# Proton Transfer in Adenine−Thymine Radical Cation Embedded in B-Form DNA

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ABSTRACT On the basis of pK_a measurements, it has been predicted that proton transfer will not occur in the radical cation of the A/T base pair (A: adenine, T: thymine). Testing this prediction, we have performed simulations as a function of time on an A/Toligomer missing one electron, in solution, using the code CP2K. We find that proton transfer occurs rapidly at temperatures below room temperature. We suggest that the difference in behavior of $(A/T)^{\cdot +}$ from that predicted on the basis of pK_a measurements is the effect of hydration. Hydration also appears to have effects not previously considered on hole motion in solution.

SECTION Biophysical Chemistry
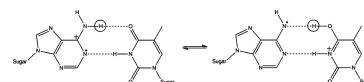
Protonation and deprotonation have been greatly studied, theoretically and experimentally, in DNA in single bases, in Watson−Crick base pairs, and in longer sequences. It has been shown experimentally, in agreement with predictions from pK_a measurements, that at room temperature proton transfer (PT) takes place in guanine−cytosine (G/C) base pairs charged positively or negatively. In the cation, a hole is captured by the guanine, which has the lowest oxidation potential of the bases, creating G^+/C. Creation of the hole causes the base pair to become acidic, resulting in PT: the proton in the central hydrogen bond linking G and C moves from its position close to the N_1 nitrogen of G to a position close to the N3 nitrogen in C.[1−4] The proton-transferred form of the cation may be written $G/(H^+)C$. As a result of proton transfer, the anion goes from G/C^- to $G(N1 −H^+)^-/C(N3 +H^+)$. PT is important because the chemical properties of the G/C base pair and the dynamic properties, in particular, the motion of the hole or the excess electron, are different in the proton-transferred form from the untransferred form.[5]

Density functional theory (DFT) calculations were in agreement with experiment for the case of an isolated $(G/C)^-$ in showing PT to be exothermic by a few kilocalories per mole and a small energetic barrier for the transfer. For $(G/C)^+$, however, until recently, DFT calculations were in disagreement with experiment, predicting PT to be endothermic by ∼1 kcal/mol, with a significant barrier for the transfer.[6] Quite recently the contradiction between theory and experiment was resolved by Kumar and Sevilla.[5] They showed, using DFT, that taking into account the first hydration layer in $(G/C)^+$ leads to agreement with experiment.

Whether PT occurs in A/T base pairs is also of interest. Sequences with a number of contiguous A/T pairs have been greatly studied since it was found, ∼10 years ago, that a hole injected into such a sequence travels with relatively little attenuation through as many as 16 A/T's.[7,8a,b] This suggested that such sequences might be used as conductors in a circuit made up of DNA. On the basis of the pK_a measurements, PT in an $(A/T)^+$ base pair (Scheme 1) was judged to be unfavorable.[1,3] There is no doubt that A^+, adenine cation, is a strong acid, but it has been considered that T, thymine, is too weak a base for much protonation to occur. Pulse radiolysis measurements of the transient spectrum of $(A/T)^-$ have shown an absorbance change that is attributed to irreversible protonation on the C_6 site of thymine.[6] However, this is most likely due to a proton or deuteron picked up from solution by the thymine, changing T^- to $T(H^+)$ or $T(D^+)$.[9] There has been no report of PT being observed in $(A/T)^+$. We report in this Letter that simulations we have carried out for an A/T radical cation in solution have shown PT in $(A/T)^+$. We suggest that the difference in the behavior of A/T from that predicted in refs 1 and 3 is due to the effects of hydration.

