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Pathways, Pathway Tubes, Pathway Docking, and Propagators in Electron Transfer Proteins

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The simplest views of long-range electron transfer utilize flat one-dimensional barrier tunneling models, neglecting structural details of the protein medium. The pathway model of protein electron transfer reintroduces structure by distinguishing between covalent bonds, hydrogen bonds, and van der Waals contacts. These three kinds of interactions in a tunneling pathway each have distinctive decay factors associated with them. The distribution and arrangement of these bonded and nonbonded contacts in a folded protein varies tremendously between structures, adding a richness to the tunneling problem that is absent in simpler views. We review the pathway model and the predictions that it makes for protein electron transfer rates in small proteins, docked proteins, and the photosynthetic reactions center. We also review the formulation of the protein electron transfer problem as an effective two-level system. New multi-pathway approaches and improved electronic Hamiltonians are described briefly as well.

KEY WORDS: Tunneling pathways; protein electron transfer; donor–acceptor interactions.

1. INTRODUCTION

Within a few picoseconds after absorbing light, photosynthetic organisms launch an electron down an efficient multi-step electron transfer chain (Feher *et al.*, 1989; Gunner, 1991). The ubiquitous single-electron transfer (ET) reaction lies at the center of cell metabolism as well. A sequence of one-electron oxidation–reduction reactions followed by proton transport generates a transmembrane electrochemical potential that energizes the synthesis of ATP. Recent developments in theoretical and experimental biophysical chemistry are beginning to indicate how proteins direct tunneling electrons to the right place at the right time (Lippard and Berg, 1994; Bertini *et al.*, 1994).

ET reactions occur throughout cell metabolism. Oxidases, peroxidases, oxygenases, hydrogenases, and nitrogenases perform key chemical transformations by the efficient coordinated transport of electrons. The enzyme nitrogenase, for example, makes ammonia from dinitrogen at atmospheric pressure and room temperature, a feat unequaled in the laboratory (Kim and Rees, 1994).

In the 1960's it was discovered that electron hops between redox groups in proteins are nonclassical (deVault, 1984). Electrons "tunnel" between spatially localized redox groups rather than being transported like delocalized electrons in metals (Hopfield, 1974). Since electron delivery to the wrong site can be lethal, quantum tunneling provides a likely means of controlling electron flow that is exquisitely sensitive to molecular architecture. Electron tunneling probabilities, zero for purely classical particles, drop roughly exponentially with distance. Recent studies indicate a richness in the mechanisms proteins might use to control electron tunneling rates. We will review some of the recent developments and challenges in this area.

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2. PROTEINS: FORMLESS OR STRUCTURED TUNNELING BARRIERS?

Most biological electron transfer rates (k_{ET}) are described successfully with a nonadiabatic formulation because the donor–acceptor distance is large (and the interaction is weak) (Hopfield, 1974), so

$$k_{ET}(\vec{R}_{DA}) \propto (\text{electronic coupling})^2 (\text{nuclear factor}) \quad (1)$$

The nuclear factor is associated with the familiar activation barrier of Arrhenius theory (Sutin and Marcus, 1985; Marcus, 1993; Levich, 1965). The limits of validity for this rate expression have been discussed extensively (Onuchic *et al.*, 1986). This barrier arises because nuclei around the redox groups adjust their positions to accommodate the charge of the redox center. The electronic factor reflects how strongly the protein allows electron amplitude to leak across the protein from donor to acceptor. A detailed understanding of how proteins might control ET rates through this tiny quantum propagation proved elusive until recently.

Simple models of long-range electron transfer treat the protein between donor and acceptor as a one-dimensional square tunneling barrier (1DSB). As such, the rate is predicted to drop exponentially with distance (Hopfield, 1974; Jortner, 1976; Moser *et al.*, 1992; Beratan *et al.*, 1992a,b):

$$k_{ET}(\vec{R}_{DA}) \propto \exp[-\beta|\vec{R}_{DA}|](\text{nuclear factor}) \quad (2)$$

Far from the well, the probability of finding the particle drops exponentially, with a constant determined simply by the well depth, and this tunneling probability enters the ET rate directly. Accounting for the role of protein-mediated tunneling at this level of theory amounts to assigning a barrier height for tunneling. This barrier is associated with the energy difference associated with moving the electron from the donor to the surrounding protein. Hopfield's 1974 prediction ($\beta = 1.44$ Å^{-1}) stimulated numerous experiments on small molecule and macromolecule bridges of fixed length and known structure. *Rigid* bridges, keeping the donor–acceptor distance fixed, were built to test these ideas (see Bolton *et al.*, 1991, for example).

