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Report

Quantum catalysis in enzymes: beyond the transition state theory paradigm. A Discussion Meeting held at the Royal Society on 14 and 15 November 2005

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How do enzymes work? What is the physical basis of the phenomenal rate enhancements achieved by enzymes? Do we have a theoretical framework that accounts for observed catalytic rates? These are the foremost questions—with particular emphasis on tunnelling phenomena—debated at this Discussion Meeting by the leading practitioners in the field.

Keywords: quantum tunnelling; electron transfer; hydrogen transfer; transition state theory; enzyme catalysis

1. BIOLOGICAL ELECTRON TUNNELLING

This Discussion Meeting was fortunate to have the presence of two distinguished colleagues who have played critical roles in the foundations and development of tunnelling mechanisms in biology, Britton Chance (University of Pennsylvania, USA) and Rudolph Marcus (California Institute of Technology, USA), both Foreign Members of the Royal Society. Moreover, 70 years ago Chance invented the stopped-flow spectroscopic instrumentation that led to the proof of the Michaelis enzyme–substrate complex. This complex was very much the focus of attention during the meeting that discussed theory, experimental pursuit and characterization of hydrogen tunnelling in a variety of oxidoreductases. Fifteen years later, Chance first revealed that quantum mechanical electron tunnelling was a critical part of biological oxidation and reduction using light-active photosynthetic bacteria at cryogenic temperatures. In the classic 1966 paper with the late Don DeVault, he not only demonstrated the tunnelling signature of temperature insensitive rate constants between 77 and 5 K for the millisecond oxidation of cytochrome *c* by the light-activated bacteriochlorophyll but also provided a theoretical basis for a long-distance electron tunnelling of over 20 Å. Fifty years ago, Rudolph Marcus (Caltech) first penned his classical theory of oxidation–reduction reactions (equation 1.1) involving electron transfer in solution. In the 1970s, these equations became the foundation of experimental

$$k_{\mathrm{et}} \propto \frac{1}{\sqrt{4\pi\lambda k_{\mathrm{B}} T}} \exp\left[\frac{-(\Delta G+\lambda)^2}{4\lambda k_{\mathrm{B}} T}\right] \tag{1.1}$$

investigations into biological electron tunnelling, again using photosynthetic systems. The early 1980s saw the first heroic experimental demonstration by John Miller and colleagues of the parabolic rise of the log rate constant with increasing driving force ($-\Delta G$) to a maximum (defining the reorganization energy, λ) followed by a fall in the 'inverted region' as predicted by Marcus. The relatively shallow fall of the inverted region was indicative of strong coupling of the electron tunnelling to quantized vibrational modes and hence the tunnelling of nuclei as well. Application of Marcus theory has become one of the foundations of transition state theory and a mainstay of those working on the Discussion Meeting's principal subject, tunnelling of the hydrogen nucleus in enzymatic oxidation and reduction.

Chance opened the meeting with an outline of the early developments by Keilin and Warburg in respiration and of Hill in photosynthesis that paved the way to the discovery of tunnelling in biological electron transfer reactions. He emphasized the early importance of purple photosynthetic bacteria in facilitating the demonstration of electron tunnelling, and the later importance of the highly robust photosynthetic reaction centre proteins that offered an unprecedented breadth of biochemical and biophysical experimental scope to explore the parameters of Marcus theory in biology. Les Dutton (University of Pennsylvania, USA) introduced the progression of work that put to test the parameters in the quantized form after Hopfield (equation 1.2*a* and 1.2*b*). In the 1970s and

$$k_{\mathrm{et}} \propto \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left[\frac{-(\Delta G+\lambda)^2}{2\sigma^2}\right], \tag{1.2a}$$

$$\sigma^2 = \lambda\hbar\omega \coth\left[\frac{\hbar\omega}{2k_{\mathrm{B}} T}\right] \tag{1.2b}$$

1980s, his laboratory's work on reaction centres made immediate use of technical opportunities such as ultra-fast lasers for activation for tunnelling kinetics over a 300 K range (rate constants and $\hbar\omega$) and replacement of cofactors and mutagenesis to change the driving force by as much as an electron volt and estimate reorganization energies (ΔG and λ). Reaction centres

