Chapter 3

Protein-Mediated Electron Transfer

The energy metabolism of living cells crucially depends on electron transfer reactions. Furthermore, electron transfer reactions are involved in a variety of anabolic and catabolic processes. The physiological importance of electron transfer reaction is the reason for the enormous attention that these processes are receiving. Nowadays, the combination of experimental and theoretical research revealed new insight in the molecular mechanism. Especially site-directed mutagenesis and structural investigations by NMR and X-ray crystallography increased the understanding of electron transfer processes in proteins. Several books and articles review the research on electron transfer in proteins in the last years (Marcus & Sutin, 1985; Canters & van de Kamp, 1992; Farid *et al.*, 1993; Bendall, 1996).

During an electron transfer reaction, an electron moves from the donor to the acceptor molecule (eq 3.1).

$$\mathrm{D} + \mathrm{A} \longrightarrow \mathrm{D}^+ + \mathrm{A}^- \tag{3.1}$$

Besides the actual electron-transfer process, the reaction in eq 3.1 involves also diffusion processes which bring the two molecules together or take them apart.

$$\mathrm{D} + \mathrm{A} \rightleftharpoons \mathrm{D/A} \xrightarrow{k_{et}} \mathrm{D}^+/\mathrm{A}^- \rightleftharpoons \mathrm{D}^+ + \mathrm{A}^- \tag{3.2}$$

In eq 3.2, k_{et} marks the rate of the actual electron-transfer reaction. The value of the rate constant that is observed in experiments depends on the experimental setup, the measurement method and on the considered system. Usually, the observed reaction involves only the formation of a bimolecular complex and the electron transfer step, since most often the change in absorption upon reduction or oxidation is measured (Bendall, 1996; Sokerina *et al.*, 1998). It is often believed that first order rate constants of photoinduced reactions reflect the rate of the electron transfer step. In proteins, however, conformational dynamics often influences the reaction rates (Kostić, 1996; Davidson, 1996). Often, not the electron transfer rates but rather the rates of the conformational dynamics are measured (Zhou & Kostić, 1992b; Zhou & Kostić, 1993b; Ivković-Jensen & Kostić, 1996; Crnogorac *et al.*, 1996; Ivković-Jensen & Kostić, 1997; Ivković-Jensen *et al.*, 1998).

The redox centers in electron-transfer proteins can be as much as 20 Å apart from each other. A detailed description of the role of the intervening medium is required to understand how proteins maintain a highly efficient electron transfer. Many recent investigations are inspired by the model that electrons take more or less specific paths in the protein (Onuchic *et al.*, 1992). More advanced models that are based on Greens functions (Larson, 1981; Larson, 1983;

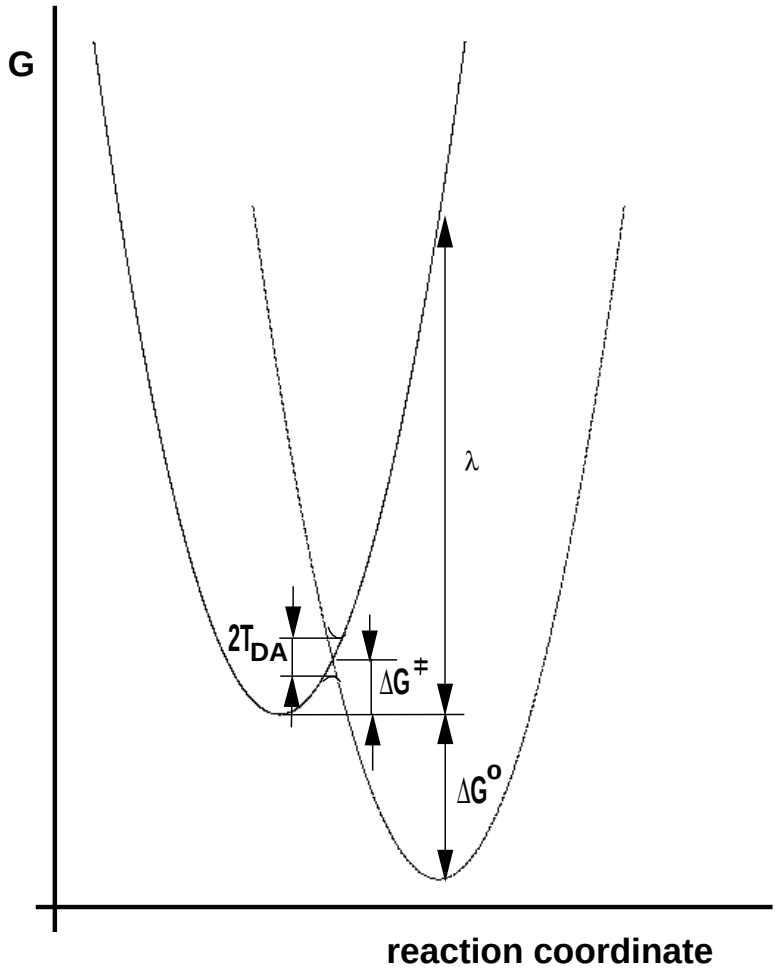

Figure 3.1: Electron Transfer. During the reaction, the reactants transit from the higher energy surface (left) to the lower potential energy surface. The rate of the electron-transfer reaction is influenced by the reorganization energy λ and Gibbs free energy ΔG^{o} as well as by the tunneling matrix element T_{DA}.

Skourtis & Onuchic, 1993; Skourtis *et al.*, 1993) or extended Hückel calculations (Siddarth & Marcus, 1993b; Siddarth & Marcus, 1993c; Siddarth & Marcus, 1993a; Stuchebrukhov, 1994; Stuchebrukhov & Marcus, 1995) support the simple *Pathways* model.

