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Electron Transfer and Proton Coupling in Proteins

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It is shown that electron transfer in proteins is not very dependent on protein structure but is considerably aided by donor/acceptor group orientations. The value of structure within proteins is very largely due to overall atomic packing, and since proteins generally are equally packed, the separation distance of donors and acceptors is usually the dominant term. The need to describe structural features in detail is, however, essential when electron/proton coupled motions are involved. The question as to the importance of small conformational oscillations and changes is examined. © 1999 Academic Press

Key Words: **electron transfer; proton transfer; proteins; Marcus equation; relaxation energy; transmission coefficient; water in proteins; bio-energetics.**

This article is dedicated to Professor P. Day on the occasion of his retirement from the directorship of The Royal Institution London. Professor Day was an undergraduate with me at Wadham College, Oxford, and then did his D.Phil. and some subsequent research also with me at Oxford. He was always an outstanding scholar and experimentalist, as shown in his work published on various aspects of spectroscopy and electron transfer in such substances as mixed valent compounds. Professor Day has gone on to analyze these and many other systems involving both magnetic and electronic properties in a most sophisticated manner. The analysis I now give of electron transfer in proteins owes much to our earlier experiments and discussions.

Electron transfer reactions are of considerable importance in the oxidation–reduction reactions of a large family of enzymes. Perhaps the most important of these reactions are in the electron transfer chains of energy transduction systems in mitochondria and chloroplasts, where electron transfer is coupled to proton transfer (1). The structures of some of these proteins are now known, and their electron transfer centers are very well described. In particular Dutton and co-workers (2) have been concerned with the basic nature of electron transfer in the reaction center of photosynthesis, while others, especially Gray *et al.* (3), have been examining electron transfer in simpler copper and heme–iron proteins with ruthenium complexes attached. Summaries of this and other research by other authors are given in a recent publication (4). It would be of great advantage if these basic electron transfer steps could be understood separately from all chemical redox reactions involving atom motions within the proteins of energy capture devices. In this paper I shall analyze electron transfer itself at first before turning to coupling to proton movements. I consider that electron transfer in these systems is understood in essence, though much detailed refinement is lacking.

First we elucidate the way electrons are able to be transferred over "distances" ranging from 5 to 25–30 Å where 25–30 Å appears to be the limiting distance in these protein systems for a rate of one per second. The interpretation of all the results has been in terms of the Marcus equation [1],

$$k_{ET} = \frac{2\pi^2}{h\sqrt{\pi\lambda kT}} \cdot H_{AD}^2 \exp\left(-\frac{(\Delta G^\circ + \lambda)^2}{4\lambda kT}\right), \qquad [1]$$

where ΔG° is the redox potential difference between donor, D, and acceptor, A, λ is the total reorganization energy, and H_{AD}^2 is the tunneling transmission coefficient, which is proportional to e^{-R}.

This temperature independent part of the equation is often written as

$$2.303 \log k_{ET} = \beta\left(\frac{1}{R}\right) \qquad [2]$$

when corrected to $\Delta G^\circ + \lambda =$ zero and where R is the "distance" between D and A which will need to be precisely defined. β is presumed to be a constant characterizing the medium between D and A.

In the literature above (2–4) $\log k_{ET}$ has been determined for some 40–50 examples, but unfortunately there appears to be a conflict between two major groups, Dutton (2) and Gray (3), as to the value of β. This apparent disagreement has carried over into a difference in approach where Dutton describes the protein space between donor and accept by a bulk constant, density of electrons, while Gray describes

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the protein space by a pathway along bonds. The models have been adjusted from time to time. Other authors, including those originally responsible for the pathway description (5), now analyze donor/acceptor distances in terms of multiple pathways (6). The purpose of this paper is primarily to clarify the experimental analysis of the electron transfer process in proteins so as to remove confusion as to the dependence of $\log k_{ET}$ on R and then to ask why some proteins may have special additional properties not included in the description above, such as coupling of electron transfer to proton transfer. The analysis starts from an examination of the donor/acceptor centers in proteins.