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were the first membrane proteins to yield high-resolution crystal structures that provided critical inter-cofactor edge-to-edge distances (R) and atomic level details of protein packing (ρ). Dutton reported on how these Marcusian tunnelling parameters are selected and varied in biology in the natural engineering of oxidoreductases. This Darwinian examination at the molecular scale yields a remarkable simplifying picture. One by one, the parameters were shown to be more or less generic components of the engineering ($\hbar\omega$, ρ and λ varying but not selected), leaving a clear and dominant selection of distance for the engineering of electron transfer chains and catalytic clusters. ΔG is sometimes selected and sometimes not, especially when the distance between the redox cofactors is small. It is also not unusual that electron tunnelling through chains encounters major endergonic redox step(s) (up to several hundreds of meV) in electron transfer chains that overall are mildly exergonic. Dutton provided test drives for the resultant Darwin-purged quantized Marcus expression (equations 1.3a and 1.3b)

$$\log k_{\mathrm{et}}^{\mathrm{exer}} = 15 - 0.6R - 3.1\frac{(\Delta G + \lambda)^2}{\lambda} \qquad (1.3a)$$

$$\log k_{\mathrm{et}}^{\mathrm{ender}} = 15 - 0.6R - 3.1\frac{(-\Delta G + \lambda)^2}{\lambda} - \frac{\Delta G}{0.06} \qquad (1.3b)$$

in several simulations of native electron transfer systems. This included (i) light-activated electron tunnelling mediated charge separation and recombination in the highly complex electron transfer chains of the reaction centres of plant photosystems; (ii) electron transfer through the long chain of nitrate reductase that includes a 600 meV thermal redox barrier that marshals multiple electrons before delivery to the catalytic site and (iii) the role of the barriers of proton pumping and dioxygen reduction in cytochrome oxidase in removing the possibility of electrons by-passing the catalytic site. Dutton also noted the power of shortened electron tunnelling distances between cofactors at the thresholds of catalytic sites. He identified the energetic limit in two-electron catalysis for two, sequential one-electron tunnelling to a thermally accessible radical transition state. Beyond this limit, when the one-electron reduction is energetically remote, a dramatic mechanistic change must occur towards the principal subject matter of the meeting, the concerted two-electron transfer and tunnelling as a hydrogen or hydride.

2. ENZYMIC HYDROGEN TUNNELLING

Measurement of competitive kinetic isotope effects and their temperature dependence is now the 'gold standard' for diagnosing experimentally tunnelling regimes in enzyme systems. Amnon Kohen (University of Iowa, USA) provided an insightful review of these approaches, now widely adopted in the field, and discussed recent data from a number of laboratories, including his own, that are consistent with environmentally coupled or vibrationally assisted tunnelling models. The more general acceptance of full tunnelling models that accommodates tunnelling of all isotopes of hydrogen, and barriers to the reaction that reflect environmental reorganization, was notable at the meeting. This is a significant departure from views held until recently by many in the field that tunnelling is restricted to a few enzyme systems, and where observed, can be interpreted using the Bell 'tunnelling correction' of semi-classical transition state theory. Kohen presented his experimental studies of H-tunnelling in dihydrofolate reductase. He emphasized the role of distal mutations on enzymic H-tunnelling probed through measurement of kinetic isotope effects and their temperature dependence. Using isotopically labelled cofactors, he presented kinetic data that are consistent with full tunnelling models for the hydrogen nucleus. His kinetic studies with mutant enzymes altered at residues distal to the active site—residues that are predicted theoretically to be part of a network of motions coupled to the active site—provide experimental support for coupling of motion to active site chemistry, and are consistent with environmentally coupled models of H-transfer. Dihydrofolate reductase featured also in the presentation of Ruedi Allemann (University of Cardiff, UK). Unlike Kohen's approach which relies on measurements in the steady-state and analysis of the commitment to catalysis to extract intrinsic kinetic isotope effects, Allemann 'isolated' the chemical step using stopped-flow methods. He emphasized studies with a thermostable dihydrofolate reductase from the hyperthermophile *Thermotoga maritima* as well as the intensively studied *Escherichia coli* enzyme and he presented kinetic data consistent with environmentally coupled models of H-tunnelling. Of interest was his finding that at physiological pH, the kinetic isotope effect data are consistent with the need to invoke 'gated motion' along the H-transfer coordinate, but at elevated pH the reaction was dominated by passive motions.

