# What Controls the Rates of Interprotein Electron-Transfer Reactions

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## ABSTRACT

Rates of electron-transfer (ET) reactions are dependent on driving force, reorganizational energy, distance, and the nature of the medium which the electron must traverse. In kinetically complex biological systems, non-ET reactions may be required to activate the system for ET and may also influence the observed rates. Studies of ET from tryptophan tryptophylquinone to copper to heme in the methylamine dehydrogenase–amicyanin–cytochrome $c$-551i ET complex, as well as studies of other physiologic redox protein complexes, are used to illustrate the combination of factors which control rates of interprotein ET reactions.

## Introduction

Long-range interprotein electron transfer (ET) is fundamental to respiration, photosynthesis, and redox reactions of intermediary metabolism. It involves donor and acceptor proteins with redox centers that are separated by relatively long distances, and the overall redox process may require several reaction steps including specific binding of proteins, protein rearrangements, and chemical transformations. As such, a complete understanding of an interprotein ET reaction requires a multidisciplinary approach that includes physical, kinetic, and biochemical analyses. The rates of many protein ET reactions have been reported in the literature. However, simple knowledge of the reaction rate tells us nothing about mechanism, and such data are often grossly overinterpreted. It is sometimes incorrectly assumed that the observed rate is a true ET rate constant ($k_{ET}$). However, the rate that is associated with the change in the redox states of the reactants is not necessarily $k_{ET}$, since the redox reaction may be rate-limited by a non-ET process. Alternatively, it is sometimes incorrectly assumed that if the reaction rate is not extremely fast, then the reaction must be rate-limited by some other process. However, this will be true only when ET reactions occur over very short distances and exhibit large driving forces. In biological systems, ET reactions typically have low driving forces, sometimes with $\Delta G° > 0$, and often span distances greater than 10 Å. For such reactions, $k_{ET}$ may be quite slow.

This Account describes the physical and kinetic parameters which determine rates of biological ET reactions. It will first review how nonadiabatic ET reactions differ from adiabatic chemical reactions, and why an alternative to transition state theory must be used to explain their thermodynamic behavior. Second, it will describe how adiabatic non-ET processes can modulate the observed rates of redox reactions, and how kinetic data from such systems must be interpreted. Last, to illustrate these points, specific examples are given of protein ET reactions whose rates are subject to different types of regulation.

## ET Theory

Unlike adiabatic chemical reactions which involve the making and breaking of bonds and proceed via a well-defined reaction coordinate, the substrates and products of a nonadiabatic protein ET reaction are often chemically indistinguishable. For an adiabatic reaction, the probability of the reaction occurring when the activation energy is achieved is approximately unity. When a chemical reaction is adiabatic, the temperature dependence of its rate may be fit to the Eyring equation to yield values for the enthalpies and entropies of activation, which describe the transition state for the reaction. For a nonadiabatic reaction, however, the probability of the reaction occurring when the activation energy is achieved is much less than one. Thus, description of nonadiabatic ET reactions requires a modified form of transition state theory (eq 1).[1] The activation free energy for the reaction

$$k_{ET} = \frac{4\pi^2 H_{AB}{}^2}{h\sqrt{4\pi\lambda RT}} \exp[-(\Delta G° + \lambda)^2/4\lambda k_B T] \qquad (1)$$

is equal to $(\Delta G° + \lambda)^2/4\lambda$, where $\Delta G°$ is the driving force determined from the redox potential difference for the reaction and $\lambda$ is the reorganizational energy. The degree of nonadiabicity (i.e., the probability of the reaction occurring in the transition state) is related to the electronic coupling between reactants and products in the transition state ($H_{AB}$). The other parameters are Planck's constant ($h$), the Boltzmann constant ($k_B$, or alternatively the gas constant), and temperature ($T$). It should be noted that quantum-mechanical effects are neglected in eq 1. To correct for this an additional term, the characteristic frequency ($h\omega/2\pi$), must be included.[2,3] However, this correction is required only when $h\omega/2\pi > k_B T$.

The definition of $\lambda$ is the energy needed to deform the nuclear configuration from the reactant to the product state. This includes the donor and acceptor pair before and after ET and is comprised of two components. The inner-sphere reorganizational energy ($\lambda_i$) reflects redox-dependent nuclear perturbations of the redox centers, such as changes in bond lengths. The outer-sphere reorganizational energy ($\lambda_o$) reflects changes in the surrounding medium, such as changes in solvent orientation.

