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New Concepts

Chemically Gated Electron Transfer. A Means of Accelerating and Regulating Rates of Biological Electron Transfer†

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ABSTRACT: Long-range protein electron transfer [ET] reactions may be relatively slow because of long ET distance and low driving force. It is possible to dramatically increase the rate of such nonadiabatic reactions by using an adiabatic chemical reaction to activate the system for rapid ET. Three such examples are discussed; nitrogenase, pyruvate:ferredoxin oxidoreductase, and the methylamine dehydrogenase−amicyanin complex. In each example, the faster activated ET reaction is gated (i.e., rate-limited) by the chemical reaction. However, the reaction rate is still orders of magnitude greater than that of the ungated true ET reaction in the absence of chemical activation. Models are presented to describe the mechanisms of activation in the context of ET theory, and the relevance of such chemically gated ET to the regulation of metabolism is discussed.

Long-range electron transfer (ET)[1] within and between proteins is fundamental to respiration, photosynthesis, and redox reactions of intermediary metabolism. These are kinetically complex processes that may require several reaction steps, including protein−protein interactions, protein rearrangements and conformational changes, chemical transformations such as proton transfer, and the actual ET event. A significant problem in applying ET theory to long range protein ET reactions is that it is often difficult to ascertain whether or not the observed rate of the redox reaction (k_{obs}) is a true ET rate constant (k_{ET}). In such reactions, a non-ET event (K_x) may be required to optimize or activate the system for ET. This applies to both interprotein (eq 1) and intraprotein (eq 2) ET reactions. We have previously developed kinetic models that define kinetically complex ET reactions as true, gated or coupled (eqs 3−5), and described criteria for categorizing such ET reactions (*1*−*4*).

$$A_{ox} + B_{red} \overset{K_a}{\rightleftharpoons} A_{ox}/B_{red} \underset{k_{-X}}{\overset{k_X}{\rightleftharpoons}} [A_{ox}/B_{red}]^* \underset{k_{-ET}}{\overset{k_{ET}}{\rightleftharpoons}} A_{red}/B_{ox} \quad (1)$$

$$A_{ox}-B_{red} \underset{k_{-X}}{\overset{k_X}{\rightleftharpoons}} [A_{ox}-B_{red}]^* \underset{k_{-ET}}{\overset{k_{ET}}{\rightleftharpoons}} A_{red}-B_{ox} \quad (2)$$

$$\text{true ET:} \quad k_{ET} \ll k_X \quad K_X(k_X/k_{-X}) \gg 1 \quad k_{obs} = k_{ET} \quad (3)$$

$$\text{gated ET:} \quad k_X \ll k_{ET} \quad k_{obs} = k_X \quad (4)$$

coupled ET:

$$k_{ET} \ll k_X \quad K_X(k_X/k_{-X}) \ll 1 \quad k_{obs} = K_X{}^*k_{ET} \quad (5)$$

Discussion of gated ET reactions often focuses on conformationally gated ET (*5*). In such reactions a protein conformational change, or rearrangement of proteins relative to each other within an ET complex, occurs prior to ET and optimizes the positions of the redox centers and their geometries for the ET event. Examples of this phenomenon

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have been reported for physiologic and nonphysiologic protein ET reactions (e.g., refs *6*−*9*). This paper focuses on an emerging class of protein ET reactions that are gated, not by conformational changes, but by chemical adiabatic reactions (i.e., making or breaking of bonds). Three specific examples are discussed to illustrate this point. What makes these reactions particularly interesting is that in the absence of the preceding chemical reaction, the true ET reaction is relatively slow. After the chemical reaction step, the ET rate constant is orders of magnitude greater. Thus, even though these reactions are gated by a chemical reaction step that is slower than the activated ET step, the overall rate of the gated redox reaction is much greater than the rate of the "ungated" true ET reaction in the absence of the chemical reaction.

ANALYSIS OF ET REACTIONS AND CRITERIA FOR GATED ET

The theoretical basis for what physical parameters control the rates of nonadiabatic ET reactions is well established (*10*). An important difference between these true ET reactions and adiabatic chemical reactions is that the probability of the reaction occurring when the activation energy is achieved is approximately unity for a chemical reaction, but for a nonadiabatic ET reaction, the probability of the reaction occurring when the activation energy is achieved is much less than one (Figure 1). Thus, description of nonadiabatic ET reactions requires a modified form of transition state theory (eqs 6 and 7) (*10*). The activation free energy for the reaction is equal to $(\Delta G^\circ + \lambda)^2/4\lambda$, where ΔG° is the driving force determined from the redox potential difference for the ET reaction and λ is the reorganization energy.

