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Resonance effects in strongly exothermic long-range electron transfer and their possible implications for the behaviour of site-directed mutant proteins

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Long-range electron transfer investigations of hemoproteins, blue copper and iron-sulphur proteins frequently rest on electronically excited metal centres. When the excitation energy approaches the oxidation or reduction potentials of intermediate residues the superexchange view normally used, however, fails and a variety of new dynamic features arise. These all involve population of the intermediate cation or anion residue states which can be partially or wholly vibrationally relaxed. We discuss suitable views and a new theoretical formalism for these phenomena. We also note some important implications for site-directed mutagenesis in long-range, strongly exothermic electron transfer processes.

Long-range electron transfer; Mutagenesis; Superexchange; Three-level electron transfer

1. INTRODUCTION

Longe-range electron transfer (ET) in modified hemoproteins [1–11], blue copper [12–14] and iron-sulphur proteins [15,16], and in native protein reactivity [17] is a key concept in protein electron transfer. The notation implies that the ET distance exceeds the donor and acceptor size and that ET is mediated by intermediate networks of organized peptide chains. The simplest formal long-range ET rate constant form is [18–20]:

$$k = \kappa \frac{\omega_{eff}}{2\pi} \exp(-\Delta G^{\ddagger}/k_B T); \quad \Delta G^{\ddagger} = (E_r + \Delta G_o)^2/4E_r \quad (1)$$

where the nuclear activation part contains the molecular and environmental reorganization free energy E_r and the reaction free energy ΔG_o ('the driving force') The pre-exponential factor incorporates the effective nuclear frequency of all reorganized 'classical' nuclear modes, ω_{eff}, and the electronic transmission coefficient, κ. The latter contains the wave functions of all electronic donor, acceptor and intermediate bridge group states, and their residual interactions. k_B is finally Boltzmann's constant and T the temperature.

A common and useful concept for the role of intermediate protein matter is superexchange [21–23]. In the way this frame is commonly used in biological ET [23] the protein matter is explicitly reflected solely in the transmission coefficient. This does not imply that the intermediate states are physically populated. By 'delocalization' of the donor and acceptor wave functions through the protein their function is instead to ensure more favourable donor–acceptor electronic overlap and therefore more facile ET than for direct overlap. This can be achieved also for off–resonance coupling, when ionized LUMO and HOMO intermediate state energies are high compared with donor and acceptor energies. Also, if the nearest neighbour overlap along the donor–protein–acceptor chain is weak compared to the excitation energy of the intermediate residues, superexchange reduces to perturbation form of the order determined by the number of intermediate residues. This condition is valid for through–space off–resonance coupling but less obviously satisfied for through–bond coupling via covalently linked residues [17].

A number of the documented cases of long–range metalloprotein ET have involved excited electronic states of the metal centres, corresponding to the sequence [1– 11,25]:

$$D\text{–}A \xrightarrow{h\nu} D^*\text{–}A \xrightarrow{k_f} D^+\text{–}A^- \xrightarrow{k_r} D\text{–}A \quad (2)$$

The donor, D is heme or another metalloporphyrin. The acceptor, A is a -$Ru(NH_3)_5^{2+/3+}$-like group attached to surface histidines [1–3,7], similarly attached -$Ru(bipy)_2^{2+/3+}$ (bipy = 2,2'-bipyridine) [4], $Co^{2+/3+}$ cage groups [5,6], or other redox groups [26]. Both forward ET from excited D* to A (k_f in Eq. 2] and reverse ET from A^- to D^+ (k_r) can in principle be followed. By variation of either heme or surface group ΔG_o can be brought to span more than one eV. Use of such a wide range, however, brings into focus new effects associated with intermediate peptide residue oxidation or reduc-

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tion for which normal superexchange formalism fails. These effects are not simply conversion of superexchange to 'sequential', i.e. independently single-step ETs. A hierarchy of intermediate state vibrational relaxation patterns rather emerges, different from both common superexchange and sequential ET [27–31].

We shall illustrate this by a single intermediate electronic state (Fig. 1). The donor and acceptor states represent metalloporphyrin, blue copper, iron–sulphur, and surface-attached metal centres. The intermediate state is a cation or anion residue radical with a redox potential, E_o, comparable to that of the photo-oxidized electron or hole such as tyrosine, histidine or tryptophane cation radicals the E_o's of which are in the range +0.5–1.5 eV (NHE) [32,33]. No data for anion radical formation are available but envisaged to be within a similar reach.