REDOX DONOR/ACCEPTOR CENTERS

The redox centers, called the donor (D) and acceptor (A), have three experimentally determined properties: (a) a redox potential difference, ΔG°, (b) an extension in liganded space which is directional due to the stereochemistry of their molecular structure, see Fig. 1, and which affects determination of β, see below, and (c) *local* reorganizational energies, λ_D and λ_A. The design of the centers often appears to meet optimal conditions for electron transfer and usually depends only on their ground state electronic properties within the proteins. Without giving detailed analysis it is obvious that the redox potential of any group can be adjusted by synthesizing an appropriate molecular framework around it, for example, the ligands for a metal site, and then further modulating the properties of the complex by situating it in a particular fixed protein surrounding. This is clearly seen in metal centers of, for example, blue copper and heme proteins (7). Recently Gray *et al.* (8) described the sites as "constrained" by the protein to generate an optimal redox potential of donor and acceptor for function. This design element for optimal values of ΔG° is not so obvious for purely organic donors and acceptors. We examine next the characters of the donor and acceptor to see how they can affect the direction of electron transfer (Table 1). It is immediately clear that all the metal centers of electron transfer have strong internal electron coupling, often in unsaturated covalent binding, over at least one and often over a considerable number of ligand atoms. Some come from a molecular unit inserted in the protein, e.g., porphyrin, and some from the protein amino acids themselves, e.g., histidine, imidazole, or cysteine thiolate (Table 1). Purely organic donors and acceptors obviously also have a considerable spatial distribution of their relevant molecular orbitals. The orbital of the electron to be transferred, from the donor, and the orbital of the acceptor clearly occupy considerable space and can be directionally oriented, which must affect the analysis of the distance between them, see below. Although the anisotropy of these orbitals is clear, its extent is quite difficult to quantify and orbital symmetry matching between donor and acceptor is also important. By way of contrast the protein consists of a variety of bond types, most of which are saturated. It is therefore essential that the distance over which an electron is transferred should be divided between the structures of the D and A centers and the protein matrix itself, Fig. 1. Third, the very fact that electron density is not highly localized on single atoms but distributed over the molecular centers also reduces the reorganization energies, λ_A and λ_D, locally, and their values will differ from those of the protein itself. There is little or no conformational change on change of oxidation state of the D and A centers either due to the rigidity of the molecular

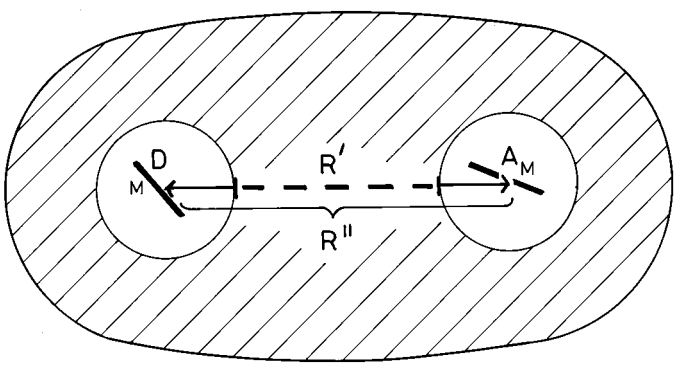

FIG. 1. A schematic representation of the relationship between donors (D) and acceptors (A) in a medium where the medium can be a linker plus a solvent or a protein plus water. There are zones around D and A which are different from the more remote regions. The zones can be ligands of a metal (M) or differentially polarized parts of the medium. The distances between D and A centers are not always long enough, >10.0 Å, in electron transfer experiments for the donors and acceptors to have no effect on the central region. Note that the shapes of D and A are not spherical in practice so that in each and every electron transfer experiment, the route, represented by a distance R, of electron transfer varies not only in the central region but also around both donor and acceptor (see text).

TABLE 1
Metal Coordination Spheres

Protein	Coordinated ligand
Cytochrome *c* (Fe)	Mesoporphyrin cross-linked to sulfur of protein Histidine, methionine
Myoglobin[a] (Fe)	Protoporphyrin, heme, histidine Fe (III) (only) water
Cytochrome b_{562}[a] (Fe)	Protoporphyrin, histidine (2)
Chlorophyll (Mg)	Chlorin, histidine
Azurin[a] (Cu)	Thiolate, histidine (2)
Note also	
Naphthoquinones	Two conjugated rings
Phaeophytin	Four conjugated rings
Chlorophyll dimer	See above

[a] Ruthenium sites have been incorporated with dipyridyl, imidazole, and histidine as ligands.

group or to the way in which the protein holds the center in a rigid clamp, whence the local reorganizational energy is minimized (Gray *et al.* (8)). We must therefore divide λ into a local λ_i and a medium λ_0 where λ in Eq. [1] is $\lambda_i + \lambda_0$ and $\lambda_i = \lambda_D$ or λ_A. The overall effect of the protein and the structure of the molecular unit may well be to optimize the overall structure for function, i.e., ΔG°, local λ_i for D and A, and orientation of D and A may all be selected.

