Electron Transfer in Proteins: Structural and Energetic Control of the Electronic Coupling

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Abstract: The available experimental data on the electron donor (D)−acceptor (A) coupling (H_{DA}) for electron-transfer (ET) reactions in proteins are re-examined. In spite of their structural and energetic similarities, the photosynthetic reaction center and other ET protein systems exhibit a marked difference of the exponential decay of H_{DA} with the distance separating D and A. A numerical study shows that this difference is explained in terms of very small variations of the energetics between these two classes of proteins.

Since the proposal of Dutton and co-workers[1] of a "universal" relationship between the electron donor (D)−acceptor (A) coupling (H_{DA}) and the distance (d) separating D and A in electron-transfer (ET) proteins, a number of experiments have shown important departures from such a simple picture.[2] The situation has come to a tendency toward a classification of ET proteins into two groups, according to their ability to fit or not to fit the "universal" law.[3,4] Proteins of the "universal" type seem to conform to a one-dimensional square-barrier (1DSB) model, for which Gamow's formula gives an exponential dependence of H_{DA} on d, with a decay constant $\beta_{1DSB} = (2mE/\hbar^2)^{1/2}$, where m is the electron mass, $\hbar$ is Planck's constant divided by 2π, and E is the energy of the tunneling electron. Dutton's compilation[1] gives $\beta_{1DSB} = 0.7$ Å^{-1} from the average exponential decay of the maximum ET rate constant k_{max} (s^{-1}) $= 10^{13}e^{-1.4(d-3.6)}$ *versus* d (Å). Other ET proteins do not show such a simple correlation of H_{DA} with d. Instead, there seems to be a more pronounced correlation of H_{DA} with the length of some specific pathways from D to A, along the protein bonds. Onuchic and Beratan and co-workers[5] (OB) associated to each of these pathways an additive contribution to H_{DA} allowing for a selection of those with the optimum coupling strengths.

The limitations of both theories are well-known. Briefly, Dutton's model simply does not rely upon a microscopic description of proteins, while, as we will show below, OB's approximation largely underestimates the contributions of through-space (TS) interactions. An accurate determination of H_{DA} must include the complete structural and energetic complexity of proteins into the electronic Hamiltonian, which has to be treated exactly.[6] To date, only a very limited number of such studies exists.[7] It is therefore difficult to appreciate the whole behavior of H_{DA} in ET proteins. A lucid discussion of the present *status* of the theory has been given by Friesner.[8]

In this paper, we re-examine the available experimental data, including the most recent ones, on the k_{max}-*vs*-d correlation. Our compilation clearly distinguishes between the photosynthetic reaction center (PRC) and other ET protein systems. By analyzing, on a variety of proteins, the possible energetic and structural origins of the observed behaviors, we show that there is no influence of the protein's structure on the *average* distance−decay rate of H_{DA}. However, experimental data support the notion of a large dispersion of the electronic energy levels of the protein as reflected by the important scatter of the data.

According to theory, k_{max} is related to H_{DA} through the semiclassical Marcus expression for the ET rate constant k:[9]

$$k = \frac{2\pi}{\hbar}|H_{DA}|^2 \frac{1}{\sqrt{4\pi\lambda k_B T}} e^{-(\Delta G^\circ + \lambda)^2/4\lambda k_B T} \quad (1)$$

where k_B is the Boltzmann constant, T is the temperature, λ is the nuclear reorganization energy accompanying ET, and ΔG° is the reaction free-energy change. k_{max} is reached at vanishing activation energy ($\Delta G^\circ = -\lambda$),

$$k_{max} = \frac{2\pi}{\hbar}|H_{DA}|^2 \frac{1}{\sqrt{4\pi\lambda k_B T}} \approx (2.575 \times 10^8)|H_{DA}|^2 \quad (2)$$

where the numerical estimate of k_{max} (in s^{-1}) as a function of H_{DA} (in cm^{-1}) holds for a typical reorganization energy $\lambda = 1$ eV at $T = 300$ K. H_{DA} reflects the influence of the intervening medium on the ET rate.