We have carried out QM/MM simulations on a 10-mer, A/T duplex of canonical B-DNA that was generated using the AMBER 9 software suite.[10] To prepare the system, 18 Na^+ counterions, matching the number of phosphates, were placed along the backbone, and the initial structure was equilibrated. Following this minimization, the system was solvated with a truncated octahedron of ∼2000 TIP3P waters such that the edge of the water box is a minimum of 8 Å from any atom in the system. The system was again minimized, restraining the DNA atoms with a 500 kcal mol^{-1} $Å^{-2}$ force constant and then minimized once more without restraints on any of the atoms. Five contiguous A/T base pairs (numbers 4 through 8) and their sugar plus backbone atoms were chosen as the quantum system, with one electron removed to introduce a hole. The code used for the simulations was CP2K, which is freely available. It provides a general framework for DFT using a mixed Gaussian and plane waves approach and classical pair and many-body potentials.[11]

Scheme 1. Schematic Diagram Showing Predicted Proton Transfer (PT) Reaction in a One-Electron-Oxidized A/T Base Pair[a]
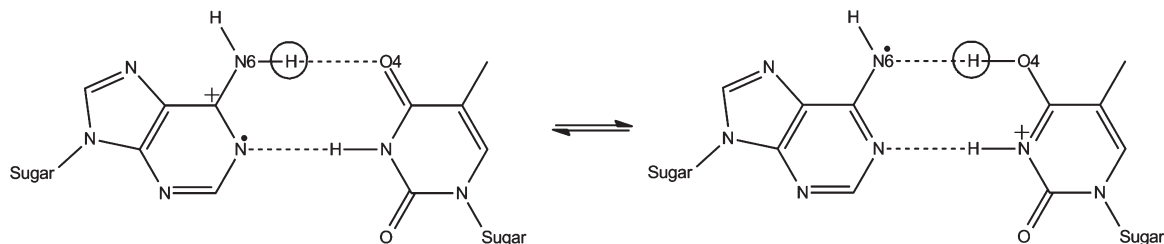
[a] Circle shows the locations of the proton in the base pair.

Following the calculations of Mantz et al.,[12] in describing the quantum system, we used the Kohn–Sham energy functional expression at the restricted open shell DFT–Becke–Lee–Yang–Parr level, ROBLYP,[13,14] corrected by applying an empirical self-energy correction as described in ref 15. The remaining energy terms describing the molecular mechanical systems in CP2K are standard.[12] We used a double-ς valence basis set with polarization functions. A later check showed that the error in using double-ς rather than triple-ς gave rise to a difference in the N–H and H–O bond lengths that we calculated to be < 2%.

Two simulations were carried out, one with an initial temperature of 20 K and the other with an initial temperature of 300 K, both with a time interval of 0.5 fs. The time length of the simulation was 600 fs for the former case and 460 fs for the latter. In both cases, the temperature was not kept constant but allowed to rise with time. To plot the wave functions, we used the calculated spin density $m(\mathbf{r})$.

In both simulations, we found, as was found in ref 12, the wave function initially spread out over the five adenines in the QM region. A delocalized wave function of four or five A's had been predicted in previous calculations that were simplified by treating the water surrounding the DNA as a continuum.[16,17] The delocalized wave function is ascribed to a polaron, which is a compound particle in the sense that the polarization of the surrounding medium by the hole must move with it. There is experimental evidence of the polaron wave function extending over about four or five A's; it is summarized in ref 18. The polaron wave function at 300 K that we obtained for very early times in our simulation is similar to that obtained in early times with the CP2K simulation of ref 12. (See figure 2 in that reference.) Both extend over 5 A's and have very small amplitude on the T's for $m(\mathbf{r}) \geq 0.001$, the value chosen for figure 2 of ref 12. In both ref 12 and our simulations, the amplitude of the polaron does not fall off monotonically with distance from its center, presumably reflecting variations under local conditions at each A. In our case, we found the amplitude variations to be somewhat smoother than those found in ref 12.

