

Protein Control of True, Gated, and Coupled Electron Transfer Reactions

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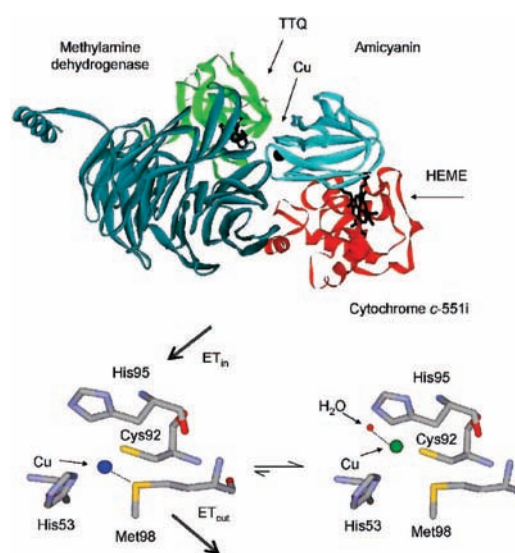
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CONSPICUOUS

Electron transfer (ET) through and between proteins is a fundamental biological process. The rates of ET depend upon the thermodynamic driving force, the reorganization energy, and the degree of electronic coupling between the reactant and product states. The analysis of protein ET reactions is complicated by the fact that non-ET processes might influence the observed ET rate in kinetically complex biological systems. This Account describes studies of the methylamine dehydrogenase–amicyanin–cytochrome *c*-551i protein ET complex that have revealed the influence of several features of the protein structure on the magnitudes of the physical parameters for true ET reactions and how they dictate the kinetic mechanisms of non-ET processes that sometimes influence protein ET reactions.

Kinetic and thermodynamic studies, coupled with structural information and biochemical data, are necessary to fully describe the ET reactions of proteins. Site-directed mutagenesis can be used to elucidate specific structure–function relationships. When mutations selectively alter the electronic coupling, reorganization energy, or driving force for the ET reaction, it becomes possible to use the parameters of the ET process to determine how specific amino acid residues and other features of the protein structure influence the ET rates. When mutations alter the kinetic mechanism for ET, one can determine the mechanisms by which non-ET processes, such as protein conformational changes or proton transfers, control the rates of ET reactions and how specific amino acid residues and certain features of the protein structure influence these non-ET reactions.

A complete description of the mechanism of regulation of biological ET reactions enhances our understanding of metabolism, respiration, and photosynthesis at the molecular level. Such information has important medical relevance. Defective protein ET leads to production of the reactive oxygen species and free radicals that are associated with aging and many disease states. Defective ET within the respiratory chain also causes certain mitochondrial myopathies. An understanding of the mechanisms of regulation of protein ET is also of practical value because it provides a logical basis for the design of applications utilizing redox enzymes, such as enzyme-based electrode sensors and fuel cells.



Introduction

While the phenomenon of electron transfer (ET) between small molecules in solution is reasonably well described,¹ the factors that influence protein ET reactions are less understood.^{2,3} Biological systems are far from ideal experimental systems for the study of a fundamental physical process such

as ET. A complete description of biological ET reactions requires not only knowledge of the physics that describes ET but also an understanding of how the biological milieu, most commonly a protein, influences this fundamental process. The studies described in this Account are patterned after the approaches that biochemists have long used to elucidate protein structure–function

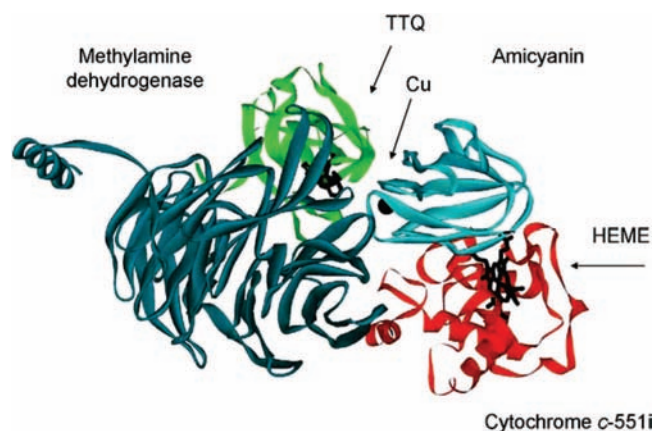


FIGURE 1. The MADH–amicyanin–cytochrome *c*-551i complex. One half of the symmetrical complex of the crystal structure (PDB, 2MTA)¹⁰ is shown.

relationships in enzymes. First one determines the rate-limiting step for the overall reaction and then the activation parameters (i.e., ΔG° , ΔH° , ΔS°) for that reaction step. In conjunction with structural studies and often site-directed mutagenesis, it is then possible to describe the roles of specific amino acid residues and features of protein structure in the catalytic process. For protein ET reactions, the same approach may be used, but there are complications. In contrast to chemical reactions, no bond making or breaking is associated with ET reactions. The redox centers that serve as electron donor and acceptor are not in direct contact, and the reactants and products may be structurally indistinguishable. As such, the reaction coordinate and transition state are poorly defined and analysis of true ET reactions requires a modified form of transition state theory with different activation parameters (i.e., λ , H_{AB}). Furthermore, when ET occurs between and through proteins, non-ET reaction steps in the overall process, including protein–protein interactions, may complicate the assignment of the rate-limiting step.

