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Assessing the Importance of Proton Transfer Reactions in DNA

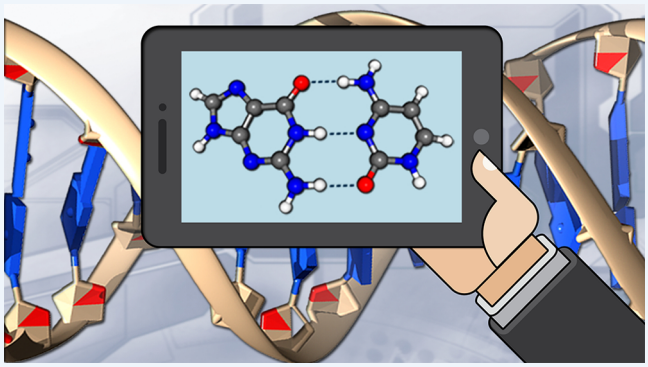
Denis Jacquemin,[†,‡] José Zúñiga,[§] Alberto Requena,[§] and José Pedro Céron-Carrasco*[,§]

[†]CEISAM, UMR CNRS 6230, Université de Nantes, 2, Rue de la Houssinière, Nantes 44322 Cedex 3, France

[‡]Institut Universitaire de France, 103 bd St Michel, Paris 75005 Cedex 5, France

[§]Departamento de Química Física, Facultad de Química, Campus de Excelencia Internacional Regional "Campus Mare Nostrum", Universidad de Murcia, 30100 Murcia, Spain

CONSPECTUS: Although engineered by millions of years of evolution, the cellular machinery is not flawless, and errors regularly appear during DNA replication. The subsequent alteration of the stored genetic message results in a *mutation* and might be the starting point of important health disorders. The question therefore is what causes DNA mutations?



All living organisms are constantly exposed to a number of external agents such as free radicals and to radiation, which may lead to induced mutations. There are also mutations happening without invoking the action of any exogenous element, the so-called spontaneous mutations. The former can be partially controlled by avoiding exposure to high-risk environments, while the latter are more intriguing because their origin is unclear and difficult to determine. As noted by Watson and Crick when they first discovered the DNA structure, the correct replication of DNA rests on the assumption that the base pairs remain in their most stable, canonical form. However, protons along the interbase hydrogen-bond network are not static entities. They can in fact interchange their positions in DNA bases through proton transfer (PT) reactions before strands unwind, giving rise to noncanonical structures defined as rare tautomers. The importance of these rare tautomers was also cleverly anticipated by Watson and Crick and some years later claimed by Löwdin to be a source of spontaneous mutations. In Watson and Crick's words: "It would be of interest to know the precise difference in free energy between the various tautomeric forms under physiological conditions." Unfortunately, rare tautomeric forms are very difficult to detect, so no direct and accurate free energy measure has been discerned. In contrast, theoretical chemistry is making good progress toward the quantification of PT reactions in DNA and their biological consequences.

This Account touches upon the theoretical studies devoted to appraising the importance of rare tautomers as promoters of spontaneous mutations. We focus in particular on the crucial role played by the biological environment on DNA stability. It has now been demonstrated that valuable macroscopic predictions require not only highly accurate theories but also refined chemical models. Hybrid quantum mechanics/molecular mechanics (QM/MM) simulations performed on short but complete DNA sequence fragments emerge in this context as the most adequate tools. In addition, these methods can be used to quantify the effect of different external agents on the PT tautomeric equilibria and, eventually, to conveniently handle them. This is the case for the possible alteration of the naturally observed mutation rate by exposure to intense electric fields. Theoretical predictions envision in this respect promising applications of ultrashort electric pulses in medicine to selectively modify the mutated/canonical ratio in DNA.

1. INTRODUCTION

As put forward by Schrödinger in his 1943 lectures at Trinity College in Dublin, a mutation reminds one of the pivotal discontinuity to the quantum theory in the sense that no intermediate occurs, so it can be viewed as a "quantum jump" between two states: the undamaged chromosome and its mutated counterpart (at that time DNA's structure remained unknown).[1] Over the two subsequent decades, Watson and Crick[2] disclosed first the double helix form of DNA in 1953, and 10 years later, Löwdin[3] proposed the link between the quantum jump concept and the interbase hydrogen bonding (H-bonding) pattern of DNA by hypothesizing a tautomeric equilibrium between the two DNA base pairs, adenine–thymine (AT) and guanine–cytosine (GC), as the driving force behind spontaneous mutations (Figure 1).