In the 1980's, a range of β values was observed for rigid electron transfer bridges. Theoretical analysis of these linkers predicts orbital energy and symmetry effects on the reaction rates. A fascinating balance of orbital interactions in model compounds seems to control the value of β, which varies with bridge structure (Bolton *et al.*, 1991; Closs and Miller, 1988; Wasielewski, 1992).

At the same time as intense work was being done on small molecules, a technology was developed to measure electron transfer rates in proteins between redox groups at fixed distance and orientation (Winkler and Gray, 1992; Therien *et al.*, 1991a,b). In proteins, just as in small molecules, donor and acceptor must be fixed in order for conclusions about bridge-mediated tunneling to be drawn unambiguously. A watershed in this field was the development of a new technology by Gray and co-workers to attach Ru complexes to proteins via surface histidines. This methodology, coupled with sophisticated electron-transfer kinetic experiments, provided the tools to probe the anisotropic nature of proteins as tunneling barriers (Therien *et al.*, 1990; Wuttke *et al.*, 1992; Karpishin, *et al.*, 1994).

Electron-transfer rates as a function of distance in model compounds and in proteins, when fitted to 1DSB models, produce a wide range of β values, ranging from 0.6 to 1.7 Å^{-1} (Mikkelson and Ratner, 1988; Therien *et al.*, 1991a,b). In small molecules, β values are consistently smaller than in proteins. This discrepancy provided the first experimental hint that the coupling mechanism in proteins might be qualitatively different from that in the smaller systems. The faster decay of rate with distance in proteins suggests that the coupling mechanism is less efficient. Looking at three-dimensional protein structures, a reasonable inference is that the mediation routes are less direct than in small molecule bridged systems.

In the late 1980's Onuchic and Beratan asked whether or not "measuring β" in proteins was a meaningful endeavor (Beratan *et al.*, 1987; Onuchic and Beratan, 1990). They argued that at physiological redox potentials *a single "universal" value of* β *does not exist.* The balance of through-bond and through-space contacts between donor and acceptor was proposed to set the coupling strength. They showed that mixing of the donor and acceptor states with the bonding orbitals of the bridge dominates the coupling process. This mechanism is referred to as "hole transfer." Their analysis suggested that ET rates in proteins should depend on the coupling pathway strength, rather than the linear distance between redox centers (Beratan *et al.*, 1991, 1992a,b).

3. TUNNELING PATHWAYS

What is the first step beyond a structureless tunneling barrier model of a protein? Electronic coupling

interactions mediated through bonds are longer range, in general, than interactions through space. Thus, it is convenient to break the electron tunneling into through-bond and through-space steps. The pathway strategy estimates the coupling as the product of decay factors along the most direct coupling route between donor and acceptor.

$$\text{(Electronic coupling)} \propto \Pi_i \epsilon_i^B \Pi_j \epsilon_j^H \Pi_k \epsilon_k^S \quad (3)$$

This expression represents a product of decay factors associated with each type of contact along a pathway (Beratan *et al.*, 1987; Onuchic and Beratan, 1990). Here the probability of tunneling between two points is determined by the *strongest coupling pathway* between those points. The through-bond decay parameters (B and H) are fixed at values estimated from model-compound experiments or from simple calculations. A critical aspect of the pathway model is the vital role of hydrogen bonds as tunneling mediators. Since the through-bond mechanism is dominated by hole transfer, hydrogen-bond mediation is particularly effective. The through-space decay factor is explicitly (and strongly!) distance dependent. Through-space decay is very costly; viable coupling pathways in proteins rarely contain more than one or two through-space steps. The strength of the strongest coupling pathways to a protein's surface from a redox center varies enormously (see Section 3.3). Pathways are easily identified using x-ray structural coordinates and a simple graph search algorithm (Betts *et al.*, 1992).

