MAURIZIO BRUNORI and FRANCESCA CUTRUZZOLÀ (*)

Electron Transfer in Biological Systems (**)

Abstract — Electron transfer in biological systems is basic to key bioenergetic processes such as light harvesting, respiration and nitrogen fixation. Electron transfer generally involves metal groups and/or organic prosthetic groups, but it is clear that the control of rates and thermodynamics is exerted by the protein.

In this article, the general theory of electron transfer is outlined and selected examples of biochemical tests of the theory are briefly reviewed. Special attention is given to the novel aspects of biological electron transfer, which are promising for the future developments in Biochemistry and Biophysics.

1. Introduction.

The role of electron transfer (ET) in biological systems is basic to bioenergetics involving, as it does, all processes which deal with light harvesting, respiration and nitrogen fixation. The initial phase of research which laid the foundations of present day knowledge, started after World War I with the pioneering work of biochemists such as O. Warburg and D. Keilin. Such a long period, which extended for over 40 years, led to the understanding of the role of enzymes in catalyzing bioenergetic processes and the characterization of the chemistry involved in the biological redox reactions. Thus it became clear that these events generally involve metals and/or organic prosthetic groups, and the control of rates and thermodynamics of ET is exerted by the protein.

In the seventhies the whole field of biological ET took a new perspective thanks to some major breakthroughs. First of all, after the threedimensional structure of hemoglobin and myoglobin became available due to the work of J.C.

(*) Dipartimento di Scienze Biochimiche «A. Rossi-Fanelli», Università di Roma «La Sapienza», P.le Aldo Moro 5, 00185 Roma, Italy. E-mail: brunori@axrma.uniroma1.it

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Kendrew and M.F. Perutz, more and more proteins were solved at the atomic level, and many of these are major actors in biological redox process; suffice to recall that the structures of the reaction center of photosynthesis and of cytochrome-c-oxidase (the two crucial membrane proteins involved in light harvesting and respiration) are now available. Second, a solid general theory of ET proposed by R. Marcus was extended to biological sytems, stimulating a large number of experimental studies in order to test its applicability to complex anisotropic objects such as redox active proteins. Finally the development of laser spectroscopy complemented other rapid reaction methods, such as stopped-flow and temperature-jump, allowing to extend the time domain down to p-sec in the realm where primary photochemical events and charge separation occur; thus structural dynamics of ET came of age.

In this brief article written on the occasion of the 100th anniversary of the discovery of the electron by J.J. Thomson, it seemed best to briefly review the novel aspects of biological ET which has been a very reach and productive field of modern Biochemistry, but promises to provide more new findings.

2. Outline of the General theory.

ET probability depends on the overlap between the electron-containing orbital (wavefunction) of the donor with that of the acceptor. This overlap can be very small since redox centres in proteins are often located 10-15 Å apart. In intramolecular ET, donor and acceptor are separated by the protein matrix, which has been assigned a low (D = 2–4) dielectric constant. In a classical picture, the energy barrier for ET is too high; however in a quantum mechanical view, the electron can tunnel through the barrier with finite probability. To relate tunnelling theory and experiments, a simple description of the ET rate is given by Fermi's Golden Rule, which applies to non-adiabatic ET occurring between weakly coupled redox centres:

$$k_{et} = (\textit{electronic factor}) \cdot (\textit{nuclear factor}) = k_{et} = 2\pi/\hbar\, H^2_{AB}\, (FC) \qquad (1)$$

The ***nuclear factor***, or Franck-Condon term (*FC*), states that during ET the nuclei do not have time to respond because of their large mass relative to that of the electron. Marcus [1] has provided a simple description of the energy terms that comprise the FC factor using harmonic potential energy curves (Figure 1). ET rate is determined by the free energy gap (ΔG°) or driving force, and the reorganizational energy (λ); ET will occur when the potential energies of reactants and products cross.

