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Mechanisms for control of biological electron transfer reactions

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ABSTRACT

Electron transfer (ET) through and between proteins is a fundamental biological process. The rates and mechanisms of these ET reactions are controlled by the proteins in which the redox centers that donate and accept electrons reside. The protein influences the magnitudes of the ET parameters, the electronic coupling and reorganization energy that are associated with the ET reaction. The protein can regulate the rates of the ET reaction by requiring reaction steps to optimize the system for ET, leading to kinetic mechanisms of gated or coupled ET. Amino acid residues in the segment of the protein through which long range ET occurs can also modulate the ET rate by serving as staging points for hopping mechanisms of ET. Specific examples are presented to illustrate these mechanisms by which proteins control rates of ET reactions.

1. Introduction

Important roles of enzyme and protein cofactors are participation in metabolic redox reactions and mediation of biological electron transfer (ET) reactions. While many natural redox centers in proteins are simply metals (e.g. copper and iron), others are organic molecules (e.g., flavins) or organometallic molecules (e.g., hemes). Some redox centers are protein-derived cofactors [1,2] such as tryptophylquinone cofactors that are formed by posttranslational modification of tryptophan residues [3]. In recent years there has been an increased understanding of how the protein environment of the cofactor influences the properties of these redox centers and the mechanisms for control of biological ET reactions. It has also become evident that unmodified residues in redox proteins can be reversibly oxidized and reduced during long range ET reactions. This can significantly accelerate the rate of ET by allowing it to occur via a mechanism referred to as hopping [4,5]. This review will concentrate on three general strategies by which proteins control the rates of biological ET reactions. The first section will provide examples of how the protein controls the magnitudes of the ET parameters; electronic coupling (H_{AB}) and reorganization energy (λ) that are associated with the ET reaction. The second section will describe how the protein can influence the rates of the ET reaction by kinetic mechanisms of gated or coupled ET. The third section will illustrate how amino acid residues in the segment of the protein through which long range ET occurs can enhance the rate of ET by serving as staging points for hopping mechanisms of ET.

2. Protein control of ET parameters

2.1. Electron transfer theory

Before discussing the ways by which the protein environment can influence ET parameters, and consequently the rate of ET, it is necessary to understand that ET reactions are not described by transition state theory (Eq. (1)). Instead they are described by a modified form of transition state theory (Eq. (2)) which is often referred to as Marcus theory or ET theory [6]. For ET reactions, the activation free energy (E_a) is equal to $(\Delta G^\circ + \lambda)^2/4\lambda$. ΔG° is the thermodynamic driving force for the reaction which is determined from the difference in the oxidation–reduction midpoint potential values (ΔE_m) for the donor and acceptor redox centers. This review will not discuss the mechanisms by which the protein environment influences E_m values of redox cofactors and metal. While this is an important consideration, this subject has been extensively studied and reviewed elsewhere [7]. Instead, this

Abbreviations: AADH, aromatic amine dehydrogenase; E_m, oxidation reduction midpoint potential; ET, electron transfer; H_{AB}, electronic coupling; k_{ET}, true electron transfer rate constant; k_{obs}, observed rate constant; λ, reorganization energy; MADH, methylamine dehydrogenase; MEDH, methanol dehydrogenase; N-quinol, aminoquinol; N-semiquinone, aminosemiquinone; PQQ, pyrroloquinoline quinone; preMADH, precursor of MADH; preTTQ, precursor of TTQ; TTQ, tryptophan tryptophylquinone.

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section will focus on the other ET parameters. The reorganization energy (λ) is the difference in energy between the reactant and product states at the potential energy minimum of the reactant state. For simplicity of presentation, the multidimensional energy surfaces that describe the reactant and product states are typically presented as intersecting parabolas (Fig. 1A). The gap at the intersection of the wavefunctions that are represented by the parabolas is a consequence of the interaction of the reactant and product states. If the gap at the intersection point is large then the probability of crossover when E_a is achieved is unity (Fig. 1B). This system is said to be adiabatic and is best described by Eq. (1). When the gap at the intersection of the wavefunctions is small (Fig. 1C), the activation energy may need to be achieved several times before the crossover from reactant state to product state occurs. This system is said to be nonadiabatic and is best described by Eq. (2). H_{AB} describes the degree to which the wavefunctions of the reactant and product states overlap (Fig. 1). The pre-exponential coefficient in transition state theory (A in Eq. (1)) is replaced in ET theory by a group of constants and variables of which the primary determinant is the H_{AB}, which in essence reflects the probability that the reaction will occur when the activation energy is achieved. As described in Eq. (3), the magnitude of H_{AB} is determined by the ET distance between donor and acceptor (r) and the nature of the intervening medium between donor and acceptor sites with respect to its ability to facilitate ET. The latter parameter is quantified as β. The other terms in Eqs. (1)–(3) are the characteristic frequency of the nuclei (k_o which is typically assigned a value of $10^{13}\ s^{-1}$), Planck's constant (h), the gas constant (R) and temperature (T).

$$k = A\exp[-E_a/RT] \quad (1)$$

$$k_{ET} = [4\pi^2 H_{ab}^2/h(4\pi\lambda RT)^{0.5}]\exp[-(\Delta G^\circ + \lambda)^2/4\lambda RT] \quad (2)$$

$$k_{ET} = k_o \exp[-\beta r]\exp[-(\Delta G^\circ + \lambda)^2/4\lambda RT] \quad (3)$$