Many biologically relevant interprotein ET reactions, as in the respiratory and photosynthetic ET chains, occur within and between integral membrane proteins. These are inherently difficult to study because the proteins are difficult to purify and cannot readily be studied under aqueous conditions. Soluble redox proteins are more amenable to mechanistic studies. Several soluble ET proteins have been characterized; however, relatively few complexes of soluble ET protein partners have been described and defined at the structural level.^{4–10} This Account will focus on results obtained from studies of the methylamine dehydrogenase (MADH)–amicyanin–cytochrome *c*-551i complex from *Paracoccus denitrificans* (Figure 1), which is perhaps the best characterized physiological protein ET complex. X-ray crystal structures are available for the

complex of MADH and amicyanin⁹ and for the ternary protein complex.¹⁰ It was demonstrated by single-crystal polarized absorption microspectroscopy¹¹ and EPR spectroscopy¹² that in the crystalline state MADH is catalytically competent and transfers electrons from its tryptophan tryptophylquinone (TTQ) cofactor to the cytochrome heme via the type 1 copper center of amicyanin. The steady-state kinetic parameters for methylamine-dependent cytochrome *c*-551i reduction by the MADH–amicyanin complex in solution have been characterized,¹³ and rates of the individual ET reactions that occur within the complex have been determined by monitoring characteristic changes in the absorption spectra of the proteins that occur during the redox reactions.^{14–16} Site-directed mutagenesis studies of MADH and amicyanin have identified specific amino acid residues that stabilize specific protein–protein interactions,^{17,18} modulate the E_m value of the copper,¹⁹ and influence ET parameters for the reactions that occur within the complex.^{20–23} It was also possible to generate mutations of amicyanin that alter the kinetic mechanisms of ET reactions within the complex by converting true ET reactions to ones that are gated or coupled.^{24,25} These studies showed that ET rates may be significantly altered by subtle changes in protein structure by a variety of mechanisms.

Electron Transfer Theory

In the classical model, ET occurs at the intersection of the potential energy surfaces for the reactant and product states. For simplicity, these multidimensional energy surfaces are typically presented as parabolas described by the free energy (ordinate) and reaction coordinate (abscissa) (Figure 2). The reorganization energy (λ) is the energy difference between the reactant and product states at the equilibrium nuclear configuration of the reactant (i.e., the minimum). When ΔG° is equal to zero the activation energy is $\lambda/4$. H_{AB} is the electronic coupling matrix element that represents the extent to which the wave functions of the reactant and product states overlap. The splitting at the intersection point is equal to $2H_{AB}$. This describes the degree of nonadiabaticity (i.e., probability of the reaction occurring when the activation energy has been achieved). When H_{AB} is zero (a diabatic system), there is no chance of the reaction occurring regardless of the energy. At the other extreme, the adiabatic system, the value of H_{AB} is so large that the probability of crossover when the activation energy is achieved is unity. Such adiabatic reactions are described by transition-state theory. In a true ET reaction, the gap represented as $2H_{AB}$ is relatively small, and this system is said to be nonadiabatic. Because of the weak coupling, the

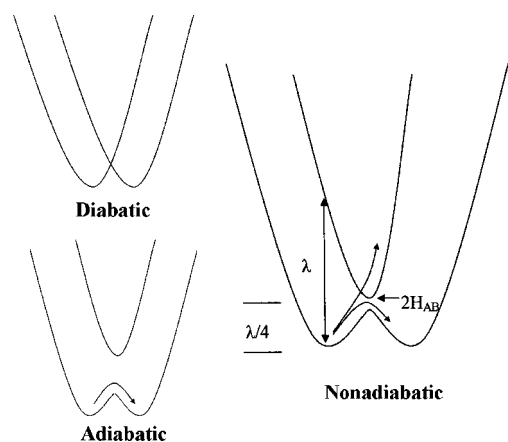
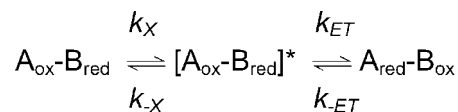


FIGURE 2. Potential energy diagrams for electron transfer reactions. In each set, the parabolas on the left and right represent the reactant and product states, respectively. In these examples $\Delta G^\circ = 0$.

