Mutations in DNA under the Effect of Electric fields: A Multiscale Study

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1. Abstract

There are many known mechanisms that lead to DNA mutations. Some occur spontaneously, but others are induced by external factors such as electric fields, intercalators, radiations, etc. One of the mechanisms that occurs spontaneously, but also gets altered by external electric fields, is the double proton transfer process between DNA base pairs. In order to understand the kinetics and thermodynamics of this mutation in the absence and presence of electric fields, work has been done at the quantum mechanics (QM) level on simplified models of DNA, and at the multiscale quantum mechanics/molecular mechanics (QM/MM) on a realistic model of DNA. The results show that the rate of tautomerization in the simplified models can be increased by a factor of *ca.* four under the effect of electric fields that are in the order of $5x10^9$ V/m. It was also shown that the tautomerization in the AT is likely to happen through tunnelling. The study on the realistic model helped explain the details of a mutation bias that was observed experimentally, namely the conversion of the GC to AT base pairs [1].

2. Introduction

The double proton transfer in DNA is a reaction that involves the hydrogen atoms of the hydrogen-bonds between the base pairs. For example, in GC, as the proton in G moves towards C, that of C moves towards G creating the rare tautomer G^*C^*. Upon DNA opening for the replication, G^* or C^* are then complemented with a nucleotide other than C or G (i.e. A or T) [2]. Then, upon the second cycle of DNA duplication, the GC is converted to an AT. This bias in the conversion of GC to AT has been also explained using QM studies [3] which showed that the DPT in GC is energetically more favourable than that in AT.

GC has three hydrogen bonds that involve thee protons (H_1, H_2 and H_3), meaning there are three possibilities of double proton transfer reactions: between H_1 & H_2, H_1 & H_3, and H_2 & H_3. However, one of the possibilities is more favourable and dominant over the other two. It is probable that only a single proton transfers (SPT) from one nucleotide to the other, leading to a zwitterion e.g. G^-C^+. The double proton transfer (DPT) can occur either synchronously or in a concerted mechanism. In the former, both protons move together and the reaction happens through only one transition state. In the latter, each proton moves separately and the potential energy surface would have more than one transition state. It is important to fully understand these reactions as the point mutations can accumulate over time and eventually lead to genetic diseases

like cancer. If controlling these reactions is well understood, then it would be possible to prevent these mutations and therefore have preventive therapy for some genetic diseases.

Exposure to high electric fields has been linked to higher prevalence of cancer cases, especially brain cancer for adults and leukaemia for kids. It is thus key to consider the DPT and SPT in the presence of electric fields. In the microenvironment of DNA, if a charge is placed halfway in its horizontal cross-section, the electric field generated at this point would be in the scale of 10^8 to 10^9 V/m, which is in the range of the fields inside a phospholipid membrane. This field range is about one order of magnitude greater than the fields used in practical medical uses. This work is meant to show the details of the kinetics of the SPT and DPT in the presence and absence of electric fields using QM, a multiscale approach (QM/MM) and molecular dynamics while quantifying the errors that result from the computational simulations.

3. Methodology

For models of single isolated base pairs (i.e. GC or AT) that are studied in the gas phase, density functional theory (DFT) models such as B3LYP was used with a Pople basis set. B3LYP performs well for hydrogen bonded systems like an isolated base pair that is not exposed to stacking pi-interactions. For accurate results in the real model of DNA, it is mandatory to account explicitly for dispersion. For the isolated base pairs, the electric field was applied using Gaussian. The fields were applied in three different directions (x, y and z) and two different orientations each (i.e. +x and -x, +y and -y, and +z and -z) where the x orientation is aligned with the movement of the double proton transfer. The applied fields ranged from 10^8 to 10^9 V/m, with five field strengths considered within this range. Vibrational analysis was completed for all optimized geometries to confirm whether the structure is a transition state of a reactant/product. To learn about the mechanism of the proton transfer reactions, intrinsic reaction coordinate calculations were done using the transition state structures as a starting point.

For the realistic model of DNA, a dodecamer was considered at a QM/MM level as it is not tractable to study such a realistic model of DNA fully at the QM level. In this model, three consecutive stacked base pairs are considered at the QM level and the rest of the system (including the explicit water solvation and sodium counterions) are considered at the MM level [4]. The advantage of this multiscale model is gaining the accuracy of the QM level (where the chemistry is taking place) while accounting for the rest of the system at a cheaper classical level. The original structure was obtained from an x-ray structure (PDB ID: 1BNA). A total of 10 simulations were completed using NAMD with AMBER *parmbsc1* forcefield to thermalize the system from 50 K to 300K over 30 ps each. A total of at 25 snapshots were collected from the MD simulation to perform on them QM/MM calculations using ChemShell 3.7 linked with NWChem 6.6 and DL-POLY. For the QM part, B3LYP was used with the explicit correction for dispersion, XDM (exchange-hole dipole moment), combined with an augmented Dunning double zeta basis set. Climbing nudge elastic bands were used to compute the reaction pathways. The vibrational analysis was obtained by calculating the Hessian in order to determine the kind of the molecule (i.e. transition state versus reactants or products).

