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   Electron-Transfer Reactions in Proteins: Electronic Coupling in Myoglobin

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13. ABSTRACT (Maximum 200 words)
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Electron-Transfer Reactions in Proteins: Electronic Coupling in Myoglobin

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Recent measurements of electron-transfer (ET) rates in Ru(NH$_3$)$_5$His myoglobin derivatives have shown the need for improved theories for treating the "path" of long-range ET. We have investigated these systems theoretically using a combined artificial intelligence (AI)--superexchange method. As in our previous articles, this model first employs an AI search technique that yields the important amino acid residues for the mediation of electrons between the donor and the acceptor. A quantum mechanical method is then used to diagonalize the orbitals of the selected protein subset and to calculate the electronic coupling. Encouraging agreement with experimental data is found, no adjustable parameters having been introduced. The results yield the relevant amino acid paths.

Introduction

Long-range electron transfer (ET) is central to many biological processes such as respiration and photosynthesis. Electron-transfer reactions in biological systems such as proteins have therefore been the subject of intense experimental and theoretical studies. In theories of electron-transfer reactions, factors which influence the rate of these reactions include the driving force ($-\Delta G^\circ$), a nuclear reorganization term ($\lambda$), the separation distance ($R$), and the nature of the medium separating the electron donor and the acceptor. In order to probe unambiguously the effect of the intervening medium on the rate of electron-transfer reactions in proteins, there have been several experimental investigations of intramolecular electron-transfer reactions in native and modified proteins. In this paper, we present results of theoretical studies on the distance and medium dependences of the rates of electron-transfer reactions in proteins, particularly the ruthenium-modified myoglobin derivatives studied experimentally by Gray and co-workers.

For nonadiabatic electron-transfer reactions, such as the long-range ET reactions in proteins, the rate constant for transfer of an electron from a donor to an acceptor can be expressed in terms of a Golden Rule type expression, namely, as the product of the square of an electronic coupling matrix element ($H_{DA}$) and a nuclear Franck-Condon factor ($FC$):

$$
\kappa_{ER} = \frac{2\pi}{h} |H_{DA}|^2 FC
$$

The Franck-Condon factor ($FC$) can be estimated quantum mechanically, semiclassically, or classically. Various expressions for FC are given, for example, in a recent review. In the classical limit, the Franck-Condon factor is given by

$$
FC = \frac{1}{(4\pi\lambda RT)^{1/2}} \exp\left(\frac{(-\Delta G^\circ + \lambda)^2}{4\lambda RT}\right)
$$

where $\Delta G^\circ$ is the standard free energy for the electron-transfer reaction at a fixed donor–acceptor separation distance $R$ and $\lambda$ is a reorganization term which contains both solvent (protein) and vibrational contributions.

The electronic matrix element, $H_{DA}$, describes the coupling of the orbitals of the donor (D) with the acceptor (A). Simple square barrier tunneling models have suggested an exponential decay of $H_{DA}$ with the distance $R$ separating the donor and the acceptor:

$$
H_{DA} = H_{DA}^{0} \exp(-\beta(R - R^0)/2)
$$

where $H_{DA}^{0}$ is the matrix element at van der Waals contact, $R^0$ ($R^0 \sim 3 \text{ Å}$), and $\beta$ is the distance decay factor. Such an exponential dependence has been experimentally and theoretically verified for several synthetic D–A systems, where $\beta$ has been found to be between 0.8 and 1.2 Å$^{-1}$. A recent analysis of ET rates in several biological systems, most particularly the bacterial photosynthetic reaction centers, by Dutton and co-workers has provided an overall support for the exponential decay model for $H_{DA}$ in biological ET reactions as well. However, in electron-transfer systems which are not structurally homogenous, such as proteins, the electronic structure of the protein medium coupling D and A, in addition to the separation distance, is expected to affect $H_{DA}$.

It has been shown that the electronic coupling in derivatives of cytochrome c does not scale uniformly with distance. The electronic coupling in these proteins is, on the other hand, satisfactorily explained by simple pathway models as well as by more sophisticated models which explicitly take into account the electronic structure of the protein medium. The model that we have previously introduced to treat electronic interactions in proteins combines an artificial intelligence (AI) approach with a quantum mechanical formulation of superexchange, without the introduction of any adjustable parameters. The relative values of electronic coupling elements obtained with this model were in good agreement with experimental results for the cytochrome c derivatives.

Recently, Gray and co-workers have experimentally studied ET in Ru-modified myoglobin derivatives. As noted by these authors, a simple pathway analysis does not adequately describe the electronic coupling in these systems. Nor did a multiple pathway model which neglected interferences between pathways. Presently, we investigate the electronic interactions in these myoglobin derivatives using our AI–superexchange method.