In general the activation barrier (ΔG^*) is given by the expression:

$$\Delta G^* = (\Delta G^\circ + \lambda)^2/4\,\lambda \qquad (2)$$

Depending on the sign of ($\Delta G^\circ + \lambda$) one can distinguish the normal, acti-

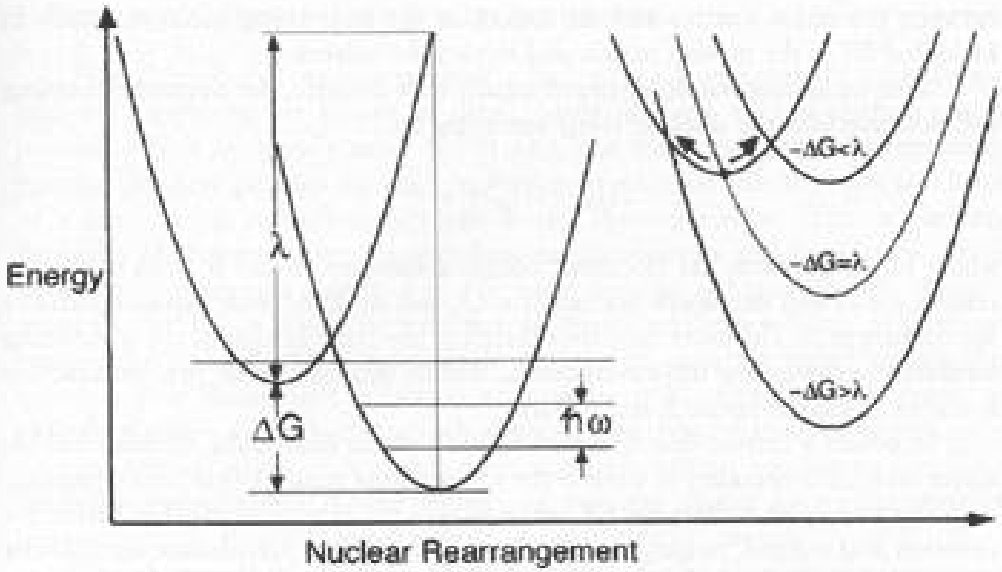

Fig. 1. (LEFT PANEL) Potential energies of the reactant (upper parabola) and product (lower parabola). The energies with their surrounding medium are approximated as harmonic oscillator potentials; the equilibrium geometry corresponds to the bottom of the potential well. In a quantized view only certain energy levels are permitted, shown as horizontal lines spaced by the quantum energy of the oscillator $\hbar\omega$. (RIGHT PANEL) The activation energy of the reaction depends upon the free energy (ΔG) relative to the reorganization energy (λ). When $-\Delta G = \lambda$, there is no activation energy and the ET rate is maximal; in the other cases, the activation energy is non-zero and the ET rate is slower.

vationless and inverted region; thus ET rate goes through a maximum for $-\Delta G^{\circ} = \lambda$ (activationless regime), and decreases on both sides (Figure 1).

Using the classical approximation for the FC factor, the Fermi equation becomes:

$$k_{et} = 2\pi/\boldsymbol{h}\ H^{2}_{AB}\ (4\pi\ \lambda k_{B}T)^{-1/2}\ \exp\ (-\ (\Delta G^{\circ} + \lambda)^{2}/4\ \lambda\ K_{B}T) \qquad (3)$$

According to equation (3) the main temperature dependence comes from the exponential factor, but when the activation energy is zero a weak temperature dependence remains because of the pre-exponential factor, causing the rate to decrease as the temperature increases. The temperature-indipendence of some ET reactions can however be explained according to Hopfield [2].

The other term influencing the ET rate, the ***electronic factor*** (H_{AB}), represents the weak coupling of the reactant and product wavefunctions; H_{AB} yields the overlap between the orbitals A and B, and is often referred as the «electronic coupling matrix element». The degree of orbital overlap depends on the distance

between the redox centres and the nature of the intervening medium, which in biological ET is the protein matrix and in part the solvent.

Since wavefunctions decay exponentially with distance, the electronic coupling will decrease with the distance $(r-r_0)$ according to:

$$H_{AB}^2 = (H_{AB}^0)^2 \, e^{-\beta(r-r_0)} \tag{4}$$

where H_{AB}^0 represents the electronic coupling between A and B when the redox centres are in van der Waals contact ($r = r_0$), and its decay with distance includes the coefficient β. The latter describes therefore the contribution of the intervening medium in propagating the wavefunction, and its dependence on protein structure is matter of intense research and debate.