The parabola model in Fig. 1 is a convenient way to describe the physical basis for ET. A challenge for those wishing to understand the regulation of biological ET reactions is to describe the protein structure–function relationships that influence the magnitudes of the ET parameters in Eq. (2) that determine k_{ET}. Sections 2.2 and 2.3 describe examples of the use of site-directed mutagenesis to selectively alter the values of H_{AB} and λ for ET reactions. These examples describe ET reactions involving quinoprotein dehydrogenases and their protein electron acceptors [8]. These include the tryptophan tryptophylquinone (TTQ)-dependent enzymes methylamine dehydrogenase (MADH) and aromatic amine dehydrogenase (AADH) and the pyrroloquinoline quinone (PQQ)-dependent methanol dehydrogenase (MEDH) [9]. Each of these cofactors participates in catalysis as well as ET. MADH from *Paracoccus denitrificans* catalyzes the oxidative deamination of primary amines, most specifically methylamine [10] and donates the substrate-derived electrons to the cupredoxin amicyanin [11] (Scheme 1A). It has been shown that MADH, amicyanin and cytochrome *c*-551i [12] form a ternary protein complex in which the oxidative deamination of methylamine is coupled to the reduction of the cytochrome via amicyanin [13–15] (Fig. 2A, and Scheme 1B). AADH from *Alcaligenes faecalis* catalyzes the oxidative deamination of aromatic amines, including tryptamine and dopamine [16] and donates electrons to the cupredoxin azurin [17] (Fig. 2B, and Scheme 1C). MEDH from *P. denitrificans* catalyzes the oxidation of methanol to formaldehyde [18] and donates electrons to cytochrome *c*-551i [19] (Fig. 2C, and Scheme 1D). These quinoprotein dehydrogenases are of particular interest because unlike the vast majority of dehydrogenases, they do not use $NAD(P)^+$ or small redox-active molecules as their physiologic electron acceptors, but instead donate electrons to other soluble redox proteins [8].

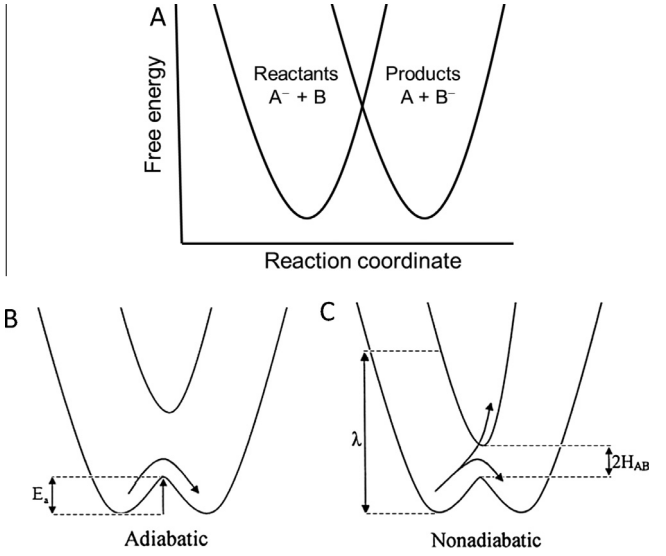


Fig. 1. (A) A simple two-dimensional representations of the multi-dimensional potential surfaces of product and reactant states. (B) A representation of a reaction that is described by transition state theory. (C) A representation of a reaction that is described by electron transfer theory.

2.2. How proteins can influence H_{AB}

As indicated in Eq. (3), the nature of the protein through which ET occurs is a determinant of k_{ET}. The protein is a heterogeneous matrix composed of a combination of secondary structures, and varying amounts of covalent bonds, hydrogen bonds, and empty space. The relative efficiency of the protein matrix in mediating ET is quantified by β in Eq. (3). Two approaches have been used to determine the effect of the intervening protein on ET. The pathways approach does not presume a single average β value to describe the protein medium between redox centers, but determines a β value for each through-bond and through-space segment of the ET pathway [20,21]. In this approach H_{AB} is proportional to the product of the β values for each segment of the pathway. An alternative to the pathways model for assessing the relative H_{AB} values for protein ET reactions is a direct distance model in which the effective β value is related to distance and the atomic packing density of the intervening protein medium [22,23]. The common theme for both approaches is that small decreases in the distances of through-space jumps in ET pathways, or increases in the atomic packing density, can dramatically increase the rate of ET. In other words, ET occurs much more slowly during jumps through space than when tunneling through bonds. Relative values of H_{AB} and β may be calculated from crystal structures of proteins or protein complexes. A useful tool for performing such calculations is the HARLEM computer program [24].

2.2.1. How protein dynamics can influence H_{AB} during ET through a protein

In principle, protein dynamics could transiently reduce the distance of through-space jumps in an ET pathway and increase atomic packing of the segment of the protein through which ET occurs. This would effectively increase H_{AB} in solution relative to the crystal state. This has been demonstrated for ET through the MADH–amicyanin–cytochrome *c*-551i complex. The crystal structure of this three-protein ET complex has been determined [13] and it was shown in solution that all three proteins must be present for ET from MADH to the cytochrome [11]. The ET reaction from the copper of amicyanin to the heme of the cytochrome in solution exhibited a k_{ET} of $87\ s^{-1}$ at 30 °C. Analysis of the

(A) Catalysis by methylamine dehydrogenase

$$CH_3NH_3^+ + 2Amicyanin(Cu^{2+}) + H_2O \rightarrow CH_2O + NH_4^+ + 2Amicyanin(Cu^{1+}) + 2H^+$$

(B) Catalysis by the methylamine dehydrogenase-amicyanin complex

$$CH_3NH_3^+ + 2Cytochrome\ c(Fe^{3+}) + H_2O \rightarrow CH_2O + NH_4^+ + 2Cytochrome\ c(Fe^{2+}) + 2H^+$$

(C) Catalysis by aromatic amine dehydrogenase

$$R\text{-}CH_2NH_3^+ + 2Azurin(Cu^{2+}) + H_2O \rightarrow R\text{-}CHO + NH_4^+ + 2Azurin(Cu^{1+}) + 2H^+$$

(D) Catalysis by methanol dehydrogenase

$$CH_3OH + 2Cytochrome\ c(Fe^{3+}) \rightarrow CH_2O + 2Cytochrome\ c(Fe^{2+}) + 2H^+$$

Scheme 1. Reactions catalyzed by quinoprotein dehydrogenases.