Judith Klinman (University of California–Berkeley, USA) developed further the theme of environmentally coupled hydrogen tunnelling by reference to her work with soybean lipoxygenase. She emphasized recent studies with wild-type and mutant enzymes that exhibit very large primary deuterium kinetic isotope effects and the notion of 'distance sampling' required to bring donor and acceptor atoms sufficiently close to effect efficient wavefunction overlap. The wild-type enzyme has a very small enthalpy of activation, and that for deuterium transfer is only slightly increased. The primary kinetic isotope effect is therefore weakly dependent on temperature and the pre-exponential factor ratio ($A_{\mathrm{H}}/A_{\mathrm{D}}$) derived from Arrhenius analysis is substantially inflated over that expected for semi-classical transfer. Klinman interprets these data to indicate an optimized active site set up to catalyse H-transfer by tunnelling. The temperature dependence of the reaction is increased in mutant enzymes altered on either side of the donor carbon atom of the substrate. The size of the kinetic isotope effect remains constant, but the $A_{\mathrm{H}}/A_{\mathrm{D}}$ prefactor ratio is reduced substantially below unity. These data are interpreted to indicate a less optimal configuration for tunnelling in the mutant enzymes, leading to increased activation energy. The Klinman analysis is based on, and fully

consistent with, the full tunnelling model for enzymic H-tunnelling and, coupled with structural analysis of lipoxygenase offers new insight into the effects of localized mutations on tunnelling characteristics.

Experimental studies of tunnelling in B_{12}-dependent systems, specifically methylmalonyl–CoA mutase, were also presented by Ruma Banerjee (University of Nebraska, USA). In this system, the transfer of a hydrogen atom from substrate to the deoxyadenosyl radical is accompanied by a very large kinetic isotope effect, a value that is well beyond the upper limit for semi-classical reactions in the absence of tunnelling. The reaction is kinetically complex involving cobalt–carbon bond cleavage, a homolysis reaction that is ‘triggered’ by conformational change in the protein. Banerjee presented an elegant computational analysis of the cobalt–carbon bond cleavage reaction which provided details of the energetics of formation of the radical products of homolysis. The simulation demonstrated the ideal geometry of the radical species for H atom transfer from substrate to the deoxyadenosyl radical following cobalt–carbon bond cleavage. Dexter Northrop (University of Wisconsin-Madison, USA) presented interesting data on the pressure dependence of isotope effects in enzymes and made a case for hydrogen not tunnelling in yeast alcohol dehydrogenase. His work has demonstrated suppression of kinetic isotope effects at high hydrostatic pressure in this enzyme system, which finds similarities with studies of chemical reactions by Isaacs that display unusually large kinetic isotope effects. Isaacs had previously shown that ‘normal’ deuterium kinetic isotope effects are insensitive to pressure, but those reactions displaying an unusually high kinetic isotope effect reflecting inferred contributions from quantum mechanical tunnelling are sensitive. Northrop originally attributed his pressure sensitivity of the kinetic isotope effect measured with alcohol dehydrogenase to tunnelling effects, but he now rules out tunnelling effects on the basis of more recent data and extrapolations of the intrinsic kinetic isotope effect to unity at pressures above the experimental range. Moreover, ^{13}C isotope effects are also suppressed at high pressure, which he uses to argue against significant tunnelling effects in the H-transfer reaction and he offers protein domain motion as a possible origin of the observed effects. Pressure effects are delivering new types of data that are seemingly challenging current frameworks for the physical basis of H-transfer. The mechanistic basis of these effects generated is as yet unclear, but generated much debate at the meeting. It is clear that studies of this type will make a major contribution to current experimental strategies based on more established methods, i.e. competitive and temperature-dependent isotope effects studies.