For both the "20 K" and the "300 K" runs, the extended wave function just described starts changing at ∼10 fs. Beginning at that time, the polaron contracts rapidly. By ∼25 fs in the "20 K" run, 20 fs in the "300 K" run, it is localized on one adenine with a small amplitude on each of the neighboring A's. A typical wave function for the contracted polaron is shown in Figure 1 for the "20 K" run with $m(\mathbf{r}) = 5 \times 10^{-5}$ to encompass

99% of the spin. Once contracted, the volume of the polaron remains roughly constant for both the "20 K" and "300 K" runs over 450 fs of the simulations, with random fluctuations up to ∼15 to 20% of its average volume.

Further details of what happens to the polaron over the "20 K" run are shown in Figure 2 for the base pair on which the polaron is concentrated, in this case, base pair 6 at the center of the QM region. Figure 2b relates to the interbase hydrogen bond with N6 at the adenine end, O4 at the thymine end, and the proton between them. (See Scheme 1.) In Figure 2a, the temperature is plotted as a function of time, whereas in Figure 2b, the bond lengths are plotted. It is seen that at the beginning the proton is in its position in the Watson–Crick conformation, ∼1.0 Å from N6 and ∼1.8 Å from O4. The bond lengths start to change almost immediately, and by 60 fs, the proton has moved so that it is 1.0 Å from O4 and 1.8 Å from N6. Therefore, proton transfer has taken place. As expected, on the other A's in the QM region, the proton has not moved from its position near N6. Figure 2a shows that by 60 fs the average temperature is 170 K. In our "20 K" run, the proton remains in the transferred position to 465 ps, where the temperature is 225 K, although the N6–H distance does vary. It appeared that the big decrease in the N–H distance at 465 fs, coupled to a small increase in the O4–H distance, could be an indication that the proton transfer was about to reverse. When the length of the run was increased by 100 fs, however, it was seen that that was not the case.

We consider now the results of the "300 K" run. In Figure 3b are plotted the variation with time of the O4–H and N6–H bond lengths for base pair 5, where the polaron was concentrated during that run. It is immediately apparent that the proton remains 1.0 Å from N6. Although there are fluctuations in the O4–H distance, there is no proton transfer on base pair 5 nor in the other base pairs, in the temperature range 300–340 K. This is in line with the relatively facile hole transport observed in a sequence of four or more A's.[19] Long experience with low-temperature transport in DNA, in irradiated samples, for example, has shown that when PT occurs, causing separation of spin and charge, the hole tends to localize. Separation of spin and charge occurs in many different systems and has been found to cause localization.[20]