The pathway model has one essential feature built into it that is missing in 1DSB models. This is the radically different distance range for tunneling through a bonded medium and through empty space. The range of tunneling through space is set by the binding energy of the tunneling electron. An electron bound in a 1D well by 10 eV decays far from the well as $\exp(-1.7R)$ with R measured in Å. In contrast, the exponential decay constant associated with tunneling through covalent bonds and hydrogen bonds is *at least one-half of this value*. This intrinsic difference between through-bond and through-space tunneling defines the landscape for electron tunneling in proteins.

The sharp distinction between through-bond and through-space tunneling in proteins has at least two important and dramatic consequences. First of all, this line of analysis predicts that hydrogen bonds should be excellent tunneling mediators because they introduce relatively small through-space gaps between lone-pair electrons and $X - H$ bonds (Beratan *et al.*, 1987, 1991, 1992a,b; Onuchic and Beratan, 1990). This expectation was confirmed by experiments in Ru-modified proteins (Therien *et al.*, 1990, 1991a,b). In the absence of facile hydrogen bond-mediated tunneling pathways, electron-transfer rates should be orders of magnitude slower than what is actually observed. The second key prediction of the pathway model is that electrons tunnel very weakly across van der Waals gaps. This prediction was recently confirmed as well (Wuttke *et al.*, 1992).

Small redox proteins, like cytochrome *c*, contain a single redox center. The anisotropic structure of this protein is mirrored in the anisotropy of coupling pathways into the protein's redox center. A simple way of visualizing this anisotropy is to examine scatter plots of amino acid to redox-center coupling vs. amino acid to redox-center distance. 1DSB models put all of these points on a single exponential curve, whereas pathway models scatter these points orders of magnitude off of any single exponential curve (Beratan *et al.*, 1991, 1992a,b).

3.1. Functional Docking and ET

In many biological electron transfer reactions, an important ET step is *inter*molecular. However, theoretical work on electronic coupling in ET reactions has been confined largely to *intra*molecular ET. The intramolecular problem is simpler because the geometry is often relatively well known and the pathway analysis is straightforward.

The pathway model was recently extended to describe *inter*molecular electron transfer (Aquino *et al.*, 1995). This problem is particularly challenging because few electron transfer complexes have been subjected to detailed structural analysis. In existing x-ray structures, it is unclear whether or not the biologically relevant docking orientation is found in the solved structures. Furthermore, even if the interprotein docking orientation is known, the electron transfer rate is expected to be strongly dependent on the through-space decay associated with noncovalent contacts between molecules.

The interprotein pathway analysis strategy taken was to analyze couplings based on the *intra*molecular coupling in each interacting species. This allows the intermolecular through-space electron transfer, which is sensitive to the docking geometry, to be treated as a parameter to be analyzed. Thus, calculation of the coupling between the surface amino acids and the redox center permits a simple estimate of interprotein electronic coupling decay for a given docked structure.

The electronic coupling coupling is factored accordingly:

$$\text{(Electronic coupling)} \propto \Pi_i \epsilon_D(i) \Pi_j \epsilon_A(j) \epsilon_{\text{inter}} \quad (4)$$

where $\Pi_i \epsilon_D(i)$ is the electronic coupling decay between the electron donor site and the surface of the protein containing the electron donor; $\Pi_j \epsilon_A(j)$ is the electronic coupling decay between the electron acceptor site and the surface of the protein containing the acceptor; and ϵ_{inter} is the electronic coupling decay between protein surfaces (i.e., a through-space or hydrogen-bond coupling between surface atoms on the two proteins). Note that in Eq. (4) $\epsilon(i)$ and $\epsilon(j)$ can be any of the three types of decay (i.e., covalent, hydrogen-bonding, or through-space). Factoring the electronic coupling decay in this way separates quantities that are well defined, $\Pi_i \epsilon_D(i)$ and $\Pi_j \epsilon_A(j)$, from a quantity that is less well defined, ϵ_{inter}. The interprotein decay is poorly defined because the distance between proteins is not well established and geometrical changes from reference X-ray structures are not easily predicted.