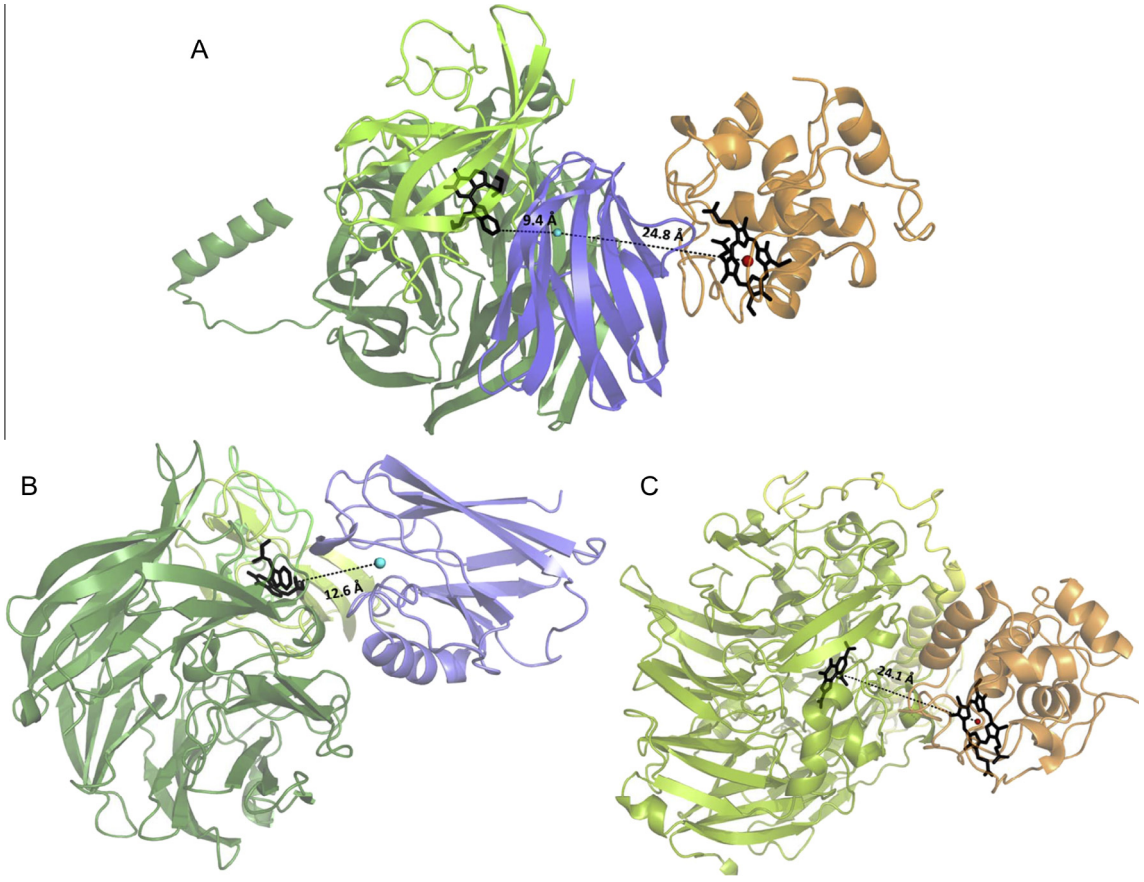


Fig. 2. Structures of protein ET complexes. (A) The structure of the MADH–amicyanin–cytochrome *c*-551i complex (PDB entry 2MTA) [13]. One half of the symmetrical complex is shown. The proteins are colored dark green for the MADH α subunit, lime green for the TTQ-containing MADH β subunit, blue for amicyanin, and orange for cytochrome *c*-551i. TTQ and the heme porphyrin ring are drawn in stick and colored black, copper is drawn as a sphere and colored light blue, iron is drawn as a sphere and colored red. (B) The structure of the AADH–azurin complex (PDB entry 2H3X) [120]. One half of the symmetrical complex is shown. The proteins are colored dark green for the AADH α subunit, yellow-green for the TTQ-containing AADH β subunit and blue for amicyanin. TTQ is drawn in stick and colored black. Copper is drawn as a sphere and colored light blue, iron is drawn as a sphere and colored red. (C) A model of the structure of the MEDH–cytochrome *c*-551i model complex [52]. This model was constructed using the coordinates of the structures of MEDH (PDB entry 1LRW) and cytochrome *c*-551i (PDB entry 2MTA) as described in reference [50]. The proteins are colored green for the PQQ-containing MEDH α subunit, yellow for the MEDH β subunit, and orange for cytochrome *c*-551i. PQQ and the heme porphyrin ring are drawn in stick and colored black. Iron is drawn as a sphere and colored red. These figures were produced using PyMOL (http://www.pymol.org/).

temperature dependence of k_{ET} using Eq. (2) yielded a value for H_{AB} of 0.3 cm^{-1} [25]. A study of this same ET reaction in crystals of the protein complex yielded a much slower k_{ET} of 3×10^{-3} $s^{-1}$and much smaller value for H_{AB} of 7.3×10^{-4} cm^{-1} [26]. The experimentally determined λ values for the reactions in solution and in the crystal state were identical, thus verifying that the same true ET reaction was being monitored in both states. An explanation for these results was provided by the results of a joint X-ray and neutron diffraction study of amicyanin [27] which revealed a dynamic nature of the protein, especially around the

copper-binding site. Furthermore, the residues in the region of the protein between the redox centers were shown to be highly dynamic, as judged by hydrogen/deuterium exchange. This evidence for the dynamic nature of amicyanin provided a reasonable explanation for the previous ET results, as the protein dynamics could increase k_{ET} in solution relative to the crystalline state by transiently shortening through-space jumps in pathways or by increasing the atomic packing density, or both. This would effectively increase the H_{AB} in solution relative to the crystalline state.