activation energy may have to be achieved several times before the crossover from reactant to product states occurs. For nonadiabatic ET, k_{ET} is described by a modified form of transition-state theory (eq 1).¹ The activation energy for the reaction is equal to $(\Delta G^\circ + \lambda)^2/(4\lambda)$, where ΔG° is determined from the redox potential difference for the ET reaction. The other parameters are Planck's constant (h), the gas constant (R), and temperature (T). In simple system, H_{AB} and, consequently, k_{ET} will decrease exponentially with distance. This is reflected in eq 2, where k_0 is the characteristic frequency of the nuclei (10^{13} s^{-1}), which is the limit for k_{ET} when donor and acceptor are in van der Waals' contact and $\lambda = -\Delta G^\circ$. The donor to acceptor distance is r , and r_0 is the close contact distance (3 Å). The parameter β is used to quantitate the nature of the intervening medium with respect to its efficiency to mediate ET.^{26,27} The challenge for those wishing to understand biological ET reactions is to make the transition from

SCHEME 1. Models for Kinetically Complex ET



$$\text{true ET: } k_{ET} \ll k_X \quad K_X \gg 1 \quad k_{\text{obs}} = k_{ET}$$

$$\text{gated ET: } k_X \ll k_{ET} \quad k_{\text{obs}} = k_X$$

$$\text{coupled ET: } k_{ET} \ll k_X \quad K_X \ll 1 \quad k_{\text{obs}} = K_X^* k_{ET}$$

the realm of parabolas to the world of proteins and to describe protein structure–function relationships that reveal how the protein influences these ET parameters. Examples of the use of site-directed mutagenesis to selectively alter the values of ET parameters for true ET reactions in the MADH–amicyanin–cytochrome *c*-551i complex (Table 1) will be discussed.

$$k_{ET} = \frac{4\pi^2 H_{AB}^2}{h\sqrt{4\pi\lambda RT}} e^{-(\Delta G^\circ + \lambda)^2/(4\lambda RT)} \quad (1)$$

$$k_{ET} = k_0 \exp[-\beta(r - r_0)] \exp[-(\Delta G^\circ + \lambda)^2/(4\lambda RT)] \quad (2)$$

Kinetic Complexity of Protein ET

For long-range protein ET reactions, it is often difficult to ascertain whether the observed rate of the redox reaction (k_{obs}) is a true ET rate constant (k_{ET}) (Scheme 1). In some reactions, a non-ET event (where K_X is the equilibrium constant for this reaction) may be required to optimize or activate the system for ET.^{2,3,28,29} This applies to both interprotein and intraprotein ET reactions. Kinetic models have been developed that define such kinetically complex ET reactions as true, gated, or coupled. ET reactions from TTQ in MADH are true or gated

TABLE 1. Electron Transfer Reactions within Native and Mutant Complexes of Methylamine Dehydrogenase, Amicyanin, and Cytochrome *c*-551i

ET donor	ET acceptor	k , 30 °C (s^{-1})	ΔG° (kJ/mol)	λ (kJ/mol) ^a	λ (eV)	H_{AB} (cm^{-1}) ^b	reaction type	ref
<i>O</i> -quinol MADH	amicyanin	9.8	-3.2	222	2.3	12 ± 7	true	31
<i>N</i> -quinol MADH	amicyanin	275	-3.2 ^c	341	3.5	23000	gated	41
<i>O</i> -quinol α F55A MADH	amicyanin	45	-3.2	174	1.8	3 ± 1	true	22
<i>O</i> -quinol MADH	M98Q amicyanin	0.2	-3.3	261	2.7	12 ± 4	true	23
<i>O</i> -quinol MADH	M98A amicyanin	9.6	-3.2	203	2.1	6 ± 2	true	23
<i>O</i> -quinol MADH	F97E amicyanin	0.2	-3.2	222	2.3	3 ± 0.1	true	21
<i>O</i> -quinol MADH	P94F amicyanin	53	-21.7	222	2.3	5 ± 1	true	39
<i>O</i> -quinol MADH	P94A amicyanin	82	-18.9	212	2.2	3.8 ± 1.2	true	25
<i>O</i> -quinol MADH	P52G amicyanin	3.0	-4.82	270	2.8	78 ± 30	gated	24
<i>O</i> -quinol MADH	M51A amicyanin	1.3	-2.8	299	3.1	142 ± 20	gated	48
amicyanin	cytochrome <i>c</i> -551i	87	+3.2	116	1.2	0.3 ± 0.1	true	32
P94F amicyanin	cytochrome <i>c</i> -551i	0.6	+21.7	125	1.3	0.3 ± 0.1	true	39
P94A amicyanin	cytochrome <i>c</i> -551i	0.4	+18.9	222	2.3	8.3 ± 5.5	coupled	25

^a λ is sometimes expressed in units of kJ/mol and sometimes as eV so both values are given. ^b Errors are listed only for H_{AB} , which is the parameter that yields the most uncertainty. For the other parameters, errors are typically much less than 10%. ^c Values of ΔG° for gated reactions refer to the ET reaction step and not the non-ET reaction, which gates ET.

