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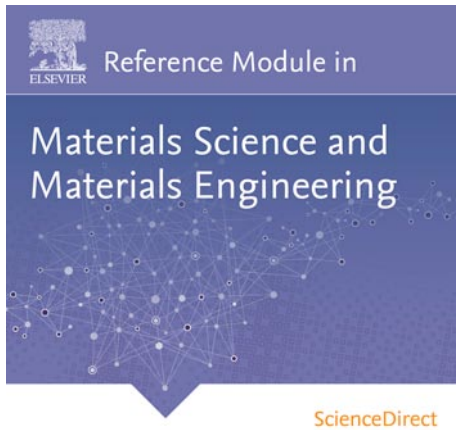

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Bizzarri A.R., and Cannistraro S., Biophysics: Electron Transfer in Metalloproteins. In: Saleem Hashmi (editor-in-chief), Reference Module in Materials Science and Materials Engineering. Oxford: Elsevier; 2016. pp. 1-10.

ISBN: 978-0-12-803581-8
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Biophysics: Electron Transfer in Metalloproteins ☆

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Nomenclature **Units** Å, eV, mV, s^{-1}

ET Electron transfer

Introduction

In the energy-capture machinery of biology, especially in photosynthesis and in mitochondria there are chains of redox enzymes containing metal ions, including iron, heme-iron, copper, manganese, arranged in order so as to assist electron flow and generate charge-separation which represents the first step in the energy-capture process (Jortner and Bixon, 2009). The ability of transition metals to exist in more than one stable oxidation state makes them suitable catalysts for biological processes that require transfer of electrons. The oxidation/reduction centers are placed in order of their potentials, expressed in terms of free-energy differences, so that the directionality of the electron flow is thermodynamically determined. Along the bioenergetic pathways, electrons flow down a gradient of potential energy that spans a range of less than 1.2 eV.

However, biological material is not constituted by regular arrays of lattice pointing to allow electrons to travel over long distances as delocalized Bloch waves; therefore, the familiar concepts of metallic conduction, through partly filled conduction bands, cannot apply. Instead, biology employs redox centers which are localized potential wells, among which electron transfer (ET) occurs through an hopping mechanism from center to center. The redox centers are nearly always found buried beneath the protein surface; the protein coating serving as an insulator which protects the redox center from short-circuiting ETs. Electrons can be transported across protein-domain interfaces or along a redox-chain which consists of a number of proteins (see for instance **Figure 1**); the related processes involving several reaction steps.

In ET intra-protein, the redox centers are held at a fixed distance and in a fixed orientation with respect to each other in order to presumably optimally adapt to physiological needs. When two redox centers, that are consecutive in the ET train, are located on different proteins, ET requires proteins first to form a docking or associative complex in which the partners assemble, transiently, through complementary contact surfaces. The mutual approach, initially, may be governed by long-range electrostatic forces reflecting the overall charges on the two partners. At shorter ranges, either hydrophobic or Coulomb interactions between opposite charge patches, or combinations of both, determines the structure of docking complex and the relative orientation of the partners. Generally, for successful ET within a docking complex, the partners should have motional degrees of freedom that allow them to perform a rolling or sliding motion with respect to each other.

The peculiarity of ET processes mediated by metalloproteins is that they occur over long distances, in a very fast, directional, and efficient way; the donor and the acceptor proteins having redox centers separated by distances between 5–25 Å. In contrast to usual chemical reactions which involve the making and breaking of bonds and proceed via a well-defined reaction coordinate, the reactants and products of a protein ET are often chemically indistinguishable.

ET reactions in metalloproteins are usually termed as outer sphere ETs, since they take place with no or very weak coupling between donor and acceptor sites of the electron.

Typical ET rate constants for biological processes lie in the order of 10^2–10^3 s^{-1}. Optimization of biological ET for speed seems to be necessary to compete with back reactions, especially to prevent charge recombination to occur.

Several factors are suggested to influence the ET efficiency, such as the distance between the redox centers, the role of the protein medium, the possible existence of conducting pathways, the role of the solvent medium, the assistance of the protein and solvent dynamics (Beratan *et al.*, 1992; Moser *et al.*, 1992; Lin *et al.*, 2002). Indeed, the dynamics of the intervening medium through which an electron passes can finely tune the ET process. In particular, a prominent role is played by low-frequency, collective vibrational modes.

wDifferent kinds of ET metalloproteins can be found in biological processes. Among others, cytochromes in which the active site is the heme consisting of iron coordinated to porphyrin group; the redox potentials for the Fe^{+3}/Fe^{+2} couple ranging from 5 to 260 mV. Iron–sulfur proteins are characterized by iron–sulphur clusters centered on the iron with redox potentials covering a range, from − 700 to 500 meV. Blue copper proteins, in which the copper ion directly coordinates to amino acid residues in a distorted tetrahedral arrangement, are characterized by peculiar spectroscopic properties and a redox potential for the Cu^{+2}/Cu^{+1} couple from 130 to 680 mV.