Table 1 presents an up-to-date compilation of k_{max} *versus* d for 23 ET protein systems studied in the literature.[10−20] These

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Table 1. Experimental Data of k_{max} *vs d* between Centers of Edge Atoms[a]

source	protein	d (Å)	k_{max} (s^{-1})	ref
calf liver	cyt *c*/cyt b_5	11.5	4.9×10^5	10
human	Hb hybrid	16	4.2×10^5	17
horse heart	Ru-cyt *c* His 72	8.4	9.8×10^5	11
	Ru-cyt *c* His 33	11.1	2.7×10^6	11
Candida krusei	Ru-cyt *c* His 39	12.3	3.3×10^6	11
Saccharomyces cerevisiae	Ru-cyt *c* His 62	14.8	1.0×10^4	11
	Ru-cyt *c* His 58	13.2	6.0×10^4	11
	Ru-cyt *c* His 66	13.3	1.1×10^6	11
sperm whale	Ru-Mb His 81	19.3	4.0×10^3	16
	Ru-Mb His 116	20.1	3.3×10^3	16
	Ru-Mb His 12	22	3.3×10^3	16
	Ru-Mb His 48	12.7	3.0×10^6	16
Sacc. cerevisiae	Flavocyt b_2/cyt *c*	14.2	6.6×10^5	12
rat liver	Ru-cyt b_5 Cys 65	12.4	1.8×10^7	19
Ps. aeruginosa	azurin	25.2	3.0×10^2	15
Ps. fluorescens	azurin	25.2	1.0×10^2	15
Alc. faecalis	azurin	25.2	1.0×10^2	15
Par. denitrificans	MEDH-cyt *c*	14.9	8.1×10^5	13
human	Hb hybrid	16	1.0×10^2	14
yeast	cyt *c*/CCP	18	8.1×10^3	18
Ps. aeruginosa	Ru-Azurin His 122	10	7.1×10^6	20
	Ru-Azurin His 124	15.6	2.2×10^4	20
	Ru-Azurin His 126	21.8	1.3×10^2	20

[a] Abbreviations : cyt = cytochrome, CCP = cyt *c* peroxydase, Mb = myoglobin, Hb = hemoglobin, MEDH = methanol dehydrogenase.

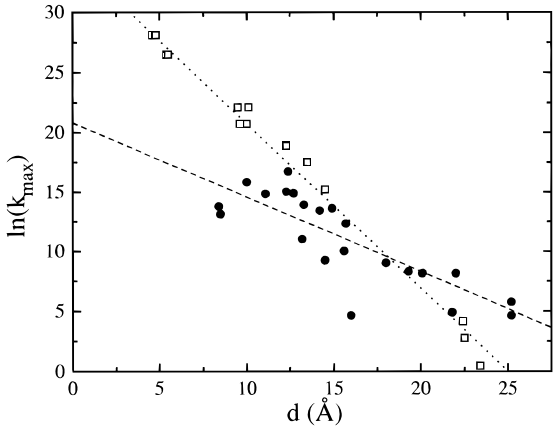

Figure 1. k_{max}-*vs*-*d* data on the ET proteins given in Table 1 (●) and on the PRC from ref 1 (□). Solid lines are linear regressions.

data are plotted in Figure 1, along with those of Dutton's group on the PRC.[1] It is seen that, for proteins other than the PRC, $k_{max}(d)$ exhibits a large dispersion from one protein to another and from one point to another in the same protein with an *average* exponential decrease of k_{max} with d of 0.66 $Å^{-1}$ which is only half the value corresponding to the PRC (1.4 $Å^{-1}$). However, before we can rely upon these observations, a great deal of experimental work remains to be done in order to verify that both the donor−acceptor distances and the reported k_{max} values can be used confidently. Indeed, the three-dimensional structures of most of the modified proteins are not known yet, while the effects of metal substitution on the structures in the heme proteins are not controlled, leading to an uncertainty of several angstroms in the physical distances. Moreover, some of the kinetic data are single-point measurements at uncertain $\Delta G°$ and λ values.