The explanation for PT occurring at low temperatures in the $(A/T)^+$ case, in contradiction with what was expected from the pK_a values, should be similar to that offered by Kumar and Sevilla for $(G/C)^+$, namely, that the contribution of the first solvation shell lowers the energy for the proton-transferred
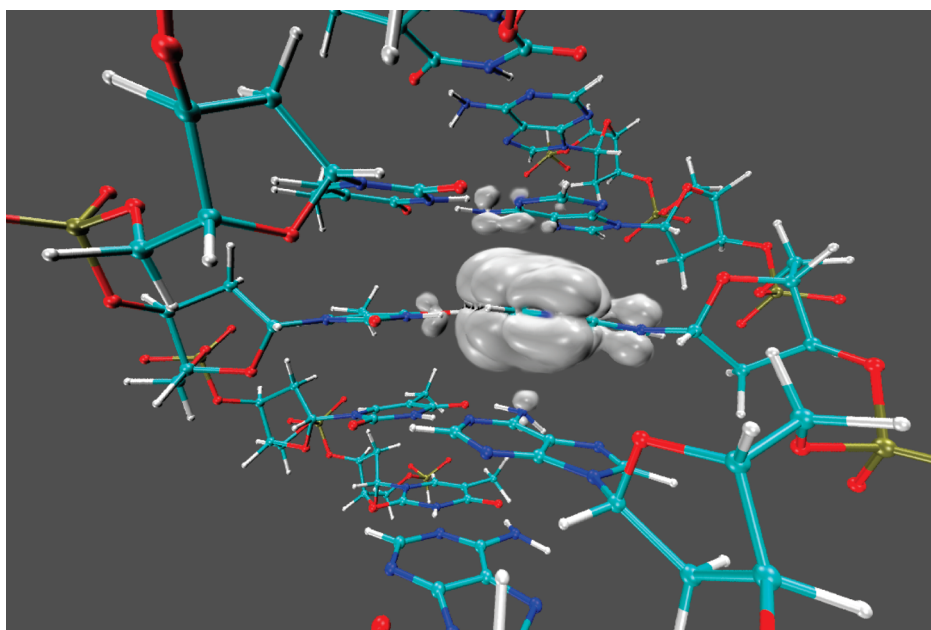
Figure 1. Contracted hole polaron wave function at "20 K" after proton transfer.
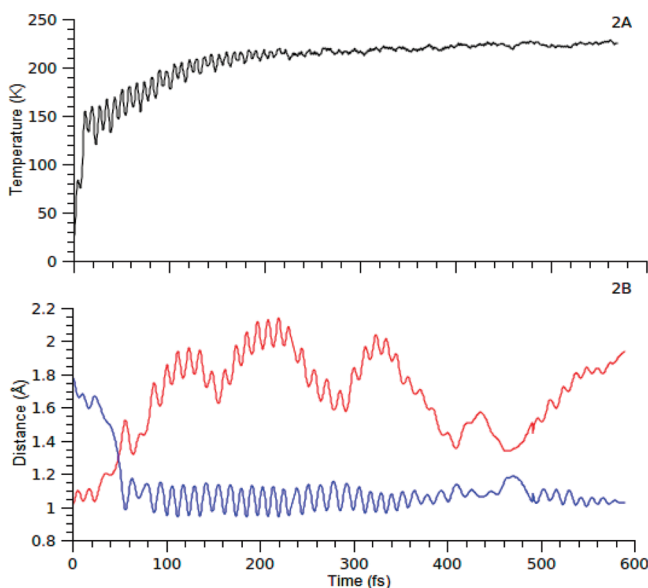

Figure 2. (a) Temperature as a function of time for "20 K" run. (b) Lengths of N−H (red) and H−O (blue) bonds as a function of time for "20 K" run.
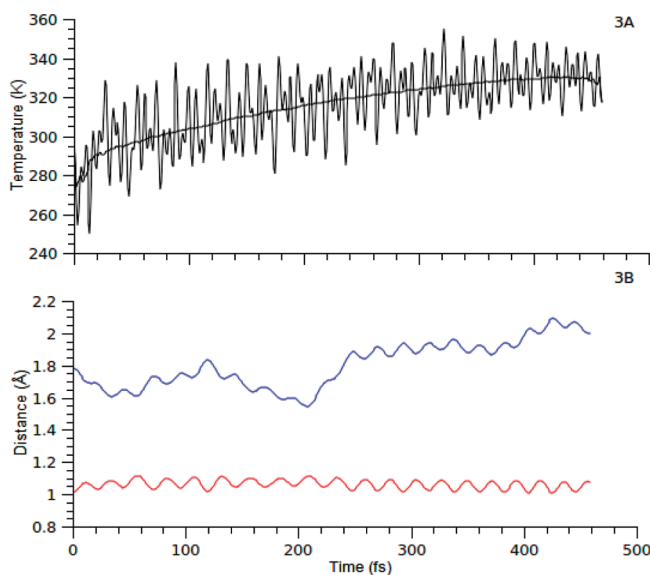

Figure 3. (a) Temperature as a function of time for "300 K" run. (b) Lengths of N−H (red) and H−O (blue) bonds as a function of time for "300 K" run.

