Introduction

Long range electron transfer (ET), i.e. the transfer of electrons across distances of 10 Å and more, is essential in all biological systems. In 1941 Szent-Györgyi reported a transfer of electrons between enzymes in the oxidation system.[1] Yet regarding the insulating properties of the peptide matrix described by Evans and Gergely,[2] with an energy gap between filled and empty bands in a polypeptide structure far too large for semiconductive properties at physiological temperatures, no simple explanation for the ability of living organisms to translocate electrons through enzymes could be found. Twenty years later, the importance of biological long range ET for the metabolism was confirmed by Mitchell, who proposed in his chemiosmotic hypothesis that during photosynthesis and respiration a flow of electrons is directed across the membrane dielectric, spanning a range of 35 Å.[3] All living cells are therefore powered by the translocation of electrons - "the flow of electrons on oxidation-reduction reactions is responsible directly or indirectly for all of the work done in living organisms".[4]

By now many examples for ET not only in enzymes involved in photosynthesis and respiration, e.g. photosystem II[5] (PSII) and fumarate reductase,[6] but also in metabolic catalysis, like prostaglandin H synthase[7] and ribonucleotide reductase,[8] are known and have been investigated in detail. Yet many questions remain to be answered regarding the factors that govern fast and selective distal ET in peptides.[9] There is an ongoing discussion about the relative importance of superexchange and sequential mechanisms in biological ET. And while some information about the identity of the "stepping stones" in sequential ET could be obtained from molecular biology experiments, there is still not much known about the specific abilities of the 20 different natural amino acids to mediate protein ET.

1 Electron Transfer

1.1 Marcus Theory

A first theoretical model for the reaction of an electron donor D with an electron acceptor A was established by Marcus in the late 1950`s.[10, 11] In this model, the factors that control the ET reaction are the driving force $-\Delta G^0$, arising from the difference in the oxidation potentials of D and A, and the reorganization energy λ needed for the nuclear rearrangements that accompany ET. The ET rate k_{ET} can then be estimated as:

$$k_{ET} = k_{ET}(0)\exp\left[\frac{-(\lambda + \Delta G^0)^2}{4\lambda RT}\right]$$

Equation 1: classical Marcus theory, ET transfer rate k_{ET} as a function of reorganization energy λ and driving force ΔG^0.

The factor $k_{ET}(0)$ represents the rate for activationless ET ($-\Delta G^0 = \lambda$) A graphical outline of the Marcus theory is given in Figure 1, showing a rate increase with increasing driving force in the Marcus normal region. A maximum rate is reached at $-\Delta G^0 = \lambda$. This contains the central lesson of the Marcus theory: for an ET event to occur in an efficient manner, available driving force and necessary reorganization energy have to be balanced. The energy needed to bring the nuclei from the equilibrium position of the reactants to the equilibrium position of the products (λ) has to be compensated by the driving force.

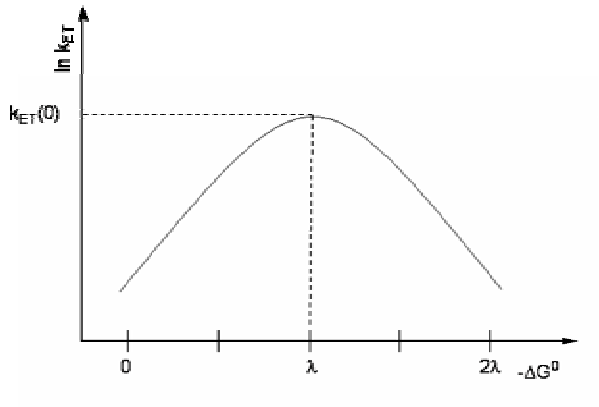

Figure 1: Dependence of ET rates on driving force and reorganisation energy according to the Marcus theory.

In addition to this intuitive coherency, the Marcus theory also predicts an "inverted region". For $-\Delta G^0 > \lambda$, the rate decreases with increasing driving force. Excess free energy has to be dissipated in order to allow ET.