More recently, understanding the mechanism of electron transduction through biological macromolecule has become of fundamental importance not only in increasing our knowledge of the ET process, ubiquitous in biology, but also in the development of novel, improved bioelectronic devices (Gray and Winkler, 2010).

☆*Change History*: February 2015. A.R. Bizzarri and S. Cannistraro updated the Section 'Perspectives and Final Remarks' and the References.

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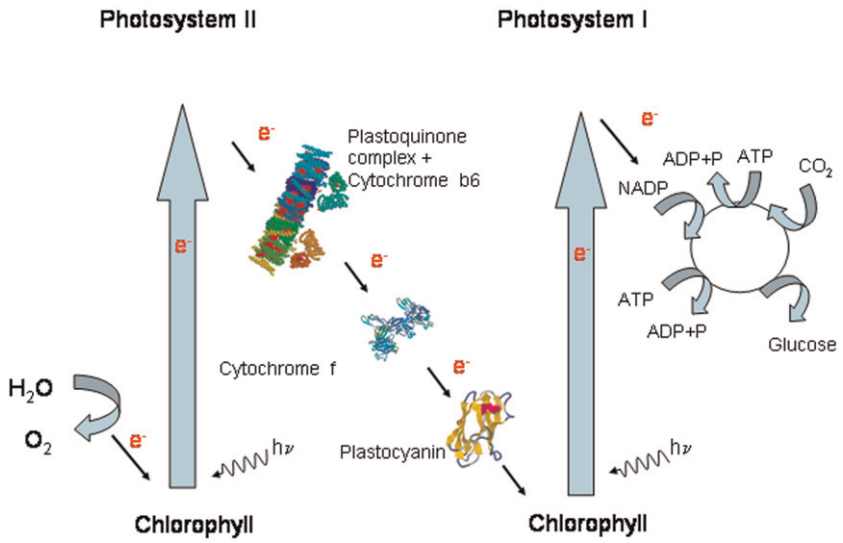

Figure 1 Photosynthesis pathways as found in green plants. Electrons are extracted from water by Photosystem II and driven through a complex coupled ET pathway to the CO_2 reduction cycle by Photosystem I.

The ET Rate: A General Overview

ET theory describes the transition of an electron from a donor D to an acceptor A; the reactant and product, DA and D^+A^-, representing the system before and after the ET process, respectively (Jortner and Bixon, 2009). The states $|DA\rangle$ and $|D^+A^-\rangle$ can be expressed by the combination of the wavefunctions for the two redox centers:

$$|DA\rangle = (\overline{\Phi}_D \Phi_A); \quad |D^+A^-\rangle = (\Phi_D \overline{\Phi}_A)$$

where Φ_D and Φ_A are the complete wavefunctions describing the nuclear and electronic motions of the two redox centers; the bar denoting the electron participating in the transition.

The traditional transition-state theory of the rates for chemical reactions involves motion along a potential energy surface in which the reactant atoms gain energy from thermal collisions, surmount an activation energy barrier to achieve a transition state and spontaneously decay into the product. In contrast to these, usually called adiabatic reactions, where formation of the transition state leads almost inevitably to the product, the probability of long-distance ET for such a transition is small. Accordingly, a non-adiabatic description for the ET reaction is more appropriate.

For non-adiabatic ET reactions, the first order rate constant k_{ET}

$$|DA\rangle \overset{k_{ET}}{\rightarrow} |D^+A^-\rangle$$

can be conveniently expressed by the Fermi's golden rule obtained from the time-dependent quantum mechanical perturbation theory:

$$k_{ET} = \frac{2\pi}{\hbar} V_R^2 \rho \qquad [1]$$

where $\hbar$ is the Planck's constant divided by 2π, V_R^2 is the square of the quantum matrix element for electronic coupling between donor and acceptor, averaged over all possible thermal fluctuations of the system; ρ is the density of states, that is the number of states per unity interval of energy.

The electronic coupling V_R^2 is the principal origin of the distance dependence of the ET constant rate. Simplest model, neglecting the role of intervening medium, predicts V_R^2 falls off exponentially with the distance R between the donor D and the acceptor A according to $e^{-\beta R}$, where β is an attenuation factor. In order to obtain ET rates in the order of 10^2–10^3 s^{-1}, R must be then in the range 10–20 Å (see also in the following).

Under the requirement of the Franck–Condon principle, stating that during the almost instantaneous ET process the nuclei do not change neither their positions nor their momenta, the ET rate can be expressed in the form:

$$k_{ET} = \frac{2\pi}{\hbar} V_R^2 F_C \qquad [2]$$

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where F_C is the Franck–Condon weighted density of states reflecting the overlap of the donor and acceptor nuclear and solvational wavefunctions.