To obtain a correct description of the role of the intervening medium and distance on k_{et}, it is necessary to work in the activationless regime ($-\Delta G^0 = \lambda$) (Figure 1).

The principles underlying ET between two proteins offer another theme for variation and control, namely molecular recognition as a prerequisite for ET. For efficient ET, diffusible redox proteins must interact in order to maximize proximity between the redox centres within a collisional complex (AB), given that most redox active proteins are asymmetric with their cofactor/metal generally located on one side of the macromolecule. The kinetic model which describes interprotein ET is:

$$A^+ + B \underset{k_{-a}}{\overset{k_a}{<=>}} (A^+B) \xrightarrow{k_{et}} (AB^+) \rightarrow A+B^+ \tag{5}$$

Under pseudo-first order conditions (e.g. [A]>>[B]), the overall rate constant k_{12} is given by:

$$k_{12} = k_a \, k_{et} \, [A]/(k_{-a} + k_{et} + k_a \, [A]) \tag{6}$$

Within this model, two limiting conditions can be envisaged where either complex formation or intra-complex ET are rate limiting. Marcus theory [1] allows to describe the overall rate constant k_{12} as a function of the equilibrium constant K_{12}, the self exchange rate constants of the two partners k_{11} and k_{22}, and the work term W_{12} involved in the configurational change of reactants and products along the reaction coordinate:

$$k_{12} = (k_{11} \, k_{22} \, K_{12} \, f_{12})^{1/2} \, W_{12} \tag{7}$$

where

$$W_{12} = \exp(w_{11} + w_{22} - w_{12} - w_{21})/2RT \tag{8}$$

These expressions have been tested experimentally with a number of well characterized redox proteins.

3. Experimental tests of the theory in biochemistry.

Several families of proteins involved in biological ET, with variable structure and catalytic complexity, are known in detail. Many of these are one-electron carrier proteins, which are usually small (≈ 100 a.a.), and display no associated enzymatic function. In these proteins the electron resides on a special cofactor, such as a heme or a metal atom, and often they can donate the electron to different partners. Examples of this group are the cytochromes, cupredoxins and ferredoxins. Other more complex proteins consist of one or more domains and may perform chemical reactions, such as oxidation or reduction of organic molecules. Some of them can convert the flow of charge from single electron to pairs (or more) using either cofactors (like flavins and quinones) by forming stable radical intermediates, or radicals of amino acid side-chains such as tyrosine or tryptophan together with metal centres. Among others, it is worth mentioning the various respiratory complexes located in organelle's and plasma membranes, the large class of detoxifying enzymes, (e.g. cytochrome P-450s), and the photosynthetic reaction centres.

The photosynthetic reaction centre proved to be an excellent test system for several reasons: (i) its 3D structure is known at atomic resolution [3], (ii) it contains several redox cofactors at different but fixed distances; (iii) the driving force can be varied experimentally; and (iv) ET can be studied over a wide temperature range. Using experimental data obtained with proteins where the primary quinone was substituted with other compounds of different redox potential, Dutton and coworkers [4] have shown a good linear dependence of ln k_{et} on distance, with $\beta = 1.4$ Å^{-1}, $r_0 = 3.6$ Å and a pre-exponential factor of 10^{15} s^{-1} (Figure 2). These Authors concluded that, in any protein, ET rate follows an exponential decay of the electronic wavefunctions with distance, implying a homogeneous intervening medium.