The transmission coefficient for a three-level reaction through a high-energy intermediate state (Fig. 1) is [18,20]

$$\kappa = (T_{DA})^2 \sqrt{4\pi^3/\hbar^2\omega_{eff}^2 E_r k_B T} \quad (3)$$

where $2\pi\hbar$ is Planck's constant. T_{DA} is the three-centre electron exchange coupling [27,31]

$$T_{DA} = V_{DB} V_{AB} \Delta U^* \quad (4)$$

where V_{DB} and V_{AB} are nearest neighbour electron exchange factors coupling the intermediate bridge group ('B') to the donor and acceptor, respectively. ΔU^* is the energy gap between the donor–acceptor crossing and the bridge group energy at this nuclear configuration (Fig. 1).

Eqn. 4 is valid when ΔU^* is large compared with V_{BD} and V_{AB}. This condition is normally satisfied for ground state centres. The equation fails as $\Delta U^* \rightarrow 0$ corresponding to an excited donor or acceptor with E_o approaching that of the bridge group. In these limits the intermediate states are temporarily populated and in principle physically detectable. Such processes must be handled by a different perturbation theory form where attention is given not only to the electronic part of the bridge group state as in common superexchange but also to electronic– vibrational coupling in this state. Such a formalism is available [27–31]. We focus on a few features bearing directly on the systems represented by Eqn. 2. We notice first that the divergency as $\Delta U^* \rightarrow 0$ is an artefact associated with the limiting form of Eqn. 4 and the one- dimensional view in Fig. 1. This is encountered also in resonance Raman scattering and other two–photon processes which are 'optical analogues' of thermal three- level ET [34]. In all cases divergency can be lifted by residual coupling to a continuous vibrational dispersion [28,34].

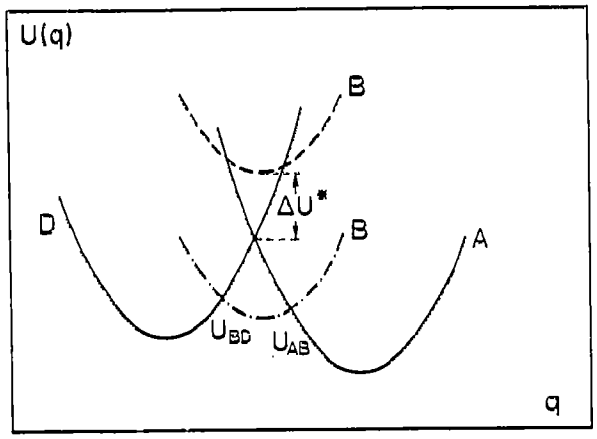

Fig. 1. Potential surfaces, $U(q)$, spanned by the nuclear coordinate q for three-level ET involving donor (D), acceptor (A) and bridge group state (B). Two bridge group positions are shown. The activation energy for high energy (upper) is given by the D/A crossing for low energy (lower) by the higher of the D/B and B/A crossings.

By such procedures a rate constant valid in the whole intermediate state energy range from high values well above the donor–acceptor crossing to low values well below, can be obtained [28,31]. The rate constant reduces to simple forms when the intermediate state is off resonance with the donor/acceptor state at the crossing. The common superexchange form thus emerges at high energies [31] (Eqns. 3 and 4). At low energies the rate constant can also be given the form in Eqn. 1 but κ and ΔG^* are now [28,31]

$$\kappa = \frac{4\pi^{5/2}}{(\hbar\omega_{eff})^2\sqrt{E_r^{BD}E_r^{AB}}} \frac{(V_{BD})^2(V_{AB})^2}{\sqrt{k_B T|U_{BD}-U_{AB}|}} \quad (5)$$

$$\Delta G^* = U_{BD} = (E_r^{BD} + \Delta G_o^{BD})^2/4E^{BD} \quad \text{if } U_{BD} > U_{AB} \quad (6)$$

$$\Delta G^* = U_{AB} = \Delta G_o^{BD} + (E_r^{AB} + \Delta G_o^{AB})^2/4E^{AB} \text{ if } U_{BD} < U_{BD}$$

where 'BD' and 'AB' refer to the transitions D→B and B→A, respectively.