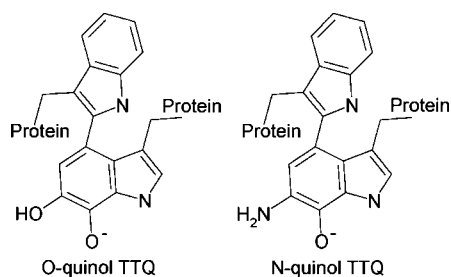


FIGURE 3. Different forms of reduced TTQ in MADH. Reduction of MADH by dithionite yields *O*-quinol TTQ, whereas reduction of MADH by the amine substrate yields *N*-quinol TTQ in which the substrate-derived nitrogen has displaced a quinone oxygen.

depending on the whether the reduced cofactor is in the *O*-quinol or *N*-quinol state, respectively (Figure 3).^{30,31} ET from copper to heme is a true ET reaction.³² Using site-directed mutagenesis it has been possible to convert these true ET reactions to gated and coupled reactions (Table 1). This has provided insight into how the protein may dictate the kinetic mechanism of the ET process, as well as control true ET parameters.

Alteration of λ by Site-Directed Mutagenesis

It is presumed that the ET event occurs only when the system is completely optimized for ET to occur in the absence of nuclear motions. The energy required to bring the reactant and product states to this common intermediate state is λ . It comprises the inner sphere, λ_{in} , which reflects changes in ligand bond lengths and bond angles that accompany the redox reaction, and the outer sphere, λ_{out} , which reflects reorientation of solvent molecules that accompanies the redox reaction. For reactions in solution between small molecules, these distinctions are reasonably well-defined, but not so for protein ET reactions. For protein-bound redox cofactors such as quinones or flavins, one does not have a simple set of metal ligands but a large number of bonds and an asymmetric electron distribution in a complex organic molecule. Even with metalloproteins, amino acid residues provide metal ligands and reorientation of the protein matrix may accompany the redox reaction. For these reasons, the distinction between λ_{in} and λ_{out} becomes blurred, and the influence of the protein environment on each becomes difficult to ascertain. Mutations of MADH and amicyanin are described that appear to selectively alter λ_{in} and λ_{out} for the true ET reaction from *O*-quinol MADH to amicyanin.

Alteration of λ_{out} . Phe55 of the α subunit of MADH is present in a substrate channel that connects the protein surface with the active site and is a key determinant of amine

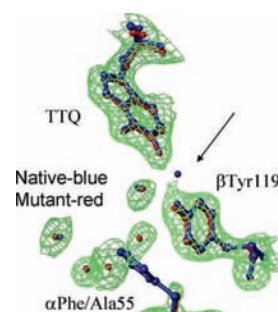


FIGURE 4. Active-site structures of MADH (blue) and α F55A MADH (red). The structures of the ternary protein complexes with native (PDB, 2MTA) and α F55A MADH (PDB, 1GM2)²² are superimposed with the electron density for α F55A MADH included. The single water present in the native active site and absent in the mutant is indicated by the arrow.

substrate specificity.³³ An α F55A MADH mutation (Figure 4) significantly increased k_{ET} from *O*-quinol MADH to amicyanin.²² Thermodynamic analysis of the ET reaction revealed that k_{ET} increased because λ for this reaction decreased by 0.5 eV as a consequence of the mutation. The crystal structure of α F55A MADH was determined in complex with amicyanin and cytochrome *c*-551i and compared with that of the native complex.²² Very little difference in the overall structure was seen, but there was a change in the solvent content of the active site and substrate channel (Figure 4). Two waters are in the native MADH active site in close proximity to TTQ and shielded from bulk solvent by α Phe55. While water fills the void left by removal of the phenyl ring in the channel, only one of the active-site waters is present in α F55A MADH. The observed decrease in λ is consistent with TTQ being less solvated in α F55A MADH than in native MADH. The dramatic influence of water on λ may explain why proteins that evolved to function solely as ET mediators usually contain redox centers that are buried within the protein matrix, thus minimizing λ_{out} . Redox enzymes with bifunctional cofactors such as TTQ, which participate in catalysis as well as ET, must be at least partially exposed to solvent at the active site, which may explain why their ET reactions tend to exhibit relatively large values of λ .