This exceptional result (Figure 2) does not prove, however, that the tunnelling barrier between the redox centres is microscopically uniform, but rather that the observed value represents an average. Gray and coworkers [5] have used ruthenated proteins (mainly myoglobins and cytochromes) as model systems to investigate the dependence of β on the structure of the intervening medium, i.e. the detailed protein structure. In this and other similar studies, a plot of ln k_{et} against distance does not always yield a linear relationship, suggesting that the protein does not always provide a homogeneous energy barrier. Starting with the intrinsically heterogenous packing inside a protein, Onuchic and Beratan [6] have proposed a model which takes into account the detailed structure of the intervening medium and views coupling between redox centres *via* pathways. In this model the exponential decay of the electronic coupling depends not only on the distance between redox sites, but also on the detailed contacts along the ET pathway(s). The electronic decay factor changes with covalent, hydrogen bond and through-space jump, which are empirically computed with an algorithm searching for potential ET pathways in

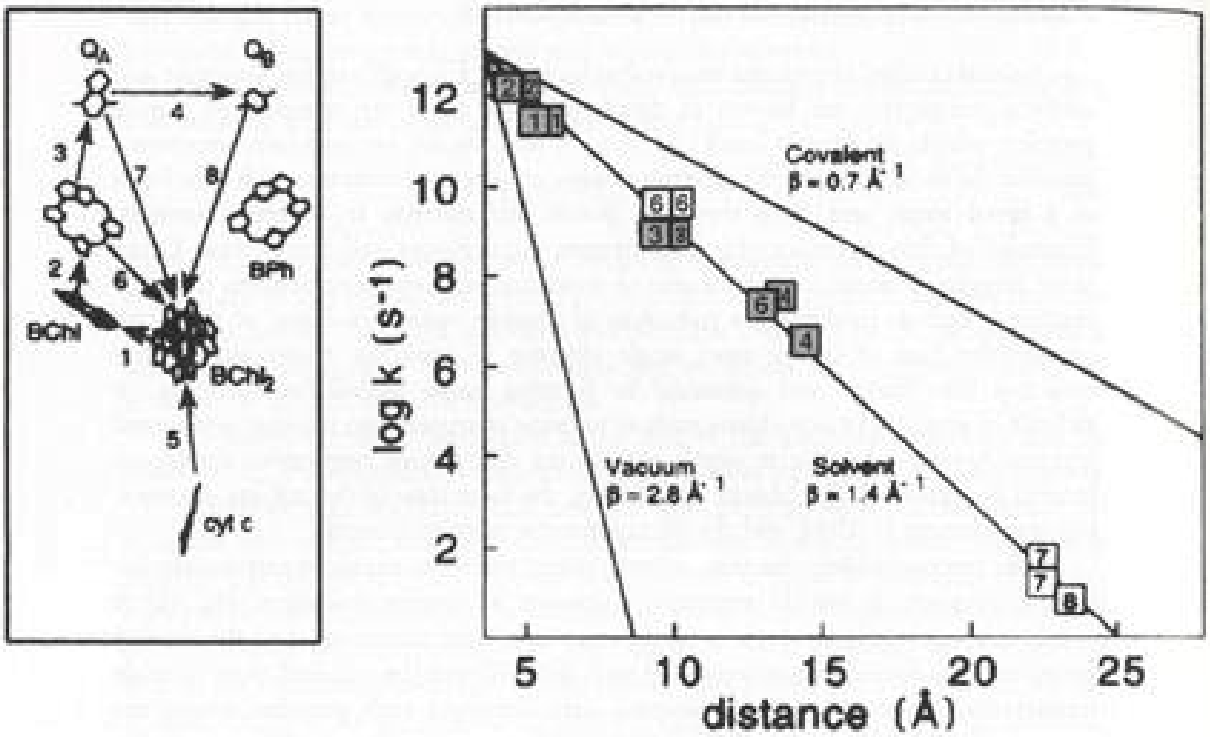

Fig. 2. Effect of distance on the intraprotein ET rate in the photosynthetic reaction centre, according to Dutton and coworkers [4]. (LEFT PANEL) Following light excitation of the bacteriochlorophyll dimer ($BChl_2$), ET progresses sequentially through bacteriochlorophyll monomer (BChl), bacteriopheophytin (BPh), primary quinone and secondary quinone (Q_A and Q_B). The resulting positively charged oxidized dimer can be re-reduced by the nearest of the four cytochrome c (cyt c). (RIGHT PANEL) The experimentally determined rates within the reaction centre fit a straight line with $\beta = 1.4$ Å^{-1}, compared to 2.8 Å^{-1} in vacuum, and to 0.7 Å^{-1} in a rigid covalent system. The line through experimental data extrapolates to 10^{13} s^{-1} at 3 Å, the van der Waals distance (Reproduced with permission from Outline of theory of protein electron transfer, Moser, C.C. and Dutton, P.L. in «*Protein Electron Transfer*», p. 1-21, Copyright BIOS Publisher Limited, 1996).