$$k_{ET} = \frac{4\pi^2 H_{AB}{}^2}{h\sqrt{4\pi\lambda RT}}\, e^{-(\Delta G^0+\lambda)^2/4\lambda RT} \quad (6)$$

$$k_{ET} = k_o \exp[-\beta(r - r_o)] \exp[-(\Delta G^\circ + \lambda)^2/4\lambda RT] \quad (7)$$

The degree of nonadiabicity (i.e., 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}). If H_{AB} is zero, then one would observe an intersection of the potential energy curves for the reactant and product states (a diabatic state, Figure 1A). When H_{AB} is finite, then there is a gap between the curves at the intersection point. When H_{AB} is small (i.e., a weakly coupled reaction), then this gap is small enough that the system may "jump the gap" so that reactants sometimes do not go to products even when the activation energy is achieved (nonadiabatic, Figure 1B). The smaller the value of H_{AB}, the more times that energy barrier has to be achieved before the ET occurs. As H_{AB} increases, the probability of the reaction occurring when the activation energy is achieved increases. When H_{AB} becomes sufficiently large, the reaction will proceed to completion each time the activation energy is achieved. Then the reaction is considered adiabatic (Figure 1C) and no longer described by eq 6. In eq 7, the probability term is described by the distance between redox centers ($r - r_o$) and β, which is related to the nature of the intervening medium. Discussions of the mathematical and physical meanings of H_{AB}, λ, and β may be found in many reviews of ET theory (*2*, *10*−*13*). Other terms in eqs 6 and 7 are Planck's constant (h), the gas constant (R), temperature (T), and the characteristic frequency of the nuclei (k_o).

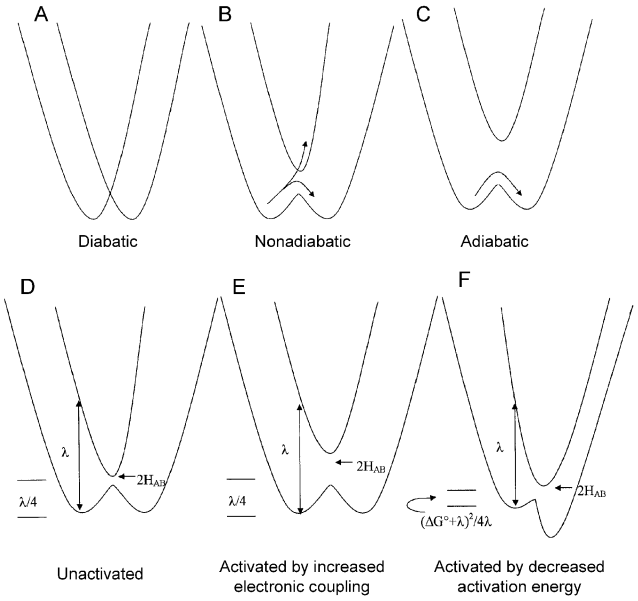

FIGURE 1: Energy profiles for electron-transfer reactions. Panels A−C describe the extent of crossover between potential energy curves for the reactant and product states in diabatic, nonadiabatic and adiabatic systems. In these examples, ΔG° is set at zero. Panel D graphically depicts the parameters λ, H_{AB}, and the activation energy $(\Delta G^\circ + \lambda)^2/4\lambda$. When ΔG° is zero, the activation energy reduces to $\lambda/4$. Panel E shows the consequences of increasing H_{AB}, as would occur after a conformational change that brings the electron donor and acceptor closer together. Panel F shows how altering ΔG° for the reaction can decrease the activation energy for the reaction as well as increase the equilibrium constant for the reaction.

In contrast to true and coupled ET reactions, the rate constant for a gated reaction will not exhibit a predictable dependence on ΔG° since the rate-determining reaction step is not driven by the redox potential difference between the reactants. When the temperature dependence of k_{obs} for a gated reaction is analyzed by eq 6, the fitted value of H_{AB} will likely exceed the nonadiabatic limit, which is often taken to be about 80 cm^{-1} (*13*). Similar analysis by eq 7 is likely to yield unreasonably small or negative values for the ET distance (*2*). Values of λ obtained from such analysis may also be unreasonably large, but this alone is not a sufficient criterion for categorizing a reaction as gated. It may be possible to confirm that an ET reaction is gated by examining the effects on k_{obs} of varying solution conditions and performing kinetic isotope effect studies. True ET rates should not be affected by buffer conditions or exhibit isotope effects, but rates of gated reactions may, depending on the nature of the reaction step which is rate-determining for the observed redox reaction.