Alteration of λ_{in} . Spectroscopic and crystallographic analysis of M98Q amicyanin revealed that this substitution of the axial ligand of copper caused a significant rhombic distortion of the type 1 site³⁴ (Figure 5). The EPR spectra of native and M98Q amicyanins exhibited $A_{||}$ values of 53 and 23 G, respectively. Comparison of isomorphous crystals of native and M98Q amicyanins at atomic resolution revealed no significant change in the distances and orientations of the three equatorial copper ligands but indicated that the mutation increased the distance of the copper from the equatorial plane

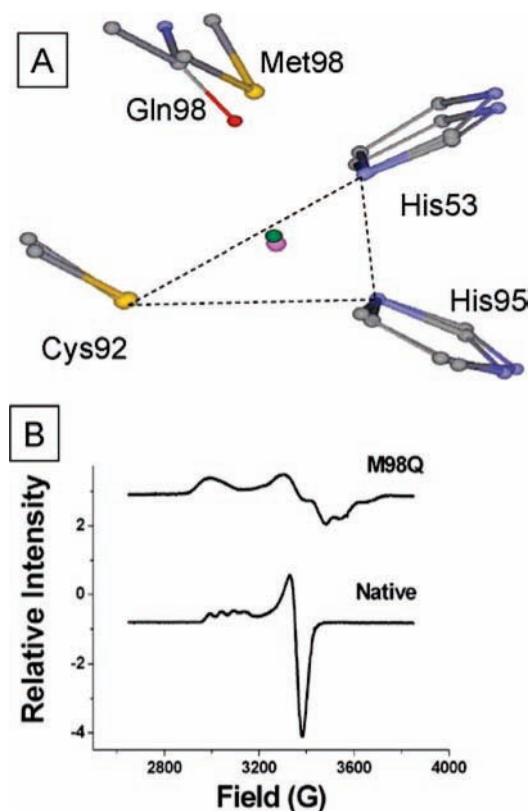


FIGURE 5. (A) Position of copper relative to the plane formed by equatorial ligands in native (purple sphere) and M98Q (green sphere) amicyanins. The superimposed structures are native (PDB, 2OV0, 0.75 Å) and M98Q (PDB, 2IDT, 1.0 Å).³⁴ (B) EPR spectra of native and M98Q amicyanins.²³

that is formed by the other three copper ligands from 0.20 to 0.42 Å. The mutation had little effect on the E_m value, but k_{ET} for the reaction from *O*-quinol MADH to amicyanin was reduced 45-fold in M98Q amicyanin.²³ Thermodynamic analysis of these ET reactions showed that the decrease in k_{ET} was due to an increase of 0.4 eV in λ . No change in the experimentally determined H_{AB} or ET distance was observed, confirming that the mutation had not altered the rate-determining step for ET and that this was still a true ET reaction. The basis for the increased λ for the reaction with M98Q amicyanin is not solely the nature of the atom that provides the axial ligand (Gln98 OE2). M98A amicyanin also uses an oxygen, from water, for the axial ligand, and no such change in λ was observed (Table 1).^{19,23} These results correlate well with results of quantum chemical calculations of λ of model compounds of the type 1 copper site with glutamine and methionine axial ligands. The calculated λ_{in} for a $Cu(I m)_2(SCH_3)(CH_3CONH_2)$ model was approximately 0.3 eV greater than that for a $Cu(I m)_2(SCH_3)(SCH_3)_2$ model.³⁵ This relationship between the extent of rhombicity and λ , most likely λ_{in} , highlights the importance of the geometry of the

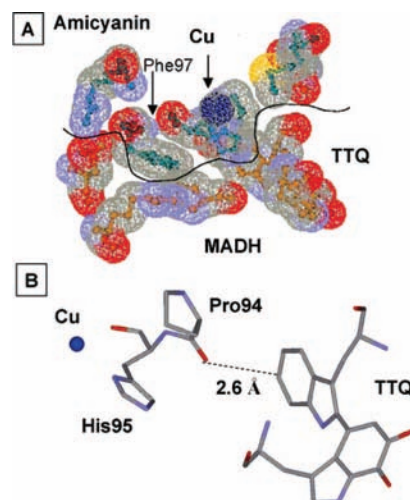


FIGURE 6. The amicyanin–MADH interface: (A) residues of amicyanin (green) and MADH (brown) near the site of interprotein ET are shown as ball and stick with their van der Waals radii colored as oxygen (red), nitrogen (blue), carbon (gray), and sulfur (yellow); (B) predicted point of interprotein ET from the backbone oxygen of Pro54 of amicyanin to TTQ.

type 1 copper site in controlling λ , consistent with the concept of “rack-induced” folding of type 1 copper proteins facilitating rapid ET by reducing λ .³⁶

