Side Chains Affect Electron Tunneling Rates across Amino Acids

Tung-Chou Tsai and I-Jy Chang*

Department of Chemistry, National Taiwan Normal University, 88 Tingchow Road Section 4, Taipei 11718 Taiwan, The Republic of China

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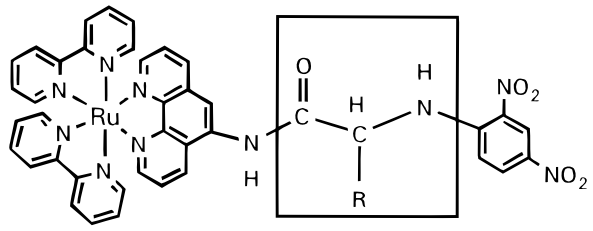
Highly efficient biological electron transfer (ET) has triggered extremely active research. Experimental studies of ET in modified proteins,[1−3] protein−protein complexes,[4,5] and enzymes[6−9] have provided a wealth of new data and many critical tests of theory. Based on the semiclassical theory,[10] the rate constant for nonadiabatic intramolecular ET (k_{ET}) is given by eq 1.

$$k_{ET} = (4\pi^3/h^2\lambda\, k_B T)^{1/2}(H_{AB})^2 \exp\{-(\Delta G^\circ + \lambda)^2/4\lambda\, k_B T\} \quad (1)$$

Experimental results indicated a small value of H_{AB}, the electronic coupling matrix element, for long-range electron transfer in proteins.[11,12] These results have prompted many theoretical investigation of long-range coupling in proteins. Kuki and Gruschus used an inhomogeneous aperiodic lattice (IAL) Hamiltonian that includes all the occupied orbitals from entire protein to calculate H_{AB}.[13] Marcus and Siddarth have developed an artificial intelligent searching to define the important parts of the protein between donor and acceptor followed by extended Hückel calculation.[14] A simple pathway model has been proposed by Beratan and Onuchic.[15] In this model, the electronic coupling can be evaluated from the product of decay factors for electron transfer through covalent bonds, hydrogen bonds, or space jumps along the pathway. A searching algorithm[16] has made the pathway model widely adapted. However, the common criticism of this model is the fact that all covalent bonds in the protein are treated the same.[14a] In particular, the side chains of individual amino acids have not been taken into account (unless they are directly involved in the main pathway).

Since the pathway model uses the product of decay factors, it would be straightforward to incorporate the difference decay factors for different side chains or C−C bond vs C−N bonds. However, lack of experimental data makes the improvement not practical. To assess the effects of side chains, we have constructed donor/acceptor complexes with single amino acid bridges



Complexes with three different amino acid bridges, glycine-, phenylalanine-, and isoleucine-, have been prepared. The absorption spectra of these complexes showed three bands. Bands around 280 and 450 nm are typical ruthenium polybipyridyl complexes and have been attributed to polypyridyl $\pi \rightarrow \pi^*$ transition and metal-to-ligand charge-transfer (MLCT), respectively.[17] The band around 350 nm is the $n \rightarrow \pi^*$ transition of the dinitrophenyl-amine moiety. Excitation into the MLCT band of these complexes results in luminescence maximizing near 605 nm. Table 1 summarizes the absorption and emission properties of these complexes.

Relative quantum yield measurements of the emission in aqueous solution are shown in Figure 1a. The emission of the donor−acceptor complexes is dramatically quenched. Since the dinitrophenyl-amine moiety does not absorb in the region of $[Ru(bpy)_2phenNH_2]^{2+}$ emission, energy transfer is not likely to be responsible for the quenching. Furthermore, the emission of the donor−acceptor complexes is not quenched in acetonitrile solution (Figure 1b). These results strongly indicate an electron transfer

