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Electron tunneling in ruthenium-modified cytochrome *c*

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Distant Fe^{2+}-Ru^{3+} electronic couplings have been extracted from intramolecular electron-transfer rates in Ru(2,2′-bipyridine)$_2$(imidazole)(histidine-*X*) $^{2+}$ (*X* = 33,39,62,72,79) derivatives of cytochrome *c*. The rates are $> 1 \cdot 10^8$ (79); $3.2(4) \cdot 10^6$ (39); $2.6(3) \cdot 10^6$ (33); $9.0(3) \cdot 10^5$ (72); $1.0(2) \cdot 10^4$ s^{-1} (62); the couplings increase according to 62 (0.006) < 72 (0.057) < 33 (0.097) < 39 (0.11) < 79 (> 0.6 cm^{-1}). The rates (and the couplings) correlate with the lengths of σ-tunneling pathways comprised of covalent bonds, hydrogen bonds, and through-space jumps from the histidines to the heme group.

The electron-transfer (ET) reactions that occur within and between proteins typically involve prosthetic groups separated by large molecular distances (> 10 Å). An understanding of how the intervening medium, driving force, and nuclear reorganization energetics and dynamics modulate these long-range protein ET reactions has been a central goal in this field [1–22]. Of particular interest has been the mechanism by which the peptide matrix promotes electronic coupling between distant redox sites [4–10]. Our investigations of electron transfer in ruthenium-modified proteins have suggested that the structure of the peptide between the donor and acceptor controls the coupling [1,12,16–20].

We have studied intramolecular electron transfer by attaching photoactive Ru complexes to protein surfaces [17]. Ru(bpy)$_2$(CO$_3$) has been shown to react with surface His residues to yield, after addition of excess imidazole (im), Ru(bpy)$_2$(im)(His)$^{2+}$ [21]. The protein-bound Ru complexes are luminescent, but the excited states ($*Ru^{2+}$) are rather short lived ($\tau \leq 100$ ns). When direct ET from $*Ru^{2+}$ to the heme ($*k_{ET}$) cannot compete with excited-state decay (k_D), ET quenchers (e.g., $Ru(NH_3)_6^{3+}$) are added to the solution to intercept a small fraction (1–10%) of the excited molecules, yielding (with oxidative quenchers) Ru^{3+}. If, before laser excitation of the Ru site, the heme is reduced, then $Fe^{2+} \rightarrow Ru^{3+}$ ET ($^bk_{ET}$) can be monitored by transient absorption spectroscopy. We have used this method to examine ET rates in five different modified cytochromes: (Ru(His33), $^bk_{ET} = 2.6(3) \cdot 10^6$ s^{-1}; Ru(His39), $3.2(4) \cdot 10^6$ s^{-1}; Ru(His62), $1.0(2) \cdot 10^4$ s^{-1}; Ru(His72), $9.0(3) \cdot 10^5$ s^{-1}; Ru(His79), $> 10^8$ s^{-1}). Only in Ru(His79) did $*Ru^{2+} \rightarrow Fe^{3+}$ ET measurably accelerate excited-state decay; the rates for this reaction in the four other Ru(bpy)$_2$-modified proteins were determined from the small yields of Ru^{3+}-Fe^{2+} detected by transient absorption spectroscopy: (Ru(His33), $*k_{ET} = 2(1) \cdot 10^5$ s^{-1}; Ru(His39), $1.4(5) \cdot 10^6$ s^{-1}; Ru(His62), $1.1(2) \cdot 10^5$ s^{-1}; Ru(His72), $3.4(7) \cdot 10^5$ s^{-1}; Ru(His79), $> 5 \cdot 10^7$ s^{-1}) [20].

According to semiclassical ET theory, rates become activationless when the reaction driving force ($-\Delta G°$) equals the reorganization energy (λ) [2]. The driving force (0.74 eV) is approximately equal to the reorganization energy (0.8 eV) estimated for the Ru(bpy)$_2$(im)(His)-cyt *c* reactions [18]. The activationless (maximum) rates are limited by an electronic factor, $k_{max} = (\pi/\hbar^2 \lambda k_b T)^{\frac{1}{2}} H_{AB}^2$, where H_{AB} is the matrix element that couples the reactants and products at the transition state. Values of k_{max} and H_{AB} for the $Fe^{2+} \rightarrow Ru^{3+}$ ET reactions are given in Table I.