Alteration of H_{AB} by Site-Directed Mutagenesis

H_{AB} depends on the ET distance and the nature of the intervening medium ($H_{AB} \sim e^{-\beta r}$). Two approaches have been used to predict relative H_{AB} values for protein ET reactions from known structures. In one, the overall H_{AB} is proportional to the product of the H_{AB} for each individual through-bond or through-space step along the ET pathway.²⁶ In the other, the overall H_{AB} is proportional to the direct distance using a single average β that is related to the packing density of the intervening protein.²⁷ Because the β value for ET through a vacuum (2.8 \AA^{-1}) is much larger than that for ET through a covalent bond (0.7 \AA^{-1}),³⁷ it follows that efficient ET through proteins would occur primarily through bonds. An F97E mutation of amicyanin decreased k_{ET} for the reaction from *O*-quinol MADH to amicyanin.²¹ The ΔG° and λ associated with the ET reaction were unaffected by the mutation, and the decrease in k_{ET} was due solely to a decrease in H_{AB} (Table 1). Phe97 is located at the MADH–amicyanin interface (Figure 6). Inspection of the structure of the protein complex reveals that an interprotein through-space jump of at least 2.6 Å is required for ET from TTQ to copper (Figure 6B). On the basis of the native structure and analysis of the ET reactions of native and F97E amicyanin, it was concluded that the F97E mutation causes an increase in this critical interprotein distance of 0.9 Å, which

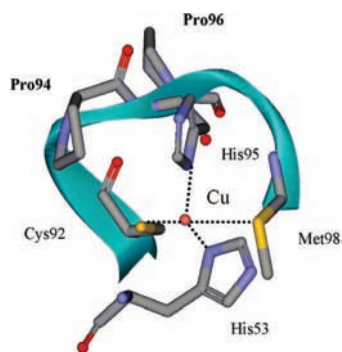


FIGURE 7. The copper center of amicyanin. The “loop” indicated as a ribbon from Cys92 to Met98 contains three of the amino acids providing copper ligands as well as two proline residues that have been targets for site-directed mutagenesis.

accounts for the observed decreases in H_{AB} and consequently k_{ET} . This demonstrates that small changes in the length of through-space segments of ET pathways, particularly interprotein gaps, can significantly alter H_{AB} .

Alteration of ΔG° by Site-Directed Mutagenesis

The ΔG° for true ET reactions depends on the ΔE_m for the donor and acceptor redox centers. Factors that influence the E_m value include the identity of ligands for metal cofactors, protein-imposed constraints on organic cofactor conformation or metal ligation geometry, H-bonding pattern around the cofactor, presence of water, hydrophobicity, and electrostatic effects. An example of the use of site-directed mutagenesis to alter k_{ET} by altering the E_m value of amicyanin is described to illustrate the predictable effect of altering ΔG° on k_{ET} for a true ET reaction.

Pro94 resides in the “ligand loop” of amicyanin, a sequence of amino acids that contains three of the four copper ligands (Figure 7). P94F and P94A mutations of amicyanin increased its E_m value by 150 and 115 mV, respectively.³⁸ Atomic resolution structures of P94F and P94A amicyanins³⁴ revealed that the bond lengths and angles of the copper ligands were unchanged as a consequence of mutation of Pro94, but in each mutant, a hydrogen bond to the copper-coordinating thiolate sulfur of Cys92 is introduced by movement of the amide nitrogens of Phe94 and Ala94 closer to the thiolate sulfur than the nitrogen of Pro94. This is the likely explanation for the increased E_m values, which result in a more negative ΔG° for ET from MADH and more positive ΔG° for ET to cytochrome *c*-551i. As expected for true ET reactions, this causes an increase in k_{ET} from TTQ to copper and decrease in k_{ET} from copper to heme³⁹ (Table 1). Analysis of the temperature dependence of these reactions indicated that the λ values for these ET reactions were unchanged by mutations, despite the

large change in ΔE_m value. Steady-state kinetic studies of methylamine-dependent reduction of heme by the three-protein complex indicated that the P94F mutation decreases k_{cat} because the now unfavorable uphill ET reaction from copper to heme becomes the rate-limiting step in the overall reaction.³⁹ This has important implications for understanding the roles of individual redox centers in regulating the rate of flux through biological ET chains.

Protein Control of the Kinetic Mechanism of ET

To investigate the kinetic control of protein ET reactions, it is desirable to obtain systems where it is possible to compare a true and a gated ET reaction between the same redox centers within the same protein matrix. A common criterion for initially classifying an ET reaction as gated is an experimentally determined H_{AB} that exceeds the nonadiabatic limit.⁴⁰ Supporting evidence includes demonstration that the reaction rate is influenced by factors that would not be expected to affect a true ET reaction, such as ionic strength or viscosity. ET reactions from TTQ in MADH will be true or gated depending on whether the reduced cofactor is in the *O*-quinol or *N*-quinol state, respectively (Figure 2). ET from amicyanin to cytochrome *c*-551i in the complex is another true ET reaction.³² By using site-directed mutagenesis, we were able to convert the true ET reaction from *O*-quinol to a conformationally gated ET reaction²⁴ and the true ET reaction from amicyanin to the cytochrome to a kinetically coupled ET reaction.²⁵