proteins of known three-dimensional structure (Figure 3). This model has been applied to different redox proteins in order to define the role of the intervening medium and of secondary structure elements on the ET rates. The model predicts: (i) that hydrogen bonds provide short-cuts for electron coupling between otherwise unconnected protein segments; (ii) that β-sheet proteins will exhibit a greater degree of coupling than α-helical proteins; (iii) that pathways are always identified in groups giving rise to the concept of «pathway tubes».

The large body of experimental data available today indicates that the two models (homogeneous and heterogeneous) are not necessarily exclusive. In some

cases the protein behaves as a homogeneous medium with «distance-dependent» exponential decay, as for photosynthetic reaction centre, ruthenated azurin and ruthenated cytochrome b_5, although different β-values were obtained. In other cases the coupling decay factor was shown to be anisotropic (for example in cytochrome c), giving rise to a model in which «hot» and «cold» spots for ET can be identified.

In the case of inter-molecular protein proteni ET, Equation (7) can be semplified given some assumption about the work terms, to become:

$$k_{12} = (k_{11}\, k_{22}\, K_{12})^{1/2} \qquad (9)$$

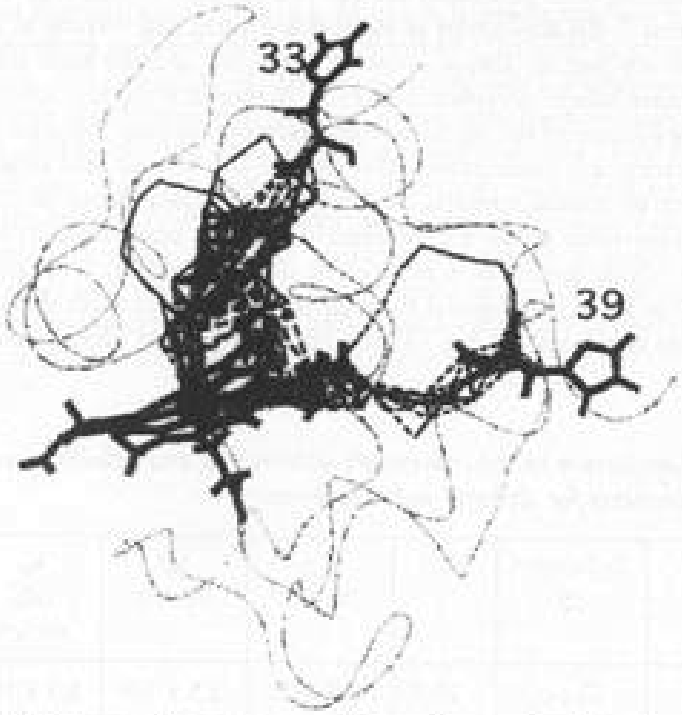

Fig. 3. Connectivity between redox centres according to Beratan, Onuchic and coworkers. The figure depicts the structure of cytochrome c with the heme highlighted and the polypeptide chain sketched as a continous line. The two histidine residues at position 33 and 39 are the sites of chemical modification with ruthenium. Dotted lines represent the calculated pathways for ET. The strongest coupling is provided by bonds at the centre of a tube of pathways that connect the Donor-Acceptor couples within the protein. The decay factors were calculated to be: covalent bonds, $\varepsilon_c = 0.6$; hydrogen bonds, $\varepsilon_H = \varepsilon_c^2\, e^{-\beta(r-r_0)}$; through-space jumps, $\varepsilon_s = 1/2\, \varepsilon_c\, e^{-\beta(r-r_0)}$, where r_0 is the reference distance for covalent bonds (1.4 Å) or hydrogen bonds (2.8 Å) and β is the decay lenght for through space jumps (1.7 Å). Reproduced with permission from Regan *et al.* (1993) J. Phys. Chem. 97, 13083-13088; Copyright American Chemical Society.