2.2.2. How the orientation of proteins within a complex can influence H_{AB} during interprotein ET

For interprotein ET a through-space jump is absolutely required for the electron to get from one protein to the other. Therefore, the nature and positions of residues at the protein–protein interface have a strong influence on the rates of the ET reaction. This was demonstrated in the structurally-characterized MADH–amicyanin complex [28]. Conversion of the interfacial residue Phe97 of amicyanin to Glu significantly decreased k_{ET} for the ET reaction from the quinol form of MADH to oxidized amicyanin [15]. The ΔG° and λ associated with the ET reaction were unaffected by the F97E mutation. The decrease in k_{ET} was due solely to a decrease in H_{AB} from 12 cm^{-1} to 3 cm^{-1}. Phe97 is located immediately adjacent to the site at which the electron is predicted to jump from MADH to amicyanin and the F97E mutation causes an increase in this critical interprotein distance within the protein complex. It was determined that an increase of approximately 0.9 Å in this interprotein through-space jump accounts for the observed decrease in k_{ET} and experimentally-determined decrease in H_{AB}.

2.3. How proteins can influence λ

λ is comprised of two components, the inner-sphere reorganization energy (λ_{in}) and the outer-sphere reorganization energy (λ_{out}). λ_{in} describes redox-dependent nuclear perturbations of the redox centers, such as changes in bond lengths and bond angles of metal ligands or organic cofactors. λ_{out} describes changes in the surrounding medium that are associated with the ET reaction, such as reorganization of solvent molecules. For intraprotein ET reactions, configurational changes of amino acids near and between the redox centers may also contribute to λ_{out}. For interprotein ET reactions, changes in the protein interface may also contribute to λ_{out}. Examples are presented of how alterations of the protein by site-directed mutagenesis can alter λ. One involves the copper site of amicyanin and the other involves the TTQ site of MADH.

2.3.1. How proteins can influence λ_{in}

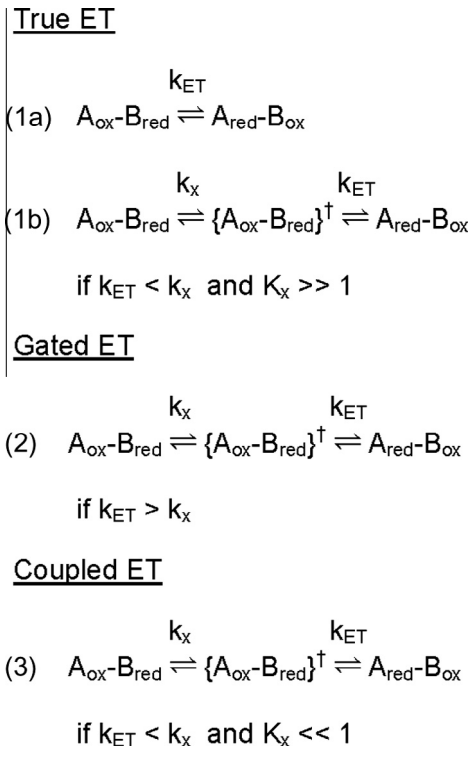
Mutation of the axial Met ligand of the type 1 copper site of amicyanin to Gln caused an increased rhombicity of the ligation geometry of the type 1 site [29,30]. The mutation had little effect on the E_m value however the k_{ET} for the ET reaction from quinol MADH to oxidized M98Q amicyanin was reduced 45-fold compared to that for native amicyanin. Analysis of the temperature dependence of these ET reactions showed no change in the experimentally-determined H_{AB} but an increase of 0.4 eV in λ. A similar mutation of the axial Met ligand of the type 1 copper site in nitrite reductase increased the λ by 0.3 eV [31]. These two results correlate well with the results of quantum chemical calculations of λ of model compounds of the type 1 copper site with Gln and Met axial ligands [32], and are consistent with the concept of "rack-induced" folding of type 1 copper proteins facilitating rapid ET by reducing λ [33]. These studies demonstrated that the geometric constraints that the protein places on the metal ligands, in this case the extent of rhombicity of the type 1 copper site, significantly influences λ. In this case it is most likely λ_{in} that is affected.

2.3.2. How proteins can influence λ_{out}

Residue αPhe55 of MADH has been shown by site-directed mutagenesis to be a key determinant of substrate specificity of MADH [34,35]. An αF55A MADH mutation also significantly increased the k_{ET} for the ET reaction from quinol MADH to oxidized amicyanin [36]. This was primarily a consequence of a decrease in the λ for this reaction of 0.5 eV. The crystal structure of αF55A MADH revealed no significant perturbation of structure of MADH except for the mutated residue which resides 7 Å from TTQ and is not in the ET path from TTQ to amicyanin. The most notable difference in the structure was a change in the solvent content of the active site and substrate channel. Two waters are in the MADH active site within 5 Å of TTQ and shielded from the bulk solvent by αPhe55. Only one of these waters is in the αF55A MADH active site. Since the reorganization of solvent is a major contributor to λ_{out} for ET reactions, the observed decrease in λ is consistent with TTQ being less solvated in αF55A MADH than in native MADH. This may also explain why the λ for the reaction with native MADH is unusually large for a true ET reaction (i.e., 2.3 eV) [37,38]. Protein ET reactions between metal redox centers that are completely buried within the protein matrix and shielded from solvent tend to exhibit λ values that are much less than this.