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For redox centers that are buried within a protein, $\lambda_o$ may also include configurational changes in the protein matrix, and for interprotein reactions the interface between donor and acceptor proteins. Direct measurement of $\lambda$ in proteins is difficult. Methods have been developed to calculate $\lambda$ in proteins.[4] While such calculations have provided a basis for beginning to understand the determinants of $\lambda$ in proteins, such as protein polarization, several issues are still unresolved for complex biological ET reactions. Little is known of the effects on $\lambda$ of redox-dependent reorientation of the interface between proteins during interprotein ET. Also, most model studies have focused on metal cofactors which function only as ET agents. The contribution to $\lambda$ from organic cofactors, such as flavins and quinones, which may be partially exposed to solvent at the active site and which may couple catalytic reactions to ET, is not well understood. As such, there is still no generally accepted benchmark as to what may be considered a reasonable $\lambda$ value for an interprotein ET reaction.

In simple systems, $H_{AB}$ and consequently $k_{ET}$ will decrease approximately exponentially with distance. This is reflected in eq 2,[1] where $k_o$ is the characteristic

$$k_{ET} = k_o \exp[-\beta(r - r_o)] \exp[-(\Delta G° + \lambda)^2 / 4\lambda RT] \quad (2)$$

frequency of the nuclei (approximately $10^{13}$ s$^{-1}$). It is the maximum rate when donor and acceptor are in van der Waals contact and $\lambda = -\Delta G°$. The donor-to-acceptor distance is $r$, and $r_o$ is the close contact distance (3 Å). The parameter $\beta$ is used to quantitate the nature of the intervening medium with respect to its efficiency to mediate ET. Values of $\beta$ have been empirically determined for certain well-defined media.[3] The distance dependence of $k_{ET}$ through proteins, however, is difficult to infer from the results obtained for nonprotein model systems because the protein is not a homogeneous medium, and because all proteins are not identical. Average $\beta$ values of 0.7–1.4 Å$^{-1}$ have been proposed for use in analyzing protein ET reactions.[3,5]

An important question is whether it is really appropriate to use a single average $\beta$ value to describe the intervening medium between redox centers. It may be more accurate to view the protein as a nonhomogeneous matrix with different $\beta$ values for different intervening segments. This viewpoint forms the basis for the so-called *Pathways* or *Greenpath* analysis,[6] which calculates the relative efficiency of all possible ET pathways according to eq 3, where $i$ ranges over the pathway steps and $\epsilon_i$ is a

$$H_{AB} \propto \Pi \epsilon_i \quad (3)$$

wave function decay factor for each step in the pathway. The values assigned for $\epsilon$ are 0.6 for transfer through a covalent bond, 0.36 e$^{-1.7(r-2.8)}$ for transfer through a hydrogen bond, and 0.6 e$^{-1.7(r-1.4)}$ for a through-space jump.[6]

Given the uncertainty in $\beta$ for protein ET reactions, it is not possible to calculate with precision an ET distance from analysis by eq 2 of the variation of $k_{ET}$ with $T$ or $\Delta G°$.
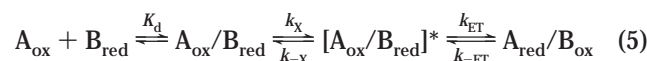
Reasonable estimates of ET distance have, however, been experimentally determined in this manner by fitting data to eq 2 using a range of $\beta$ values (discussed later).

## Kinetic Complexity of Protein ET Reactions: When Is an ET Rate Constant Really an ET Rate Constant?

Having addressed the question of what physical parameters determine $k_{ET}$, we now address another difficult question. How does one know if the measured rate for a redox reaction between proteins is really the rate of the actual ET event? For redox reactions involving proteins, the actual meaning of the kinetically determined limiting first-order rate constant for the ET reaction ($k_3$ in eq 4) must be interpreted with caution. It may not be a true

$$A_{ox} + B_{red} \overset{K_d}{\rightleftharpoons} A_{ox}/B_{red} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} A_{red}/B_{ox} \quad (4)$$
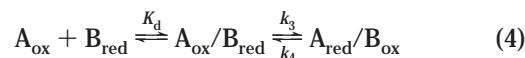
$k_{ET}$.[7] Protein dynamics (i.e., transient formation of unstable conformational intermediates) or catalytic events (e.g., protonation/deprotonation) may contribute to the observed rate. In kinetic models that are used to analyze these data, any spectroscopically invisible or otherwise undetectable events, after binding and preceding the spectral change associated with the redox reaction, will be reflected in this rate constant. In this case, eq 4 is more correctly written as eq 5, where $k_X$ is the rate constant for

$$A_{ox} + B_{red} \overset{K_d}{\rightleftharpoons} A_{ox}/B_{red} \underset{k_{-X}}{\overset{k_X}{\rightleftharpoons}} [A_{ox}/B_{red}]^* \underset{k_{-ET}}{\overset{k_{ET}}{\rightleftharpoons}} A_{red}/B_{ox} \quad (5)$$

this prerequisite adiabatic reaction step. Three situations are considered below in which the ET event is preceded by some reversible prerequisite non-ET event, and specific examples of each are presented in Table 1 and discussed later.

