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Energetics of Direct and Water-Mediated Proton-Coupled Electron Transfer

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Supporting Information

ABSTRACT: Proton-coupled electron transfer (PCET) is an elementary chemical reaction crucial for biological oxidoreduction. We perform quantum chemical calculations to study the direct and water-mediated PCET between two stacked tyrosines, $TyrO^{\bullet} + TyrOH \rightarrow TyrOH + TyrO^{\bullet}$, to mimic a key step in the catalytic reaction of class Ia ribonucleotide reductase (RNR). The energy surfaces of electronic ground and excited states are separated by a large gap of ~20 kcal mol^{-1}, indicative of an electronically adiabatic transfer mechanism. In response to chemical substitutions of the proton donor, the energy of the transition state for direct PCET shifts by exactly half of the change in energetic driving force, resulting in a linear free energy relation with a Brønsted slope of $^1/_2$. In contrast, for water-mediated PCET, we observe integer Brønsted slopes of 1 and 0 for proton acceptor and donor modifications, respectively. Our calculations suggest that the π-stacking of the tyrosine dimer in RNR results in strong electronic coupling and adiabatic PCET. Water participation in the PCET can be identified perturbatively in a Brønsted analysis.

Proton-coupled electron transfer (PCET), the stepwise or concerted transfer of a proton and an electron, is involved in many enzymatic and organic radical reactions.[1] In biological energy transduction,[2] PCETs catalyzed by the oxidoreductases of cellular respiration and the photosynthetic machinery result in an electrochemical proton gradient across a biological membrane that is coupled to the synthesis of ATP and to other endergonic processes.[3] PCET processes have been studied experimentally and theoretically in simple model systems and enzymes.[1,4] As both electrons and protons are elementary particles, many PCET processes involve strong quantum mechanical effects even at ambient conditions,[5] including tunneling, as witnessed by often high deuterium kinetic isotope effects (KIEs).[6] The quantum nature of the two transfer processes and their coupling pose unique challenges for the development of accurate theoretical descriptions. Generalizations of Marcus theory of electron transfer[7] provide useful theoretical frameworks[1] to describe PCET, to distinguish different mechanisms, and to quantify the degree of electronic adiabaticity.[1,8,9] Adiabatic proton transfer (pT) is expected if the coupling V_{DA} (defined as half of the energy gap between ground-state and electronically excited-state potential energy surfaces at the transition state) is large compared to the thermal energy, k_BT.[1,8,9]

Here we use quantum chemical calculations to study the ground and excited-state energetics of direct and water-mediated PCET in a tyrosine-dimer model (net reaction $TyrO^{\bullet} + TyrOH \rightarrow TyrOH + TyrO^{\bullet}$) that mimics part of the radical-transfer chain of ribonucleotide reductase (RNR). RNRs catalyze the conversion of nucleotides to deoxynucleotides in all organisms.[10,11] The long-range radical transport between the two enzyme subunits[10] is thought to be mediated in part by PCET between two stacked tyrosine residues adjacent in sequence.[1,10,11] PCET in chemically similar phenoxy/phenol systems has previously been studied using theoretical approaches.[9,12−14]

A model of the *Escherichia coli* ribonucleotide reductase (RNR) tyrosine dimer, Tyr-730/Tyr-731, together with the connecting peptide backbone was constructed based on the crystal structure of subunit R1 (PDB ID: 2X0X).[15] Structures with and without a water molecule intervening between the phenol oxygens served as models for water-mediated and direct PCET. Hydrogen atoms in 2′, 3′, and (2′,5′) positions of Tyr-730 were substituted with nitro (NO_2), methyl (CH_3), chloro (Cl), and fluoro (F) groups to obtain different driving forces for the PCET reaction. All models were structure optimized in the doublet state, using the dispersion-corrected hybrid density functional, D-B3LYP, and a def2-SVP basis on all atoms.[16] Transition state structures were subsequently optimized, starting from structures with the proton constrained halfway between tyrosine donor and acceptor oxygens O_1 and O_2. Single-point energy calculations were performed on the optimized structures using the def2-TZVPP basis set[16d] and the D-B3LYP[16a,b,e,f] level of theory. Reaction profiles for PCET reactions were obtained by constrained optimizations, with fixed differences in distance of the transferred proton(s) to the tyrosine donor and acceptor oxygen atoms as a reaction coordinate, $r = d(O_2H_2) - d(O_1H_n)$, with $n = 1$ and 2 for direct and water-mediated transfer. Kinetic isotope effects were estimated from zero-point vibrational energies (ZPEs) using an Eyring-like expression[6a] and Wigner's semiclassical correction,[6b] in a simplified treatment of quantum dynamical effects.[5] Vibrational frequencies were determined using the AOFORCE module in TURBOMOLE. Vertical excitation energies were computed by linear response-time-dependent density functional theory (TDDFT) at the B3LYP/def2-TZVPP level of theory[16g] and approximate second-order coupled cluster theory (CC2).[16i] All calculations were performed using TURBOMOLE v. 6.2.[17]