case. In the optimum configuration for PT, the waters in the first solvation shell form a ring around the G where the hole is localized.[5] It is of interest in this context that our simulation showed that PT did not occur in $(A/T)^+$ until the hole wave function has changed from being spaced out along the line of A's to the contracted form, where it is almost entirely on one base. The change in geometry would make it possible for the waters in the first solvation shell to make a ring around the adenine similar to that formed around the guanine in $(G/C)\cdot^+$ in reducing the energy of the proton-transferred state. Note also that Sevilla et al., in studying single strand adenine

oligomers, have found that base stacking sets up a significant barrier to deprotonation.[21]

According to the calculations of ref 5, with the contribution of 11 waters, the energy of the proton-transferred (G/C) radical cation is lower than that of the radical cation with the proton in the position for the Watson−Crick conformation by ∼1.2 kcal/mol. They find the barrier height between the "normal" state and the transition state to be ∼1.4 kcal/mol. Therefore, there is a barrier for a transition of the proton from the transferred position to the normal position of ∼2.6 kcal/mol, which is large enough to make the transferred state the dominant one

at room temperature. For the (A/T) cation, the fact that we see the untransferred "normal" state at room temperature indicates that the barrier for return to the "normal" state is smaller than that of $(G/C)^+$.

The results cited above give rise to some questions. An important one is that of the contradiction between the various experiments indicating that the polaron is delocalized, spread over four or five A's, at room temperature,[18] and the "300 K" simulation gives from 15 to 20 fs up to ~0.5 ps a contracted polaron limited to about one A. The extent of the polaron clearly varied somewhat with time during the simulation but certainly did not recover its full delocalized extent. Undoubtedly, if the simulations were carried out to times of about microseconds at room temperature and above, then the delocalized form of the polaron should be the one seen for the majority of the time.

Theoretical calculations suggest that motion of the water molecules could result in hole wave function changes from contraction to delocalization or expansion and vice versa. Mantz et al. suggested that at the root of these changes is the fact, demonstrated by them, that an electronegative nitrogen (N3 or N7) on a neutral adenine can form a strong bond with a water hydrogen, whereas such a bond does not form on a charged adenine.[12] The latter is not unexpected; the positive charge on the A would repel a proton. One can imagine a scenario in which, starting from a delocalized polaron in a region with a relative dearth of water or hydrogens facing the polaron, water floods into the region supplying hydrogens that could form bonds with N3 or N7 of the adenine at sites where the polaron amplitude is small. This would drive those portions of the polaron into the central sites where the polaron amplitude is larger. It is reasonable that such a process could end up with the hole contracted to one or two A's. If, subsequently, the water ebbed away, then the reverse process could occur and the polaron could be reconstituted.

Another example of the important effect of water on the distribution of hole charge among adenines is provided by calculations of Adhikary et al.[21] They show that for an adenine dimer cation radical surrounded by water molecules, optimization of the wave function leads to 73% of the spin (and charge) of the hole on one adenine and 27% on the other. This provides another effect that might contribute to the contraction of the polaron.

In summary, we conclude that, in contradiction with predictions based on pK_a measurements, proton transfer from A^+/T to A/(H^+)T occurs at low temperatures. Our simulations suggest that the latter form is dominant up to perhaps 225 K, and the former is dominant at room temperature and above. We suggest that stability of the proton-transferred form is due to the water that solvates the A/T cation. In our "300 K" simulation, it is found that initially the hole polaron wave function extends over ~5 As, which is in agreement with the prediction of previous calculations based on a simplified model (continuum water)[16,17] and with the simulations of Mantz et al.[12] The same extended wave function was initially found in the simulation we carried out in the "20 K" run. For our simulations in both the lower temperature and higher temperature ranges, after 10 to 15 fs, the extended wave function of the polaron contracts, initially to a single A. Mantz

et al. suggest that the contraction is due to the water hydrogens interacting with the adenines. In the "20 K" run, proton transfer takes place, completed ~50 fs after the polaron contraction. The contraction in polaron size should have a considerable effect on the polaron mobility. However, because it does not entail separation of spin and charge, it is not expected to cause the immobilization or near-immobilization found due to proton transfer.

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