**True Electron Transfer.** The ET step is the slowest step in the overall redox reaction, so $k_3 = k_{ET}$. When analyzed by eq 1, the experimentally determined $\lambda$ includes no contributions from non-ET reaction steps. It reflects nuclear displacements in the redox centers, protein matrix, and solvent. $H_{AB}$ will be within the nonadiabatic limit (often taken to be <80 cm$^{-1}$),[8] and a reasonable ET distance may be estimated from the data using eq 2 (Table 1).

**Coupled Electron Transfer.** The preceding adiabatic reaction step influences the experimentally determined rate constant, even though $k_{ET}$ is rate-limiting for the overall reaction. This occurs when the relatively fast reaction step which precedes ET is very unfavorable (i.e., $K_X = k_X/k_{-X} \ll 1$). In this case, $k_3$ will be influenced by the equilibrium constant for that non-ET process such that $k_3 = k_{ET}K_X$. It follows that the experimentally derived $\lambda$ ($\lambda_{obs}$) will contain contributions from both the ET event and the preceding reaction step (i.e., $\lambda_{obs} = f[\lambda_{ET}, \lambda_X]$). The experimentally determined $H_{AB}$ may also be an apparent value if ET is coupled.

It is noteworthy that many of the nonadiabatic ET reactions listed in Table 1 exhibit $\lambda$ values which are large

**Table 1. Physiologic Interprotein Redox Reactions That Have Been Analyzed by ET Theory[a]**

| proteins | donor | acceptor | $r$ (Å) from structure | $r$ (Å) calculated | $H_{AB}$ (cm$^{-1}$) | $\lambda$ (eV) | reaction type | ref |
|---|---|---|---|---|---|---|---|---|
| O-quinol methylamine dehydrogenase/ amicyanin | TTQ[b] | Cu$^{2+}$ | 9.4 | 9.5 | 12 | 2.3 | nonadiabatic | 22 |
| N-quinol methylamine dehydrogenase/ amicyanin | TTQ | Cu$^{2+}$ | 9.4 | −4.9 | 12 000 | 3.3 | gated | 24 |
| amicyanin/ cytochrome $c$-551i | Cu$^{+}$ | heme | 23 | 13−24 | 0.3 | 1.1 | nonadiabatic | 27 |
| O-quinol aromatic amine dehydrogenase/ azurin | TTQ | Cu$^{2+}$ | nd[c] | 15.2 | 0.13 | 1.6 | nonadiabatic | 28 |
| N-quinol aromatic amine dehydrogenase/ azurin | TTQ | Cu$^{2+}$ | nd | −1.6 | 820 | 3.1 | gated | 28 |
| nitrogenase (wild type) | 4Fe/4S cluster | 8Fe/7S P-cluster | 14 | −84 | $7 \times 10^{12}$ | 4.4 | gated | 29 |
| nitrogenase (L127D) | 4Fe/4S cluster | 8Fe/7S P-cluster | 14 | 14 | 0.9 | 2.4 | nonadiabatic | 29 |
| methanol dehydrogenase/ cytochrome $c$-551i | PQQ | heme | nd | 15 | 0.07 | 1.9 | nonadiabatic | 9 |
| rubredoxin reductase/rubredoxin | FAD | Fe$^{3+}$ | nd | −12 | $2 \times 10^{6}$ | 4.0 | gated | 32 |

[a] For each of these reactions the temperature dependence of $k_{ET}$ was analyzed according to eqs 1 and 2. [b] The different redox forms of TTQ are shown in Figure 2. [c] nd, not determined because a crystal structure of the complex is not available.

relative to what has been reported for nonphysiologic model systems. It may be that kinetically coupled ET is a common feature of physiologic interprotein redox reactions. It is difficult to distinguish between true and coupled ET reactions from only the results of thermodynamic analysis. Alternative biochemical approaches, such as those described later for the methanol dehydrogenase−cytochrome $c$-551i system,[9,10] and use of site-directed mutagenesis with the goal of selectively altering $\lambda$ will be necessary to better understand the physical and kinetic basis for the large $\lambda$ values that are associated with many physiologic ET reactions.

