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Enol Tautomers of Watson−Crick Base Pair Models Are Metastable Because of Nuclear Quantum Effects

Alejandro Pérez,[†] Mark E. Tuckerman,[‡] Harold P. Hjalmarson,[¶] and O. Anatole von Lilienfeld*,[¶]

Department of Chemistry, New York University, New York, New York 10003, Department of Chemistry and Courant Institute of Mathematical Sciences, New York University, New York, New York 10003, and Sandia National Laboratories, Albuquerque, New Mexico 87185

Received March 9, 2010; E-mail: oavonli@sandia.gov

Abstract: Intermolecular enol tautomers of Watson−Crick base pairs could emerge spontaneously via interbase double proton transfer. It has been hypothesized that their formation could be facilitated by thermal fluctuations and proton tunneling, and possibly be relevant to DNA damage. Theoretical and computational studies, assuming classical nuclei, have confirmed the dynamic stability of these rare tautomers. However, by accounting for nuclear quantum effects explicitly through Car−Parrinello path integral molecular dynamics calculations, we find the tautomeric enol form to be dynamically metastable, with lifetimes too insignificant to be implicated in DNA damage.

Introduction

In their seminal paper, Watson and Crick highlighted the importance of the occurrence of pyrimidine and purine bases in their most plausible tautomeric form, that is, their keto- rather than their enol form, to produce correct matching.[1] A decade later, however, it was hypothesized[2] that proton tunneling could significantly enhance the formation of the rare tautomeric enol-forms, and thereby cause mismatch in the interstrand pairing of nucleobases, possibly leading to errors in DNA replication. These rare tautomeric enol-forms could spontaneously emerge through the antiparallel and concerted transfer of two hydrogen-bonded protons between adenine (A) and thymine (T), and between cytosine (C) and guanine (G). This hypothesis rests on the assumption that rare tautomers are dynamically stable and would persist on a time scale sufficiently long as to alter the cell's genetic information via the formation of mispairs. This conjecture was further supported by several computational studies suggesting that at least the GC tautomers would have sufficient lifetime to induce DNA damage through base pair mismatches.[3−12]

Experimental techniques, such as NMR, have failed to produce conclusive evidence for intermolecular tautomers of DNA bases.[13] Experimental results were only reported for the intermolecular tautomeric equilibrium of nucleobase pair analogues in the electronic excited state, which seems to favor rare tautomers: Kasha and co-workers detected the product tautomers in solutions of 7-azaindole dimer after photoexcitation.[14] More recently, Zewail et al. followed in real time the tautomerization of the same system using femtosecond techniques.[15] The product tautomer was detected and monitored using time-of-flight mass spectrometry, and these experiments provided insight into some mechanistic aspects of the decay.

Although the bonding pattern in 7-azaindole dimer (two N···H−N intermolecular hydrogen bonds) differs slightly from the canonical DNA base pairs (N−H···O and N···H−N), the authors noted the importance of quantum tunneling for this process. Using isotope substitutions in the intermolecular H-bonds, a dramatic increase in lifetime of the rare tautomer was found upon deuteration.[15] Experimental studies on other tautomerizable nucleobase analogues have also been reported.[16−19] Intramolecular proton transfer was studied spectroscopically in

[†] Department of Chemistry, New York University.

[‡] Department of Chemistry and Courant Institute of Mathematical Sciences, New York University.

[¶] Sandia National Laboratories.

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isolated nucleobase guanine[20,21] or uracil.[22] However, to date, no direct experimental evidence has been reported in support of the existence of the rare enol tautomers of DNA base pairs. It is, therefore, an open question whether nuclear quantum effects facilitate or hamper their formation.

The goal of this study is to investigate the role of nuclear quantum effects in the double proton transfer between Watson−Crick base pairs. To this end, we performed a computational experiment in which we simulated the double proton transfer with and without nuclear quantum effects. For the former, the Feynman path integral formalism was used.[23,24] Several other methods have been developed to study nuclear quantum effects in the calculation of molecular properties, including a wave packet methods for quantum dynamics,[25] generalized transition state theory,[26−28] the dispersed polaron method,[29] nuclear orbital and molecular orbital methods,[30,31] the nuclear-electron orbital method,[32,33] non-Born−Oppenheimer DFT,[34] and multicomponent DFT.[35]