3.1 Marcus Theory

According to Marcus theory, the rate constant (k_{et}) for a non-adiabatic electron-transfer reaction is proportional to the square of the tunneling matrix element (T_{DA}) between the donor (D) and the acceptor (A) and to the density of vibrational states weighed by their Franck-Condon factors. The symbols λ and ΔG^{o}, respectively, stands for the reorganization energy and the Gibbs free energy of the reaction (Marcus & Sutin, 1985).

$$k_{et} = \frac{2\pi}{\hbar} \mid T_{\mathrm{DA}} \mid^{2} \frac{1}{\sqrt{4\pi\lambda RT}} \exp\left(-\frac{(\Delta G^{o}+\lambda)^{2}}{4\lambda RT}\right) \tag{3.3}$$

The argument of the exponent in eq 3.3 corresponds to the free energy that is required to reach the transition state, i. e., to the activation free energy of the electron-transfer reaction. If the

potential energy of the reactant state and the product state is assumed to be harmonic and the coupling between the reactant and the product state is small, i. e., the reaction is non-adiabatic, then the activation free energy is given by the argument of the exponent in eq 3.3. The reorganization energy λ is the energy required to reach the equilibrium geometry of the product state at the potential energy surface of the reactant state and vice versa. The Gibbs free energy of the reaction ΔG^{o} is related to the redox potential difference between the reactant state and the product state. If the two molecules of the same type form a redox couple, the electron-transfer reaction is called self-exchange reaction. In this case the Gibbs free energy of the reaction is zero and the activation free energy is $\lambda/4$. Therefore self-exchange reactions can be used to determine reorganization energies experimentally.

The parameters in eq 3.3 are accessible to theoretical calculations. The reaction free energy is related to the redox potential difference of the reactants. The difference between the redox potential of a redox-active center in a protein and a proper model compound can be calculated by continuum electrostatic methods or by free energy simulations (see Section 4). The redox-potential of model compounds can be obtained from quantum chemical calculations (Mousca *et al.*, 1995). Also several approaches exist to access the reorganization energy λ from molecular dynamics simulations using a linear response approximation (Zhou *et al.*, 1995a; Muegge *et al.*, 1997), from continuum electrostatic models (Zhou *et al.*, 1995b; Basu *et al.*, 1998a; Basu *et al.*, 1998b), or from density matrix methods (Chernyak & Mukamel, 1998). The theoretical estimation of the tunneling matrix element T_{DA} is a special challenge. The simple *Pathways* model described in Section 3.2 provides reasonable estimates of relative electronic couplings. More advanced, but computationally more expensive methods used a Green's function approach (Skourtis & Onuchic, 1993; Skourtis *et al.*, 1993) or the extended Hückel theory (Larson, 1981;Larson, 1983; Siddarth & Marcus, 1993b; Siddarth & Marcus, 1993c; Siddarth & Marcus, 1993a; Stuchebrukhov, 1994; Stuchebrukhov & Marcus, 1995).

3.2 Pathways Model of Electron Transfer in Proteins

The theoretical basis and the algorithm of the *Pathways* method are described elsewhere (Beratan *et al.*, 1990; Onuchic *et al.*, 1992; Onuchic & Beratan, 1990; Beratan *et al.*, 1991; Beratan *et al.*, 1992; Betts *et al.*, 1992; Regan *et al.*, 1993). Here I briefly explain only the salient features of the method.

An electron-tunneling path is a trace of connected covalent bonds, hydrogen bonds, and van der Waals contacts (interactions through space), that links the donor with the acceptor. The respective decay parameters for attenuation of electronic coupling via these bonds and contacts are the unitless quantities ε_C, ε_H, and ε_S, defined in eq 3.4 and are calculated with the standard parameters α, β, and r_{eq}; r is the distance between the interacting atoms.

$$\varepsilon = \alpha \exp(-\beta(r - r_{eq})) \tag{3.4}$$

Coupling within aromatic rings of heme, histidine, phenylalanine, tyrosine, and tryptophane and within the guanidinium group of arginine was defined in two ways. (1) Bonds were treated as usual covalent bonds (ε=0.6). (2) The enhanced coupling was recognized by neglecting the attenuation (ε=1.0). The tunneling matrix element t_{DA} for a single path is proportional to the relative coupling, according to eq 3.5.

$$t_{DA} \propto \prod_i \varepsilon_C(i) \prod_j \varepsilon_H(j) \prod_k \varepsilon_S(k) \tag{3.5}$$

The path providing the strongest coupling between donor and acceptor is searched by a graph theoretical algorithm called depth-first (Betts *et al.*, 1992).

The electronic coupling via a given path depends on the degree of covalency of the iron-ligand and copper-ligand bonds included in that path. As in a previous study, in which consideration of anisotropic covalency was introduced into the Pathways algorithm (Ullmann & Kostić, 1995), the relative coupling was scaled by coefficients (γ) representing the contributions of the relevant ligands (L) to the redox molecular orbitals of the electron donor (D) and acceptor (A) (Newton, 1988, Newton, 1991); see eq 3.6.

$$t_{DA} \propto \gamma_{DL}^2 \gamma_{AL}^2 \prod_i \varepsilon(i) \tag{3.6}$$

This scaling is based on the reasonable assumption that the expansion coefficients γ are independent of the relative coupling. The values of γ were 0.68 for Cys84, 0.11 for His37, 0.11 for His87, and 0 for Met92 in plastocyanin (Lowery *et al.*, 1993; Ullmann & Kostić, 1995) and 1.00 for the porphyrin ligand and 0.6 for each axial ligand in cytochrome c_6.