The interbase hydrogen atoms are essentially transferred as positively charged particles, so the tautomeric equilibria are usually interpreted as proton transfer (PT) reactions rather than as hydrogen atom exchanges.[3] It is therefore convenient to classify the PT reaction products, the so-called rare tautomers, according to the electrical charge of the resulting mutated isomer. Let us consider first a single proton transfer (SPT). In the AT and GC base pairs, the protons most prone to be

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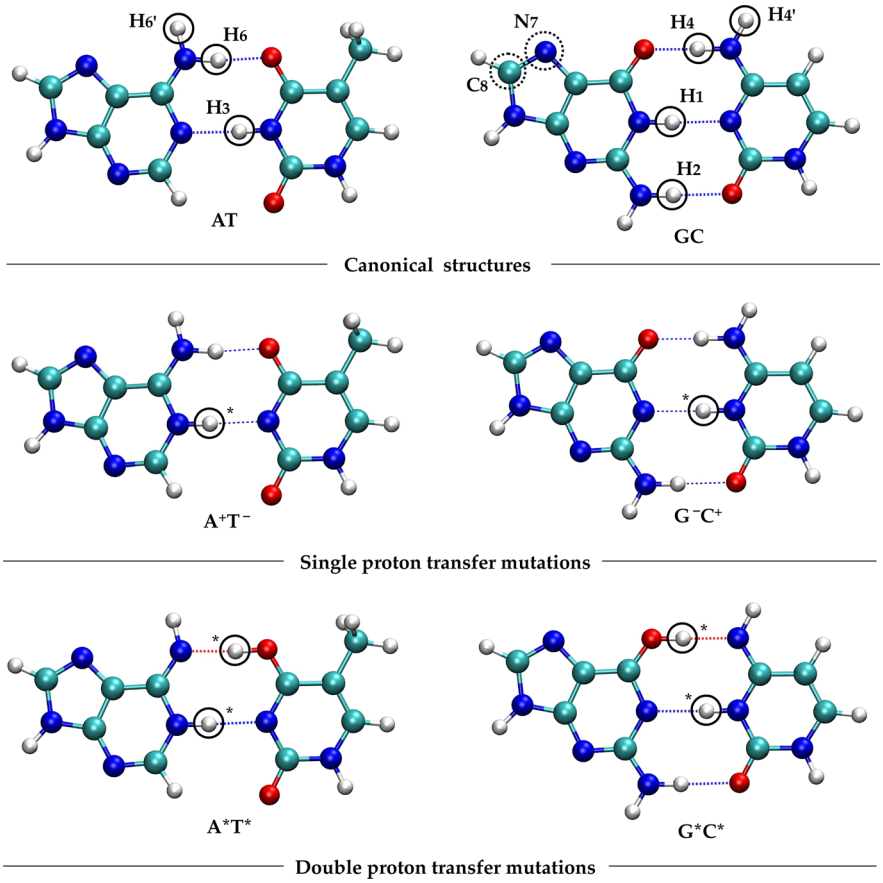

Figure 1. Chemical structures of the canonical AT and GC base pairs compared with their most relevant rare tautomeric forms: on the one hand, A^+T^- and A*T* and, on the other hand, G^-C^+ and G*C*. In the top panel, all protons undergoing possible PT reactions are circled (see text). The classical atomic numbering in DNA is used. The shifted protons in the rare tautomeric forms are marked with asterisks.