1.2 Distance Dependence of Electron Transfer

The Marcus theory explains ET in cases, where electron donor D and electron acceptor A are in close contact. For cases, in which D and A are separated by a distance r, the occurence of non-adiabatic ET can be explained by electronic coupling of D and A. An additional factor, the electronic coupling matrix element H_{AD}, was introduced into the Marcus theory by Levich.[12] This changes Equation 1 to the following expression:

$$k_{ET} = \left(\frac{4\pi^3}{h^2 \lambda k_B T}\right)^{\frac{1}{2}} H_{AD}^2 \exp\left[\frac{-\left(\lambda + \Delta G^0\right)^2}{4\lambda RT}\right]$$

Equation 2: Marcus-Levich equation for non-adiabatic ET, describing the rate of electron transfer k_{ET} as a function of reorganisation energy λ, driving force ΔG^0 and electronic coupling between donor and acceptor H_{AD}.

with the square of the electronic coupling H_{AD}^2 giving the probability that an electron tunnels through the potential barrier between D and A. Since the height of the barrier is dependent on D/A-distance, the electronic coupling matrix element introduces a distance dependence into ET theory. The strength of electronic coupling of D and A decays exponentially with increasing D/A separation (Equation 3):

$$H_{AD} = H_{AD}^0 e^{-\beta(r_{DA} - r_0)}$$

Equation 3: Distance dependence of the electronic coupling element H_{AD}, with r_0 representing the close-contact donor/acceptor distance.

The importance of the separating medium for the strength of electronic coupling is expressed by the exponential factor β in Equation 3. When D and A are separated by a matrix, the ability of this matrix to mediate the electronic coupling influences the efficiency of ET. The importance of the exponential factor β increases with increasing D/A-distance.

1.3 Electron Transfer through Peptides

As could be seen in paragraph 1.2, the characteristics of a matrix separating electron donor (D) and electron acceptor (A) are of great importance for the efficiency of ET processes between D and A. The distance decay factor β in the extended Marcus theory for non-adiabatic ET is a measure for the ability of the matrix to mediate electronic coupling between D and A. In vacuum β is estimated to 3 – 5 $Å^{-1}$, in water to 1.65 $Å^{-1}$.[13] With β-values of this order, the tunneling of electrons across distances of 10 Å would take several million years, according to the classical theory. Since electrons are transferred through active enzymes across distances of 20 Å and more with rates in the millisecond range,[14] it can be concluded that the peptide matrix is able to support ET between distal redox-partners in a highly efficient manner. Two major models are discussed to explain the specific ET properties of peptides: the superexchange and the hopping model.[15]

1.3.1 Superexchange

Already in 1949 Evans and Gergely[2] pointed out that semiconductivity can be excluded as an explanation for ET through peptides since the energy gap is far too large to be overcome at physiological temperatures. An alternative explanation for efficient ET through enzymes was suggested by DeVault and Chance, who measured the temperature dependence of cytochrome oxidation in *Chromatium vinosum,*[16] and found the reaction rates to show a very weak temperature dependence. They proposed a mechanism based on electron tunneling. A model for the kinetics of ET between proteins by a thermally activated tunneling process was then described by Hopfield,[17] who predicted a distance decay constant β of 1.44 $Å^{-1}$.

The superexchange - or tunneling - model is based on the idea that the orbitals of the bridge molecules are mixed with the donor and acceptor orbitals to increase electronic coupling between donor and acceptor. A simple model for ET across a bridge consisting of n identical repeat units was developed by McConnell. In this model, the charge transfer from the electron donor D to the electron acceptor A occurs in a single-step via a virtual intermediate state, constructed by mixing of the orbitals of D, bridge elements B, and A (Figure 2). The coupling element H_{DA} (see 1.2) thus is a function of the coupling between the redox sites and the bridge, the coupling between the bridge elements, and the energy gap between the tunneling electron and the reduced bridge states δE.[18]

Mixing of all available electronic states lowers the potential barrier and thereby increases the tunneling probability H_{AD}^2. The ET rate is increased, as compared to the transfer rate in vacuum, by an improved electronic coupling, which is expressed by a lowered value of the distance-decay factor β (see Equation 3).