A surface-to-redox center coupling map is obtained for each protein by calculating the electronic coupling decay, $|\Pi_i \epsilon(i)|^2$, between each surface atom and the redox center. These maps identify regions of the protein that can efficiently couple electron transfer from the redox site. Matching of strongly coupled regions on the two proteins will result in the maximal intermolecular electron transfer coupling (assuming ϵ_{inter} is not too small) and can be used as a criterion for evaluating putative docked structures. This criterion can supplement other criteria (e.g., electrostatic energy) for evaluating the overall functional importance of a docked structure.

Central to this strategy is the definition of protein surface. The main assumption is that an electron must proceed through a surface residue to leave the protein. We define a surface residue as one that is in contact with the molecular surface; the surface traced out as a spherical probe is rolled over the van der Waals surface of the protein. However, unlike the standard method, which uses a 1.4 Å radius spherical probe, we use a 3.0 Å radius probe. This generates a smoother surface and prevents atoms that border invaginations in the protein from being considered surface residues. This strategy is used because residues that line clefts in a protein are unlikely to be in contact with an atom on another protein in a docked structure.

From this analysis we can conclude that by generating surface coupling maps, we can learn a considerable amount about the coupling between proteins, but one still has to deal with the problem of the coupling between their surface residues. The functional docking strategy tells us which surface regions of the proteins one should put together in order to optimize the rate without addressing the question of docking stability. However, the length of the jump between the surface residues will be strongly dependent on the docking configuration, directly influencing the final coupling. Another important point to keep in mind is that all the calculations presented here have been performed for static structures. However, through-space jumps may be sensitive to dynamical motions. Increased attention to the dynamics of these contacts is certainly needed. The recent availability of x-ray crystal structures of docked electron transfer proteins is stimulating further work in this direction (Pelletier and Kraut, 1992; L. Chen *et al.*, 1994; Z. Chen *et al.*, 1994). Other studies are providing additional information about the dynamics of docking and ET as well (McLendon, 1991; Stemp and Hoffman, 1993).

3.2. Exponential and Nonexponential Distance Dependence in Biological ET

An essential feature of pathway analysis is the considerable scatter expected in log(rate) vs. distance plots (Beratan *et al.*, 1991, 1992a,b). This scatter was indeed seen in Ru protein systems. However, in the photosynthetic reaction center (PRC) of *Rps. viridis* (Deisenhofer and Michel, 1989), it was recently observed that scatter (correcting as well as possible for free energy and reorganization energy differences) is less pronounced (Moser *et al.*, 1992) than was found in the Ru-modified proteins.

We have calculated the tunneling pathway strengths in *Rps. viridis* (Fig. 1, Table I). Figure 2 shows a scatter plot of the pathway couplings squared (proportional to the ET rates after correction for free energy and reorganization energy differences (Moser *et al.*, 1992). A number of observations can be made that explain the lack of scatter in the rate data. The pathways possess two key features. First, the paths are "taut"; they fall nearly on a straight line of sight between the donor and acceptor pairs. Second, the early paths consist of limited through-space contacts (Table I). That is, the paths for the early reactions have similar through space-distances in the strongest paths. These paths are homologous to one another.

The later paths in the PRC (between quinones and from quinones back to the special pair) all have

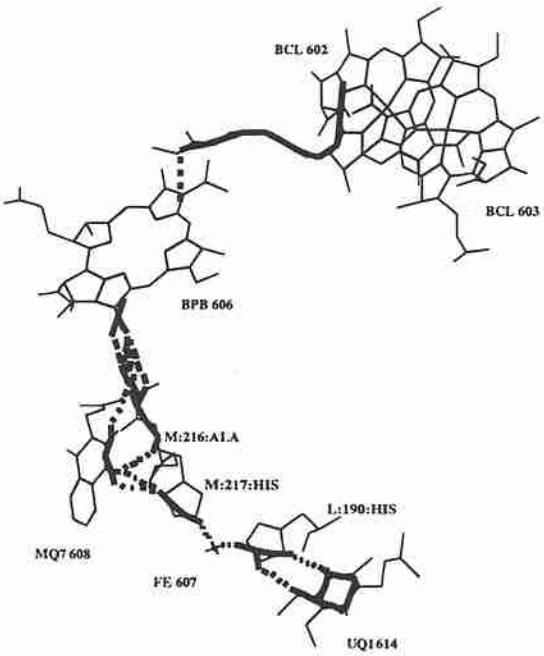

Fig. 1. Tunneling pathways (within 65% of the strongest) in the photosynthetic reaction center of *Rps. viridis* are shown for the forward ET reactions (see Table I).