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Table 1. Absorption and Emission Maximum of Donor−Acceptor Complexes

	absorption, nm ($\epsilon \times 10^{-4}$, M^{-1} cm^{-1})		emission, nm	
complexes	aqueous[a]	CH_3CN	aqueous[a]	CH_3CN
$Ru(bpy)_2phenNH_2^{2+}$	285 (6.9), 275sh (6.0)	285 (6.89)	608	608
	457 (1.5), 418sh (1.3)	457 (1.5), 420sh (1.3)		
D-gly-A	284 (5.9), 271sh (5.6)	284 (6.3), 275sh (6.4)	605	605
	345 (1.93)	339 (2.5)		
	450 (1.7),[b] 420sh (1.72)[b]	450 (1.75),[b] 420sh (1.8)[b]		
D-phe-A	284 (6.1), 271sh (5.9)	284 (7.4), 275sh (7.5)	605	605
	344 (2.3)	336 (3.2)		
	450 (1.7),[b] 420sh (1.75)[b]	450 (1.8),[b] 420sh (1.82)[b]		
D-ile-A	285 (7.4), 271sh (7.2)	285 (8.3), 275sh (8.4)	605	605
	346 (2.4)	339 (3.1)		
	451 (2.1),[b] 420sh (2.2)[b]	451 (2.2),[b] 420sh (2.25)[b]		

[a] Napi buffer (pH = 7, μ = 0.1 M). [b] Due to the mixing of the nπ* transition, the maximum of the MLCT bands were obtained from excitation spectra.

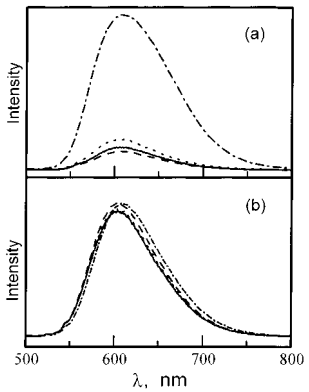

Figure 1. Emission spectra of $Ru(bpy)_2phenNH_2^{2+}$ (- − - − -), D-gly-A (···), D-phe-A (—), and D-ile-A (- - -) complexes in (a) Napi buffer (pH = 7, μ = 0.1 M) and (b) CH_3CN. The absorbance of all samples at the excitation wavelength (470 nm) were 0.14.

is responsible for the emission quenching of donor−acceptor complexes in aqueous solution. Acetonitrile is less polar than water and apparently cannot stabilize the charge separated species as evident in the absorption spectra of these complexes (Table 1). In acetonitrile solution, the n → π* transition exhibits large blue-shift, while the MLCT remains the same. Harder to populate the π* level on the dinitrophenyl-amine moiety results in lower ET reaction driving force. In this case, the driving force is not sufficient for ET reaction in acetonitrile solution; therefore, no quenching observed. This result implies that the driving force for ET reaction in aqueous solution is very small.

Similar results are found in excited-state lifetime measurements. Single-exponential decay rates for glycine-, phenylalanine-, and isoleucine-bridged complexes are $2.2(\pm0.1) \times 10^7$, $3.2(\pm0.1) \times 10^7$, and $3.7(\pm0.2) \times 10^7$ s^{-1}, respectively, in sodium phosphate buffer solution (pH = 7, μ = 0.1 M), while the decay rate of model compound, $[Ru(bpy)_2phenNH_2]^{2+}$, is $1.3(\pm0.2) \times 10^6$ s^{-1}. Decay rates for the donor−acceptor complexes in acetonitrile solution are in the proximity of 1.3×10^6 s^{-1}. Nanosecond transient absorption studies of the donor−acceptor complexes showed no detectable intermediate. The thermal back electron transfer is likely to be much faster than the photoinduced ET reaction. This result can be rationalized by the higher driving force for the back reaction.

From energy minimized structures of the complexes by using Insight II, the closest donor/acceptor distances through space are 4.4, 5.1, and 4.7 Å for gly-, phe-, and ile-bridged complexes, respectively. At such distances, ET by direct space jump cannot be responsible for the observed fast rates. Therefore, ET in these complexes must be through bonds. Although the electron-transfer rates do not vary considerably, they do correlate with the electron donating ability of the side chain for each amino acid. These results are consistent with a σ-hole tunneling coupling mechanism for electron transfer in metalloproteins. Noticeably, the electron-transfer rate across phenylalanine is slower than the isoleucine bridge. The π-bonds of the aromatic ring are energetically more accessible than the σ-bonds of the aliphatic amino acid, and aromatic groups have been postulated as important factors for long-range electron transfer in metalloproteins.[18,19] However, our results show that in σ-bond dominated electron-transfer pathways, remote π-orbitals do not facilitate electron transfer.

The ET rates reported in this paper strongly support a σ-tunneling superexchange mechanism for electron transfer across amino acids. Decay factors for individual amino acids can add precision to the pathway model without sacrificing its speed and ease of application for estimating long-range coupling in proteins.

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