Theory

For donor–bridge–acceptor (D–B–A) electron-transfer systems, where the distance between D and A spans 10–20 Å, the direct
The proteins considered here are the ruthenium-modified myoglobin derivatives studied experimentally by Gray and coworkers. Three mutant human myoglobins were modified by replacing the heme by Zn mesoporphyrin (ZnP) and coordinating a pentaaaminoruthenium complex to a surface histidine residue (Ru HisXYM, where X = 70, 48, and 83). In the resulting singly ruthenated myoglobin derivatives, ET rates from ZnP to Ru were measured using transient absorption spectroscopy. The driving force for the reaction is 0.82 eV, and the reorganization energy, λ, has been estimated to be 1.3 eV from a study of the driving force dependence of ET rates in Ru His 48 Mb. Activationless ET rates, kact, were extracted from the observed ET rates, assuming λ = 1.3 eV for all the three derivatives.

In the calculational procedure, the donor is the Zn porphyrin group and the acceptor is the ruthenium histidine group. The coordinates of the Ru-modified myoglobins were obtained from the refined protein coordinates by performing a search of the ruthenated histidine conformations. The two side-chain dihedral angles, ψ2-Cα and Cβ-Cα, were each rotated by 1° to 360° to find the most stable conformer. The resulting structure files were used in the AI search.

For each of the three derivatives, His 70 Mb, His 48 Mb, and His 83 Mb, the search was allowed to select several paths, as described earlier. A preset threshold value for the net electronic coupling of any path was used to exclude particularly unimportant paths. Figures 1–3 show the amino acid residues that were selected by the search for each of the three myoglobin derivatives.
The experimental data. (In ref 8 and here, the simplest model, the porphyrin ring, and, hence, extended Hückel theory was also used to calculate HDA values of extended Hückel calculations are more reliable for relative values.)

The calculated values are (in s$^{-1}$) $8.0 \times 10^7$, $1.2 \times 10^8$, and $1.1 \times 10^8$ for the His 70 Mb, His 48 Mb, and His 83 Mb derivatives, respectively. The maximum rate constant for ET in these three derivatives as follows: The convergence of the search procedure, more tolerant cutoff values were used in the AI search enabling the selection of more amino acid residues.

Only 5–10 of the 153 possible amino acid residues are determined by the AI search as important in these protein-mediated ET reactions.

A quantum mechanical calculation of the electronic coupling matrix element was then performed using, as the bridge, the amine acid residues picked by the AI search. As in earlier papers, extended Hückel theory was used to calculate HDA values. These electronic couplings, given in Table I, are similar to the experimental values.

TABLE I: Calculated Electronic Couplings and Electron-Transfer Rates

<table>
<thead>
<tr>
<th>f (deriv)</th>
<th>$R$ (Å)</th>
<th>$H_{DA}$ (cm$^{-1}$)</th>
<th>(k&lt;sub&gt;max&lt;/sub&gt;/k&lt;sub&gt;max&lt;/sub&gt;)</th>
<th>calc</th>
<th>exp*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (His 70)</td>
<td>11.1</td>
<td>5.9 $\times$ 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>26</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>2 (His 48)</td>
<td>13.8</td>
<td>2.0 $\times$ 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3 (His 83)</td>
<td>17.6</td>
<td>7.0 $\times$ 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>0.03</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

* Reference 8.

The maximum rate constant for nonadiabatic ET reactions occurs when the driving force equals the reorganization energy, i.e., when the exponential factor in eq 2 is unity. The maximum or activationless rate constant is then given by eq 6 (using a classical approximation):

$$k_{max} = \frac{2\pi H_{DA}^2}{\hbar^2} \frac{1}{(4\pi \lambda RT)^{1/2}}$$

The calculated $k_{max}$ values are (in s$^{-1}$) $8.0 \times 10^7$, $1.2 \times 10^8$, and $1.1 \times 10^8$ for the His 70 Mb, His 48 Mb, and His 83 Mb derivatives, respectively. The square root of $k_{max}$ was 18, 1, and 0.01 for His 70 Mb, His 48 Mb, and His 83 Mb, respectively.