**Gated Electron Transfer.** The adiabatic reaction step that precedes ET is rate-limiting, so the observed rate is actually that of a non-ET event (i.e., $k_3 = k_X$ ). In contrast to true and coupled ET reactions, the rate constant for a gated reaction will not exhibit a predictable dependence on $\Delta G°$ since this reaction step is not being driven by the redox potential difference between the reactants. The reaction will still vary with temperature, but if the dependence of rate on temperature is analyzed by eq 1, the values which are obtained for $\lambda$ and $H_{AB}$ will be unrelated to the ET event. Since it is often not possible to vary $\Delta G°$ for physiologic interprotein ET reactions, analysis of the temperature dependence of the rates of these reactions by eq 1 may be useful for determining whether the ET reaction is gated. This is not necessarily obvious. When the temperature dependence of a rate constant for a gated reaction is analyzed by eq 1, the fitted value of $H_{AB}$ will likely exceed the nonadiabatic limit. In fact, analyses of gated ET reactions by eqs 1 and 2 have yielded unreasonably large values for $H_{AB}$ and $\lambda$, and in some cases negative fitted values for the ET distance (Table 1).

## ET Reactions in the Methylamine Dehydrogenase−Amicyanin−Cytochrome $c$-551i Complex

Methylamine dehydrogenase (MADH), amicyanin, and cytochrome $c$-551i from *Paracoccus denitrificans* form one of the best-characterized ET complexes of proteins. It has provided a powerful system with which to study mechanisms of interprotein ET. The crystal structure of this ternary protein complex has been determined.[11] The orientation of the three redox centers in the crystal structure and the direct distance which separates tryp-tophan tryptophylquinone [TTQ] of MADH, copper of amicyanin, and heme of cytochrome $c$-551i are shown in Figure 1A. A *Pathways* analysis of the crystal structure is shown in Figure 1B. In the crystalline state, the complex catalyzes methylamine oxidation and subsequent ET from TTQ to heme via copper, as demonstrated by substrate-dependent spectral changes viewed by single-crystal polarized absorption spectroscopy.[12] This is a physiologically relevant complex in which amicyanin is an obligatory mediator of ET from MADH to the cytochrome. The amicyanin gene is located immediately downstream of that for MADH, and inactivation of the former results in loss of the ability to grow on methylamine.[13] MADH, amicyanin, and cytochrome $c$-551i are isolated as individual soluble proteins, but they must form a ternary complex to catalyze methylamine-dependent cytochrome $c$-551i reduction.[14,15] Although it is thermodynamically favorable, MADH does not reduce cytochrome $c$-551i in the absence of amicyanin, presumably because the proteins do not interact in an orientation that allows for productive ET. Reduced amicyanin will not efficiently donate electrons to cytochrome $c$-551i in the absence of MADH at physiologic pH because the redox potential of amicyanin is more positive than that of the cytochrome. The redox properties of amicyanin are altered on complex formation with MADH so as to facilitate the reaction.[16] Other structurally similar type I copper proteins, plastocyanin and azurin, do not effectively substitute for amicyanin.[14,17]