It is easy to measure precisely the redox potentials of all the local sites, and this has been done. We shall not refer to these data in this paper. It is not so easy to quantify the reorganization energies of the sites but very good consistent values have been obtained by several methods (2, 8). They lie inside the range 100–200 millivolts (see Table 2). The value for isolated ruthenium complexes is similarly small (9) but in contrast that for hydrated high-spin ions such as Fe^{2+} and Fe^{3+} is large, >1 volt. (Ions such as these, which are expected to change structure either in bond length or bond angle with redox state, are very rarely if ever used in electron transfer reactions in biological systems.) In passing it is worth noting that some centers contain two or more metals and are of mixed valence. Some fall in different classes of mixed valence as described by Day. Examples of exchange-averaged valence are found in Fe_4S_4 and Cu_2 centers while some Fe_2S_2 and Fe_3S_4 centers have identifiable metal atoms of different valence. All such systems add to the complication of deciding upon the description of the distance between donor and acceptor "centers," see below.

We shall now turn our attention to the examination of the proteins which form the matrix between the donors and acceptors, since we would like to know if in evolution special transfer modes have been selected.

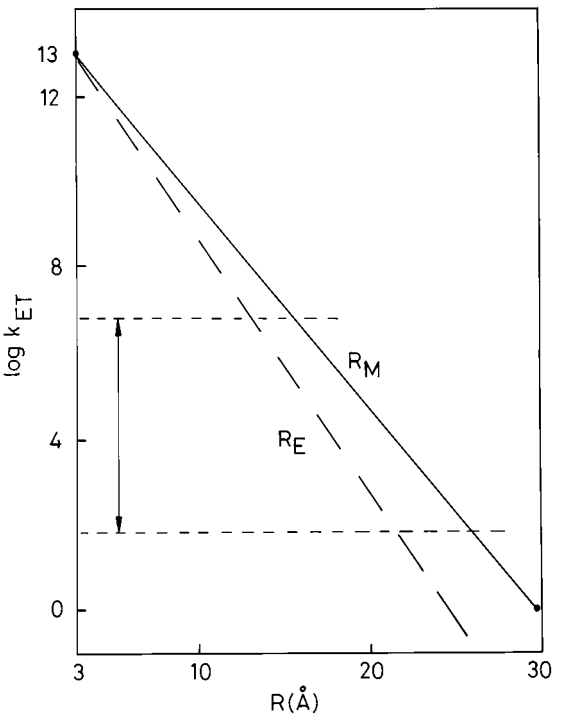

FIG. 2. The effect on the slope of a plot of $\log_{10}$ (electron transfer rates) versus distance where the distance is defined either by measuring the edge to edge, $R_E(R')$, or metal center to metal center, $R_M(R'')$, distances between donors (D) and acceptors (A).

PROTEINS

The proteins which have been examined are grouped here into three classes: (a) the reaction center (2), myoglobin and cytochrome b_{562} (10), (b) the blue copper protein, azurin (3), and (c) cytochrome *c* (3). To a good approximation the proteins in the first group are α-helical, in the second the copper protein is a β-sheet in the form of a barrel, and in the last protein the part of the protein which will concern us is structured in a not too obvious fashion although much of the rest of cytochrome *c* is helical, see Fig. 3. Measurements of protein mobility show that the β-sheet is the most rigid framework, the α-helices of cytochrome b_{562}, myoglobin, and possibly parts of the reaction center are only somewhat less rigid. They change structure only very slightly on oxidation state change. In cytochrome *c* one or two regions in and around the nonhelical parts, near the heme, are quite mobile while the helical part of cytochrome *c* is relatively rigid due in part to the cross-linking to the heme. Thus the part of this protein that concerns us (see Fig. 3) is in fact not very rigid and changes with oxidation state (11). This information on structure and mobility will be shown to be relevant to the analysis of electron transfer and to proton coupling to electron transfer within the protein matrices. The proteins bind all the groups in Table 1 internally so that they are free from solvent with the exception of the heme of myoglobin and the ruthenium complexes. Between all the centers listed in Table 1 there are strands of protein matrix through which the electron must pass. We shall characterize it by experimentally determined β_0 and λ_0 values, see Fig. 1. Note that this manner of space-filling is irregular and nonuniform. In

TABLE 2
Reorganization Energies of Groups (millivolts)[a]

Group	Reorganization energy (λ)	
	(a) In water	(b) In proteins
Heme iron	~200	<150
Copper complexes	>1000	<200
Ruthenium complexes	~150	<150
Chlorins, quinones, phaeophytins	Small?	<150
Iron aquated ions	>1000	

[a] Data from references in Refs. (2), (3), and (8).