Eqns. 5 and 6 show, first that the bridge group is physically populated as ΔG^* is now given by the highest of the two crossings of this state with the donor and acceptor state. The appearance of two, instead of a single electronic factor shows, however, secondly that the process is a single act, and not merely two sequential processes. This is because Eqns. 5 and 6 rest on only a single reactive attempt towards the bridge/acceptor crossing. Multiple attempts or frictional damping modifies this result [35], but are less important for the present discussion.

The vibrationally unrelaxed nature of the intermediate state is also reflected in the pre-exponential energy denominator $\sqrt{k_B T|U_{BD}-U_{AB}|}$ which represents the nuclear velocities at the two crossings. These are given by

thermal velocity only at the higher crossing – providing the factor $\sqrt{k_B T}$ while the difference $|U_{BD} - U_{AB}|$ is converted to kinetic energy at the lower crossing, giving a larger velocity [27,28]. Eqns. 5 and 6 formally diverge when $U_{BD} \rightarrow U_{AB}$, but detailed analysis shows this to be in reality a finite, 'mild' resonance [28,31].

We conclude by some observations on the importance of these views for long–range, strongly exothermic ET with a photo-oxidized metalloporphyrin donor as in myoglobin or cytochrome c, and surface–attached Ru(III)-acceptor centres (Eqn. 2). A suitable ionizable aromatic bridge group could be Trp–14 in – $Ru(NH_3)_5^{2+/3+}$ modified cytochrome *c*. The following intermediate state population patterns could then in principle occur (Fig. 2).

(A) ET from photo-excited metalloporphyrin via the bridge group LUMO to Ru(III). This would occur for suitably matched excited metalloporphyrin and bridge group LUMOs, even slightly endothermically due to the large exothermic energy gap in the following ET from bridge anion radical to acceptor.

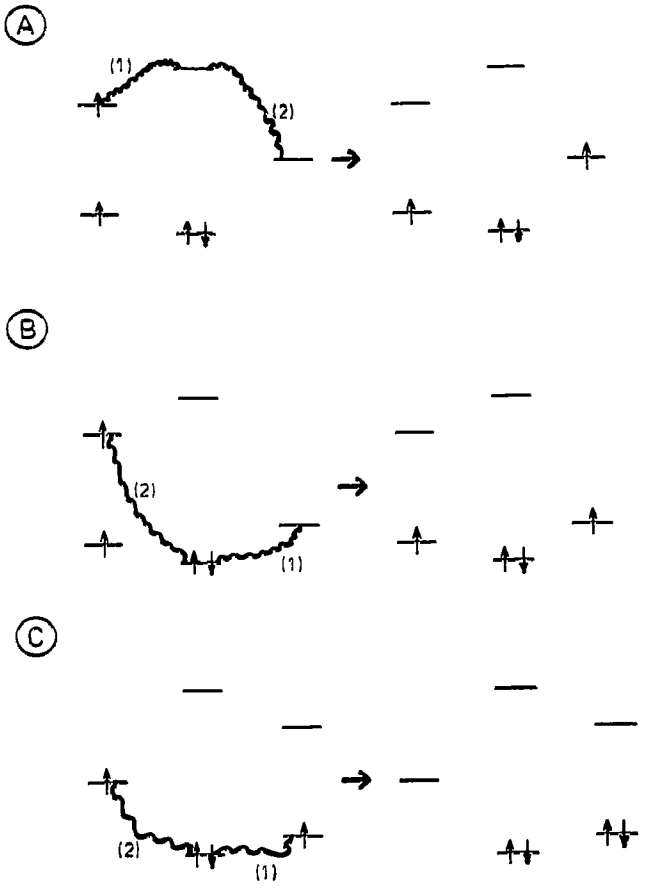

Fig. 2. ET sequences corresponding to (A)–(C) in the text. Ground and excited levels of donor, bridge and acceptor to the left, middle, and right, respectively. Numbers 1 and 2 indicate the ET order, and vertical level positions reflect the relative values. (A) Forward ET from excited donor via bridge LUMO to acceptor. (B) Forward ET from filled bridge group HOMO to acceptor, followed by ET from excited donor to bridge group. (C) Reverse ET from filled bridge group HOMO to oxidized donor, followed by ET from reduced acceptor to bridge group.