Chemically Gated ET from *N*-Quinol MADH to Amicyanin. The phenomenon of “chemically gated” ET²⁹ is one in which a prerequisite chemical reaction activates the system for ET that is so rapid that the reaction becomes rate-limited by the chemical step, yet the chemical step is much faster than the corresponding true ET reaction in the absence of activation. ET from *N*-quinol MADH to amicyanin is chemically gated by the transfer of a solvent-exchangeable proton as indicated by the large observed solvent kinetic isotope effect on the apparent k_{ET} for the reaction.^{30,41} Analysis of the temperature dependence of the reaction rate yielded unreasonable values of H_{AB} of 23000 cm^{-1} and an ET distance of -4.9 \AA , which provided strong evidence that the rate did not describe a nonadiabatic ET reaction and is more appropriately described by standard transition-state theory. The rate was also dependent on pH and the concentration of monovalent cations. The pH dependence was attributed to an ionizable group that is involved in binding the cation that stabilizes a negatively charged transient reaction intermediate that is

formed by the rate-limiting deprotonation of the *N*-quinol to generate the activated ET complex. This model provided a detailed description of how a chemical reaction that occurs at an enzyme active site can gate an ET reaction that occurs at the protein surface.³⁰ Chemically gated ET was also described for another TTQ-dependent enzyme, aromatic amine dehydrogenase [AADH], which catalyzes the oxidative deamination of phenylethylamines⁴² and donates substrate-derived electrons to the copper protein azurin.⁴³ As with MADH and amicyanin, ET from the *O*-quinol AADH to azurin was a true ET reaction, but ET from the substrate-reduced *N*-quinol AADH to azurin was gated by deprotonation of the *N*-quinol TTQ.⁴⁴ Other protein ET reactions described in the literature that may be considered examples of chemically gated ET controlled by chemical reactions other than deprotonation include the ET reaction between the iron protein and molybdenum–iron protein of the nitrogenase complex^{45,46} and the ET reaction from thiamine pyrophosphate to an iron–sulfur cluster in pyruvate–ferredoxin oxidoreductase in the presence of CoA.⁴⁷

Conformationally Gated ET from MADH to P52G and M51A Amicyanins. For interprotein ET, if the ideal orientations of the proteins for binding and ET are different, then some rearrangement of proteins within the complex must occur to maximize k_{ET} . This phenomenon has been elucidated by site-directed mutagenesis of amicyanin residues Met51 and Pro52, which are present at the MADH–amicyanin interface (Figure 8).^{24,48} A portion of Pro52 is in close proximity to β Val127 of MADH, and the side chain of Met51 makes close contacts with β Val156 of MADH. A P52G mutation both increased the K_d for complex formation and decreased k_{ET} for the true ET reaction from *O*-quinol MADH.²⁴ Thermodynamic analysis of k_{ET} yielded an increase in H_{AB} from 12 to 78 cm⁻¹ with a corresponding decrease in ET distance of 3 Å, which is physically impossible given the known structures. A significant increase in the experimentally determined λ further suggested that the ET reaction was now gated by a slower non-ET process. Analysis of the crystal structure of P52G amicyanin revealed that in addition to the loss of three carbons of Pro52 the position of the side chain of Met51 was altered such that contacts with the side chain of β Val156 of MADH were lost. When Met51 was mutated to alanine to mimic the loss of the side chain, a decrease in k_{ET} and increased values of λ and H_{AB} were observed, similar to what was seen with P52G amicyanin (Table 1). In contrast to P52G amicyanin, the K_d for complex formation between M51A amicyanin and MADH was the same as that for native amicyanin.⁴⁸ Therefore, the loss of the interactions involving Pro52 were primarily responsible for the change in K_d for P52G amicyanin, while the inter-

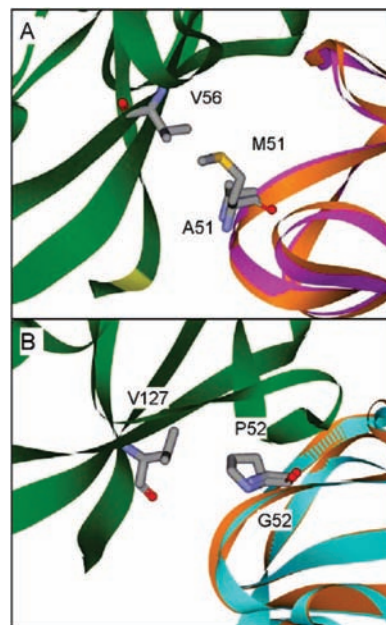


FIGURE 8. Interactions involving amicyanin residues Met51 and Pro52 at the MADH–amicyanin interface. Protein backbones of the MADH β subunit (green), native amicyanin (orange), M51A amicyanin (purple), and P52G amicyanin (cyan) are shown as solid ribbons with amicyanin residues 51 and 52 and residues on MADH with which they interact shown as sticks. Structures (A) M51A amicyanin (PDB, 2QDV)⁴⁸ and (B) P52G amicyanin (PDB, 2GB2)²⁴ are shown in relation to the MADH structure after alignment with the amicyanin portion of the native complex structure (PDB, 2GC4).