We compare results from Car−Parrinello molecular dynamics (CP-MD) and Car−Parrinello path integral molecular dynamics (CP-PIMD)[36,37] simulations of corresponding DNA base pair models at ambient temperature in the gas-phase. The CP-PIMD method has been recently applied to model the nuclear quantum behavior of proton transfer in hydrogen-bonded systems, both in the gas phase[38,39,41] and in the bulk.[40,42,43] The PI (path integral), or quantized classical path methods, were also shown to be useful for studying biological systems such as enzymes[44] for which methods capable of dealing with nuclear quantum effects have recently been reviewed.[45]

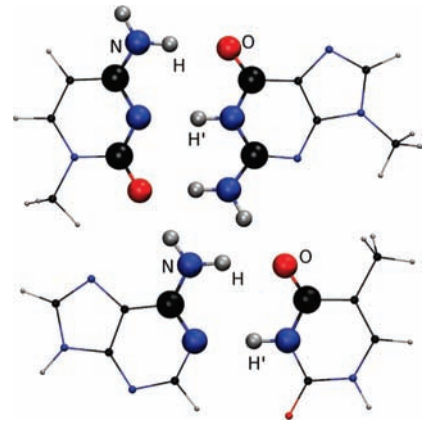

Figure 1. Canonical Watson−Crick base pairs superimposed on model systems. H′ is the most acidic proton and the reaction coordinate $\xi(\mathbf{r}) = r_{HN} - r_{HO}$ for the double proton transfer involves the less acidic proton, H. (Top) GC model (guanidyl-formaldehyde−methanimidamidyl-formide, big spheres) superimposed on GC (smaller spheres). (Bottom) AT model (formamide−formamidine, big spheres) superimposed on AT (smaller spheres). To saturate valency for the calculations, hydrogen atoms were added to both models.

In first principles PIMD, the nuclei are quantized following Feynman's path integral formalism[23,24] and the many-body potential is obtained "on-the-fly" from electronic structure calculations performed as the paths are sampled.[46,47] Thus, CP-PIMD accounts not only for thermal fluctuations and bond rearrangements, but also for nuclear tunneling and zero point energy effects. Our findings are in agreement with recent approximate quantum dynamics calculations by Shigeta et al. who investigated the isotope effect in the double proton transfer in DNA base pairs at 0 K using a two-dimensional fit of a reduced potential energy surface.[48] However, we note the importance of quantizing all degrees of freedom. As previously reported by one of us,[38] not only the transferring proton species, but also the heavy-atom skeleton should be quantized as the latter has significant nontrivial effects on proton tunneling. To the best of our knowledge, a full path-integral description of the double proton transfer in DNA base pair models has not previously been reported.

Computational Methodology

Model Systems of DNA Bases. We have performed a set of Car−Parrinello molecular dynamics calculations with classical (CP-MD) and quantized nuclei (CP-PIMD) for two model systems that closely mimic the hydrogen-bonding pattern of the two DNA Watson−Crick base pairs. These model systems are depicted in

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Table 1. Relative Energies (kcal/mol) for Structures Corresponding Approximately to Reactants (R), the Transition State (TS), and Products (P) for GC and AT Model Systems

	GC model			AT model		
method	ΔE_{P-R}	ΔE_{TS-P}	ΔE_{TS-R}	ΔE_{P-R}	ΔE_{TS-P}	ΔE_{TS-R}
RHF	13.50	14.65	28.15	11.80	9.47	21.27
MP2	9.37	4.04	13.41	8.62	4.37	12.99
CC2	**10.24**	**2.67**	**12.91**	**9.24**	**3.08**	**12.32**
PBE0	10.95	3.00	13.95	8.93	2.81	11.74
PBE	10.63	0.55	11.18	8.56	1.19	9.75
B3LYP	11.39	4.78	16.17	9.47	3.52	12.99
BLYP	**11.16**	**3.15**	**14.31**	**9.30**	**2.44**	**11.74**

Figure 1. The AT model was previously investigated[5] using classical nuclei, whereas the GC model is proposed here for the first time and has been validated (to be discussed later) by comparing its energetics to the full GC base pair results.[4] We assume these models to be sufficiently representative for the description of the double proton transfer in their full base pair counterparts.

The effects of the phosphate backbone, or the $\pi-\pi$ stacked neighboring base pairs, are second-order effects as recently shown by Zoete and Meuwly,[8] and are unlikely to affect our findings qualitatively. These authors found similar reaction barriers for the double proton transfer in an isolated GC pair as well as the GC pair embedded in a DNA strand (see Figure 3 in ref 8). The consideration of all these secondary effects is prohibitive in the path integral setting and our aim here is to quantify the contribution of nuclear quantum effects on the double proton transfer within nucleobase pairs.