**Gated and Ungated ET from TTQ to Copper.** ET between MADH and amicyanin was studied in solution by stopped-flow spectroscopy. The reactions of four redox forms of MADH were characterized (Figure 2): dithionite-reduced O-quinol and O-semiquinone forms,[18] and substrate-derived N-quinol[19] and N-semiquinone[20] forms. The reactions of three of these redox forms with amicyanin exhibited a predictable dependence on $\Delta G°$. Analysis of the $\Delta G°$ dependence of $k_{ET}$[21] (Figure 3A) yielded values of $\lambda$ and $H_{AB}$ that were identical to those obtained from analysis of the temperature dependencies of $k_{ET}$ for the reactions with amicyanin of the O-quinol[22] and N-semiquinone[20] (Figure 3B). These analyses also predicted an ET distance that closely matched that seen in the crystal structure (Table 1). In contrast, analysis of the temperature dependence of the reaction between amicyanin and the
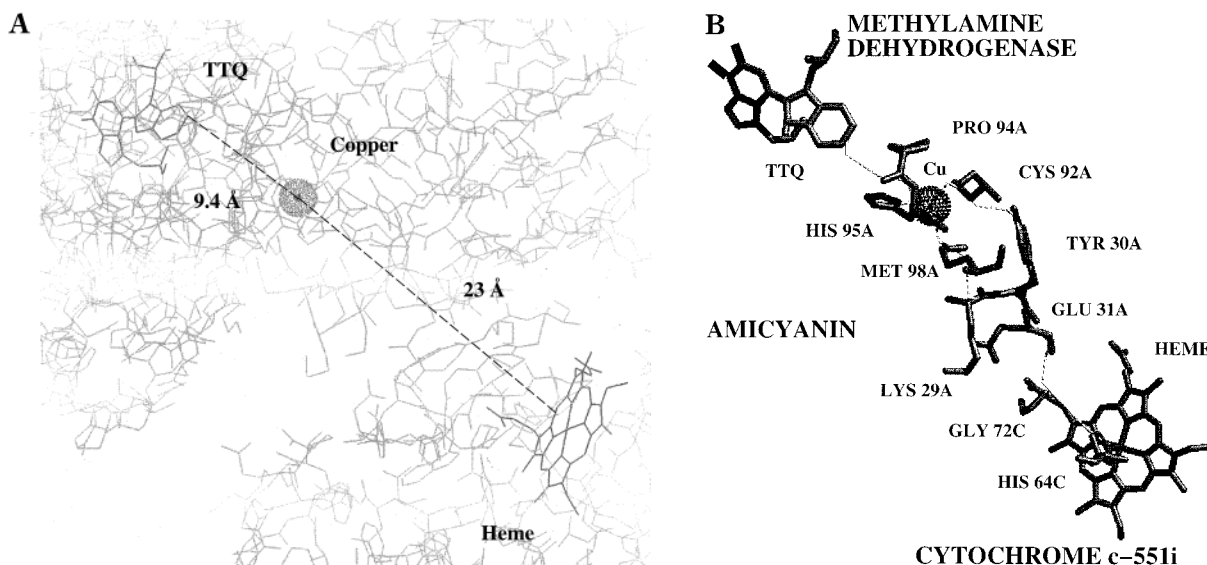
**FIGURE 1.** (A) Orientation of redox cofactors in the MADH—amicyanin—cytochrome *c*-551i complex. A portion of the crystal structure is shown with the direct distances between the cofactors indicated. Coordinates are available in the Brookhaven Protein Data Bank, entry 2MTA. (B) Results of a *Pathways* analysis of the crystal structure.[22,27] The most efficient predicted ET pathways between redox centers are shown (i.e., at least an order of magnitude more efficient than alternative pathways). The pathway from TTQ to copper involves a through-space jump (dashed line) from the surface-exposed Trp108 indole ring of TTQ to the carbonyl O of Pro94 of amicyanin, followed by passage through six covalent bonds (solid line) via the His95 ligand to copper. Two dominant sets of pathways were predicted from copper to the heme iron. In each, the point of intermolecular ET was from the backbone O of Glu31 of amicyanin to the backbone N of Gly72 of cytochrome *c*-551i, and the entry of electrons to iron occurred via either the porphyrin ring or the His61 ligand. In one, the exit of electrons from copper occurred via the Cys92 copper ligand, and the phenolic side chain of Tyr30 was an intermediate between Cys92 and Glu31. In the other, the exit of electrons from copper occurred via the Met98 copper ligand, and the backbone of Lys29 was an intermediate between Met98 and Glu31.
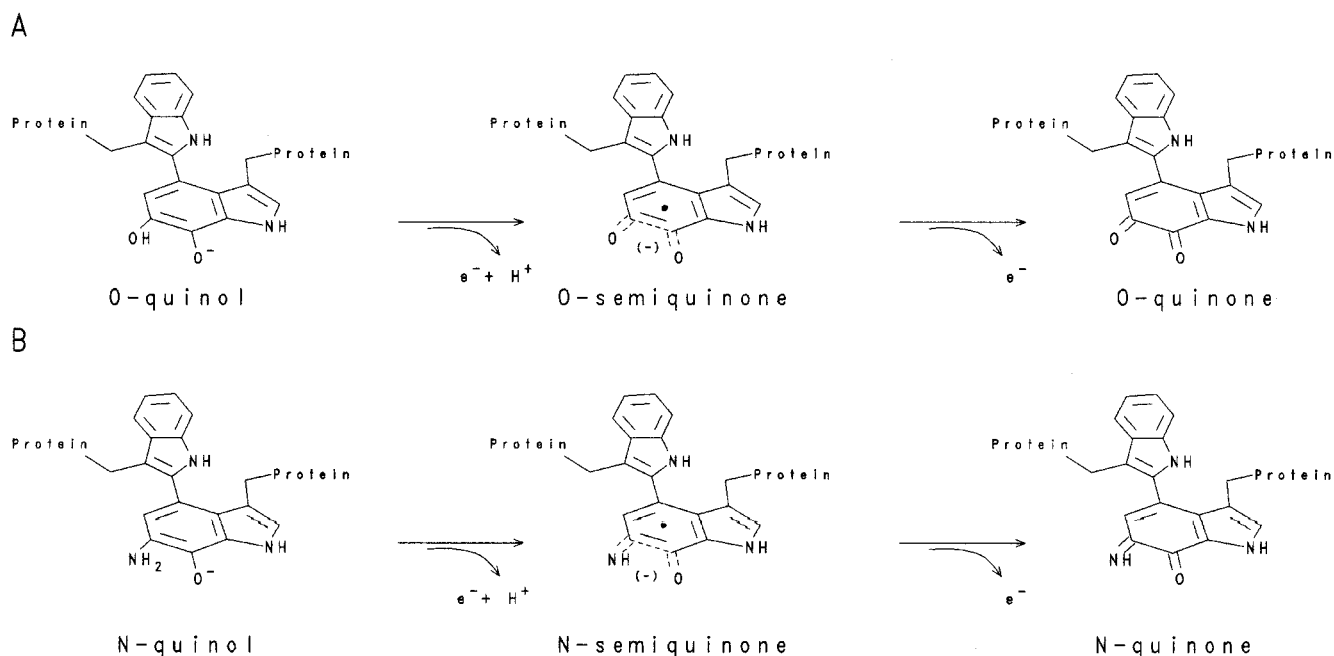