EXAMPLES OF CHEMICALLY GATED ET REACTIONS

Nitrogenase. The ET reaction between the iron protein and molybdenum−iron protein of the nitrogenase complex was

[1] Abbreviations: ET, electron transfer; H_{AB}, electronic coupling; λ, reorganization energy; PFOR, pyruvate:ferredoxin oxidoreductase; TPP, thiamine pyrophosphate; MADH, methylamine dehydrogenase; TTQ, tryptophan tryptophylquinone.

Table 1: Enhancement of k_{ET} in Chemically Gated ET Reactions

experimental system	k (s^{-1})	H_{AB} (cm^{-1})	λ (eV)	r (Å) calcd	r (Å) structure	reaction type
nitrogenase (L127Δ)[a]	0.18	0.9	2.4	14	14[b]	nonadiabatic
nitrogenase (wild type) + MgATP[a]	150	7×10^{12}	4.4	−84	14[c]	gated
PFOR + pyruvate[d]	0.001	0.07	2.1	14.6	12[e]	nonadiabatic
PFOR + pyruvate + CoA[d]	140	499	2.6	1.6	12[e]	gated
O-quinol MADH/amicyanin[f]	10	20	2.4	9.5	9.4[g]	nonadiabatic
N-quinol MADH/amicyanin[f]	275	23 000	3.5	−4.9	9.4[g]	gated

[a] Ref *14*, k_{ET} values shown here were measured at 23 °C. [b] Ref *16*. [c] Ref *15*. [d] Ref *17*, k_{ET} values shown here were measured at 10 °C. [e] Ref *25*. [f] Refs *21*−*22*, k_{ET} values shown here were measured at 21 °C in 10 mM potassium phosphate, pH 7.5. [g] Ref *26*.

shown to be gated by events associated with either MgATP binding or hydrolysis (*14*). In this system, binding of MgATP to the iron protein induces a conformational change that is necessary for proper docking of that protein with the molybdenum−iron protein (*15*). Deletion of residue Leu127 of the iron protein produced a mutant which adopted a conformation that mimicked the MgATP-bound state (*16*) and allowed the study of ET within the nitrogenase complex in the absence of MgATP. The ET rate for the reaction of the L127Δ protein complex in the absence of MgATP was approximately 1000-fold smaller than that of the wild-type complex in the presence of MgATP. Analysis of these reactions by ET theory (Table 1) indicated that the slow reaction in the absence of MgATP was a true ET reaction, whereas the faster reaction in the presence of MgATP was gated (*14*). Thermodynamic analysis of the faster reaction yielded an H_{AB} which is well in excess of the nonadiabatic limit, and a negative ET distance. The slower reaction of the L127Δ mutant, however, yielded parameters that were characteristic of nonadiabatic ET and a distance similar to what is seen in the crystal structure. While the precise mechanism of activation of this system for rapid ET is not known, it is clear that MgATP binding or hydrolysis dramatically influences the ET rate, even though the faster reaction is gated.

Pyruvate:Ferredoxin Oxidoreductase (PFOR). PFOR catalyzes the CoA-dependent oxidative decarboxylation of pyruvate. It contains a thiamine pyrophosphate (TPP) cofactor as well as three iron−sulfur clusters. Addition of pyruvate generates a hydroxyethyl−TPP intermediate. The rate of the ET reaction from this cofactor adduct to the iron−sulfur cluster in the absence of CoA is 10^5-fold smaller than that in the presence of CoA (*17*). Analysis by ET theory (Table 1) indicated that the slow reaction in the absence of CoA was a true ET reaction, whereas the much faster reaction in the presence of CoA was gated. The mechanism of this activation is not known, but it was suggested that CoA may facilitate the formation of a highly reducing hydroxyethyl−TPP radical intermediate which increases the driving force for ET.