Accordingly, ET will occur at nuclear configurations for which the total potential energy of the reactants and surrounding medium is equal to that of the products and the surrounding medium. The quantity F_C is a sum of square of the overlap integrals $S_{V_{DA}V_{D^+A^-}}$ of the vibrational wavefunctions of the reactants (ν_{DA}) with the corresponding ones of the products ($\nu_{D^+A^-}$), weighted by Boltzmann factors:

$$F_C = \sum_{\nu_{DA}} \sum_{\nu_{D^+A^-}} S_{\nu_{DA}\nu_{D^+A^-}}{}^2 p(\nu_{DA}) \qquad [3]$$

with

$$S_{\nu_{DA}\nu_{D^+A^-}} = \int \chi_{\nu_{DA}} \chi_{\nu_{D^+A^-}} \, dx$$

where $\chi_{\nu_{DA}}$ and $\chi_{\nu_{D^+A^-}}$ are the wavefunctions for the states ν_{DA} and $\nu_{D^+A^-}$, respectively, x being the oscillation coordinate; $p(\nu_{DA})$ is the equilibrium Boltzmann probability of finding the system in the vibrational state ν_{DA}. The sum is over any given set of the vibrational quantum numbers (ν_{DA}, $\nu_{D^+A^-}$) of the reactant DA and the product D^+A^-, including the solvent; such a sum being however limited by the fact that only a small number of states have a finite overlap and hence contributes to the final term.

The calculation of F_C can be extremely problematic and different expressions for the Frank–Condon factor can be obtained depending on the approximations done. Classical, semiclassical and quantum mechanical approaches, according to the treatment of the nuclear motions, have been followed to work out useful expressions for F_C.

When all the vibrational frequencies are relatively small, for example, $\hbar\omega \ll k_B T$, the vibrational manifold of DA and D^+A^- can be treated as a continuum and the passage across the activation barrier can be described classically in terms of the activated complex theory, properly modified for non-adiabatic reactions. Accordingly, the ET rate is related to the free activation energy, ΔG^*, of the reaction as follows:

$$k_{ET} = k_{ET}(0) e^{-\Delta G^*/k_B T} \qquad [4]$$

where the pre-exponential factor $k_{ET}(0)$ defines the limiting rate of the reaction.

Generally, classical results are valid only at temperatures high enough that the vibrations are fully excited. The classical approach is discussed in the next session where the expression worked out by Marcus for F_C, and hence for the ET reaction rate, is presented. When $\hbar\omega \geq k_B T$, the discrete nature of the vibrational manifold must be taken into account. The ET rate may become temperature independent and a quantum mechanical view is more appropriate. Moreover, under these conditions, electron tunneling may become important. This aspect will be briefly presented in the following.

Marcus Theory of ET

The simplest theoretical treatment of the rate of ET in metalloproteins, is due to Marcus using a classical harmonic oscillator model, which generates parabolic potential energy curves (Marcus and Sutin, 1985). Indeed, this approach represents the most insightful and used theoretical framework to interpret experimental ET results in metalloproteins.

The ET process can be seen as an electron jump from DA to D^+A^- and requires to match the Franck–Condon principle, which implies the nuclear configuration to be the same immediately after the ET as before, and, in addition, the conservation of energy. Under these requirements, the electron jump takes place in the vicinity of the crossover, nuclear configuration C (**Figure 2**).

Thermal fluctuations and/or vibrations in some coordinates will be required for reaction to occur. Since the charge distribution of the protein matrix is different before and after the ET process, the surrounding medium will be polarized differently in the two states. Therefore, the coordinates involved into the ET reaction include vibrational coordinates of the protein and the vibrational and orientational coordinates of the surrounding solvent. All the fluctuating nuclear coordinates relevant to the ET reaction are usually lumped together into the so-called reaction coordinate Q. The dependence of the potential energy curves of the reactant DA and the product D^+A^- on Q is assumed to be quadratic, according to the harmonic approximation, and unchanged by the ET reaction (**Figure 2(a)**).

In the non-adiabatic case, the passage through C does not usually cause transition from DA to D^+A^-. Once the system reaches the intersection of the potential curves C, the probability of going from DA to D^+A^- depends on a number of factors such as the extent of coupling of electronic orbitals of the two reactants, which in turn depends on the separation distance of the two reactants. The separation between the two potential energy curves over a vertical distance being given by $2V_R$, where V_R is the electronic coupling element between DA and D^+A^- (**Figure 2(b)**). If the gap is large enough, as it occurs in adiabatic reactions, the transition DA to D^+A^- will take place each time the crossing point is reached. Conversely, for small V_R as in non-adiabatic ET reactions, the electron, for most of the times it reaches the crossing point, continues its motion along the curve DA (upwards running arrow in