3. Kinetic mechanisms of protein ET reactions

Given the complex nature of the structure and function or redox enzymes and proteins, one must acknowledge that the observed rate of the overall redox reaction (k_{obs}) may not necessarily be a true k_{ET}. The k_{obs} may describe a non-ET event that precedes ET. Such events include binding of redox partners, reorientation of redox proteins within a complex, a protein conformational change or a chemical event such as deprotonation. In such cases, the preceding event is required to optimize or activate the system for ET [39–41]. This could be true of both interprotein and intraprotein ET reactions. In this respect, the protein plays a major role in determined the rate of ET reactions.



Scheme 2. Kinetic mechanisms of true, gated and coupled electron transfer reactions.

A simple kinetic model (Scheme 2) has been used to define, identify and analyze the kinetic mechanisms of protein ET reactions. In this model k_x is the rate of a reaction step that precedes ET and is necessary to optimize the system for ET. K_x is the equilibrium constant for that reaction step. In order for k_{obs} to be k_{ET}, the ET event must either be a single-step reaction (1a in Scheme 2) or be the rate-determining step for the overall reaction (1b in Scheme 2). In the latter case, in order for k_{obs} to equal k_{ET}, K_x must also be greater than 1. If a kinetically-indistinguishable reaction step precedes ET and is slower than k_{ET}, the k_{obs} will be the rate constant for that non-ET event (k_x) (2 in Scheme 2). This scenario describes a gated ET reaction [42,43]. It is also possible that a preceding reaction step may be faster than k_{ET}, but still affect k_{obs} if K_x is much less than 1 (i.e., the preceding reaction is thermodynamically very unfavorable). In this scenario k_{obs} will be equal to the product of K_x and k_{ET} (3 in Scheme 2) and this is described as a coupled ET reaction [44]. Experimentally distinguishing whether an ET reaction is true, gated or coupled is challenging. If the experimentally-determined ET distance from analysis using Eq. (3) is similar to the distance between redox centers observed in a crystal structure of the protein or protein complex, then this would be compelling evidence that k_{obs} is k_{ET}. If the experimentally-determined ET distance is unreasonably small, or correspondingly if the H_{AB} is unreasonable large, then this would suggest that k_{obs} is not k_{ET}. Furthermore, if reaction conditions such as ionic strength, viscosity or pH influence k_{obs}, then it is not likely k_{ET}, which should be unaffected by such conditions. Examples of ET reactions that are gated by and coupled to a variety of non-ET events are presented.

3.1. Gated ET

3.1.1. An example of conformationally-gated ET

In a conformationally-gated ET reaction the non-ET event that precedes the true ET step is a conformational rearrangement. In the case of interprotein ET, if the ideal orientations of the proteins for binding and ET are different, then some rearrangement of proteins within the complex after binding must occur to maximize k_{ET}. This phenomenon has been elucidated by site-directed mutagenesis of Met51 of amicyanin which resides at the MADH–amicyanin interface in the protein ET complex [45,46]. When Met51 was mutated to Ala, k_{obs} for the reaction from O-quinol MADH to oxidized amicyanin decreased. Analysis of the reaction using Eqs. (2) and (3) yielded an increase in the apparent values of λ and H_{AB}, and a corresponding value for ET distance that was impossibly small. These data suggested that k_{obs} was not k_{ET}. The K_d for complex formation between M51A amicyanin and MADH was the same as that for native amicyanin indicating that it was not the initial binding event that was perturbed and that the relative orientations of the proteins immediately upon binding are probably the same. It was concluded that this mutation slows the rate of a conformational rearrangement that occurs rapidly within the native amicyanin-MADH complex subsequent to binding and prior to ET, thus causing ET to become gated by the slower rearrangement [46]. 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.

3.1.2. An example of chemically-gated ET

It has been possible to study four different ET reactions between MADH and amicyanin, ET to amicyanin from the O-quinol and O-semiquinone forms which are generated by reduction with dithionite [38], and ET to amicyanin from the aminoquinol (N-quinol) and aminosemiquinone (N-semiquinone) forms which are generated by reduction with methylamine [47] (Scheme 3). The reactions of the O-quinol, O-semiquinone and N-semiquinone exhibit the same values of H_{AB} and λ, and a predictable variation of k_{ET} with $\Delta G°$. As such, these may be considered to be true ET reactions. In contrast, ET from N-quinol MADH may be considered to be an example of a chemically-gated ET reaction. The k_{obs} for the reaction of N-quinol MADH with amicyanin was dependent on pH and the concentration of monovalent cations [48]. It also exhibited a large solvent kinetic isotope effect [49]. In contrast to the reactions of the other forms of MADH, thermodynamic analysis of the reaction of the N-quinol MADH yielded an unreasonably large value of H_{AB} of 23,000 cm^{-1} and corresponding impossible negative ET distance of −4.9 Å. These observations provided strong evidence that the k_{obs} for this reaction did not describe a true nonadiabatic ET reaction and that this reaction is more appropriately analyzed by transition state theory (Eq. (1)).

A model was presented to describe the role of the protein in catalyzing this chemically-gated reaction (Scheme 3). The dependence of the reaction rate on pH and cations was attributed to an ionizable amino acid side chain that is involved in binding the cation that in turn stabilizes a negatively charged transient reaction intermediate that is formed by the rate-limiting deprotonation of the N-quinol amino group 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 [50]. Very similar results were obtained for the reaction of N-quinol AADH with azurin, including a solvent kinetic isotope effect on k_{obs} for the ET reaction [51]. Thus, this seems to be a common feature in the reaction mechanism of TTQ-dependent redox enzymes.