The Methylamine Dehydrogenase (MADH)−Amicyanin Complex. MADH is a tryptophan tryptophylquinone (TTQ)-dependent enzyme that catalyzes the oxidative deamination of primary amines. The electron acceptor for MADH is a type I copper protein, amicyanin (*18*). Addition of methylamine to MADH reduces TTQ and yields an aminoquinol form of TTQ with the substrate-derived amino group covalently attached (*19*). It was shown that ET from this reaction intermediate to amicyanin is gated by the deprotonation of the substrate-derived amino group on TTQ. This was confirmed by demonstrating a significant deuterium kinetic solvent isotope effect on k_{obs} (*20*). Analysis of this reaction by ET theory (Table 1) indicated that the ET reaction was gated. The rate of this gated ET reaction is also dependent on pH and the presence of monovalent cations (*21*). It is possible to generate a nonphysiologic quinol form of reduced MADH which lacks the substrate-derived amino group. ET from this form of MADH to amicyanin is much slower than the gated reaction, and it exhibits no kinetic isotope effect or cation dependence. Analysis of this slower reaction by ET theory indicated that this reaction from the quinol is a true ET reaction (*22*, *23*) and yielded a reasonable ET distance (Table 1). Studies of analogous ET reactions of another TTQ enzyme, aromatic amine dehydrogenase, and its electron acceptor, azurin, yielded similar results (*24*).

MECHANISMS OF CHEMICAL ACTIVATION

When ET is chemically gated, one cannot determine the true k_{ET} for that reaction. Only a lower limit for that value is known, the rate of the chemical reaction that precedes ET. For a slow "unactivated" true ET reaction (eq 8), $k_{obs} = k_{ET}$. For the corresponding fast "activated" gated ET reaction (eq 9), $k_{obs}^* = k_X$. While this reaction is gated, the observed reaction rate is much greater than k_{ET} for the ungated reaction in the absence of chemical activation ($k_{obs}^* = k_X \gg k_{obs}$).

slow unactivated true ET:
$$A_{ox}-B_{red} \underset{k_{-ET}}{\overset{k_{ET}}{\rightleftharpoons}} A_{red}-B_{ox} \quad (8)$$

fast activated gated ET:
$$A_{ox}-B_{red} \underset{k_{-X}}{\overset{k_X}{\rightleftharpoons}} [A_{ox}-B_{red}]^* \underset{k_{-ET}}{\overset{k_{ET}^*}{\rightleftharpoons}} A_{red}-B_{ox} \quad (9)$$

This means that the chemical reaction step has increased k_{ET} in the activated system by several orders of magnitude ($k_{ET}^* \gg k_X \gg k_{ET}$). It is worth revisiting ET theory to discuss how this enhancement of k_{ET} may occur. As seen in eq 6 and Figure 1D, k_{ET} depends on H_{AB}, and $(\Delta G^\circ + \lambda)^2/4\lambda$. To increase k_{ET}, the activation step must increase H_{AB}, or decrease the activation energy, or both. If the activation step is linked to a conformation change that brings the redox centers closer together, thereby increasing H_{AB}, then the consequences of this would be described by Figure 1E. A decrease in activation energy accomplished by altering ΔG° is described in Figure 1F. In theory, the activation step could also alter λ, but this has not yet been demonstrated for a protein ET reaction. When activation occurs as in Figure 1F, not only is k_{ET} increased but the equilibrium constant for the ET reaction increases as well. Thus, the reaction will not only be faster, but will also occur to a greater extent. In

the cases of PFOR and MADH the activation appears to occur by altering the redox potential of the electron donor, thus decreasing the activation energy as in Figure 1F. In nitrogenase it is not clear whether activation is as in Figure 1E or 1F, or perhaps a combination of both.

CONCLUSION

When considering the evolution of proteins and reaction mechanisms, one must note important differences between long range ET reactions and catalytic reactions with respect to metabolism. Metabolically important long range ET reactions are often between proteins. Electrons are initially extracted from a metabolic substrate and are then transferred to a site of energy conservation (e.g., the membrane-bound respiratory chain). This process should be fast, but must also be specific. If the electron is transferred to the wrong acceptor protein or molecule, then energy will be lost and damage may result from the formation of reactive oxygen species or free radicals. By using a chemical reaction to activate and greatly accelerate the rate of an otherwise very slow and unfavorable ET, an important element of metabolic regulation is introduced. Chemical gating allows the rate of an ET process to be controlled by concentrations of specific metabolites, effector molecules, or pH. By having large differences in rates of the slow ungated true ET, and the very rapid gated ET, flow of electrons through the system may be effectively regulated. This strategy can prevent rapid ET when it is not necessary due to the energy state of the cell, and prevent mis-direction of electrons when adequate amounts of the natural electron acceptor are not available. While relatively few examples are cited here, this does not mean that these are isolated phenomena. Few protein ET reactions have been as systematically studied as have these, to ascertain the ET parameters for the reaction, and to determine whether the reaction is gated. Hopefully more of such studies will be forthcoming so that the extent to which chemically gated ET occurs and its significance to metabolism can be more fully appreciated.

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