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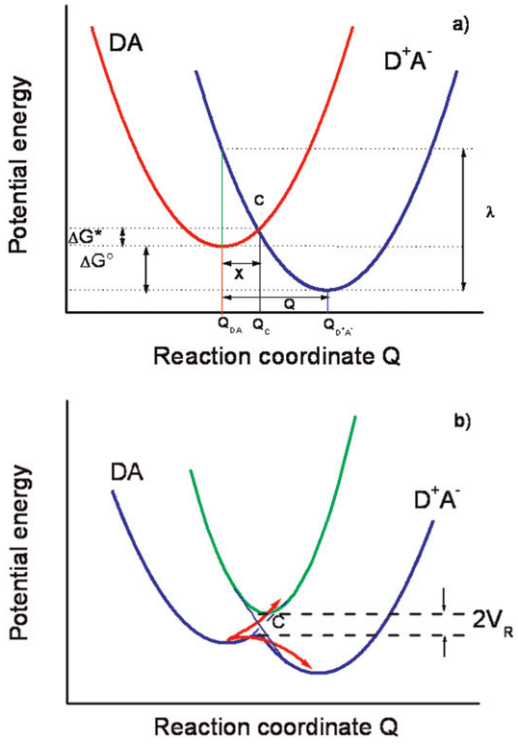

Figure 2 (a) The harmonic potential energy of the reactant (DA) and product (D^+A^-) as a function of the reaction coordinate *Q*. ΔG° is the standard free energy, λ is the reorganization energy and ΔG^* is the free energy of activation. (b) Influence of the electronic coupling on the potential energy curves. In both cases, the ordinate represents the potential energy of the nuclei of the whole system: donor + acceptor + medium.

Figure 2(b)). Only once in a while, the electron will make the transition to D^+A^- when the crossing is reached (downwards running curve in Figure 2(b)).

In the framework of the Marcus theory, the electron jumping from the equilibrium coordinate Q_{DA} of DA to the equilibrium coordinate Q_{DA}^{+-} of D^+A^-, can be described in terms of the free energy of activation, ΔG^* which is related to two experimental observables, ΔG°, the standard free energy and λ, the reorganization energy. ΔG^* is the energy required to reach the point C from DA, overcoming the activation barrier. The standard free energy or driving force, ΔG°, is the energy difference between the ground states of DA and D^+A^-, respectively and can be expressed as:

$$\Delta G^\circ = zF(E_A{}^\circ - E_D{}^\circ) \qquad [5]$$

where z is the number of electrons transferred, F is the Faraday constant and $E_A{}^\circ$ and $E_D{}^\circ$ are the midpoint potentials of the donor and acceptor centers.

The reorganization energy λ is the free energy required to move all the atoms from their equilibrium positions before the ET to the equilibrium positions they would have after the ET without transferring the electron. By referring to Figure 2(a), the activation barrier ΔG^* and the reorganization energy λ are:

$$\Delta G^* = \frac{1}{2}k_H X^2; \quad \lambda = \frac{1}{2}k_H Q^2 \qquad [6]$$

where X is the distance between the equilibrium coordinate Q_{DA} and the coordinate of the crossing point C, and Q is the distance between the equilibrium coordinates Q_{DA} and $Q_{D^+A^-}$; k_H being the force constant of the harmonic potential energy corresponding to both DA and D^+A^- .

The difference between the free energy and the activation energy can be expressed as:

$$\Delta G* - \Delta G^\circ = \frac{1}{2}k_H(Q-X)^2 = \frac{1}{2}k_H Q^2 + \frac{1}{2}k_H X^2 - k_H QX \qquad [7]$$

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By rearranging eqns [6] and [7], it comes out:

$$X = \frac{\lambda + \Delta G^{\circ}}{k_H Q} \quad [8]$$

and finally:

$$\Delta G* = \frac{1}{2} k_H \frac{(\lambda + \Delta G^{\circ})^2}{{k_H}^2 Q^2} = \frac{(\lambda + \Delta G^{\circ})^2}{4\lambda} \quad [9]$$

Therefore, from eqn [4], the ET rate can be expressed by:

$$k_{ET} = k_{ET}(0) e^{-\frac{(\Delta G^{\circ} + \lambda)^2}{4\pi\lambda k_B T}} \quad [10]$$

where the pre-exponential factor $k_{ET}(0)$ assumes the form:

$$k_{ET}(0) = \frac{2\pi}{\hbar} V_R^2 \left(\frac{1}{4\pi\lambda k_B T}\right)^{1/2} \quad [11]$$

leading to the final expression:

$$k_{ET} = \frac{2\pi}{\hbar} V_R^2 \left(\frac{1}{4\pi\lambda k_B T}\right)^{-1/2} e^{-\frac{(\Delta G^{\circ} + \lambda)^2}{4\pi\lambda k_B T}} \quad [12]$$

The ET rate can be modulated by thermodynamic (ΔG°) and intrinsic (λ) factors and, in addition, it varies with the temperature. Notably, the optimal rate is obtained when $-\Delta G^{\circ}$ matches λ such a condition having being exploited to extract information, from k_{ET} values, on V_R and λ (see below).