**FIGURE 2.** Sequential one-electron oxidations of dithionite-reduced (A) and substrate-reduced (B) TTQ in MADH. TTQ is formed by a posttranslational modification of two gene-encoded tryptophan residues of the polypeptide chain.[36] The protonation state of the oxygens in the quinol and semiquinone forms of TTQ was determined by redox studies.[18] The distribution of spin density in the semiquinone forms actually extends throughout the quinolated indole ring and into the second indole ring but is asymmetric and should not be inferred from this figure. The substrate-derived N on the N-quinol remains bound and is not released until after the complete oxidation of TTQ.[19] The physiologic acceptor for each electron is a molecule of amicyanin.

N-quinol form of MADH yielded unreasonable values of $\lambda$ and $H_{AB}$ and a negative ET distance[23] (Table 1). The rate of this reaction also exhibited a primary deuterium kinetic solvent isotope effect and a pronounced dependence on pH,[23] consistent with the adiabatic reaction step that gates the ET reaction from N-quinol MADH being the transfer
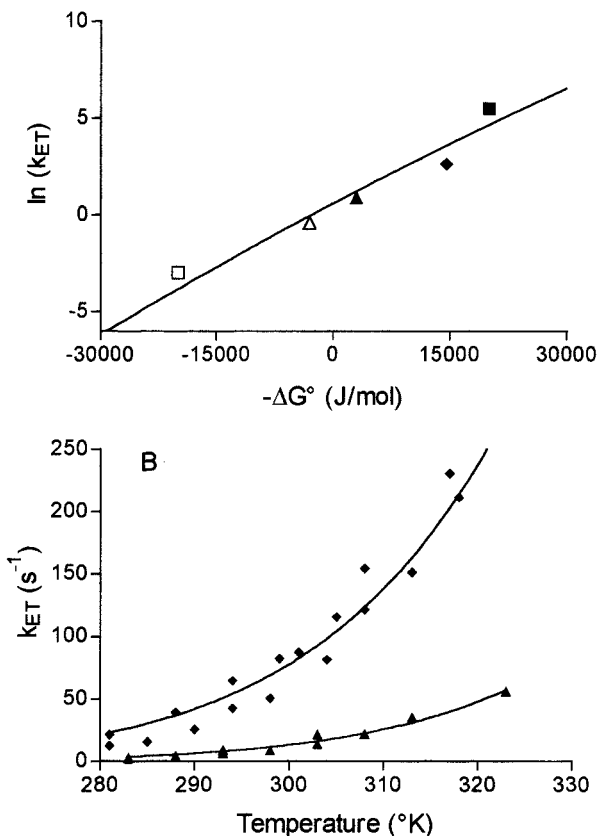
**FIGURE 3.** ET reactions between MADH and amicyanin. (A) $\Delta G^\circ$ dependence of $k_{ET}$.[20,22] Values of $k_{ET}$ were determined for the reactions of different redox forms of MADH with amicyanin shown in Figure 2A: O-quinol (▲), O-semiquinone (■), N-semiquinone (◆). Rate constants were also obtained for the reverse reactions of the O-quinol (△) and O-semiquinone (□). The solid line represent fits to eqs 1 and 2, which are superimposable. (B) Temperature dependence of $k_{ET}$.[21,22] Values of $k_{ET}$ were determined at different temperatures for the reactions of O-quinol MADH with amicyanin (▲) and N-semiquinone (◆) with amicyanin. The solid lines represent fits of each data set to eqs 1 and 2, which are superimposable. The three sets of fits shown in panels A and B yielded values of fitted parameters that were within error of each other with approximate values of $\lambda = 2.3$ eV, $H_{AB} = 12$ cm$^{-1}$, and $r = 9.5$ Å when $\beta = 1.0$.

of an exchangeable proton. While pH may affect the rate of a true ET reaction by altering $\Delta G^\circ$, in this case it was shown that pH influenced the binding at the active site of a monovalent cation which was required to facilitate the rate-limiting deprotonation of the N-quinol.[24] The latter is the reaction step that gates the ET reaction. Thus, for this system it is possible to modulate the rate for the ET reaction, which occurs over the same pathway or distance, either by changing $\Delta G^\circ$ for the reaction or, in the case of the N-quinol, by modifying the TTQ cofactor so that a chemical reaction (i.e., deprotonation) becomes rate-limiting for ET.