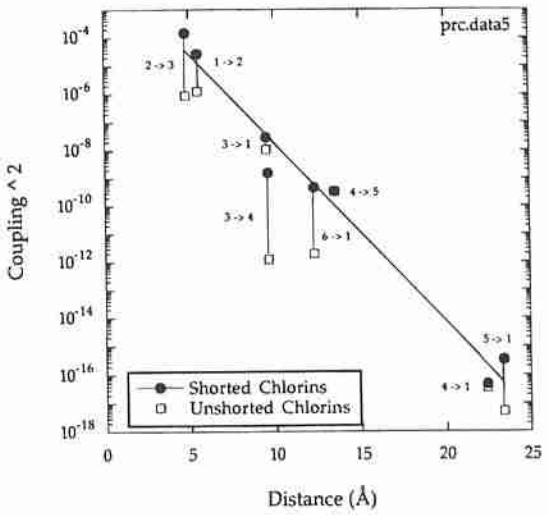

Fig. 2. Scatter plot of pathways couplings squared for charge separation and recombination pathways in the PRC. Solid dots are probably the most relevant calculation, corresponding to no penalty for tunneling across a chlorin ring (ring "shorting"). The best fit exponential to the solid dots is proportional to $e^{-1.47\,R}$, remarkably close to the experimental value (recall that the decay factors in the pathways method were taken to be as realistic as possible, based on model compound data and simple theoretical estimates). Rate nomenclature is given in Table I and in Moser *et al.*, 1992.

considerably longer nonbonded contacts than do the early reactions. The first ET reaction in the PRC competes with radiative decay to the ground state. The early charge shift steps compete with charge recombination. As such, smaller through-space distances in early pathways may be essential to overcome undesirable reactions. Through-space gaps in paths associated with later reactions are considerably larger. Such gaps presumably help to prolong the lifetime of the charge-separated states.

Is the decreased scatter of coupling in the PRC really a result of homologous pathways or related, in part, to the ET chromophores (chlorins and quinones)? A possible mechanism for decreasing scatter would arise from the integration of many pathways (each with a different strengths) by a large donor or acceptor. We have tested the idea of pathway integration in cytochrome *c* by averaging pathways to each surface atom within a region the size of a *second* heme on the protein's surface. We have averaged the absolute values of couplings within 4.6 Å of each surface atom (Fig. 3b) and compared them to the unprocessed coupling data (Fig. 3a). As such, we can determine whether docking a second large redox center would "average out" pathway effects. The degree of scatter in coupling vs. distance plots is not appreciably changed in this simple analysis. More complex analysis (e.g., adding random phase factors to the coupling data prior to summing and squaring) does not seem to decrease scatter either.

3.3. Single Paths, Multiple Paths, and Quantum Interference Between Pathways

The simple pathway product expression [Eq (3)] for electronic coupling suppresses quantum mechanical interference between pathways. A more general way of formulating the ET problem is to calculate the coupling as a function of a bridge "propagator" or Green's function that describes the electron mediation properties of the protein. In this case, the donor–acceptor coupling is

$$\text{(Electronic coupling)} \propto \text{(D to bridge coupling)} \times \text{(bridge propagator) (A to bridge coupling)} \quad (5)$$

Here no perturbation theory or pathway assumptions need to be made. Recently, there has been considerable interest in calculating and analyzing the protein propagator for a variety of Hamiltonians and at various levels