which is frequently used and is generally referred to as the «cross relation». For redox reactions between inorganic metal ions or complexes, Marcus theory [1] has been experimentally verified many times, either in the general or in the «cross relation» formulation. It has also been used to calculate reaction rates or equilibrium constants which are difficult to determine experimentally. The «cross relation» approach has also been used to interpret protein-protein ET: Table 1 reports a few of the experimentally determined rate constants for some representative copper and heme proteins, together with the calculated values. When comparison with known equilibrium and self exchange rate constants was possible, the agreement between calculated and measured values was found to be better than one order of magnitude (which is considered good). When larger deviations were observed (>100 folds), efforts have been made in order to rationalize this divergency, possibly reconsidering the applicability to the system of some of the theoretical assumptions described above. The obvious assumption which may not apply is related to the anysotropy of the redox protein and thereby to the contention that ET with any partner always occurs through one and the same contact surface.

In the case where complex formation is rate limiting the solvent cage effect increases the lifetime of the so-called «encounter complex», because after collision, the two proteins are momentarely trapped by the solvent and thus experience a large number of mutual contacts by 2D diffusion. Long range electrostatic forces come into play since many ET proteins display an anisotropy in surface charge distribution which results in a permanent dipole moment, favouring association. This effect has been investigated through (i) the ionic strength dependence of the second order rate constant k_{12}, (ii) the effect of surface charges modification by

TABLE 1 - *Comparison of experimentally determined and calculed second order rate constants for different redox proteins.*

Oxidant [a] (1)	Reductant [a] (2)	K_{12} [b]	k_{12} [c] ($M^{-1}s^{-1}$)	k_{22} [c] ($M^{-1}s^{-1}$)	k_{12} calc. ($M^{-1}s^{-1}$)	k_{12} exp. ($M^{-1}s^{-1}$)
Azurin	Cyt c	19	8×10^5	2.5×10^2	5.5×10^4	6.4×10^4
Plastocyanin	Cyt c	40.4	10^5	2.5×10^2	3.2×10^3	1.5×10^4
Stellacyanin	Cyt c	0.06	1.2×10^5	2.5×10^2	1.3×10^3	3.5×10^2

[a] Proteins: *Pseudomonas aeruginosa* azurin, horse heart cytochrome c (Cyt c) and parsley plastocyanin.
[b] Equilibrium constants were calculated on the basis of midpoint redox potentials ($E_{m,7}$).
[c] Electron self exchange rates at 20 °C used in the calculation of k_{12} according to Marcus «cross-relation».

chemistry or protein engineering, and (iii) the influence of the computed electrostatics of the redox partners in simulated docking experiments. An interesting generality related to the electrostatic make-up of ET proteins is that often the docking surfaces show a «loose» specificity, which also explains the high degree of cross-reactivity observed both *in vitro* and *in vivo* between donors and acceptors. This was shown to be the case for the widely studied redox couple cytochrome c and cytochrome-c-oxidase of the respiratory chain, where the complex between the two redox partners is stabilized by multiple electrostatic interactions and the ET pathway crucially depends on two aromatics inside a patch of negative charges on the cytochrome-c-oxidase binding site [7].

4. Outlook.

It is clear that biological ET is a field which is productive and promissing for future developments in Biochemistry and Biophysics. In spite of some reservations outlined above, the possibility of describing a pathway for coupling the Donor/Acceptor orbitals is extremely rich and stimulating; now the potentialities of protein engineering based on the use of molecular genetics and assisted by computer modeling, can display their full power. The opportunity to control by site directed mutagenesis the rate(s) and the pathway(s) of ET within a complex object such as a protein, coupled to single molecule spectroscopy (which is being progressively developped) may really open the way to a rich field of biomolecular sciences hiterto unexpected. Here biology, chemistry and physics cooperate in understanding the mechanism controlling the destiny of the electron within a protein.

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