Electronically adiabatic ground and excited state profiles for direct and water-mediated PCET processes are shown in

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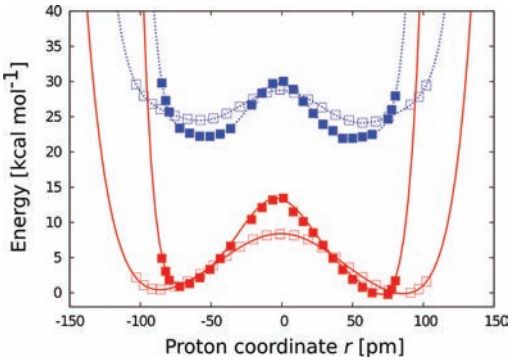

Figure 1. Ground (red) and excited state (blue) reaction energy profiles for direct (open symbols) and water-mediated PCET (filled symbols) obtained by constrained ground state geometry optimizations and DFT/TDDFT single-point calculations.

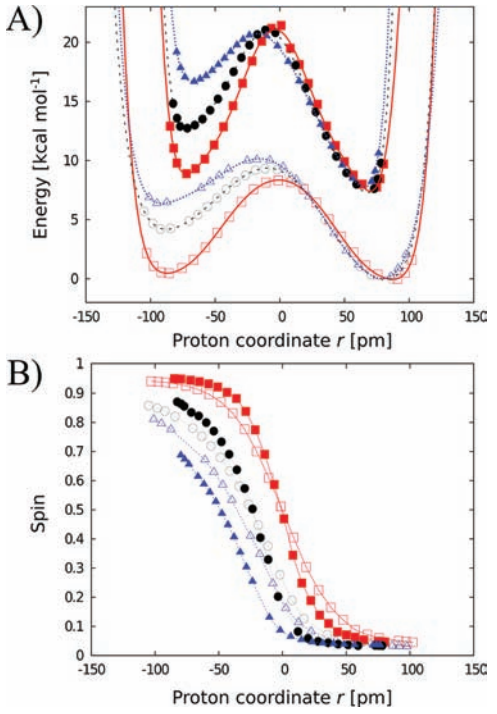

Figure 2. (A) Reaction energy profiles for direct (open symbols) and water-mediated (filled symbols) PCET with different driving forces obtained by constrained geometry optimizations (red squares, unsubstituted system; black circles, 2′-nitro substituted system; blue triangles, 2′,5′-nitro substituted system). Energies are relative to the donor minimum (right), with reaction profiles of water-mediated PCET shifted up for clarity. We note that the vertical excitation energies for direct and water-mediated PCET remain nearly unchanged upon chemical modification (20.1 and 16.3 kcal mol^{-1}, respectively, for 2′,5′ nitrosylation). (B) Integrated spin on the proton donor tyrosine as a function of the proton reaction coordinate.