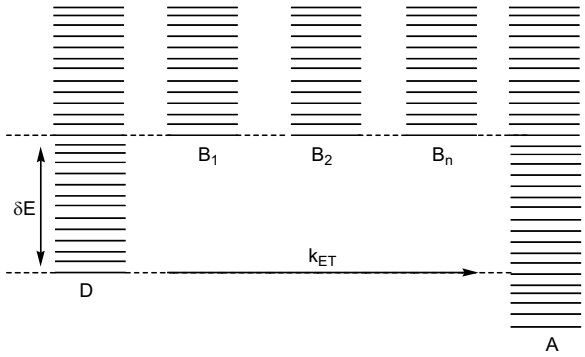

Figure 2: energy diagram for superexchange ET across a bridge of n identical units B_n (McConnell)

Since peptides can be built up from 20 different amino acids, the simple McConnell model cannot be applied in this case. A semiempirical approach by Dutton is based on the observation that ET rates through different types of proteins (reaction centres of different photosynthetic bacteria and several synthetic and semisynthetic proteins) are almost identical for similar D/A-distances. Dutton therefore concludes that the influence of peptide sequence is small and that ET pathways are optimized only in terms of distance, not in terms of peptide structure. In consequence, he regards the peptide matrix as a uniform barrier and estimates the β-value to 1.4 $Å^{-1}$.[19, 20] A rough estimate of structural differences has been taken up into a refined version of Duttons uniform barrier model, which includes the packing density of the protein matrix as an additional factor.[21] Yet bioinformatic investigations revealed no major difference in protein packing density between long-distance and short-distance electron transfers. This leads back to the central hypothesis of Dutton`s model: the belief, that in the course of evolution large effects of structural changes in ET proteins would have been highly unfavourable and that nature therefore preferred ET-proteins to be robust when it comes to structural and sequence changes. A a consequence of this demand, the peptide matrix is equipped with a mainly uniform ET behaviour, controlled by cofactor distances, and not optimized with respect to ET properties in special enzymes.[22]

This view has been challenged by Gray and Winkler, who measured ET rates in modified proteins.[14, 23] They used naturally occuring metalloproteins with copper (azurin)[24] or iron (cytochrome)[25] cofactors and with known crystal structures and attached a ruthenium-complex to specific histidine residues at defined distances. ET was then induced by photoexcitation of the ruthenium chromophore, and subsequent rapid oxidation by an external acceptor (flash-quench-technique). The rates of ET from the metal cofactor to the oxidized ruthenium complex were measured by absorption spectroscopy of the transient metal complexes. The original oxidation state was then recovered by a slow reaction with the reduced quencher (Figure 3). This method spans a wide time frame for the measurement of ET rates ranging from sub-microseconds to seconds.[13]

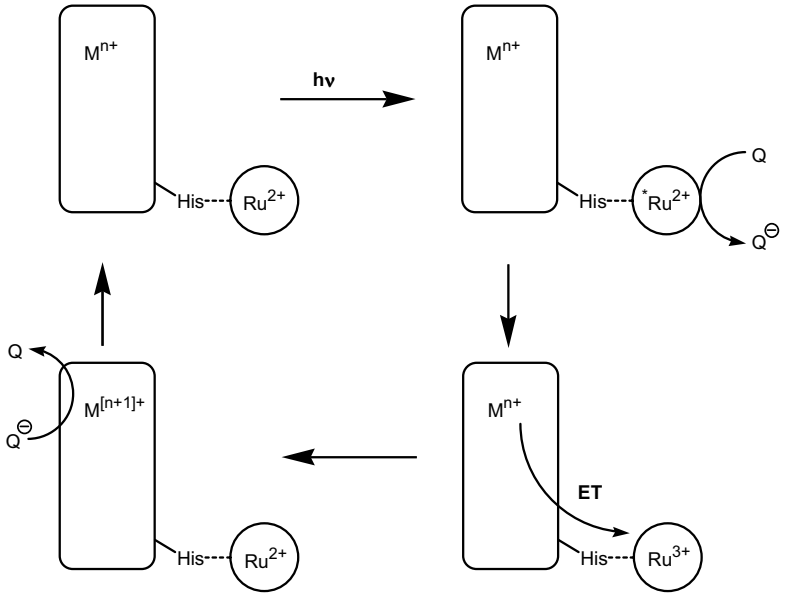

Figure 3: Flash-quench technique for the induction and observation of ET in Ru-modified metalloproteins

The method was used to gain a large collection of experimental data from different classes of modified metalloproteins. From the azurin series, a timetable for ET through proteins that proceeds according to the tunneling model, with rates in the microsecond range for ET across 15-20 Å and a β-value of 1.1 $Å^{-1}$ for a β-strand, was derived.[13] Nevertheless, in several cases, ET velocities cannot be explained by D/A distances alone: ET rates for similar D/A distances can differ by several orders of magnitude and similar rates can be observed for distances that differ by as much as 5 Å.[26] Gray and Winkler therefore concluded, that the structure of the peptide matrix is the main factor controlling ET rates.