As already mentioned, the final Marcus expression provides a sound approach to describe the ET processes in metalloproteins. However, while it well takes into account the temperature dependence of the ET rate at high temperature, it fails at low temperatures, at which deviation from an Arrhenius-like behavior, or even temperature-independence (at very low T), has been observed.

Quantum Mechanical Theories of ET

A semiclassical approach has been followed by Hopfield to derive an expression for the ET rate (Hopfield, 1974). He treated oscillators classically, but assumed quantized energy levels. By introducing the probability distributions $D_D(E)$ and $D_A(E')$, corresponding to the energy required to remove an electron from the donor and to give an electron to the acceptor, respectively, the density of states in eqn [1] can be expressed by:

$$\rho = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} D_D(E) D_A(E') \mathrm{d}E \mathrm{d}E' \quad [13]$$

He assumed, moreover, that both the distributions follow a Gaussian form:

$$D_D(E) = \frac{1}{(2\pi{\sigma_D}^2)^{1/2}} e^{\left[\frac{-(E + E_D - \lambda_D)}{2{\sigma_D}^2}\right]}; \quad D_A(E') = \frac{1}{(2\pi{\sigma_A}^2)^{1/2}} e^{\left[\frac{-(E' + E_A + \lambda_A)^2}{2{\sigma_A}^2}\right]} \quad [14]$$

with standard deviations σ_D and σ_A, respectively; E_D is the energy of the ground state of the reduced form of the donor and E_A is the reduced form of the acceptor; $D_D(E)$ is centered below E_D by an amount λ_D, because ordinarily the nuclear configuration in the initial reduced state will not be an equilibrium configuration for the final, oxidized state; for the same reason, the center of $D_A(E')$ is displaced upwards from E_A by an amount λ_A.

By taking into account the Franck–Condon principle and the conservation of energy in the process ($E = E'$), eqn [13] becomes:

$$\rho = F_C = \int_{-\infty}^{+\infty} D_D(E) D_A(E) \mathrm{d}E = \frac{1}{(2\pi\sigma^2)^{1/2}} e^{\left[\frac{-(\Delta E - \lambda)^2}{2\sigma^2}\right]} \quad [15]$$

where $\sigma^2 = \sigma_D^2 + \sigma_A^2$, $\lambda = \lambda_D + \lambda_A$ and $\Delta E = E_A - E_D$ being the energy gap of the reaction.

By assuming that both the donor and the acceptor have a quadratic dependence on the nuclear coordinate with the same force constant k_H, and a spacing between energy levels equal to $\hbar\omega_D$ and $\hbar\omega_A$ for the donor and the acceptor respectively, it comes out

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that:

$$\sigma_D^2 = \hbar\omega_D\lambda_D \coth(\hbar\omega_D/2k_HT) \quad \sigma_A^2 = \hbar\omega_A\lambda_A \coth(\hbar\omega_A/2k_HT)$$

Therefore, the resulting ET rate becomes:

$$k_{ET} = \frac{2\pi}{\hbar} V_R^2 \left(\frac{1}{2\pi\sigma^2}\right)^{-1/2} e^{\frac{-(\Delta E-\lambda)^2}{2\sigma^2}} \quad [16]$$

Notably, in the limit of high temperatures, σ approaches k_BT and eqn [16] results formally similar to the Marcus expression, once $-\Delta E$ is identified by ΔG^*. Conversely, at very low temperature, the Frank–Condon factor F_c becomes temperature independent, in agreement with some experimental data.

However, the limiting expression at low temperature is not in complete agreement with experimental data. Significant improvements can be reached by a full quantum mechanical approach taking into account also the vibrations coupled to changes of electronic state (Devault, 1980). A variety of quantum mechanical treatments, with different degrees of approximations, have been developed. Under the assumption that the system consists of a single group of harmonic oscillators and that there is a single prevailing mode, from eqn [3], the Levich–Degonadze–Jortner expression can be derived for F_C:

$$F_C = \frac{1}{\hbar\omega} e^{-S(2n+1)} \left(\frac{n+1}{n}\right) \frac{\Delta E}{2\hbar\omega} I_P\left[2S\sqrt{n(n+1)}\right] \quad [17]$$

where $\hbar\omega$ is the characteristic frequency; ΔE is the energy gap of the reaction; S is equal to $\lambda/\hbar\omega$; I_p is the modified Bessel function of order P and n is given by:

$$n = \left[e^{\frac{\hbar\omega}{kT}} - 1\right]^{-1}$$

The ET rate can be then expressed by:

$$k_{ET} = \frac{2\pi}{\hbar} V_R^2 \frac{1}{\hbar\omega} e^{-S(2n+1)} \left(\frac{n+1}{n}\right) \frac{\Delta E}{2\hbar\omega} I_P\left[2S\sqrt{n(n+1)}\right] \quad [18]$$

In the limit of very high temperature, eqn [18] again reduces to the Marcus expression. In addition, it well reproduces the experimental trend with temperature. However, it does not include the coupling of low-frequency vibrational modes of the redox center to the ET process. To take into account for low-frequency modes, a correction to eqn [18] has been done by including the oscillator zero-point energy taking into account the change of frequency, leading to an expression similar to eqn [12], containing $-\Delta G^\circ$ instead of ΔE.