Dan Nocera (Massachusetts Institute of Technology, USA) presented experimental studies of proton-coupled electron transfer (PCET)—intrinsically a quantum mechanical effect since both the electron and proton tunnel. Nature has resolved the fundamental difference in transfer distances by the evolution of enzymes to control proton transfer (shorter) and electron transfer (longer) on very different length scales. Model systems containing orthogonal electron transfer and proton transfer pathways—thereby allowing the electron and proton tunnelling events to be disentangled—were presented. The lessons learnt from these model systems enabled PCET to be exploited in biomimetic mono-oxygenases—utilizing the ‘Hangman’ porphyrin architecture—which displayed unprecedented multifunctional catalytic activity. The flash activation method developed on the model systems was used to resolve the proton coupling in an enzyme system—the radical-based quantum catalysis in class I *E. coli* ribonucleotide reductase. This enzyme utilizes both co-linear and orthogonal PCET to transport charge from an assembled diiron–tyrosyl radical cofactor to the active site over 35 Å away via an amino acid radical hopping pathway spanning two protein subunits. Mårten Wikstrom (University of Helsinki, Finland) presented a study of PCET in cytochrome oxidase. Recently, it has been suggested the proton pumping in cytochrome oxidase is not mechanistically coupled to electron transfer, but instead to proton consumption at the active O_2 reduction site. Time-resolved optical spectroscopy was used to address this, showing that electron transfer from the low-spin haem *a* to the active O_2 reduction site is in fact kinetically coupled to the primary event of the proton pumping mechanism—an internal proton transfer from a conserved glutamate to an as yet unidentified site above the haem groups.

3. THEORETICAL CONSIDERATIONS AND COMPUTATIONAL SIMULATION

Further detailed insight, particularly at the atomic level, comes from theory and simulation—such studies are key to corroborating and extending findings that emerge from experimental studies. Sharon Hammes-Schiffer (Pennsylvania State University, USA) gave a clear exposition of how theory and simulation can give valuable insight into the impact of enzyme motion on enzymic hydride and PCET. Studies of PCET were illustrated for the reaction catalysed by lipoxygenase, using a formulism that treats the active electrons and transferring protons quantum mechanically. These showed that the small overlap of the reactant and product proton vibrational wavefunctions gives rise to the highly elevated experimentally determined proton/deuterium kinetic isotope effect. The study illustrated that proton donor–acceptor vibrational motion plays a vital role in PCET. The role of motion in the hydride transfer reaction catalysed by dihydrofolate reductase was also investigated. The hybrid quantum/classical molecular dynamics simulations—using the empirical valence bond approach—showed that nuclear quantum effects such as zero point energy and hydrogen tunnelling are significant in these reactions, where they lower the free energy barrier significantly. The simulations also identified a network of coupled motions extending throughout the enzyme—these correspond to conformational changes along the collective reaction coordinate. Importantly, these coupled motions facilitate hydride transfer by reorganizing the environment—decreasing the donor–acceptor distance, orienting the substrate and cofactor appropriately, and providing

a favourable electrostatic environment for hydride transfer. It was also shown that mutations distal to the active site cause non-local structural changes that impact significantly on the probability of sampling configurations conducive to hydride transfer, thereby altering the free energy barrier and the rate of hydride transfer.

The role of protein motion in promoting tunnelling also featured in the presentation by Mike Sutcliffe (University of Manchester, UK). He amply demonstrated the power of experiment (Nigel Scrutton, University of Manchester, UK) and theory pursued in close partnership in the same laboratory. His simulations of proton tunnelling steps—based on atomic resolution structures of reaction intermediates and a variational transition state theory approach—confirmed that tunnelling dominates in these reactions. Good agreement with experiment was obtained for both calculated kinetic isotope effects and calculated phenomological activation energies when the contribution of tunnelling is included. Protons were shown to tunnel over relatively short distances. Sutcliffe also discussed the role of long-range coupled motions and short-range motions in promoting tunnelling through modulation of proton-acceptor distance. Tunnelling in the related enzyme, methylamine dehydrogenase, was presented by Neil Burton (University of Manchester, UK). With methylamine as substrate, different tunnelling paths were investigated across an ensemble of reactive enzyme configurations. The results are suggestive of two pathways, one of which is dominated by tunnelling and the other dominated by a classical 'over-the-barrier' mechanism. The influence of a number of active site amino acids on proton tunnelling and the utility of enzyme mimics were also discussed. Hans Limbach (Freie Universität Berlin, Germany) presented results illustrating the application of the one-dimensional Bell–Limbach tunnelling model to several different hydrogen transfer reactions in small molecule systems for which experimental kinetic data are available, calculating the temperature dependencies of rates and kinetic isotope effects. This study suggests that analysis of Arrhenius curves using the Bell–Limbach tunnelling model is a useful approach for a first screening of experimental data prior to more advanced physical descriptions and theoretical interpretations.