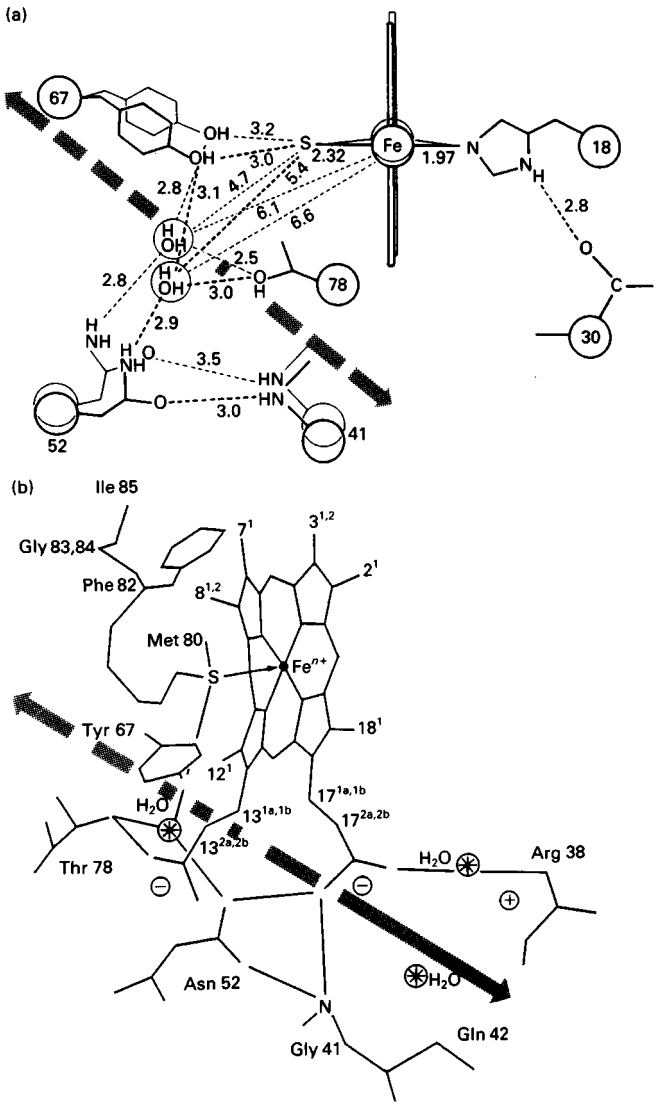

FIG. 3. A possible pathway of proton transfer in cytochrome *c*. Note that H_2O and propionate/arginine side chains as well as NH and –OH of several centers can be involved. The pathway is different in the two oxidation states, illustrating gating. It is very similar to the pathway proposed for the case of proton pumping in cytochrome oxidase (27–30).

some other proteins no strands pass between electron transfer centers and we refer to them later.