The calculated variation of $k_{max}$ is similar to the experimentally found variation, both of them showing an approximately exponential dependence with distance. However, the calculated values exhibit a steeper fall off with distance. In order to test the convergence of the search procedure, more tolerant cutoff values were used in the AI search enabling the selection of more amino acid residues per derivative. The additional amino acids selected were His 64 for the His 70 Mb derivative; Asp 44, Leu 49, Asp 60, His 64, and His 93 for the His 48 Mb derivative; and His 82, Ser 92, and Phe 138 for the His 83 Mb derivative. The values of $H_{DA}$ obtained with this more comprehensive calculation were $6.6 \times 10^2$, $3.6 \times 10^2$, and $4.2 \times 10^2$ cm$^{-1}$ and the ratio of the square root of $k_{max}$ was 18, 1, and 0.01 for His 70 Mb, His 48 Mb, and His 83 Mb, respectively (normalized with respect to the His 48 Mb derivative).

Comparing the calculated values of $H_{DA}$ in Table I, it is seen that there is no dramatic change in $H_{DA}$ due to the addition of more amino acid residues.

The resulting "extrapolation" from $k_{ET}$ to yield $k_{max}$ is not a large one—a factor of about 5.) Hence, it is preferable to compare the relative values of $k_{max}$ for these various derivatives, and, therefore, in Table I we give the ratios of $k_{max}$ with respect to the His 48 Mb derivative. We have also plotted the variation of both the calculated and experimental $k_{max}$ values with distance in Figure 4.

![Figure 3. Amino acid residues determined by the AI search for the His 83 derivative of myoglobin.](image)

![Figure 4. Variation of calculated and experimental $k_{max}$ with distance in the Ru-modified myoglobin derivatives. The filled circles refer to the experimental values, the hollow circles refer to the present calculations, the filled triangles refer to the multiple pathway results of ref 8, and the unfilled triangles refer to their single pathway results. The experimental and calculated $k_{max}$ for the His 70 Mb derivative ($R = 11.1$ Å), 7.2 $\times$ 10$^7$ and 8.0 $\times$ 10$^7$ s$^{-1}$, respectively, are too close to be distinguished in this plot. The $k_{max}$ obtained by the single pathway analysis for His 83 Mb ($R = 17.6$ Å) is 7.5 $\times$ 10$^7$ s$^{-1}$, which is off this graph. As noted in the text, it is more important to consider the variation of these $k_{max}$ values rather than their absolute values.](image)
without further correction, was taken to be the energy of the donor orbital, which is equal to that of the acceptor orbital in the transition state. The present work also neglects any effects due to conformational fluctuation of the protein coordinates at the electronic coupling.

For comparison, the values of $k_{\text{max}}$ from the Beratan-Onuchic pathway analyses of these same derivatives from ref 8 are also plotted in Figure 4. A B-O pathway is a single chain of atoms connecting the donor and the acceptor and the electronic coupling provided by a pathway is estimated by the number of links where each bond, hydrogen bond and through-space link contributes a parametrized decay factor. The two B-O pathway analyses in ref 8 differ, in that one of them uses a single pathway to estimate the electronic coupling while the other uses many such pathways and sums the electronic couplings of the individual pathways to yield the total coupling, neglecting interference between pathways. Again, it is important to note that in the pathway analyses of ref 8 only the variation of $k_{\text{max}}$ can be compared, since those analyses do not include the interaction between the donor (acceptor) with the first (last) bond of the pathway, which can cause a shift in the absolute value. As was pointed out by the authors of ref 8, while the inclusion of multiple pathways improves the agreement, neither of the two pathway analyses results in satisfactory agreement with the experimental data. Our procedure of estimating the electronic coupling automatically incorporates all possible amino acid paths within the subset of protein selected and further includes all possible interferences between these paths. It has been shown that inclusion of both the interferences between multiple paths and the non-nearest-neighbor interactions are important for describing electronic coupling in model complexes and proteins.23,24

It is of interest to compare the amino acid residues selected by the present AI search with the (atoms of the) amino acid residues involved in the pioneering pathway analyses of Beratan and coworkers.21 At present, only for the His 48 derivative have the amino acid residues from the pathway approach been identified. For this derivative, the pathway analysis yields 211 paths. All of the amino acid residues involved in these 211 paths, except one, are selected by the AI search (see Figure 2). The additional amino acid present in the pathway treatment is Asp 44. We find that our AI search proceeds from Phe 43 directly to Phe 46 or Lys 45 via through-space connections rather than involving Asp. R. H.; Schwarz, H. A.; Oevering, J.; Padden-Rose, M. N.; Heppener, M.; Oliver, A. M.; Cotsaris, E.; Verhoeven, J. W.; Hush, N. S.; Clossen, G. L.; Calabrese, L. E.; Green, N. J.; Penfield, K. W.; Miller, R. J.; Photoprocesses in Transition Metal Complexes, Biostructures and Other Molecules: Experiment and Theory: Kochanavage, E.; Ed.; Kluser: Norwaille, M.A.; 1992: p 49.