In contrast to the results presented by the speakers above—reflecting current controversy in the field—Arieh Warshel (University of Southern California at San Diego, USA) presented results that challenge not only a role for dynamical effects in enzymic reactions, but also the very existence of tunnelling in such reactions. Simulation of the lipoxygenase reaction reproduces the very large observed kinetic isotope effect and the observed rate constant without quantum mechanical tunnelling. The results suggest that transition state theory provides a quantitative framework for studies of enzyme catalysis, and that the key questions remaining to be addressed relate to the nature of the factors that lead to transition state stabilization. However, in contrast Steve Schwartz (Albert Einstein College of Medicine, New York, USA) presented results illustrating that transition state theory could not fully describe the dynamics of enzyme-catalysed reactions. Using a model system comprising a double well coupled to an environment and to a symmetrically coupled promoting vibration, it was shown that reactive trajectories pass through a wide range of points, most of which are not the saddle point. A study of lactate dehydrogenase addressed the question of how motions, such as promoting vibrations, which are on the picosecond time-scale can be reconciled with the millisecond time-scale of enzymatic turnover. The study illustrates the answer lies in the large conformational space that has to be searched to find the rare conformation needed to produce the required dynamic effects—only a small minority of conformations appear to be catalytically competent.

José Onuchic's (University of Southern California at San Diego, USA) presentation illustrated how recent theoretical advances have shed light on the impact of protein dynamics on electron tunnelling and biochemical reactivity. The original Pathway model has been generalized to understand how protein dynamics modulate not only the Franck–Condon Factor but also the tunnelling matrix element. Dynamical effects are of critical importance when interference—particularly destructive interference—among pathways modulates the electron tunnelling interactions in proteins. Such dynamical modulation of the electron transfer rate has been able to explain and/or predict a number of experimental rates that were subsequently confirmed by experiment. Results were also presented that illustrated how global transformations, which control protein functions such as allostery, may involve large-scale motion and possibly partial unfolding during the reaction event.

4. UNANSWERED QUESTIONS

The summing up of, and presenting the future perspectives for, such a lively and stimulating Discussion Meeting requires someone with both a broad and deep knowledge of the topics covered. Rudolph Marcus (Caltech; Nobel Laureate and Foreign Member of the Royal Society)—having played a critical role in laying the foundations of and subsequently developing our conceptual framework for understanding tunnelling mechanisms in biology—very ably and wisely fulfilled this role. He perceptively identified that an important component of the continued successful advancement of the field is the need to develop a common language, e.g. when referring to dynamical effects. We need to agree how we define transition state theory—is extra dynamics required in such a formulism and how important is re-crossing the barrier? He also pointed out that the introduction of new experimental techniques—for instance the use of pressure effects—heralds the need for a generally accepted working framework to accommodate, explain and advance them experimentally and theoretically. It was evident from Marcus' summary that further advancement into the realm of hydrogen tunnelling is nevertheless still wrestling with long-standing but non-trivial theoretical and experimental obstacles toward mechanistic

understanding. These include questions as basic as how enzymes achieve catalysis and, in this regard, how important is it for reactant and product wells to be symmetric for tunnelling to occur and, not least, how can the effects of the common mutagenesis approach be interpreted and used without ambiguity. This meeting served the broad biochemistry and biophysics fields well in presenting the view beyond the transition state theory paradigm of enzyme catalysis, and in bringing many—including some new—key questions to the fore. Very evident in the lectures and vigorous discussions was the message that future progress and insights into this challenging interface of chemistry, physics and biology can benefit from the synergy evident in a close partnership of experimental techniques and simulation and theory.