Figure 1, with ground-state barriers of 8.5 and 14.1 kcal mol^{-1}, respectively. Electronic vertical excitation energies at the transition states are ∼20 and ∼17 kcal/mol, indicating that the pT processes are likely adiabatic ($V_{DA} \approx 14-17 k_B T$, at $T = 310$ K), proceeding on the electronic ground state surface. Spin density differences in the ground and excited states show that vertical excitations lead to electron transfer between the tyrosines, producing

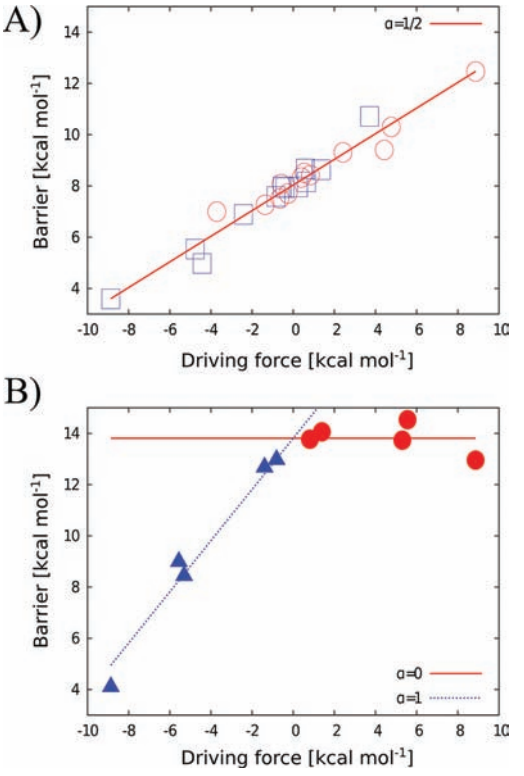

Figure 3. Response of the transition state energy to perturbations in the energetic driving force by modifications of the proton donor (red) and acceptor (blue) for (A) direct and (B) water-mediated PCET. The direct and water-mediated processes result in linear energy relations with Brønsted slopes of $^1/_2$ and 1 or 0, respectively.

$TyrO^-/TyrO^{+/\bullet}$ states (SI Figure 1A,C). At the transition state, the excitation process is linked to a small spin redistribution in the π-electron cloud (SI Figure 1B). In contrast to the likely adiabatic pT observed for the stacked tyrosines, a nonadiabatic pT was recently inferred for a chemically similar phenoxy/phenol system,[13] in which the rings were opposed in C_2 symmetry instead of stacked. For that system, our TDDFT approach predicts an excitation energy of 12 kcal mol^{-1} ($V_{DA} \approx 10\ k_B T$) at the transition state that is larger than the ∼4 kcal mol^{-1} ($V_{DA} \approx 3\ k_B T$) obtained with the complete active space self-consistent field approach (CASSCF(3,6)/6-31G) of ref 13, but close to the 9 kcal mol^{-1} ($V_{DA} \approx 7\ k_B T$) obtained from the second order-coupled cluster theory (CC2/def2-TZVPP),[16i] which incorporates more dynamical electron correlation effects. These results suggest that here the tyrosine-dimer excitation energies might be slightly overestimated at the TDDFT/B3LYP level, which otherwise has a tendency of underestimating, e.g., charge transfer excitation energies.[18] Nonetheless, our calculations indicate that the π-stacking in the RNR tyrosine dimer considerably increases the adiabaticity of the pT process in the PCET reaction.

Chemical modifications of the proton donor or acceptor affect both the ground and transition state and globally perturb the reaction profiles for direct PCET (Figure 2A, bottom curves). We find that the energy of the transition state shifts by exactly half of the perturbation in the energetic driving force, ΔE, resulting in a Brønsted slope of $^1/_2$ (Figure 3A). This linear energy relation holds over a broad range, $-10 < \Delta E < 10$ kcal mol^{-1}. In contrast, donor modifications in water-mediated PCET perturb only the product well (Figure 2A, top curves), with the transition state

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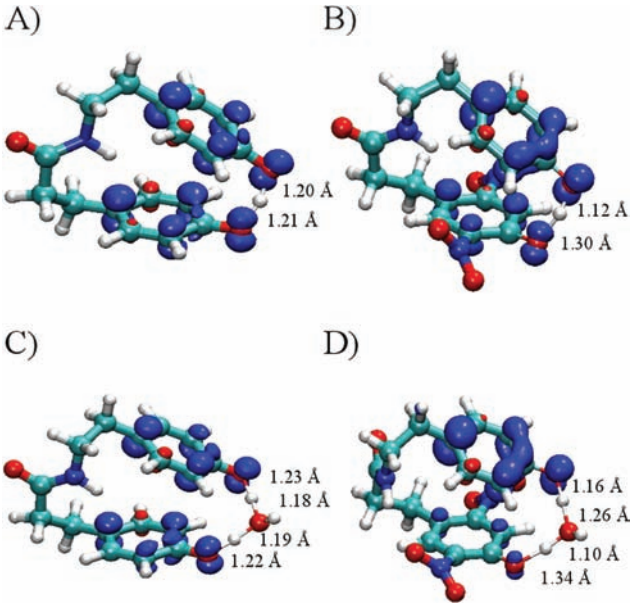