ELECTRON TRANSFER RATE, Log k_{ET}, IN PROTEINS

All the data we shall use, with but one or two exceptions, have been obtained by the two groups referred to above (2–4, 10; see also 12). Note that $\log k_{ET}$ (rate of optimal electron transfer where $\Delta G^\circ = \lambda$) to be discussed is related to the transmission coefficient H_{AD}^2, as in Eq. [2]. As is conventional the authors have therefore plotted $\log k_{ET}$ against distance (R) between the electron transfer centers. However they have chosen different ways of determining the distance (see Fig. 1). Dutton *et al.* (2) plot the edge to edge distance (D to A) minus 3.6, here called R', against $\log k_{ET}$ and obtain a good straight line of slope, β_0, in the Marcus equation (here β_0 is assumed to relate to protein only) of 1.4 Å^{-1} and an intercept deliberately plotted through the expected $\log k_{ET} = 13$ at $R' = 0$ (Fig. 2). The intercept is for van der Waals contact distance at the edges of D and A. Such a plot apparently excludes any effect of the donor/acceptor or their structures on β so long as their "edges" have been chosen correctly. The difficulty clearly lies in the choice of the edge of D and A since for a heme group it might be thought that the metal itself was the edge or that porphyrin alone or other ligand groups gave proper edges. Note that the reaction center is very largely a helical protein. Gray *et al.* (3, 10) plot from the center of each group, always a metal, a distance now called here R'' (D to A). This distance is very well defined. For both the β-sheet protein (azurin) and the α-helical protein (cytochrome b_{562}) there is one good straight line through the data using the intercept for $\log k_{ET} = 13$ at $R'' = 3.0$ Å. Here $R = 3.0$ Å is the van der Waals contact for two metal ions. They obtain a value of β (not β_0) of 1.05 ± 0.05 Å^{-1} for several proteins (Fig. 2). It is clear that such β-values do not depend greatly on whether the protein is helical or is a β-sheet. It is necessary, however, to consider how the metal ligands and their extension in space affect β, see below, since β does not relate directly to β_0 for the protein but to a sum of effects including D and A extension. Now we can choose to replot the data given by Gray *et al.* using the Dutton edge to "edge" distances, i.e., not including the immediate ligands of the metals when the value of β_0 approaches the value given by Dutton *et al.* for the helical reaction center protein (see 12). We then readily explain the value of $\beta = 1.00 \pm 0.05$ Å^{-1} for center to center (metal to metal) distances, R'', by considering the sum of electron transfer over the conjugated path of the metal chelate plus that of the transfer over the protein itself. Taking as an example $\frac{1}{4}$ of the distance to be over a conjugated path with $\beta_i = 0.2$ and $\frac{3}{4}$ of the distance as protein with $\beta_0 = 1.4$, we would have the following equations for the metal to metal condition:

$$\beta = \tfrac{1}{4}\beta_i + \tfrac{3}{4}\beta_0,$$

$$\beta = \tfrac{1}{4}(0.2) + \tfrac{3}{4}(1.4) = 1.10.$$

(Dutton's procedure effectively puts $\beta_i = 0.0$ and shortens R' by around 3–5 Å). Obviously the division of space used to give the β value is critical to the discussion. Taking all the different proteins into account we consider that there may not be a unique value for β_0 for all proteins but it lies in the range $\beta_0 \sim 1.25 \pm 0.15$ Å^{-1} which characterizes the vectors in a protein though we note that β_0 may vary a little from protein to protein, and inside individual proteins. This is

confirmed by studies of other proteins and suggests that either strictly limited paths through bonds (a chemical model (3)) and general media (a physics model (2)) will give quite good matching between theory (see below) and experiment. (Note immediately that all theories will involve some approximations or adjustable parameters.)

In the discussion above, we have so far excluded the data on myoglobin and cytochrome *c* since they do not generate information that gives good straight lines between $\log k_{ET}$ and R, no matter how R is chosen. The wide scatter in the data could be due to (i) the looseness of the proteins giving rise to uncertainty in R (we consider larger nuclear motion later), or (ii) the inhomogeneity of their folds (note: it is known from model studies, see below, that k_{ET} is somewhat solvent dependent), or (iii) the directional character of porphyrin in the protein. The other proteins, azurin, the reaction center and cytochrome b_{562} are more homogeneous in all these respects. Apart from these caveats the cytochrome *c* and myoglobin data scatter around the plots of $\log k_{ET}$ against R (consistently defined) for the other proteins reasonably well so that we do not need to alter our assumption that, to a good approximation, and once the structures of the donor and acceptor have been taken into account *the protein matrix whether a β-sheet, an α-helix or even any other well structured secondary structure, does not influence greatly* k_{ET} *in the range* $R = 5$ to 20 Å *taking* $\beta_0 = 1.25 \pm 0.15$ Å^{-1}. We shall also assume that this is true of the protein sidechains since the directional vector R crosses all kinds of groups inside the proteins. We note too that while the interior of proteins is largely hydrophobic, it often includes some charged residues and water which seemingly do not greatly affect $\log k_{ET}$ either. A more extensive discussion of β will be given elsewhere (13). If, for the moment, we liken the interior of the protein to a partially frozen half-structured solvent, an ill-determined medium surrounding the distance vector, in Fig. 1, then direct comparison with studies of β in model systems and the theories which have been developed for them can be analyzed. First we turn to the experimental value of λ_0, the re-organization energy of the protein.

VALUE OF λ_0 IN PROTEINS

The value of the total re-organization energy λ has been determined in the above proteins for electron transfer between different groups and at very different distances (Table 3). A value of 750 ± 100 millivolts is found at all distances (center to center) greater than 10 Å. The value of λ at shorter distances where little or no protein strand intervenes may fall toward 200 millivolts which would be readily explained by the required relaxation energy of the donor or acceptor sites themselves, i.e., $\lambda_i < 200$ millivolts (Table 2). There is no doubt therefore that the λ_0 for the protein electron transfer at longer range has a value of approximately 500 millivolts. It is this value we must explain. Myoglobin may have a higher value of overall λ due to the water at the heme center. The analysis and discussion of λ will be put on a firm basis in a paper by Dutton *et al.* (13) and see (8). Later in this paper we shall be concerned about this rather large value of λ_0.