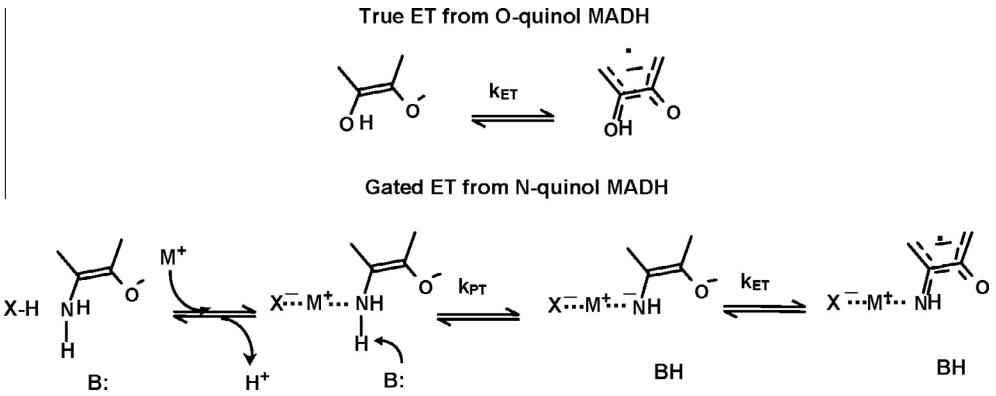
3.2. Coupled ET

3.2.1. An example of conformationally-coupled ET

An example of a conformationally coupled ET reaction is the reaction of the reduced PQQ cofactor of MEDH with the heme of oxidized cytochrome *c*-551i. Analysis of this reaction using Eqs. (2) and (3) yielded values of λ of 1.9 eV and H_{AB} of 0.07 cm^{-1} with a corresponding predicted a distance between redox centers of 15 Å [19]. This distance was somewhat less than that which was predicted from a computational docking model of the putative MEDH–cytochrome *c*-551i complex [52] (Fig. 2C). It was noted that the experimentally-determined k_{obs} for the ET reaction and the K_d for complex formation each varied with ionic strength, but to a different extent. The ionic strength dependence of each of these parameters was analyzed by van Leeuwen theory [53], 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 [54]. It was concluded that the ionic strength dependence of k_{obs} was a consequence of the ionic strength of the pre-equilibrium rearrangement process, K_x, which is ionic strength dependent. The ionic strength dependencies of this process and the initial binding (K_d) are different, which explains the experimental results.

3.2.2. An example of kinetically-coupled ET

Pro94 which lies within the ligand loop of amicyanin which contains three of the four copper ligands, Cys92, His95 and Met98 [55]. When Pro94 was converted to Ala the crystal structure of oxidized (Cu^{2+}) P94A amicyanin was relatively unaffected but the structure of reduced (Cu^{+}) P94A amicyanin exhibited two alternate conformers with the positions of the copper 1.4 Å apart [56]. One conformation was similar to that of the native protein. In the other a water replaced Met98 as the fourth copper ligand and the ET distance to the heme of the cytochrome was increased by 1.4 Å, which makes ET from this conformation much less favorable. Analysis of the ET reaction from P94A amicyanin to cytochrome *c*-551i suggested that this true ET reaction had been converted to a



Scheme 3. Chemically gated electron transfer from N-quinol MADH.

coupled ET reaction [57]. A kinetic mechanism was proposed to explain these data. Within the MADH–amicyanin–cytochrome *c*-551i complex (Fig. 2A), after the reduction of Cu^{2+} by MADH ET from the conformation with the most efficient ET pathway from Cu^{+} is coupled to an unfavorable equilibrium with the unfavorable conformation, thus limiting the availability of the optimized state and decreasing k_{obs} for the ET reaction.

4. Protein-mediated hopping

As discussed above, proteins have tuned their internal environments, cofactors, and interfaces to maximize the efficiency of ET. However, a critical determinant of k_{ET} that remains is the ET distance. The rates of ET versus ET distance have been extensively examined in a variety of protein systems to illustrate the exponential decay of k_{ET} with increasing distance between the donor and the acceptor [58–60] (see Eq. (3)). It is evident that there is a limit to the distance over which protein ET transfer reactions can occur at a rate which is sufficient to support the biological activity. Since proteins cannot always rearrange the distances between their redox active sites, a solution that nature has evolved is to shift from a single-step electron tunneling process to a multi-step process, which is referred to as hopping [4,5].

When amino acid residues mediate electron tunneling through the protein, the intervening residues are not oxidized or reduced but simply provide a conductive matrix. During hopping certain amino acid residues are reversibly oxidized and reduced and serve as discrete intermediate points that act as relays between the electron acceptor and donor. Two alternative hopping mechanisms are possible. If the electron donor reduces the intermediate which in turn reduces the electron acceptor, then this would be electron hopping. If the electron acceptor oxidizes the intermediate which in turn oxidizes the electron donor, then this would be hole hopping. By adding relay sites, the distance the hole or electron has to cross decreases stepwise. Thus, one long slow electron tunneling event is replaced by a cascade of shorter and much faster steps. In the simplest case, the rate of ET equals that of the slowest hop, which is still much faster than direct tunneling over the entire distance.

There are only a few amino acids with biologically accessible redox potentials (~1 V or less) that produce stable radicals by either the gain or loss of an electron. Of these redox-active amino acids, Trp and Tyr are best-suited to be relay sites. These residues are heterocyclic aromatic amino acids which are capable of stabilizing both charged and neutral radicals. Upon the loss of an electron, the initial result is a charged cation radical, which is stable in some environments but may be a destabilizing force within a hydrophobic protein environment. Since Trp and Tyr contain more electron negative heteroatoms within their aromatic rings, the radical cation can easily deprotonate leaving a stable neutral radical. These neutral radicals are more stable in protein environments, but are less likely to be reduced given that the resulting species will once more be charged. The neutral radicals are the terminal catalytic site for some proteins [61,62]. In order to utilize the charged radical cation as an intermediate step, proteins have evolved to either stabilize the radical cation or provide a proton donor/acceptor within the vicinity of the aromatic ring. The E_m values associated with the oxidation of Trp and Tyr residues in proteins is very sensitive to their environment, thus providing the protein with another mechanism of controlling ET rates. Trp residues have been reported with potentials as low as 0.6 V and as high as a volt [63–67]. Tyr residues are typically reported to have higher potentials of 0.9–1.4 V [64–68]. Some other amino acid residues are known to form radical species but do not seem best-suited to serve as hopping relays. For example, glycine radicals typically act as catalysts rather than a relay [69–72]. Cysteine is capable of both oxidation and reduction, but has been shown to act as an initial or terminal site for ET [73–76].