Figure 4. Spin density distributions of transition state structures of (A, B) direct and (C,D) water-mediated PCET, without (A,C) and with (B, D) chemical modifications to increase the driving force of the PCET process. Isocontour surfaces for $+0.005/Å^3$ and $-0.005/Å^3$ are shown as blue and red surface representations, respectively. Chemical modifications perturb the symmetric spin distribution (0.5/0.5) at the transition state to 0.33/0.67 (direct) and 0.15/0.85 (water-mediated), indicating reduced concertedness in proton−electron transfer. The figure was prepared using VMD.[23]

region almost unaffected. Remarkably, the resulting reaction barriers (Figure 3B) again depend linearly on ΔE, but now with slopes of 1 and 0, respectively, when the proton acceptor and proton donor groups are modified.

This sensitivity to substitutions of the acceptor, but not the donor, is a complete reversal of our earlier results for water-mediated pT.[19] The contrasting behavior here reflects the fact that in PCET both pK_a and redox potential (E_m) values are relevant, and their shifts tend to be coupled. In aqueous environments, the measured pK_a/E_m values of phenol and 4-nitrophenyl are 10.0/0.63 V and 7.2/0.92 V, respectively.[20] As shown below, at the transition state the proton is transferred to the intervening water and half an electron is on the acceptor tyrosine. Using bulk values, nitrosylation is expected to change the PCET barriers by $2.3k_BT\Delta pK_a + {}^1/_2ze\Delta E_m = -0.6$ kcal mol^{-1} and ${}^1/_2ze\Delta E_m = -3.4$ kcal mol^{-1}, respectively, from the reactant (donor) and product (acceptor) sides, in nearly quantitative agreement with the calculated values of 1.1 (−0.3) kcal mol^{-1} and −3.5 (−4.1) kcal mol^{-1} for 2′ (3′) substitutions.

Chemical modifications strongly affect the coupling of proton and electron transfer. In Figure 2B we plot the integrated spin density along the reaction as a probe of the electron localization[21] on the donor tyrosine. With increasing energetic driving force in the nitrosylated systems, the fraction of the electron transferred at the transition state drops from ∼0.5 to ∼0.15 (∼0.35) in the water-mediated (direct) process (Figure 2B). Added bias thus energetically favors stepwise over concerted transfer, in particular in water-mediated PCET.

The change from a concerted to a stepwise mechanism is reflected in the transition state structures (Figure 4). For direct PCET, the proton is tightly shared between the two phenolic oxygen atoms, with short hydrogen−oxygen distances of ∼1.20 Å. The spin population associated with the transferred proton is only −0.01, indicating that, based on the transition state on the ground-state surface, the reaction is characterized by a PCET rather than a hydrogen atom transfer, in agreement with previous studies.[12,13] Half a spin each resides in the tightly coupled π-electron systems of the two tyrosine residues, indicating a mixed valence state. An expectation value of $\hat{S}^2$ of ∼0.76 at the transition state, close to the ideal $S(S+1) = {}^3/_4$, indicates insignificant spin contamination.[22] In water-mediated PCET, a "double Zundel" ion is formed between the phenolic oxygen atoms, with short O−H distances of 1.22 Å and water O−H distances of 1.18 Å (Figure 4). As in the direct PCET, half a spin resides on each tyrosine residue, with only a small spin population of +0.02 on the transferred proton. Figure 4 also shows transition state structures and spin density distributions for direct and water-mediated PCET with a large chemical driving force of ∼10 kcal mol^{-1}. Compared to the systems without a driving force, the chemical substitutions move the proton 0.1 Å closer to the unsubstituted tyrosine. In the water-mediated reaction, this motion is partially compensated by concomitant changes in the O−H distances on the central protonated water molecule (Figure 3D).