**Selective Alteration of $H_{AB}$ by Site-Directed Mutagenesis.** The interactions between specific amino acid residues that appear in the crystal structure to stabilize the protein−protein interface between MADH and amicyanin were proven to be important by site-directed mutagenesis.[25] The $K_d$ for the MADH−amicyanin complex was significantly increased either by conversion of Phe97 to
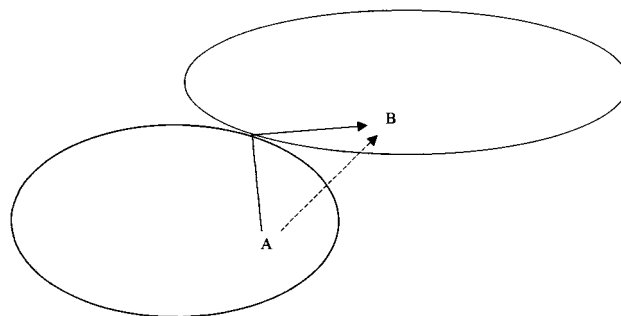


**FIGURE 4.** Schematic representation of interprotein electron transfer. This illustrates that the pathway, or overall ET distance, from a donor (A) on one protein to an acceptor (B) on another protein will depend on the location of the two redox centers relative to the protein−protein interface. In this example, the longer indirect distance (solid line) is more realistic than the shorter direct distance (dashed line) because the direct distance requires a prohibitively large through-space jump.

Glu, which prevents stabilization of the complex by van der Waals' contacts with MADH, or by conversion of Arg99 to Asp or Leu, which prevents stabilization by ionic interactions with MADH. The F97E mutation also caused a 24-fold decrease in $k_{ET}$ for the nonadiabatic ET from O-quinol MADH. The decrease in rate was due solely to a decrease in $H_{AB}$,[26] with no change in $\Delta G^\circ$ or $\lambda$. This was attributed to an increase in the interprotein distance within the complex caused by the mutation at the protein interface. Application of eq 3 indicated that an increase of 0.9 Å in the through-space jump segment of the predicted ET pathway, where the electron jumps from TTQ to amicyanin (see Figure 1B), could account for the observed decreases in $H_{AB}$ and $k_{ET}$.[26]

These results do not resolve the question of whether specific pathways are critical for protein ET reactions, but they demonstrate an important point. While it may be argued whether a protein is a homogeneous matrix for ET, for interprotein ET, the intervening space between proteins must be considered separately from the protein matrix. A caveat to this would be if ordered solvent molecules were able to bridge such a space to form part of a pathway. For an interprotein ET, if the most direct distance between redox centers includes a large through-space segment, then this direct-distance-dependent ET (dashed line in Figure 4) cannot be a relevant option. Even if one does not believe that pathways are important, one must consider the overall distance to be a sum of the two distances from each redox center to the position on the surface of each respective protein that allows for minimization of the through-space jump between proteins (solid line in Figure 4). The relatively very poor efficiency of ET through space demands this.

**Nonadiabatic ET from Copper to Heme.** ET from copper to heme within this protein complex has also been characterized by transient kinetic and thermodynamic analyses.[27] The reaction exhibited a $\lambda$ of 1.1 eV and an $H_{AB}$ of 0.3 cm$^{-1}$. Depending upon the value of $\beta$ which is used (1.4−0.7 Å$^{-1}$), analysis by eq 2 yielded a distance of 13−24 Å. The direct copper-to-heme distance observed in the crystal structure is approximately 23 Å (see Figure

1A). Since the $\beta$ of 0.7 Å$^{-1}$ appears to best describe the system, it was concluded that ET through this segment of the protein complex was relatively well-coupled, given the large distance. It was also concluded that nonadiabatic ET is rate limiting for the observed redox reaction.

