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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].

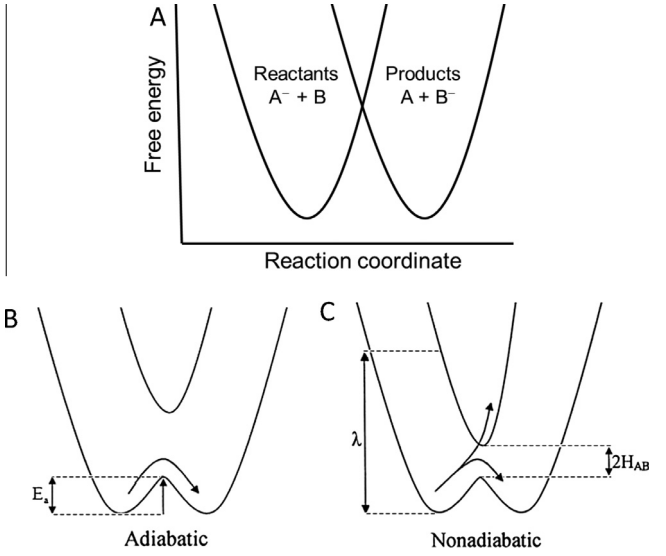

Fig. 1. (A) A simple two-dimensional representations of the multi-dimensional potential surfaces of product and reactant states. (B) A representation of a reaction that is described by transition state theory. (C) A representation of a reaction that is described by electron transfer theory.

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