Table 1. Zero-Point Vibrational (ΔZPE‡) and Entropic Corrections ($T\Delta S^‡$ at T = 310 K) to the Reaction Free Energy Barriers ($\Delta G^‡$) in kcal mol^{-1} Calculated Using D-B3LYP/def2-TZVPP[16a]

	$\Delta E^‡$	ΔZPE‡	$T\Delta S^‡$	$\Delta G^‡$	KIE$_{H/D}$	KIE$_{H/D,W}$
Direct	8.5	−3.0	−1.0	6.5	4.6	6.6
Water-mediated	14.1	−4.3	−1.2	11.0	9.3	13.9

[a] Deuterium kinetic isotope effects (KIE$_{H/D}$) are obtained from a harmonic approximation and account for shifts in ΔZPE‡. Wigner's semiclassical corrections (KIE$_{H/D,W}$) are estimated based on the imaginary frequencies of the transition state Hessian (see text).

Zero-point vibrational energy corrections (ΔZPE‡) and entropic contributions ($T\Delta S^‡$) lower the free energies of activation by 2 and 3 kcal/mol, respectively, for direct and water-mediated PCET (Table 1). The larger deuterium KIE of ∼14 calculated for water-mediated PCET, compared to ∼7 for the direct process, arises from both vibrational and tunneling corrections. A classical limit for deuterium KIEs is often considered to be ∼7.[6] We emphasize that, without quantization of the nuclear motions, our corrections provide only crude estimates of the quantum dynamical effects.[5]

Dispersion interactions lower the reaction barriers for direct and water-mediated PCET by 1.1 and 1.7 kcal/mol, respectively. For ground and transition state structures optimized with and without empirical dispersion corrections,[16e,f] we find that the added dispersion interactions bring the phenol rings 0.2 Å closer together and stabilize the constrained transition state.

To examine possible solvent dielectric effects, we used the conductor-like screening model (COSMO).[16h] In contrast to pure pT in single file water chains, which was found to be strongly favored by a low dielectric environment ($\Delta E^‡$ was reduced by ∼4 kcal/mol when going from ε = 80 to 1),[19] here the PCET barriers for both direct and water-mediated processes with a small driving force are nearly independent of the medium dielectric constant. We observe only small shifts of 0.1 kcal mol^{-1} in

$\Delta E^{\ddagger}$ and 0.7 kcal mol^{-1} in ΔE upon increasing the medium dielectric constant from ε = 1 to 80 (SI Table 1). This weak dependence on the surrounding dielectric suggests that in the concerted PCET the oppositely charged electron and proton are electrostatically shielded from the environment. Indeed, for the water-mediated process with a large driving force and a more stepwise PCET process (Figure 2), we obtain larger shifts of ~2 kcal mol^{-1} in $\Delta E^{\ddagger}$. Additionally, we estimated the effect of the electric field created by the surrounding protein medium, as represented by point charges, and found maximal shifts of 0.3 and 0.6 kcal mol^{-1} from the gas phase values of $\Delta E^{\ddagger}$ and ΔE, respectively (SI Table 1). Polarization effects of the surrounding medium are thus overall small.

Jointly, our findings of possible electronic adiabaticity and of Brønsted slopes of $^1/_2$, for direct transfer, and 1 or 0, for water-mediated transfer, suggest that chemical modifications (e.g., through mutations of nearby residues) can be used to probe for water participation in PCET by measuring reaction rates and equilibria. In our RNR model system, we expect pT to proceed as an adiabatic process on the ground-state energy surface, due to the large separation from the electronically excited state. The qualitatively different response of the activation barriers of direct and water-mediated PCET to perturbations should thus be observable in kinetic measurements. Our results could provide a basis to probe perturbatively for water molecules serving as proton wires in biological PCET reactions, including those of cytochrome *c* oxidase and the oxygen-evolving center of photosystem II.[2]

■ ASSOCIATED CONTENT

Supporting Information. Additional data on spin density differences and environmental polarization effects. This material is available free of charge via the Internet at http://pubs.acs.org.

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