In view of this experimental situation, it is important to know whether the above-mentioned discrepancies between the PRC and other ET protein systems may be supported or understood from a theoretical ground. It is our aim here to tackle this problem by looking at the energetic and structural conditions of the ET process that lead to these distinct behaviors. More precisely, we have performed a study on a variety of ET protein systems, adopting a crude, yet very common, electronic modelization of proteins. This model is based on the pure TS coupling, for which sites i and j, located at positions $\mathbf{r}_i$ and $\mathbf{r}_j$, respectively, interact with one another according to

$$V_{ij} = V e^{-\beta|\mathbf{r}_i - \mathbf{r}_j|} \tag{3}$$

We recall that, for similarly large three-dimensional homogeneous media, this coupling law (with $V/E < 0$) defines a unique dimensionless parameter Γ/E that controls the distance−decay rate β_{HOM} of the electronic coupling,[21] given by

$$\beta_{HOM} = \beta\sqrt{1 - \sqrt{|\Gamma/E|}} \tag{4}$$

Here, E is the tunneling energy (defined as the energy of the protein's orbitals with respect to that of the redox site orbitals), $\Gamma = 8\pi\rho|V|\beta^{-3}$ is the electronic band width of an associated continuous medium,[21] and $\rho = 0.05$ $Å^{-3}$ is the density of non-hydrogen sites. Remarkably, we note that this density is about the same for all proteins, including the PRC. Adopting the values $V = -6$ eV and $\beta = 1.7$ $Å^{-1}$,[3] we also note that the OB through-space attenuation factor [$\epsilon_S = 0.3e^{-1.7(r-1.4)}$] may be recovered using $E = 1.85$ eV. In the following, however, E is not restricted to this single value, but we use it as a free parameter that will be systematically varied. Note in passing that the case $V/E > 0$ was also considered in previous studies,[21] although it is usual to take $V/E < 0$ in proteins. The reader is referred to these studies for a discussion concerning the meaning of the sign of V/E, especially in how this relates to the hole-*versus* electron-transfer mechanisms.

Figure 2 shows the *average* effective decay coefficient β_{eff} as a function of Γ/E for different protein structures obtained from the Brookhaven Protein Data Bank (PDB). β_{eff} is deduced from the numerical calculation of the Green's function matrix element $G_{DA}(E)$, which is linearly related to H_{DA}.[21] The insert of Figure 2 shows the typical correlation between G_{DA} and d for selected values of E. We see that, on the average, all ET proteins, including the PRC, exhibit the same behavior as the reference continuous medium. The difference between proteins is significant only for large values of Γ/E (∼1), when the electron's energy is close to the protein's electronic band.

As we find from Figure 2, the values $\Gamma/E_{PRC} = 0.7$ and $\Gamma/E_{other} = 0.84$ correspond to the decay constants 0.7 and 0.33 $Å^{-1}$ deduced from experiment for the PRC and other ET protein systems, respectively. With our choice of V and β, the tunneling

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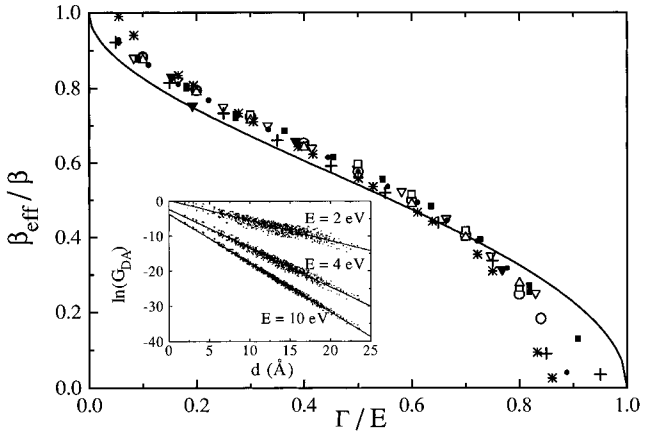