TABLE 3
Reorganization Energies (λ in millivolts) of Protein Centers

Centers D, A	Local group λ	Overall λ	Protein λ	Ref.
Ru, hem	~200	700	~500	9, 12
Ru, Cu	~200	700	~500	8
Q_A, Q_B				
Chl_2,[a] Pheo	Very small	> ~500	~500?	2
BPL^-, Q_A		~700	~500	2
Q_A^-, $BChl_2^+$	Small	700	~500	2

[a] Excited state.

ELECTRON TRANSFER RATES IN MODEL COMPOUNDS, SOLVENTS, AND MONOLAYER FILMS, AND H_{AD}^2

Electron transfer rates have been carefully studied in many model systems using rigid linkers between large conjugated donors and acceptors, see data and summaries in Refs. (14–17). We consider the use of model systems in solvents (see Fig. 1) before we consider those involving films. The linkers prepared are of different length so allowing a connection between $\log k_{ET}$ and R. These systems have an extra variable the chosen solvent. The same division of overall parameters are of interest to us, namely β and λ, which reflect the linker plus the solvent proportion of the system, λ_M, β_M (M stands for medium + linker) as well as the properties of donors and acceptors, β_A, β_D and λ_A, λ_D, already discussed. The distances, R''', measured here are usually from the centers of atoms *at the edges of the donor and acceptor*. A contact correction of about 3 Å is usually applied to R''' to allow for the structures of the groups directly joining linkers to the donor and acceptor. The data give rather poor straight lines $\beta_M = 1.0 \pm 0.15$ Å^{-1} which are somewhat solvent dependent and somewhat curiously the data do not extrapolate to $\log k_{ET} = 13$ at contact (15–17). The suggestion has been made that the solvent structure changes at short distances of separation of donor and acceptor (see Fig. 1), so that $\log k_{ET}$ is not just linearly dependent on R. (This problem also appears in the study of proteins which do not seem to be "homogeneous" at very short distances, see below.) This value of β_M can be compared directly with that for proteins, β_0.

It is also possible to study electron transfer between a donor organic molecule on the end of close-packed hydrocarbon chains which form a film attached to a metal

surface. The chains can be simple saturated fatty alkyls $-(CH_2)_n-$ or fluorinated chains $-(CF_2)_n-$. Values of β are for $-(CH_2)_n-$ chains 1.0 ± 0.2 Å^{-1} (18) and for $-(CF_2)_n-$ chains, 2.2 Å^{-1} (19).

Now the description of Fig. 1 is simplistic in that it assumes that the best electron transfer distance between D and A is along a straight line which could match a linear rigid linker taken to be the path of the electron. By making curved rigid linkers the distance through linker is longer than that through the solvent. Experiments now show that the rate of electron transfer using curved linkers can only be accounted for if it is assumed that the shorter pathway through solvent is in fact used (14). Again a value of β through the bridge of *saturated* bonds or through solvent is approximately 1.0 ± 0.15 Å^{-1}, where the spread of data is not different from that in proteins.

REORGANIZATION ENERGY, λ, IN MODEL SYSTEMS

Where the studies of electron transfer have been carried out in liquid solvents the value of λ is found to be in excess of 1.0 volt, much higher than in proteins (17). There is however some dependence of λ on the solvent. This λ includes relaxation of the donor and acceptor groups as well as of the solvent (Table 4). However, given the choices of D and A, it is very likely that the internal λ_i values are less than 200 millivolts, see above, so that λ_M (for the solvent, M = medium including the rigid connecting links from donor to acceptor) is greater than 1.0 volt. When measurements are made in frozen solutions λ_M (overall) drops to the value of around 600 millivolts (18), and similar values are found in condensed films attached to electrodes (19). The reason for the low value of λ_M in a frozen matrix is again the absence of the part of the dielectric constant dependent upon reorientation of solvent molecular dipoles. The value of 600 millivolts is close to the value observed for protein systems. The simplest theories of λ_M using a bulk dielectric model, as mentioned below and calculated in relation to energy requirements using the optical dielectric constant for charge changes on the donor or acceptor complex, then give reasonable agreement for λ_M.