TABLE I

Electron-transfer parameters for Ru(bpy)$_2$(im)(HisX)-cytochromes c

X	[Fe^{2+}-Ru^{3+}]			
	k_{max} (s^{-1})	H_{AB} (cm^{-1})	*d* (Å)	$\sigma\ell$ (Å)
79	$> 1.0 \cdot 10^8$	> 0.6	4.5	11.2
39	$3.3 \cdot 10^6$	0.11	12.3	19.6
33	$2.7 \cdot 10^6$	0.097	11.1	19.5
72	$9.4 \cdot 10^5$	0.057	8.4	24.6
62	$1.0 \cdot 10^4$	0.006	14.8	28.8

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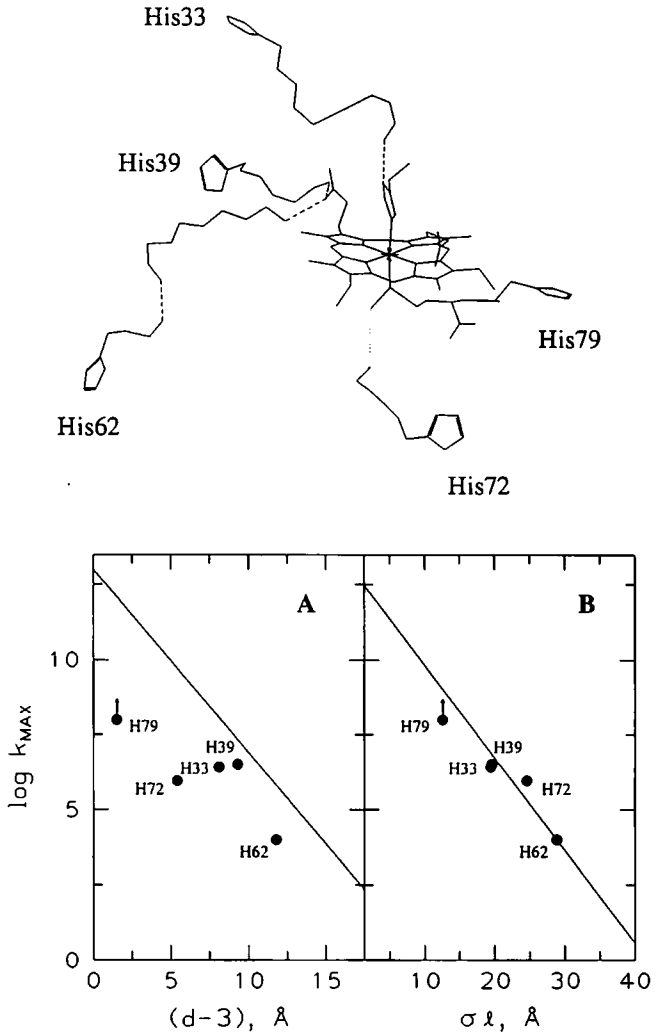

Fig. 1. Top: electronic-coupling pathways to the heme from Ru-modified residues in cytochrome *c*. (A) Maximum ET rates vs. *d* minus 3 Å (van der Waals contact). Exponential-decay line with $1 \cdot 10^{13}$ s^{-1} intercept and 1.4 $Å^{-1}$ slope. (B) Maximum ET rates vs. $\sigma\ell$: 0.71 $Å^{-1}$ slope; $3 \cdot 10^{12}$ s^{-1} intercept.

Activationless intraprotein ET spanning 12 orders of magnitude in rate and nearly 19 Å in redox-site separation has been interpreted in terms of edge-edge distance (d) exponential decay with 1.4 $Å^{-1}$ slope and $1 \cdot 10^{13}$ s^{-1} intercept [22]. Implicit in this interpretation is the assumption that the intervening polypeptide can be treated as a homogeneous medium. This correlation, then, serves as a reference line; deviations from this line indicate situations in which inhomogeneities of the intervening peptide must be considered. Since at least one ($X = 72$) of our k_{max} values falls over three orders of magnitude below this edge-edge exponential-decay line (there will be two rates in this category if, as is likely, the $X = 79$ k_{max} is below 10^{10} s^{-1}) (Fig. 1A), and two others ($X = 33,62$) deviate from the line by more than a factor of 50, analysis in terms of the structure of the intervening medium is called for.

Several models that take into account the inhomogeneity of the protein have been developed [5–11]. Beratan and Onuchic describe the coupling between redox centers in a protein in terms of pathways comprised of covalent, H-bonded, and through-space contacts [5,6]. An algorithm has been developed that searches a protein structure for the best pathways coupling two sites in a protein (the pathways between the histidines (33,39,62,72,79) and the heme are shown in Fig. 1). A given coupling pathway consisting of covalent, H-bonded, and through-space links can be described in terms of an equivalent covalent pathway with an effective number of covalent bonds (n_{eff}). Multiplying the effective number of bonds by 1.4 Å/bond gives σ-tunneling lengths ($\sigma\ell$) for the five pathways (Table I) that correlate well with the maximum ET rates (one-bond limit set at $3 \cdot 10^{12}$ s^{-1}; slope of 0.71 $Å^{-1}$) (Fig. 1B). The 0.71 $Å^{-1}$ decay accords closely with related distance dependences for covalently coupled donor-acceptor molecules [23].

We conclude that the structure of the intervening medium influences distant electronic couplings in cytochrome *c*. Importantly, Gruschus and Kuki have made inhomogeneous-aperiodic-lattice-model calculations on three derivatives ($X = 33,39,62$) that are in good agreement with our experimentally derived values (Gruschus, J.M. and Kuki, A., unpublished data).

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