Table I. Tunneling Pathway Couplings in the Photosynthetic Reaction Center

D → A[a]	Coupling	Through-bond steps[b]	Hydrogen bonds	TS distances (Å)	Straight line distance (Å)[c]
1 → 2	5.286 E-03	3	0	3.28	5.5
2 → 3	1.235 E-02	4	0	2.48	4.8
3 → 4	3.999 E-05	7	1	3.44	9.6
4 → 5	1.839 E-05	12	0	7.20	13.5
3 → 1	1.728 E-04	10	0	3.19	9.5
4 → 1	6.911 E-09	17	1	8.63	22.4
5 → 1	1.832 E-08	16	0	8.66	23.4
6 → 1	2.148 E-05	11	1	2.79	12.3

[a] 1 = Special pair, 2 = chlorophyll (L), 3 = pheophytin (L), 4 = quinone A, 5 = quinone B, 6 = cytochrome.
[b] Number of covalent bonds in pathway.
[c] Shortest edge-to-edge distance.

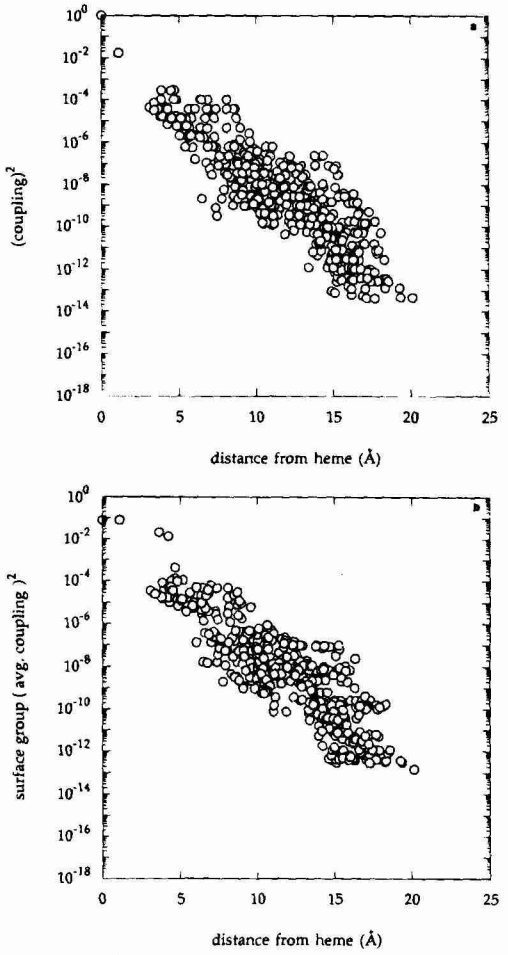

Fig. 3. (a) Standard scatter plot of coupling squared vs distance for cytochrome *c*. (b) Scatter plot of couplings squared for average coupling found within a 4.6 Å radius of each atom on the protein surface. Note that the degree of scatter is *not* decreased compared to (a).

of approximation (Regan *et al.*, 1993, 1995; Skourtis *et al.*, 1994; Gruschus and Kuki, 1993; Siddarth and Marcus, 1993). These methods capture, in principle, all multiple pathway and interference effects

There exists an infinite number of tunneling pathways that can be enumerated (including paths that retrace steps) in any protein. The electronic coupling can be written as a sum over contributions from these paths, and the summation should converge if the donor and acceptor states are sufficiently well localized:

$$(\text{Electronic coupling}) \propto \sum_{\text{paths}} \text{Coupling(path)} \quad (6)$$

Coupling pathways may interfere with one another constructively or destructively because this is a quantum problem. If multiple path effects are important, one anticipates that the secondary and tertiary folded motif, in addition to the strength of the largest coupling pathway, could be of importance in determining the electronic coupling. Recent work (Regan *et al.*, 1993, 1995) shows that interference effects can be divided into two classes and interpreted within the framework of pathway analysis. The first kind of interference arises from orbitals that interact strongly with the bonds on the dominant pathways (nearest neighbors and next nearest neighbors). This collection of paths creates a pathway family or tube that dominates the coupling in many cases. The interference of these similar paths with the main pathway is trivial in the sense that the single pathways can be viewed as simply having scaled parameters to account for these extra interactions. As different model compounds give rise to different β's, different secondary structures give rise to different types of pathway tubes.