THEORETICAL APPROACHES

There are two kinds of theoretical approaches using either bulk dielectrics (20) or using basic atomic orbitals (21). We shall follow Newton (21) in his theoretical examination of simplified model systems, where he considers the molecular orbitals of *ordered* atomic assemblies. The work leads to the conclusion that when chains of atoms, C or O, are placed between a donor and an acceptor in a fixed array, and treating the problem as one of electron superexchange, then it is of little consequence if the atoms are joined by saturated covalent bonds, hydrogen bonds (pathways) or are not linked provided their occupation of space is not altered, see also Ref. (28). Newton concluded that the value of β is around 1.0 Å^{-1} for units such as $(CH_2)_n$, $(CH_4)_n$, $(H_2O)_n$, and so on, provided that they are equally close-packed. The essence of the description is that electron density is an important criterium. It is then a matter of defining how many such atoms, which lie in the space between donor and acceptor, are approximately of equal value, and whether or not the symmetries of atom orbitals contribute optimally to the possibility of electron transfer. A guide to the first factor is the ionization energies of small molecules made from H, C, N, and O. In fact there is little difference in most combinations of C, N, O atoms, while perfluorinated compounds give values and larger β (19). These observations make the problem of describing both model systems and proteins in simple bulk terms somewhat easier. Interestingly the value of the ionization potential is lower in solid water with stronger H bonds than for gaseous water (22) suggesting that structuring the medium, not using covalent bonds, could be important.

TABLE 4
Reorganization Energy in Model Compounds

Model				λ (millivolts)			
Donor	Acceptor	Linker	Solvent	Total	Complex	Solvent	Ref.
4-Biphenyl	π-Aromatic	Steroid	MTH[a]	1220			15, 17
			Isooctane	900			
Porphyrin	π-Aromatic	Tryptycene	MTH	1200	300	900	16
			MTH (frozen)	~600	300	300	16
			Toluene	~900			16
Ir complex	Pyrazole	Phosphinate	Acetonitrile	1060	200	860	35
Porphyrin	Quinone	Cyclooctyl	Acetonitrile	1310	300	1010	15, 36
			Benzene	1010	300	710	

[a] MTH is 2-methyltetrahydrofuran.

It is an interesting feature of proteins that they all have similar densities not very different from that of water. On this ground alone we might well expect that the precise fold of the protein was immaterial, and in this case we might expect that a dielectric field model would give as good results as any. However, we have observed that β_0 for proteins averages around 1.25 $Å^{-1}$ whereas for the model systems using covalent linkers of similar composition the value is close to 1.0 $Å^{-1}$ both by experiment and theory. The possibility arises that the local density close to the trajectory of the electron between donor and acceptor in a protein is less than for the best close packing of atoms as in a covalent linker and that locally the electron density is variable. Inspection of proteins generally makes this more obvious. The implication is that proteins would make the best electron transfer routes along well-directed bonded systems (covalent or H bond), i.e., the densest packing, provided that the donor/acceptor groups are also bonded into the route and that the route is as close as possible to a straight line connection between the two (21). Of course H bonds bring N and O atoms into close electronic contact so that these paths are to be taken into account. However, H bonds in proteins are not easily defined and vary from place to place. Now, unfortunately, for this best route scenario the proteins provide only much elongated sequential C, N, O path between donors and acceptors relative to a direct vector R (Fig. 1). This makes the inclusion of hops between chains, via less densely packed regions, less disadvantageous. Thus we can see that an empirical treatment of the protein between centers in terms of a crudely averaged electron density or a treatment of multipaths through bonds, H bonds, and some jumps between chains are really different descriptions, one bulk the other an atomic sum, of the same blurred path. Moreover we should not expect β_0 to be an exactly fixed constant for all the vectors R, even within one protein. An example where R is very small illustrates the problem.

The protein, *Clostridium pasteurianum* ferredoxin, has two Fe_4S_4 clusters close together, and the shortest edge to edge distance is no more than 7 Å (23). The space around the direct vector and between the edges does not contain many C, N, and O atoms but mostly H atoms. The observed electron transfer rate is some 10^3 to 10^4 orders of magnitude lower than expected judged by proteins with a denser packing. We could say that

(i) the value of β is very high, ~ 1.6 $Å^{-1}$, due to the low electron density (2), or that

(ii) the electron is forced to use a lengthy indirect route through bond connection (4, 5)

A similar problem appears to arise in cytochrome oxidase (24).