Choice of the DFT Exchange-Correlation Functional. We have investigated the performance of several DFT functionals by comparing to second-order approximate coupled cluster theory (CC2) on various geometries on the potential energy surface. The comparison is displayed in Table 1 which also includes the values of other single Slater determinant methods, such as Hartree−Fock and second-order Møller−Plesset perturbation theory. All values were computed using the code TURBOMOLE[54] and the basis set *def-TZVPP*.

Table 1 shows that Hartree−Fock performs poorly and grossly overestimates all the barriers. Inclusion of electronic correlation is expected to improve the energetics. Indeed, hybrid functionals PBE0[55] and B3LYP[56] perform very well but their associated computational cost for first principles MD calculations is prohibitive. As one would expect for a generalized gradient approximated functional, PBE[57] seems to slightly underestimate barriers. While also known to underestimate barriers,[58] for this process, BLYP deviates from coupled cluster at most by 1.5 kcal/mol and therefore appears to represent an acceptable compromise between accuracy and computational cost. Our BLYP values also agree well with earlier quantum chemical estimates of the full base pairs in gas phase computed at the MP2/*6-31G***[4,6] and B3LYP/*6-311++G***[7,59] level. We also confirmed the TURBOMOLE BLYP values in Table 1 with the ones obtained from our CP-MD setup (not shown).

In summary, the BLYP functional represents the best compromise between accuracy and cost for our model systems, and it is the functional adopted in our Car−Parrinello MD calculations. We note, however, that the point of this study is not to investigate BLYP's suitability for energy barriers but rather to demonstrate the importance of nuclear quantum effects on free energy barriers at a given level of theory.

Car−Parrinello Path Integral Molecular Dynamics. All molecular dynamics simulations were performed at 300 K using the empirical generalized gradient approximation BLYP[60−62] within the Kohn−Sham (KS) density functional theory (DFT).[63] All calculations were carried out at the Γ-point under isolated molecule boundary conditions as formulated by Martyna and Tuckerman[64] in CPMD.[65] The fictitious mass employed to propagate the electronic orbitals was set to 400 au. Normal mode variables were used for the discrete path integral, together with massive Nosé−Hoover chain thermostats[66] to ensure adequate canonical sampling.[36,37] A Trotter number of 16 beads was used for the quantization of all nuclei.

For the AT model complex, valence electronic orbitals were expanded using a plane wave kinetic energy cutoff of 100 Ry in an isolated box of $12.5 \times 10.5 \times 6.5$ Å^3. Core electronic orbitals were represented by Goedecker et al.'s pseudopotentials[67] as published by Krack.[68] For the GC model system, a plane wave cutoff of 75 Ry was used in an isolated box of $15 \times 15 \times 8$ Å^3. Core electronic orbitals were represented by Troullier−Martins pseudopotentials.[69]

Free-Energy Sampling of Rare Events. We compute the classical potential of mean force,

$$F(\xi') = -\frac{1}{\beta}\ln\left[\frac{C}{Q(\beta)}\int d\mathbf{r}\, e^{-\beta U(\mathbf{r})}\delta(\xi(\mathbf{r}) - \xi')\right] \qquad (1)$$

for the classical free-energy profiles along the proton transfer reaction coordinate, $\xi = \xi(\mathbf{r})$ (see Figure 1). In this expression, $Q(\beta)$ is the classical canonical configuration partition function, C is a normalization constant, and $U(\mathbf{r})$ is the KS-BLYP potential energy of the system at the microstate $\mathbf{r}$.

Direct sampling of eq 1 for rare events is difficult in ordinary MD and special techniques are required to enhance the exploration of inaccessible regions. Since the double proton transfer in AT and GC has barriers well above the thermal energy, we have implemented a combination of umbrella sampling[70] with CP-PIMD and standard CP-MD to compute the free-energy profiles with and

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without nuclear quantum effects, respectively. Within umbrella sampling, one restrains the position of a selected reaction coordinate ξ to a certain window i (defined by a reference value ξ_i) typically using a harmonic bias potential, $U_i(\xi) = (K/2)(\xi - \xi_i)^2$. The harmonic force constant K was set to 0.1 Hartree/Bohr^2. In total, 14 and 11 umbrella windows were collected for AT and GC models, respectively. The length of each umbrella window was 3.6 ps and the time step was 0.072 fs. The free-energy profile is thereafter reconstructed from the biased (or non-Boltzmann) MD simulations using the weighted histogram analysis method.[71,72]