Dependence of ET Rate on the Protein Matrix

A crucial aspect of the ET process is represented by the dependence of k_{ET} on the medium between the redox centers. To obtain information on the variation of k_{ET} with the nature of the medium, first it is necessary to eliminate, or at least to minimize, the dependence of k_{ET} on both the driving force and reorganization energy. In the framework of the Marcus theory, this can be achieved by extrapolating k_{ET} when $\Delta G^o = -\lambda$. According to eqn [12], the exponential trend on driving force and reorganization energy disappears. The resulting rate remains only weakly dependent on λ ($k_{ET} \sim 1/(\lambda)^{1/2}$) and its change with the medium is mainly reflected by V_R.

The dependence of V_R on the intervening medium can be cast in the form:

$$V_R^2 = V_R^{o2} f_M^2 \quad [19]$$

where V_R^o represents the electronic coupling between DA and D^+A^- when the redox centers are in van der Waals contact, and f_M is a dimensionless attenuation factor which varies between 1 (van der Waals contact) and 0 (infinite distance). The dependence of f_M on the detailed structure of the medium connecting the two redox centers has been widely investigated. Only two cases will be considered in the following.

In the first approach, the protein intervening medium is pictured as an organic glass; the random, disordered connections between the two redox centers constituting the overall path of ET (Moser *et al.*, 1992). In this framework, the distance between the donor and the acceptor centers is the parameter governing the ET rate. The dependence of f_M^2 on the distance, R, between the center

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of edge atom of donor and that of acceptor can be expressed by:

$$f_M^2 = e^{-\beta(R-R_o)} \quad [20]$$

where the exponential coefficient of decay, β quantitatively describes the nature of the intervening medium with respect to its efficiency to mediate ET process, for instance through the propagation of the relevant wavefunctions. Variation of ET rates over 12 orders of magnitude can satisfactorily be accounted for by a distance dependence as in eqns [19] and [20], with $\beta = 1.4$ Å^{-1} and $R_o = 3.6$ Å. More generally, values of β in the range 0.7–1.4 Å^{-1} are found to reproduce the experimental k_{ET} values of metalloproteins.

An alternative description of the dependence of f_M^2 with the nature of the medium invokes the so-called pathway model which is in some sense based on the mechanism of super-exchange, owing to the fact that the electronic coupling between the donor and acceptor wavefunctions is mediated by a third center that connects the two wavefunctions (Beratan *et al.*, 1992). A pathway is defined as a combination of interacting bonds that link donor and acceptor. Three types of steps are distinguished, depending on whether the transfer occurs between atoms that are connected through a covalent bond (C), a hydrogen bond (H), or not connected at all (S); in the latter case, the electron must be transferred through space. The corresponding attenuation factor for a transfer path, can be expressed as:

$$f_M = \prod_i^{N_C} \varepsilon_i^C \prod_j^{N_H} \varepsilon_j^H \prod_k^{N_S} \varepsilon_k^S \quad [21]$$

where N_C, N_H, N_S are the number of C, H and S paths, respectively; ε are the individual attenuation factors corresponding to a particular step in the transfer path. Semiempirical expressions for the ε-factors are:

$$\varepsilon_C = 0.6; \qquad \varepsilon_H = 0.36e^{[-1.7(R-2.8)]}; \qquad \varepsilon_S = 0.6e^{[-1.7(R-1.4)]}$$

in which R is the distance between the two atoms exchanging the electron. Such an approach predicts that α-helices are characterized by a lower conductivity than β-sheets.

Reorganization Energy in the ET Process

When an electron is transferred in an intermolecular process, through the protein matrix, the distribution of charge is different before and after the transfer, as already mentioned. These changes have to be accommodated by the local dielectric properties contributing by polarizability, local bonds, reorientation of polar side chains, dissociation or association of proteolytic groups, movements of ions in the solvent, reorientation of solvent dipoles, etc. Additionally, the local structure of the redox center might undergo changes in configuration. All these physical effects are accounted for by the reorganization energy λ.