A somewhat disappointing conclusion is that very much experimental work seems to indicate that the protein over a distance R of around 10–20 Å is roughly a homogeneous medium and that the only considerable advantage to be gained for electron transfer is the choice of metal ligands and their directional character. The conclusion is reinforced when we observe that we know of no example in which real protein chains used by organisms can certainly be described as a molecular wire. A very different approach has to be present for proton transfer which in bioenergetic devices is coupled to electron transfer.

PROTON TRANSFER

This is not the place to review proton transfer in general. We note that it requires translational hops of H^+ from nonmetal to nonmetal centers and can be long range through a Grötthus-type H-bond rearrangement mechanism. Involved hops can only be proton tunnelling of less than 1.0 Å. Thus a long network of H-bonded structures is required to move a proton cooperatively over distances commensurate with membrane thicknesses (20–30 Å). Moreover to maintain proton flow the central atoms of O– or N–bonded acid/base centers must rotate to realign for subsequent transfer after the first proton transfer (25). To be able to analyse the pathway of protons and the making and breaking of such a pathway a protein structure must have a structure determined to about 1.5 to 1.0 Å resolution. No such protein structures, known to be involved in long-range protein travel, have been resolved below 2.0 Å. Hence we must use model proteins to appreciate possibilities. Fortunately such work has been done both by X-ray crystallography and NMR analyses of one protein: cytochrome *c* (11).

The studies of cytochrome *c*, a protein which has helices and some looser structural units, but no β-sheets shows that the change of redox state Fe(II) to Fe(III) involves a considerable rearrangement of hydrogen bonds along a distance vector of some 15 Å (11) (Fig. 3). The motion includes many small atomic movements and even lateral motion of one or two water molecules by 1.0 Å. The H/D exchange rate between an H bond to N or O even in the center of the protein is $100\times$ faster in the oxidized state (11). We can make two safe conclusions.

(i) The H-bond network in helical redox active proteins is likely to be oxidation state dependent.

(ii) Small rearrangements of groups within these proteins of the order of 1.0 Å can be expected at considerable distances from the redox active centers on change of oxidation state.

In studies of other small cytochromes we also found in several cases that similar proteins to cytochrome *c* showed changes of acid dissociation constants of up to 2–3 units in pK_a on change of redox state. One important group of residues was the propionates of the porphyrins (26).

We conclude that there is no difficulty in conceiving a gated and energized long-range proton pathway in helical

redox proteins (25, 27). Gating arises of course from the breaking of one H-bond path and the making of a disconnected second one with redox state. Where the proton flow is anisotropic then the switches in pK_a represent energy storage not at the redox center but by localised protons in the membrane (1). Notice that if these acid/base groups are not in free exchange with the aqueous solution this is the storage of proton energy localized in a membrane (1) before a chemiosmotic steady state develops.

Now all the above features of the model proteins are present in the electron transfer chains of energy capture devices in biological membranes. These proteins are invariably helical. There are now known to be several isolated water molecules and possible molecular channels of H-bonds in both the reaction center and in the oxidases as well as in ATP-synthetase (28–30). The heme groups carry propionic acid side-chains. Redox state changes are known to alter metal centers in oxidases by 0.5 to 1.0 Å and larger changes are seen in the positions of some lighter elements (28, 29). The exact route of protons in these matrices may, however, be impossible to discover since we cannot be sure that the H-bond networks will be seen by structural studies.

All of these observations leave two possible explanations for redox (electron)/proton couplings. The simplest is the indirect coupling in which electron flow precedes proton flow or vice versa (28–30). We may call this thermodynamic coupling. An alternative intriguing possibility we wish to propose here is that electron and proton movements are directly kinetically coupled. Here a vibrational mode involving the cooperative movement within a set of H-bonds could simultaneously allow the initiation of proton flow while generating new values of ΔG and λ in Eq. [1] so bringing about electron flow. The equation is not then directly applicable since the Franck–Condon principle is jeopardized. To separate these two coupling modes will require much detailed kinetic and structural analysis and we cannot expect definitive knowledge for some time. In conclusion we point to the recent development of theoretical models involving vibrational couplings to electron transfer (31–35), including circumstances in which the Franck–Condon assumption fails, and to the possible pathways of protons in cytochrome oxidase as indicated by site-directed mutagenesis (30). Are electron transfer and proton transfer mutually coupled to helix–helix relaxation?

This paper has been written after very extensive discussion with Professors L. Dutton and H. B. Gray and is largely a joint effort. The three of us will describe in more technical detail our conclusions in a forthcoming paper.

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