The second type of pathway interference arises from interactions between distinct pathway tubes. Such

interactions are the subject of current investigation. Experiments have been performed to measure the rate of ET between the two metals in Ru-modified azurin (Langen *et al.*, 1995). Figure 4 shows a model of a section of azurin, highlighting the region between the copper and a $Ru(bpy)_2(im)$ probe bound to a histidine at position 83 (Day and Rees, 1995). In some experiments, the donor was placed on histidines on the 120's section of the protein chain, with a clear backbone pathway to the copper. This figure shows a contrasting case, where the placement of an acceptor at 83 provides no obviously dominant route between the metals. The intervening β-sheetlike structure, with its hydrogen bonds (shown as dashed lines), is a more complicated bridge than a simple backbone route. The "best path" apparently leaves the copper via 112 and takes a hydrogen bond (112:CYS:SG → 47:ASN:N-H) to get to 47, avoiding the long detour through the length of 46:HIS. It then takes one of two H bonds (48:TRP:N-HN → 84:THR:OG1, 48:TRP:O → 84:THR:N-HN) to get to the 80's section of the chain, to finally enter the HIS at 83, and thus the probe. Additional tubes are made possible by an H bond connecting the 120's chain to the 110's chain (121:MET:O → 112:CYS:N), and a second H bond connecting the 110's chain to the 40's chain (111:PHE:O → 49:VAL:N-HN). These alternative tubes all interfere with one another in the confluence of hydrogen bonds, and sum to give the resulting electronic coupling. The nature of this interference is not obvious; elimination of tubes by blocking certain H bonds in this structure can actually *increase* the coupling.

In addition to the work of Gray and co-workers, many others have provided considerable information about ET pathways in other proteins as well (Jacobs *et al.*, 1991; Sykes, 1991; Farver and Pecht, 1994; 1993; Moreira, 1994). A growing number of model compounds that contain biologically relevant bridging units, including hydrogen bonds and specific secondary motifs, is now being built as well (Anglos *et al.*, 1994; Ogawa *et al.*, 1993; Conrad *et al.*, 1992; Turro, 1993).

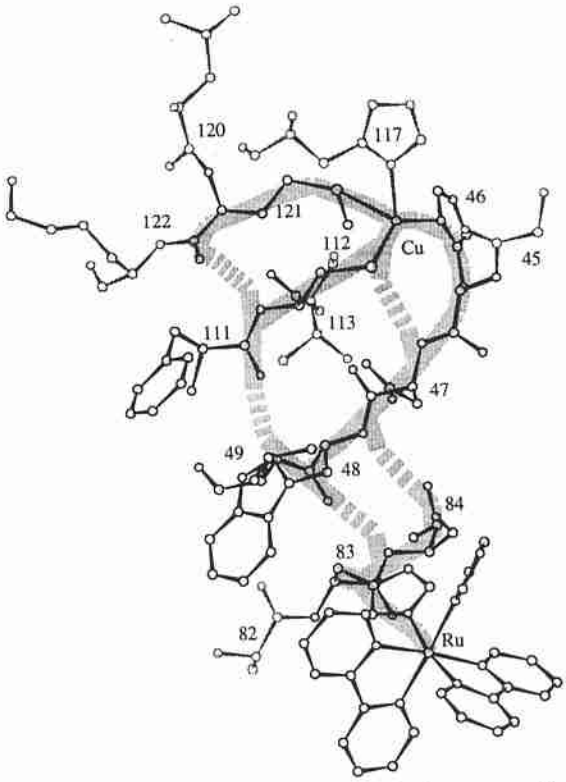

Fig. 4. Multiple pathway tubes traverse a β-sheet to provide the ET coupling from Cu to $Ru(bpy)_2(im)$(His 83) in modified azurin. Hydrogen bonds (dashed lines) are seen to play a crucial role in this coupling (Regan *et al.*, 1995).

4. TUNNELING ENERGIES AND THE TWO-LEVEL APPROXIMATION

A key assumption of nearly all ET rate theories is that the donor–protein–acceptor complex can be approximated as a two-level system (Larsson, 1981). That is, one assumes that the electron is well localized near the donor (acceptor) before (after) the electron transfer reaction. The validity of this approximation depends upon the size of the donor–acceptor interaction relative to the energy gap between donor/acceptor and mediating protein states (Skourtis and Onuchic, 1993; Skourtis *et al.*, 1993).