Marcus originally divided the reorganization energy into changes occurring at redox center (inner sphere) and those occurring in surrounding protein/water matrix (outer sphere). Accordingly, λ can be separated into: $\lambda = \lambda_i + \lambda_o$ where λ_i is the contribution to the reorganizational energy of the inner shell of atoms, close to the redox center, while λ_o refers to atoms further out, generically called 'solvent.'

The inner sphere reorganization energy λ_i, which reflects redox-dependent nuclear perturbations of the redox centers, such as changes in bond lengths and angles, can be expressed in terms of the inner shell normal vibrational modes:

$$\lambda_i = \frac{1}{2}\sum_j k_{Hj} Q_j^2 \quad [22]$$

where Q_j is the displacement from the equilibrium position of the j-th normal coordinate caused by the ET; the constant k_{Hj} being given by:

$$k_{Hj} = \frac{f_j^{DA} f_j^{D^+A^-}}{f_j^{DA} + f_j^{D^+A^-}} \quad [23]$$

where f_j^{DA} and $f_j^{D^+A^-}$ are the force constants at the equilibrium for DA to D^+A^-, respectively.

The outer sphere reorganization energy, λ_o, which reflects changes in the surrounding medium, such as changes in solvent orientation, can be estimated from the polarizability of the solvent, as considered to be a continuous polar medium:

$$\lambda_o = \frac{Ne}{4\pi\varepsilon_o}\left[\frac{1}{2R_1} + \frac{1}{2R_2} - \frac{1}{R}\right]\left[\frac{1}{D_{OP}} - \frac{1}{D_S}\right] \quad [24]$$

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where *Ne* is the charge transferred from the donor to the acceptor; R_1 and R_2 are the radii of the two spherical reactants when in contact and $R = R_1 + R_2$; D_{OP} is the square of the refractive index of the medium and D_S is the static dielectric constant; ε_o is the permittivity of space.

For redox centers that are buried within a protein, λ_o may also include configurational changes in the protein matrix and, for interprotein reactions, in the interface between the donor and the acceptor proteins. We note that the higher is the dielectric constant of the solvent, the larger is the value obtained for λ. Furthermore, for non polar solvent, λ_o vanishes and such a condition can be exploited to obtain information on λ_i.

In the framework of the Marcus theory (see eqn [12] and **Figure 2(a)**), a decrease in the driving force, for constant λ, will displace the product potential energy upward, causing ΔG^* to increase and consequently the ET rate to decrease. Similarly, at constant $-\Delta G^\circ$, an increase in λ will increase the horizontal displacement of the product from the reactant, imposing a higher activation barrier.

Depending on the sign of the quantity $(\Delta G^\circ + \lambda)$, one can distinguish the so-called normal region $[(\Delta G^\circ + \lambda) > 0]$ the activation-less region $[(\Delta G^\circ + \lambda) = 0]$ and inverted region $[(\Delta G^\circ + \lambda) < 0]$. These conditions are qualitatively illustrated in **Figure 3**.

In the normal region $[(\Delta G^\circ + \lambda) > 0]$, an increase in driving force accelerates the ET process, while it slows down in the inverted region; this representing one of the most celebrated predictions of the Marcus expression. Notably, when $[\Delta G^\circ + \lambda = 0]$, the ET rate, is least affected by variations of *T*, and λ. For $[(\Delta G^\circ + \lambda) < 0]$, the slower is the ET rate, the greater the energy liberated in the reaction; such a condition being involved into the phenomenon of chemiluminescence. Furthermore, in the inverted region, the extent of electron tunneling may become more relevant.

The reorganization energy λ constitutes a crucial parameter in ET process. Many evaluation by ab-initio quantum mechanical calculations of λ have been done. However, despite its importance, direct and precise measurements of the reorganization energy λ are quite difficult. Generally, it can be extracted by measuring the dependence of the ET rate upon $-\Delta G^\circ$ and using the fact that, from the Marcus theory, the rate is expected to be maximal when $-\Delta G^\circ = -\lambda$ Values for λ around 0.7 and 1 eV have been commonly found for intra- and inter-protein ET, respectively.