In PIMD simulations, the umbrella potential is applied on the centroid mode of the ring polymer, defined by $\xi = (1/P)\sum_{s=1}^{P}\xi_s$, where ξ_s correspond to the reaction coordinate values of the different imaginary time slices of the ring polymer, and P is the Trotter number.[24,73] The quantum potential of mean force is computed according to eq 1 where the potential energy $U(\mathbf{r})$ and the classical partition function Q are substituted by their quantum analogues, the effective classical potential and the quantum partition function, respectively.[24] These forces bias the sampling of the centroids so the generalized reaction coordinate ξ remains near the reference value ξ_i defined for umbrella window i.

The choice of a single reaction coordinate is a nontrivial task for double proton transfer events, and in principle a complete characterization of this process would require a prohibitive full two-dimensional map. Here, the relative distance involving N, O, and the less acidic proton H ($\xi(\mathbf{r}) = r_{HN} - r_{HO}$, see Figure 1) was chosen as a reaction coordinate for the GC and AT models. This reaction coordinate was used to drive the proton transfer reaction from the nitrogen of one moiety to the oxygen of the other,

$$-\mathrm{N} - \mathrm{H}\cdots\mathrm{O}- \Leftrightarrow -\mathrm{N}\cdots\mathrm{H} - \mathrm{O}- \qquad (2)$$

According to previous work,[5] for the case of the AT model, the complete proton transfer of H from N to O (see Figure 1) marks the appearance of products. By the time H migrates to O, the other proton (H′) has already been transferred to preserve electroneutrality. Thus, this one-dimensional coordinate appears sufficient as it approximates well the rate-determining step of the reaction. Similar arguments hold for the GC model dimer. We note in passing that in contrast to the neutral system, single proton transfer is the favored mechanism in charged DNA bases (which may be generated by radiation or by oxidizing agents), and it has been investigated computationally by Bertran and co-workers.[9]

Results

Free-Energy Profiles. Figure 2 (top) displays the classical and the quantum free energy profile for the intermolecular double proton transfer in the GC model at 300 K. The profiles feature a prominent asymmetric barrier characteristic of a late transition state. The geometries of the product and transition state are very similar, in agreement with the Hammond postulate.[74] Classically, an equilibrium free-energy difference and a reverse barrier of approximately 10 and 4 kcal/mol, respectively, are predicted. The agreement with the literature results[4,6,7,11,59] for classical nuclei suggests that our model system captures well the fundamental features of the double proton transfer of the complete DNA base pair system despite its lack of aromatic cores. Upon quantization of the nuclei, the reaction free energy difference increases approximately by 1 kcal/mol due to zero point motion with respect to the classical case. More interestingly, the reverse barrier becomes negligible (less than 1 kcal/mol), and the quantum system shows no clear minimum structure on the rare tautomer side. According to transition state theory, such a decrease in the barrier would speed up the reverse reaction by at least 2 orders of magnitude. A path integral formulation of quantum transition state theory can be found in references.[75–77]

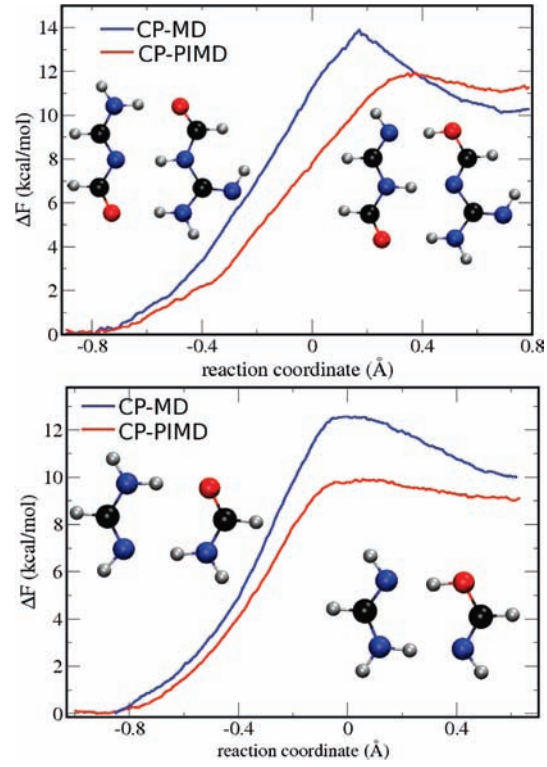

Figure 2. Classical (blue) and quantum (red) free-energy profiles for the double proton transfer in GC (top) and AT (bottom) models at 300 K. The insets show the reactants (left) and the rare tautomer products (right).

