tr>
<td>[bpyRu-F8bp-Im]$^{2+}$</td>
<td>$8.3 \times 10^{-5}$</td>
<td>$2.4 \times 10^{-4}$</td>
<td>0.32</td>
<td>$1.1 \times 10^7$</td>
<td>$4 \times 10^7$ [6]</td>
</tr>
<tr>
<td>[tmbpyRu-F8bp-Im]$^{2+}$</td>
<td>$8.3 \times 10^{-5}$</td>
<td>$2.4 \times 10^{-4}$</td>
<td>0.45</td>
<td>$4.7 \times 10^7$</td>
<td>$3 \times 10^7$ [6]</td>
</tr>
<tr>
<td>[bpyRu-F8bp-Ad]$^{2+}$</td>
<td>$2.6 \times 10^{-6}$</td>
<td>$1.1 \times 10^{-6}$</td>
<td>0.32</td>
<td>$1.3 \times 10^4$</td>
<td>NA</td>
</tr>
</tbody>
</table>

Fig. 4. Experimental [1,6] and computed ET rates for P450:wire conjugates.
superexchange interactions are dominated by coupling through unoccupied electronic states of the aromatic bridge (probably because of the inductive effect of F-substitution, which lowers bridge energy levels). For the saturated hydrocarbon bridge C13–Im, the main contributor to donor–acceptor coupling is hole superexchange. The hole and electron contributions to the superexchange coupling differ by about a factor of two.

It is interesting to note that calculations predict slower electron transfer through the [bpyRu-F9bp-Ad]^{2+} wire. The Ru(II) excited state likely decays back to the ground state before electron transfer can occur. There are two main differences between this wire and F9bp analogues. The first is that it has an adamantyl terminal group that does not coordinate directly to the heme. The absence of terminal group–heme coordination likely reduces the overall donor–acceptor coupling. The second difference is that coupling for [bpyRu-F8bp-Ad]^{2+} is primarily due to hole superexchange, as is the case for all the sensitizer wires with aliphatic bridge groups. We calculate that [bpyRu-F9bp-Ad]^{2+} ET is slower than [bpyRu-C11-Ad]^{2+} ET, ΔG° is less than λ for [bpyRu-F9bp-Ad]^{2+}, while −ΔG° is equal to λ for [bpyRu-C11-Ad]^{2+}.

We turn now to comparisons of our work on P450cam with related sensitizer-wire experiments involving indoluc nitric oxide synthase (iNOS) [7–12]. Photoinduced Fe(III) heme reduction in the iNOS systems is much faster than in P450cam even though the donor–acceptor distances are similar (Table 2). For conjugates of iNOS with [Re-Im-F6bp-Im]^{1+} and [Re-Im-C5-F8bp-Im]^{1+}, we proposed that the redox process is initiated by rapid reduction of Re(II) by a nearby tryptophan residue, analogous to the first step in an experimentally validated azurin [Cu(1)-Trp-Re(1)] hopping model system [8,46]. We also found that an iNOS Fe(III) heme can be reduced rapidly by a surface-bound [bpyRu-F9bp]^{2+} sensitizer wire (k = 2 × 10^7 s^{−1}) [12] which could represent another case where multiple pathways couple the sensitizer to the edge of the heme [22].

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References