actions involving Met51 are entirely responsible for the change in ET parameters seen with both the P52G and M51A amicyanins. Because the K_d is unaffected by the M51A mutation, the relative orientations of the proteins immediately upon binding are likely the same. Therefore, this mutation probably slows the rate of an existing but previously unrecognized conformational rearrangement that normally occurs rapidly in the native amicyanin–MADH complex subsequent to binding and prior to ET, thus causing ET to become gated. This demonstrates that subtle perturbations of protein–protein interactions may have significant effects on the rates of interprotein ET by altering the kinetic mechanism for the overall reaction. Thus, surface residues of redox proteins not only dictate specificity for their redox protein partners but also may be critical to optimize the orientations of the redox centers and intervening media within the protein complex for the ET event.

Kinetically Coupled ET from P94A Amicyanin to Cytochrome c-551i. As stated earlier, mutation of Pro94, which resides in the “ligand loop” of amicyanin (Figure 7), to alanine alters ΔG° for the reaction with *O*-quinol MADH, but it remains a true ET reaction with values of H_{AB} and λ similar to those for the reaction with native amicyanin. In contrast, the parameters for the ET reaction from reduced P94A amicya-

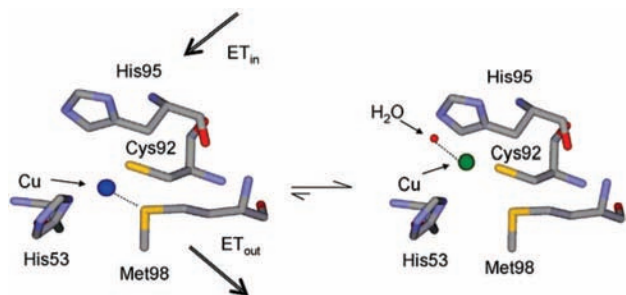


FIGURE 9. Model for coupled ET from alternate conformations of reduced P94A amicyanin to cytochrome *c*-551i.

nin to cytochrome *c*-551i were significantly altered as a consequence of the mutation. These values of H_{AB} and λ are 8.3 cm^{-1} and 2.3 eV , respectively, compared with values of 0.3 cm^{-1} and 1.2 eV for the reaction of native reduced amicyanin.²⁵ The crystal structure of reduced P94A amicyanin exhibits two alternate conformations (Figure 9) with the positions of the copper 1.4 \AA apart.¹⁹ In one of these conformations, a water has replaced Met98 as a copper ligand and the ET distance to the heme of the cytochrome is increased by 1.4 \AA . The k_{ET} from the copper in this conformation to heme is expected to be significantly diminished for three reasons. The overall ET distance is greater, the predicted ET pathway from Cu^{1+} via Met98 is disrupted, and the presence of water is likely to increase the λ associated with the ET reaction. To explain these data, a kinetic mechanism was proposed in which after the reduction of Cu^{2+} by MADH, ET from the favored conformation is coupled to an unfavorable equilibrium with the unfavorable conformation (Figure 9).

It is also possible for an interprotein ET reaction to be conformationally coupled. An example of this is the ET reaction from pyrroloquinoline quinone in methanol dehydrogenase to heme in cytochrome *c*-551i. It was shown that the optimal orientation for ET was different from that for binding such that k_{ET} was reduced by the K_{eq} for the rapid but unfavorable conformational rearrangement.⁴⁹ Thus, the most stable conformation of the redox protein or protein complex is not necessarily the optimum conformation for ET. This can lead to coupled ET.

Conclusion

The application of nonadiabatic ET theory to protein ET reactions is a challenging task. Redox enzymes are structurally, chemically, and kinetically complex. Kinetic and thermodynamic studies, coupled with structural information and biochemical data, are necessary to fully describe protein ET reactions. Site-directed mutagenesis may then be used to elucidate structure–function relationships. When mutations selec-

tively alter ΔG° , H_{AB} , or λ , then one can determine how specific amino acid residues and features of protein structure influence k_{ET} by influencing the magnitude of these ET parameters. When mutations alter the kinetic mechanism for ET, one can determine the mechanisms by which adiabatic non-ET processes control the rates of ET reactions and how specific amino acid residues and features of protein structure influence these non-ET reactions.

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BIOGRAPHICAL INFORMATION

Victor Davidson received his B.S. in Biochemistry from the University of Illinois (1973) and Ph.D. in Chemistry from Texas Tech University (1982). After postdoctoral training at Purdue University (1982–1984) and a research position at University of California, San Francisco (1984–1988), he joined the faculty at the University of Mississippi Medical Center, where he is currently Professor of Biochemistry.

FOOTNOTES

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