4. Results and Discussion

It was found that the kinetics of the isolated GC can increase with stronger fields applied along the double proton transfer movement. The electric fields helped slightly stabilize and increase the half-life of the tautomer forms of the GC base pair (i.e. the G*C*) making the mutations slightly more probable. For the AT base pair, the energy of the products was consistently higher than that of the transition states suggesting that, either the reaction goes through a different transition state that cannot be located or the reaction happens through tunnelling where no barriers are overcome to reach the products [3].

Using the QM model alone on an isolated GC base pair, it was found that, in the absence or presence of electric fields, the reaction would happen synchronously with the existence of one and only one transition state. The higher field strengths promoted the tautomerization reaction kinetically with lower barrier heights and faster reaction rates, and thermodynamically where the product would be more stabilized under the effect of stronger fields. These findings confirm that these point mutations will eventually facilitate the conversion of GC to AT.

The QM/MM analysis of the proton transfer reactions in GC was repeated 25 times using different starting geometries. The statistical distribution of mechanisms was 75%, 18% and 7% for the stepwise (with an average forward barrier height of *ca.* 10.5 kcal/mol), concerted, and concerted synchronous mechanisms, respectively [4]. The forward rate of tautomerization of GC was congruent with the experimental rate at *ca.* 10^5 s^{-1} [5]. Both stepwise and concerted mechanisms had similar equilibrium constants, but the stepwise mechanism had greater rate constants. The fast conversion to the rare tautomer G*C* is followed by an even faster conversion back to the canonical GC, therefore keeping the DNA fidelity to its original non-mutated form.

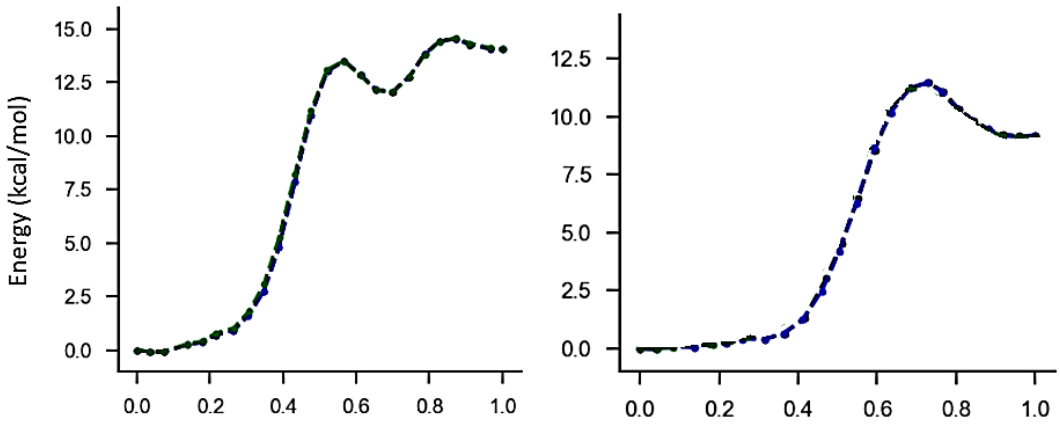

Figure 1 *Energy profile from nudge elastic band simulations of the proton transfer reactions in the GC base pair in the absence of electric fields. The energy profiles for the QM/MM simulations of some of the snapshots from the MD simulations showed a stepwise mechanism (left) and others showed a concerted mechanism (right).*

Using the realistic model of DNA, the strongest field applied along the proton transfer in one direction promoted the formation of the $G^{-}C^{+}$ zwitterion, while the same field applied in the opposite direction promoted the formation of the rare $G^{*}C^{*}$ tautomer. The lifetime of the rare species is very small (in the ps range), thus electric fields up to $1x10^{9}$ V/m will likely not cause substantial changes to the probabilities of point mutations in DNA.

5. Conclusions

In conclusion, point mutations in DNA are studied in the absence and presence of electric fields in various orientations, directions, and strengths. The results of the proton transfer reactions were presented for simple isolated DNA base pairs studied at the QM level, and for base pairs in a realistic model of DNA studied using multiscale QM/MM simulations. Overall, the fields do affect the kinetics of the reaction in its forward and reverse directions, but the effect on the thermodynamics is less pronounced. In all cases, it turns out that the DNA keeps its fidelity in restoring the canonical forms of GC, except for minor scenarios where the GC gets eventually replaced with an AT.

6. References

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