In the case of the AT model, the double proton transfer takes place via a concerted and asynchronous mechanism.[5] Figure 2 (bottom) shows the free-energy profile for the isolated AT model at 300 K. This profile is similar to the one for the GC model dimer, although the shape of the classical barrier is smoother. Classical simulations predict a free-energy difference of approximately 10 kcal/mol between two well-defined minima and a reverse barrier of approximately 2.5 kcal/mol. The classical free-energy barrier at 300 K agrees well with the potential energy barrier computed at 0 K (see Table 1). Also for this base pair model, nuclear quantum effects have a dramatic impact on the reverse barrier, which is reduced to less than 1 kcal/mol. In contrast to the GC base pair model, the free-energy difference between the tautomers decreases by ~1 kcal/mol.

In both models, the inclusion of nuclear quantum effects decreases the forward barrier significantly, indicating that the rare tautomers are more frequently visited than within the classical picture, in accord with the original tautomeric hypothesis.[2] However, the virtually complete suppression of the reverse barrier suggests that the rare tautomers are dynamically metastable and exhibit insignificant lifetimes. This prediction is in agreement with the recent work of Shigeta et al. who investigated the isotope effect in DNA base pairs using a two-dimensional fit to a reduced potential energy surface.[48]

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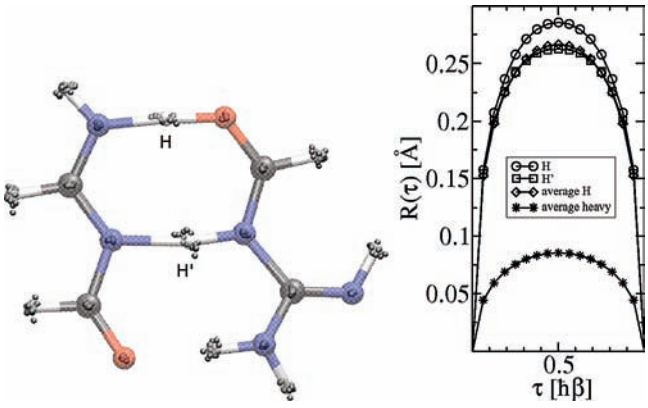

Figure 3. (Left) Snapshot of the transition state of the double proton transfer in the GC model from a typical PIMD run as presented in ref 78. H is the restrained and less acidic proton. (Right) Root mean square displacement correlation function $R(\tau)$ as a function of the normalized imaginary time, $\tau = (s-1)/P$ (in units of $\beta\hbar$ and for $s = 1, ..., P+1$) for the transition state window of the double proton transfer in the GC model.

Nuclear Quantum Nature. To better illustrate the quantum delocalization of the nuclei, a snapshot[78] from typical PIMD runs at the transition state umbrella window is shown in Figure 3 (left) for the GC model. The beads correspond to the discretized path in imaginary time, and their spread is evident for the light hydrogen atoms. To quantify the quantum delocalization of different nuclei, root-mean-square displacement correlation functions, $R(\tau) = \langle|\mathbf{r}_1 - \mathbf{r}_\tau|^2\rangle^{1/2}$ (angular brackets denote canonical average) are displayed in Figure 3 (right) as a function of the normalized imaginary time τ. Here, $\tau = (s-1)/P$ is given in units of $\beta\hbar$, and $s = 1, ..., P+1$. The quantity $R(\tau = 0.5)$ is largest for the restrained proton (H) and reaches up to 0.286 Å The quantities $R(\tau)$ for all other protons, hardly distinguishable, have been averaged, and the maximum averaged value reaches ~0.02 Å less than for H. Interestingly, the quantum nature of the other proton involved in the transfer (H′) is slightly smaller than for the average of all other protons. As one would expect, the maximum average value of $R(\tau)$ for all heavier atoms, exhibiting less quantum nature, is significantly smaller, and reaches only 0.086 Å. For the AT model, entirely analogous behavior is found (not shown).

Discussion

On the basis of our findings it seems unlikely that the rare tautomer exists sufficiently long to contribute in any significant amount to base pair mismatch during DNA replication or translation. For externally generated (e.g., through radiation or oxidative damage) charged or radical DNA base pairs, recent computational studies[7,9,59] using classical nuclei have shown that charged protonated base pairs display smaller activation barriers. As mentioned in the Introduction, the electronic structure in the excited state also appears to stabilize rare tautomers.[15] CIS calculations in the low-energy excited single electronic state by Moreno and co-workers[79] have shown that the double proton transfer in the AT pair becomes more facile than in the ground state. Whether nuclear quantum effects similarly influence the cases of externally generated (e.g., through radiation or oxidative damage) electronically excited, charged, or radical DNA base pairs will be a subject of future studies.