4.1. Model systems of hopping through proteins

Experimental systems have been developed in order to characterize the ability of Trp and Tyr to function as hopping intermediates. A peptide construct was developed with a Tyr as an electron donor and a modified amino acid as an initiator [77,78]. In this system it was possible to demonstrate that Tyr was a viable electron donor and that a peptide chain could utilize a multi-step ET process through a modified Tyr localized in the interior of the peptide chain. Other peptide models have been used to determine spectroscopic characteristics of the amino acid-based radicals. For example, a rhenium(I) tri-carbonyl diimine complex was synthesized with a peptide ligand and photo-triggered Trp radical cations were analyzed using time-resolved IR and emission spectroscopy [79].

Hopping models have also been generated within proteins by engineering the Type 1 copper protein, azurin. A rhenium or ruthenium photoactive label was attached to the protein surface to act as the acceptor and donor. It was demonstrated that the sole Trp residue in azurin could serve as a hopping intermediate for intraprotein ET between the label and the native copper site [80] and intermolecular hopping during ET between two rhenium photolabeled azurin mutants [81]. Hopping via an engineered nitrotyrosinate was also demonstrated in the azurin system [82].

4.2. Natural examples of hopping through proteins

4.2.1. DNA photolyase and cryptochromes – molecular wires

Hopping through proteins with molecular wires refers to proteins that contain a series of closely spaced and properly oriented

aromatic residues for very rapid hopping-mediated ET. This terminology may be applied to the DNA photolyase and cryptochrome families of proteins. Each of these proteins contains a FAD cofactor which functions in two distinct light dependent reactions: catalysis utilizing a wavelength tuned antenna and photoreduction of the FAD. The photoreduction cycle utilizes a triad of Trp residues which are highly conserved and provide an ET pathway between the FAD and a Trp on the protein surface (Fig. 3). The reaction is initiated by photoreduction of FAD followed by rapid ET that generates Trp radicals.

DNA photolyases repair damaged DNA substrates. The cyclobutane pyrimidine dimer photolyases repair lesions in single and double stranded DNA; while the 6-4 photolyases cleave (6-4) pyrimidine–pyrimidone dimers. All photolyases are monomeric proteins with two domains. They bind their substrates in the dark and turnover upon exposure to the wavelength of light for which their individual antennae are tuned [83,84]. The cryptochromes are also monomeric with two domains and contain a FAD cofactor and Trp triad, and have high sequence homology within their photoactive domains near the N terminus [85,86]. While a few cryptochromes are capable of DNA repair this class of enzyme has a broad range of non-DNA substrates and a greater range of physiological functions that include signaling and circadian rhythms [87,88]. The mechanism of hopping through the tryptophan triad seems to be universally conserved among these enzymes [83,84,89–93] although some have alternative pathways in place, including other Trp or Tyr residues that can substitute if the triad is disrupted [94,95].

4.2.2. Hopping-mediated proton-coupled ET in ribonucleotide reductase

Hopping systems that employ proton-coupled ET pathways utilize aromatic residues that rely on a proton acceptor/donor pair or participate in proton shuttling. Tyr residues more readily deprotonate than Trp and so Tyr radicals are relatively sensitive to the pH of their micro environment. This sensitivity affects the E_m value associated with the redox reaction which is also dependent on protonation state. The neutral/radical cation potential of Tyr in water is about twice that of the deprotonated/neutral radical potential [96]. This pH dependence implies that the oxidization of Tyr is a proton-coupled ET. In other words, the loss of an electron is inherently dependent or related to a change in protonation. In order to avoid the penalty in energy surrounding the irrevocable loss of the Tyr proton, having a proton acceptor and/or donor nearby allows the protein to compensate for the energy difference. The role of Tyr residues in this process has been studied extensively in ribonucleotide reductase (RNR).

There are three classes of RNR; each determined by the initial metal cofactor which oxidizes the catalytic thiyl radical [97,98]. The class I protein always contains a metal di-nuclear site, but the metal located within the cofactor often determines the subclass of the RNR; additionally a radical initiator located by the metallocofactor is also a determination of sub class. For class Ia and Ib this radical initiator is tyrosine [99–101]. Class II contains an adenosylcobalamin and the class III utilizes a glycyl radical generated by an iron sulfur cluster [102].

For the class I RNR's the dinuclear metal site utilizes an extended ET chain containing multiple Tyr residues and possibly a Trp. This extended aromatic chain stretches two subunits. The β subunit contains the metal cofactor and a few residues who shuttle their protons orthogonally, to proton acceptors; while the α subunit contains two Tyr residues who shuttle their protons collinearly and the catalytic thiyl radical. The proton-coupled ET pathway has been studied intensely in the class Ia RNR. By incorporating nitro-tyrosine, amino-tyrosine, or DOPA as replacements for individual Tyr residues in the predicted hopping pathway, it was possible to determine how modifications of properties of the hopping relays, such as pK_a value and E_m value, effected the hopping process [103–106]. It was also possible to replace a portion of the hopping pathway with an artificial peptide containing a photo-sensitizer on the terminal end [107,108]. With this system the photo-activated RNR could be probed spectroscopically to determine ET rates and characterize the native and modified Tyr hopping intermediates.