Perspectives and Final Remarks

In the last years, metalloproteins have registered an increasing interest thanks to the possibility of exploiting their ET capability to develop innovative hybrid nano-bio-devices with potential applications in the fields of biosensing, bioelectronics, conversion and storage of energy, etc. (Bonanni *et al.*, 2007). Indeed, metalloproteins, appropriately anchored to conductive substrates, can constitute the core of innovative nanodevices with the capability to perform multiple functions. For successful operation of these molecular-scale devices, it is essential to control the electron flow through the protein matrix and even between the protein and the external electrodes (Andolfi *et al.*, 2006; Zhang *et al.*, 2012). This requirement has significantly boosted interest in the study of mechanisms underlying the ET process in metalloproteins. With such an aim, scanning probe techniques, and in particular scanning tunneling microscopy and conductive atomic force microscopy, are extremely useful tools, that can provide new insights into the intra- and inter-molecular ET mechanisms through the measurement of current at single molecule level. The possibility of coupling scanning probe with other techniques, such as Raman spectroscopy, interfacial electrochemistry, even operating in aqueous solution, can provide further insights on localized (at the nanoscale) chemical and physical properties, to reach a deeper knowledge on the underlying ET processes. Additionally, these new approaches could address some exciting fundamental aspects of the ET process, such as the role of protein matrix dynamics, the interplay between protein and water dynamics, and the evaluation of the reorganization energy. In the field of advanced spectroscopies, laser ultrafast spectroscopy, with femtosecond resolution, offers the possibility to investigate the coupling between the excitation of the optical bands with the vibrational modes of the proteins, in order to elucidate the subtle relationships among structure, dynamics ,and functionality of these metalloproteins. Furthermore, recent advances in room-temperature single molecule fluorescence spectroscopy open pathways to access real-time observations of conformational motions and chemical reactions, at biologically relevant time scales. This can provide further understanding of aspects that are difficult to be probed in ensemble-averaged experiments, such as the synchronization of conformational motions involved in the biological function. It should be mentioned that single molecule studies can be fruitfully combined with mutagenesis strategies to map how individual residues can regulate the ET efficiency and even the binding capability with other molecules, thereby providing tools for fine tuning of the ET properties. The continuous improvements in computational and calculation capabilities, together with the development of more and more sophisticated and reliable theoretical models, provide new insights into the ET processes of these proteins that are not easily accessible by existing experimental techniques. Among other computational approaches, molecular dynamics simulations possibly combined with quantum chemical calculations, offer the opportunity to evaluate some important quantities, such as the ET rate between the electron donor and acceptor, the ET pathways, the reaction free energy, the reorganization energy, the interplay between the structural and dynamical behavior of the protein matrix, and the role of interfacial water. Finally, the insights gained from all these studies, in connection with protein engineering, could be exploited through improved rational design of synthetic ET systems, incorporating biological and abiological redox cofactors, with predictable structures and enhanced ET rates and efficiencies. The nanoscale dimensions of

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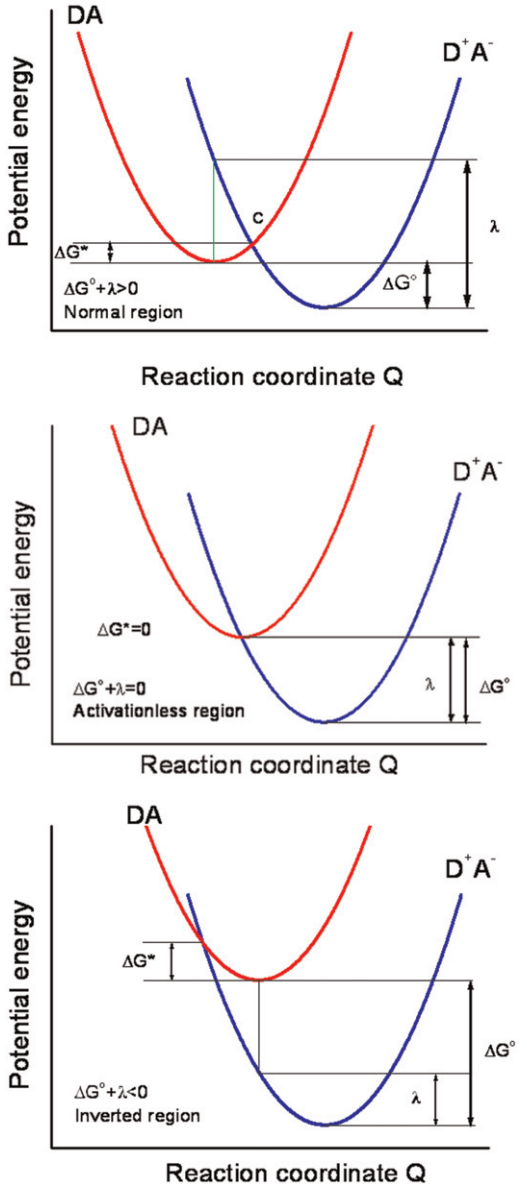

Figure 3 The harmonic potential energy of the states DA and D^+A^- as a function of the reaction coordinate Q, according to the Marcus theory, for three representative cases: $\Delta G^\circ + \lambda > 0$, normal region; $\Delta G^\circ + \lambda = 0$, activation-less region; $\Delta G^\circ + \lambda < 0$ inverted region.

these new molecules could be integrated in hybrid systems with the perspective of creating nanodevices and nano-biosensors with dramatically improved performance compared to the existing state-of-the-art.

Acknowledgments

This work was partly supported by a grant from the Italian Association for Cancer Research (AIRC No: IG 10412) and by a PRIN-MIUR 2012 Project (No. 2012NRRP5J).

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