The models employed here have a rather small entropic contribution to the barrier in gas phase owing to the small molecular reorganization, that is, thermal fluctuations are small in a rigid heavy-atom-skeleton, playing a minor role in the proton hopping mechanism. In solution, this may not be the case, and solvent molecules may reorganize to stabilize the transition state, leading to a reduction in entropy. However, one would expect a competing effect of favorable enthalpic interactions with the solvent. The degree to which our results would change if the full base pairs, hydration effects,[12] coordination to a metal cation,[6] or molecular environment were included remains to be elucidated. This effect has recently been shown to be small for classical nuclei.[8]

Solvent can affect the tautomeric equilibrium between DNA bases due to the different H-bonding patterns and dipole moments of the tautomers. X-ray studies on oligonucleotides with mispairs have suggested that water could have a stabilizing effect on the nucleobase mispair via the formation of favorable interactions with hydrophilic regions of the nucleobases.[49] In a recent study, Zewail and Kwon reexamined 7-azaindole in solution and found the rate of proton transfer to be significantly dependent on polarity and on the isotopic composition in the pair.[50] The presence of solvent could also induce the formation of tautomers between water and a single nucleobase, which would be relevant in the replication mechanism when DNA is unwound by the enzyme helicase. The solvent effect on the various tautomeric equilibria of isolated DNA bases was investigated by Hobza and Kabeláč[51] who studied computationally microhydrated complexes of DNA bases. Although the inclusion of solvent is currently computationally prohibitive in PIMD calculations, we remark, however, that the environment of nucleobases in DNA is different than that in free solution. The interbase hydrogen bonds are embedded in the DNA groove, protected from solvent molecules, and the DNA interior has a low dielectric constant.[52,53] Furthermore, the attack of a water molecule on the reaction site is impeded owing to the high directionality of the hydrogen bonds.

Finally, another pathway that would further reduce the lifetime of unusual tautomers would be through a nonradiative decay into heat (e.g., via collision deactivation). However, our model systems, due to their isolated nature, lack the necessary coupling to other degrees of freedom for this conversion. Investigation of a more complex system is currently prohibitive within the CP-PIMD methodology.

Conclusion

CP-PIMD and standard CP-MD calculations have been carried out to study the double proton transfer reaction in isolated model systems of DNA Watson−Crick base pairs AT and GC at 300 K. Our results suggest that nuclear quantum effects, including tunneling and zero point motion, play a decisive role. Their inclusion leads to a near complete suppression of the reverse barrier from the rare enol to the canonical keto tautomer, thereby rendering the former dynamically metastable. This finding is in agreement with a recent approximate quantum dynamics calculation using a two-dimensional fit of a reduced potential energy surface.[48] At the transition state, the restrained proton is found to exhibit a larger root-mean-square displacement correlation function than all other protons, underscoring its delocalized quantum nature. On the basis of energetic

(78) For a path integral animation of the double proton transfer reaction in AT and GC models, see Supporting Information. The movies do not show any real time dynamics but only the sampling of different microstates of the system, restrained to the transition state umbrella window, at 300 K.

(79) Guallar, V.; Douhal, A.; Moreno, M.; Lluch, J. M. *J. Phys. Chem. A* **1999**, *103*, 6251–6256.

arguments, our results are expected to carry over to the true Watson−Crick base pairs, unless external factors help to selectively stabilize the enol rare tautomers. In this regard, it will be important to examine the role of the DNA polymer and the solvent. We conclude that nuclear quantum effects appear to enhance the dynamic stability of the canonical DNA base pair model keto tautomers, rather than to facilitate the formation of the tautomeric enol forms. This interpretation is consistent with the aforementioned lack of experimental evidence for the rare intermolecular tautomers.

In a broader context, we support the general observation[45] that even at room temperature nuclear quantum effects can qualitatively affect the expected outcome of processes that involve hydrogen-bonding, a ubiquitous feature of many biological systems, such as DNA or the active sites of enzymes.

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Supporting Information Available: Structures used in Table 1 and path integral animations of our DNA base pair models. This material is available free of charge via the Internet at http://pubs.acs.org.

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