In the protein ET problem the donor–acceptor interaction is much smaller than the energy gap (ratio of about 10^{-6}), and the two-state approximation is valid. For the primary charge separation reaction in the PRC (and for some small molecule ET compounds) intermediate bridge states may be strongly coupled to donor and acceptor, rendering this approximation invalid. In such cases, it is still possible to describe the ET process in terms of pathways (Hu and Mukamel, 1989; Skourtis and Mukamel, 1995). It is, however, necessary to distinguish between pathways that visit the population of the intermediate bridge states (sequential mechanism) from pathways that do not (superexchange mechanism).

5. QUANTUM CHEMICAL CALCULATIONS OF ELECTRON TUNNELING IN PROTEINS

Electronic coupling can, in principle, be calculated by solving the Schrödinger equation for a molecular electronic wave function of the donor, acceptor, and intervening protein system using techniques of quantum chemistry. While accurate *ab initio* methods (Szabo, 1989) have been rather successfully applied to study electronic coupling in small model compounds (Jordan and Paddon-Row, 1992; Shephard *et al.*, 1993; Curtiss *et al.* 1993; Newton, 1991), it is not possible currently to implement these methods for proteins because the required computational time grows very rapidly with the size of the system.

Existing semiempirical quantum chemical methods (like extended Hückel) technically permit the study of systems with several hundreds of atoms, and have been applied to calculate electronic couplings in proteins (Broo and Larsson, 1991; Siddarth and Marcus, 1993; Regan *et al.*, 1993). These simple methods do involve some adjustable parameters which were originally set to reproduce heats of formation and optical transition energies of chemical compounds (ground and excited electronic state properties of the neutral systems).

The electronic propagator mentioned in Section 3.3 [Eq (5)] describes the coupling mediated by the protein. This propagator depends on properties of electronic states of the system with one extra electron added (N + 1 electron states), one electron removed (N − 1 electron states), and on the transitions energies between the ground (N electron) state and the N + 1 and N − 1 electron states (i.e., electron affinities and ionization potentials, respectively). Thus, we expect that standard semiempirical methods will be of limited use for proteins and entirely new parameter sets must be developed that properly reproduce these quantities. One attempt of this kind was reported by Gruschus and Kuki (Gruschus and Kuki, 1993). The authors developed a simple Hamiltonian for electronic coupling in proteins based upon tailored site energies that neglected some of the chemical features of the amino acids but reproduced experimental ionization potentials for amino acids and built the intersite interactions to be consistent with *ab initio* calculations on model compounds.

We are currently parametrizing an extended Hückel-like Hamiltonian to study ET in proteins based upon accurate *ab initio* calculations of the electron propagator matrices of amino acids. This new method works well on model ET compounds, and we hope that it will provide an improved quantitative description of ET coupling in proteins (Kurnikov and Beratan, 1995).

6. CONCLUSIONS

In summary, theory and experiment are in agreement that protein structure can be used for gross (distance) and fine (pathway) control of ET rates. In some proteins, the anisotropy of the paths emanating from a redox center is very large indeed. However, in specific redox reactions, the anisotropy may be less apparent because pathways between centers sample very special subregions of the protein. This may be the case in the photosynthetic reaction center. The tunneling pathway model succeeds because it correctly captures the distinct difference between electron propagation through bond (including hydrogen bonds) and through space. ET rates in Ru-labeled cytochromes *c* that are inconsistent with 1DSB models are understood in the context of the pathway model. Quantum interference between pathways is the subject of current theoretical (Regan *et al.*, 1995; Skourtis *et al.*, 1995) and experimental investigation, and these studies should lead to a deeper understanding of protein-mediated redox chemistry. Current research is also progressing toward more reliable Hamiltonians, and that should allow one to address detailed chemical questions about energetics, symmetry, and bonding in the mediation of long-range electron transport (Regan *et al.*, 1995; Kurnikov and Beratan, 1995). Stimulated by recent experiments, theoretical studies are now being aimed at ET in docked interprotein complexes as well.

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