Figure 2. Normalized exponential distance−decay coefficient β_{eff}/β as a function of Γ/E for selected proteins: (*) *Rhodopseudomonas viridis* photosynthetic reaction center (1PRC); (▽) Baker's yeast cytochrome *c* (1YCC); (○) tuna cytochrome *c* (5CYT); (□) sperm whale myoglobin (1MBC); (■) bovine liver cytochrome b_5 (1CYO); (△) *Pseudomonas aeruginosa* mutant azurin (1AZN); (▼) *Proteus mirabilis* catalase (1CAE); (●) pig liver microsomes cytochrome b_5 (1NDH); (+) cytochrome P-450. PDB numbers are given in parentheses. The solid line shows the theoretical prediction of β_{HOM}/β (eq 4). Insert: Illustration of the typical case of tuna cytochrome *c* for selected values of *E*: plot of $\ln(G_{\text{DA}})$ against the site-to-site distance *d*. Each point corresponds to a different location of the acceptor spanning the entire protein. Note the increase of the dispersion when lowering *E*. Solid lines are linear regressions giving β_{eff}.

energies are $E_{\text{PRC}} = 2.2$ eV and $E_{\text{other}} = 1.83$ eV, respectively. We see that these energies differ only by about 20%, while at the same time H_{DA} varies by many orders of magnitude between these two classes of systems (Figure 1). In a qualitative viewpoint, this conclusion is independent of the numerical values given to *V* and β, because of the universal character of Γ/E.[21] However, one expects the introduction of a more complete modelization of the protein's electronic structure, by taking into account covalent couplings, to modify appreciably the numerical value of the tunneling energy. Nevertheless, the main conclusion that a small variation of *E* could account for an important departure between similar systems would apply equally. In essence, this is because the intervening medium would remain under the wide-electronic-band regime.[22] The short-range nature of the TS interactions is largely compensated by the huge number of tunneling pathways they allow, producing a large and continuous spreading of the protein's electronic levels that cannot be accounted for in a perturbative way as in the OB single/few dominant pathway approximation (we note that TS interactions produce an important renormalization of the decay constant from the bare value $\beta = 1.7$ Å^{-1} to values $\beta_{\text{PRC}} = 0.7$ Å^{-1} and $\beta_{\text{other}} = 0.33$ Å^{-1}).

The distance−decay coefficient characterizes the *average* medium and, as such, it concerns only its global structural properties (mainly reflected by the density). This average behavior is controlled by the energetic parameter Γ/E. However, for large enough Γ/E values, there is room for an additional structural control. This is illustrated in Figure 1, for systems other than the PRC, by the large departures from the average behavior due to local structural details.

The importance of the protein's structure is qualitatively captured by the present TS coupling model (see insert of Figure 2). Moreover, the concept of an average behavior is seen to lose its meaning as the system comes closer and closer to resonance. This can be seen in Figure 2 from the increasing dispersion of β_{eff} as Γ/E increases toward 1, which reflects the large uncertainty associated to the determination of the average decay coefficient. Again, as for the average properties, the introduction of covalent couplings would be crucial for any quantitative discussion of the H_{DA} fluctuations.

In view of the presently available experimental data, the behavior of the PRC markedly differs from that of other ET protein systems; the distance−decay constant β_{PRC} is higher (by a factor of 2) than β_{other}, while the H_{DA} fluctuations are smaller (by more than one order of magnitude). Although these differences rest on an uncertain experimental basis, we have shown that they can be supported from a theoretical analysis by assuming only a small variation in the energy of the tunneling electron. Clearly, any further progress in resolving the origin of these differences would have to extend the theoretical analysis to include through-bond as well as through-space interactions, and would also greatly benefit from an extension and a critical re-examination of the experimental data.

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