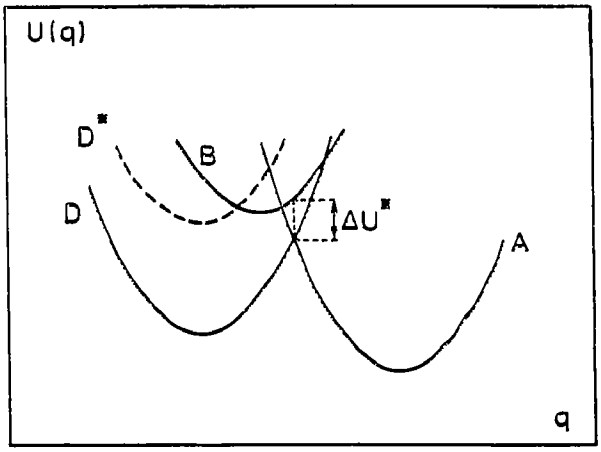

Fig. 3. Potential surface configuration where ET from ground state donor is by 'normal' superexchange, while excited donor ET proceeds via a populated, vibrationally unrelaxed bridge intermediate state.

(B) ET from bridge HOMO to Ru(III) followed by ET from photo-excited metalloporphyrin to the 'hole' in the bridge group HOMO. This is sometimes viewed as 'hole' transfer and might be feasible for - $Ru(biby)_2^{2+/3+}$ surface groups but not for - $Ru(NH_3)_5^{2+/3+}$-like groups with much lower E_os. Interference from double exchange ET from bridge group to photoexcited metalloporphyrin hole followed by ET from the excited donor electron to bridge cation radical, could here be envisaged.

(C) Reverse ET, from bridge group HOMO to oxidized porphyrin, followed by ET from Ru(II) to HOMO hole in the bridge group cation radical. This is

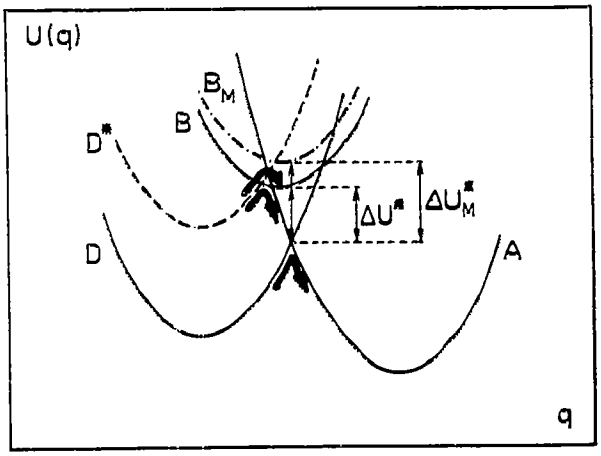

Fig. 4. Potential surface configuration showing two intermediate states representing wild–type (B) and a mutant (B_M). ET from ground state donor gives a small mutant effect reflected primarily in different energy gaps ΔU^* and ΔU_M^* (Eqn. 4). In excited donor ET the mutant follows Eqn. 4 while the lower–lying wild–type bridge group is now populated along the ET path. The mutagenic effect is therefore large and reflected also in the activation energy. The trajectories for the two cases are indicated by the arrows.

feasible due to the high negative E_os of the oxidized metalloporphyrins [1–7].

ET from Ru(II) via the bridge group LUMO is not feasible due to poor redox potential match. The other three cases can be framed by the formalism above and are illustrated by Fig. 3.

These views are finally suggestive as to expectable site– directed mutagenic effects in the through–bond amino acid sequence [36]. The high–energy superexchange form (Eqn. 3) suggests that mutation effects in through–bond chains are small provided that ΔU^* is significant and the mutant and wild–type proteins structurally similar. On the other hand, if a residue of high E_o (say Tyr, His or Trp) is replaced by a residue with a notably lower value, or vice versa, *and* the overall ET is strongly exothermic (i.e. involves excited donor states), then the repiacement may involve a potential surface shift as in Fig. 4. The implication is that the relatively low–energy intermediate state in the wild–type protein is populated, whereas mutant protein ET proceeds by normal high–energy superexchange (or vice versa). Such effects would be reflected in both electronic factor and activation free energy, and could be much larger than purely electronic superexchange effects. These considerations might be useful in free energy and mutagenesis interpretation of strongly exothermic metalloprotein ET.

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