## Other Protein Complexes That Exhibit Both Gated and Ungated ET Reactions

**Aromatic Amine Dehydrogenase (AADH) and Azurin.** Another TTQ-dependent enzyme, AADH, uses the blue copper protein azurin as an electron acceptor.[17] As was observed with MADH, the oxidation of the chemically reduced O-quinol AADH by azurin was rate-limited by ET, whereas oxidation of the substrate-reduced N-quinol AADH by azurin was gated[28] (Table 1). As with the MADH−amicyanin reaction, the adiabatic reaction step that gates the latter ET reaction is the transfer of an exchangeable proton.[28]

**Nitrogenase.** The ET reaction between the iron protein and molybdenum−iron protein of the nitrogenase complex was converted from one which was gated to one which was not by site-directed mutagenesis.[29] The reaction of the native protein is believed to be gated by conformational events associated with either MgATP binding or hydrolysis. Thermodynamic analysis yielded an $H_{AB}$ which is well in excess of the nonadiabatic limit and a negative ET distance. The reaction of an L127D mutant, however, yielded parameters that were characteristic of nonadiabatic ET and a distance similar to what is seen in the crystal structure (Table 1).

## Kinetically Coupled ET from Methanol Dehydrogenase (MEDH) to Cytochrome *c*-551i

Methanol dehydrogenase (MEDH) and cytochrome *c*-551i from *P. denitrificans* form a physiologic complex in which electrons are transferred from pyrroloquinoline quinone (PQQ) to heme. Thermodynamic analysis of $k_{ET}$ yielded values of $\lambda = 1.86$ eV, $H_{AB} = 0.071$ cm$^{-1}$, and an ET distance of 15 Å.[9] Although the structure of the complex of MEDH and cytochrome *c*-551i is not known, this is a reasonable estimate of the minimum ET distance, given the known structures of each protein. PQQ is buried approximately 10 Å from the protein surface, and docking studies suggest that the nearest the surface of a globular protein such as the cytochrome could be positioned is about 15 Å from PQQ.[9] Both $k_{ET}$ and the association constant for complex formation varied with ionic strength.[10] The ionic strength dependence of each was analyzed by Van Leeuwen theory,[30] which takes into account monopole−dipole, dipole−dipole, and monopole−monopole forces, to predict the orientations in which macromolecules interact. These analyses indicated that the optimal orientations for binding and ET were similar but slightly different.[10] This is consistent with the model of coupled ET discussed earlier, where rapid but unfavorable (i.e., $K_{eq} \ll 1$) rearrangement of the proteins after binding is required to produce an optimal orientation for ET.

## Other Physiologic ET Complexes

Although kinetic data have been reported for many bimolecular protein ET reactions, the experimental approaches described here have been applied to relatively few physiologic interprotein ET reactions, or even intramolecular ET reactions within complex redox enzymes. Nonphysiologic ET reactions between cytochrome *c* and plastocyanin have been analyzed by ET theory.[31] ET from rubredoxin reductase to rubredoxin was shown to be gated on the basis of results of the analysis of temperature dependence data using ET theory[32] (Table 1). The only other physiologic ET complex of soluble proteins for which a crystal structure of the complex is known is that of cytochrome *c* and cytochrome *c* peroxidase.[33] Interpretation of results of ET studies with this system has been controversial, as it has been suggested that multiple binding sites may be operative in solution. Recently, temperature and viscosity dependence studies of ET from the cytochrome to the Trp radical cation of the peroxidase provided evidence that the nonphysiological reaction with horse cytochrome was gated by configurational changes in the complex, whereas the physiologic reaction with yeast cytochrome was not.[34] Crystal structures have also recently been determined for three of the four protein ET complexes of the membrane-bound respiratory chain:[35] cytochrome *c* oxidase, cytochrome *bc*₁, and fumarate reductase (succinate dehydrogenase). Hopefully, kinetic and thermodynamic studies of ET reactions in these proteins can be analyzed by ET theory and interpreted in the context of these new structures to provide an understanding of the regulation and mechanism of the respiratory chain at a molecular level.

## Conclusion

The meaningful application of nonadiabatic ET theory to interprotein ET reactions is problematic. Redox enzymes are structurally, chemically, and kinetically complex. It is critical that one be able to discern the contributions to the observed reaction rate from adiabatic non-ET processes (e.g., protein conformational changes and proton-transfer reactions). This Account attempts to summarize and illustrate the potential pitfalls in measuring ET rates in complex biological systems, and describes experimental approaches for addressing these problems and interpreting the results which are obtained from such systems. A meaningful interpretation is possible only if the kinetic mechanisms of these complex systems are rigorously characterized. Kinetic and thermodynamic studies, coupled with structural information and biochemical data, are necessary to develop meaningful hypotheses. Site-directed mutagenesis studies will be required to test hypotheses that arise, especially if mutations can be made which selectively alter either $H_{AB}$ or $\lambda$. If such studies are done correctly, then it will be possible to answer critical questions in this field, such as whether specific pathways mediate ET and how specific features of protein structure contribute to the $\lambda$ values associated with true and coupled interprotein ET reactions. It will also help to

identify when ET reactions are gated so that we can better understand the mechanisms by which adiabatic non-ET processes control the rates of ET reactions.

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