4.2.3. Hopping-mediated long distance hole transfer through MauG

MauG [109], which is encoded by the *mauG* gene, is a diheme containing enzyme that catalyzes the final steps in the biogenesis of the posttranslationally-derived TTQ cofactor of MADH [110]. The substrate for MauG is a precursor of MADH (preMADH) that contains preTTQ, a hydroxylated residue βTrp57 and an unmodified βTrp108 [111]. MauG catalyzes the crosslinking of the indole rings of these Trp residues, the addition of a second oxygen to βTrp57, and the final oxidation to quinone, in that order [112,113] to form the protein-derived TTQ cofactor. The heme irons of MauG are approximately 40 Å and 19 Å from the edge of

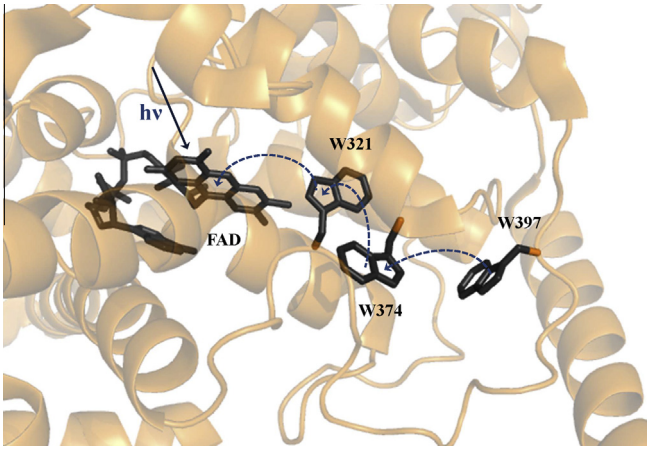


Fig. 3. Proposed hopping pathway in a cryptochrome. The relevant portion of the structure of *Aribidopsis* cryptochrome 2 (PDB entry 1U3C) [121] is shown. FAD and the tryptophan triad are drawn in stick. The dashed lines indicate the flow of electrons following photoreduction (*hv*). This figure was produced using PyMOL (http://www.pymol.org/).

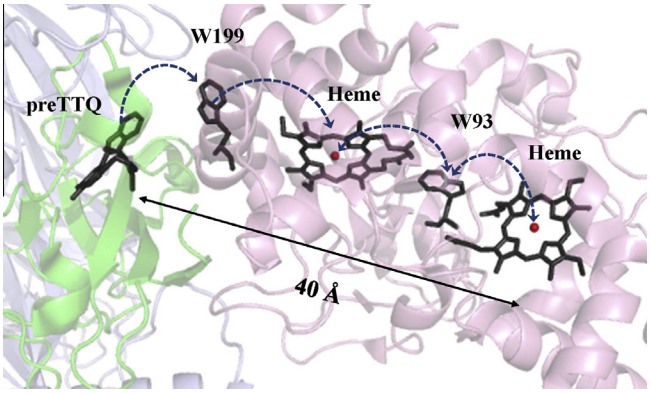


Fig. 4. Proposed hopping pathway from preMADH to the hemes of MauG. The relevant portion of the structure of the preMADH–MauG complex (PDB entry 3L4O) [114] is shown. The protein is colored pink for MauG, green for the MADH β subunit and blue for the MADH α subunit. The hemes, Trp93 and Trp199 of MauG, and βTrp108 and βTrp57 of preMADH (preTTQ) are drawn in stick. The dashed lines indicate the flow of electrons from preMADH to the *bis*-Fe^{IV} hemes of MauG. The lines to and from each heme to Trp93 have two arrows in indicate charge resonance stabilization of the *bis*-Fe^{IV} state. The nearest distance from preTTQ to the Fe of the oxygen-binding heme of MauG is indicated. This figure was produced using PyMOL (http://www.pymol.org/).

βTrp108, which is the closest of the residues that are modified to form TTQ [114]. Therefore long range ET is required for the catalysis (Fig. 4). The redox center of MauG that serves an electron acceptor for the three two-electron oxidation reactions is a *bis*-Fe^{IV} species [115,116] comprised of a five-coordinate ferryl heme and a six coordinate His-Tyr ligated-heme. The *bis*-Fe^{IV} species exhibits charge resonance stabilization which essentially distributes two "holes" over the two hemes and a bridging Trp93 residue via a mechanism of ultrafast ET between hemes that is mediated by hopping through Trp93 [115]. A residue of MauG on the protein interface, Trp199, was shown to be essential for the oxidation of the substrate [117,118]. Mutation of Trp199 resulted in loss of the catalytic activity while not affecting the structure of the MauG-preMADH complex or the redox properties of the hemes. Furthermore, a kinetic and thermodynamic analysis of the ET reaction from the MADH precursor to *bis*-Fe^{IV} MauG yielded experimentally-determined values of H_{AB} and ET distance that were consistent k_{ET} describing ET through a hopping segment involving Trp199 and not descriptive of a single-step electron tunneling reaction [119].

5. Conclusions

There are several different mechanisms by which proteins can modulate the rates of ET reactions to and from protein-bound redox centers. Proteins can shield the redox center from water and optimize the ligation geometry of metal centers to minimize λ to increase k_{ET}. Protein dynamics can transiently increase H_{AB} to increase k_{ET}. Protein conformational changes or chemical reaction steps involving amino acid residues can be used to optimize the system for ET. In gated or coupled ET mechanisms the k_{obs} is less than the true k_{ET}, but the ET reaction might not occur at all without the preceding reaction step that optimizes the system for efficient ET. Amino acid residues, in particular Trp and Tyr, may be reversibly oxidized and reduced and allow hopping mechanisms that will significantly increase k_{ET} relative to the rate of a single step electron tunneling process.

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