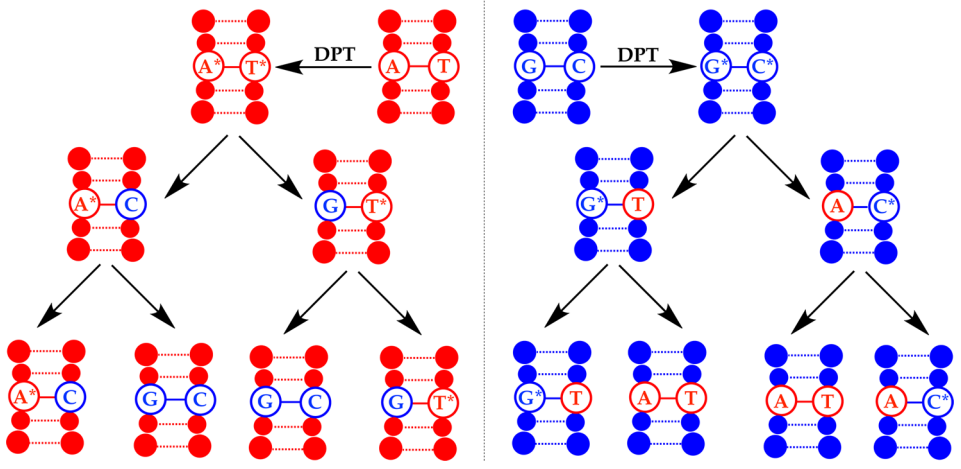

Figure 2. Logical tree of possible genetic errors induced by the A*T* and G*C* rare tautomeric forms: A* (imino) pairs with C, T* (keto) pairs with G, G* (enol) pairs with T, and C* (imino) pairs with A.

exchanged are, respectively, H3 and H1[4] (see Figure 1 for numbering). Consequently one expects SPT reactions mainly to yield the A^+T^- and G^-C^+ zwitterionic forms. Neither of these structures fit the canonical Watson–Crick DNA base pairs, and their presence during the replication process is expected to cause severe damage in the DNA code by causing "deletion" of the complementary base pair.

Of course, there are mechanisms to recover the electrical neutrality while avoiding such dramatic alterations in the genetic code. The simplest possibility consists of going back, with the proton simply returning to its original position leaving the DNA sequence intact. Alternatively, a double proton transfer (DPT) might be induced through the migration of a second proton, H6 in AT and H4 in GC, in the opposite direction to that of the SPT. This mechanism yields the A*T* and G*C* rare tautomers, in which the twin rearrangement restores the neutrality of the bases but alters their properties. The A*T* and G*C* mutations also have a common feature.

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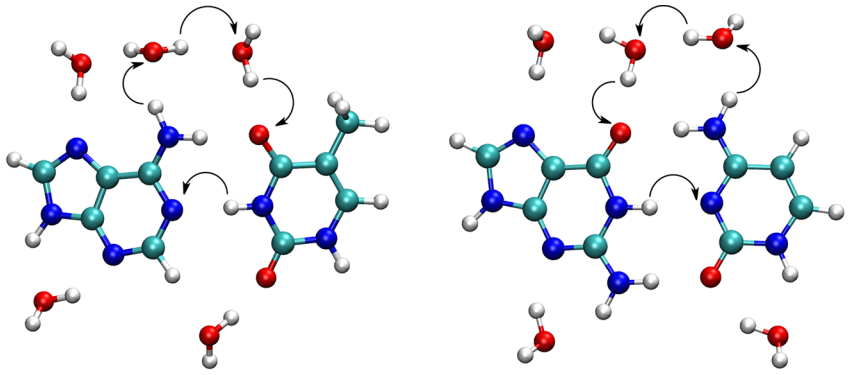

Figure 3. Proposed paths for water-assisted DPT in the hydrated AT and GC base pairs. Arrows show how water molecules simultaneously accept and donate protons during the tautomeric reaction.

One of the carbonyl groups (−C═O) in them undergoes a tautomerization to become an enol (═C−OH) group, while the amino moiety ($-NH_2$) is transformed into the corresponding imine (═NH). DNA stability can therefore be interpreted as the preponderance of keto/amino (canonical) tautomers versus the imino/enol (rare) tautomers. Figure 2 illustrates how DPT may initiate mispairs (A*C, T*G, G*T, and C*A) after the first replication to yield a net base pair swap (A*T* → GC and G*C* → AT) in the daughter strands in the following generations.

If the PT reaction leads to a stable rare tautomer, the error introduced might affect some important biochemistry processes such as those associated with cellular aging and cancer.[3] To rationalizing these phenomena, one has to know the energetic profile connecting the keto−enol and amino−imino forms during cell replication. Unfortunately, this information is quite difficult to get *in vivo* due to the limited sensitivity of the experimental methods. Such a major drawback has stimulated the theoretical chemistry community to assess the importance of SPT and DPT mechanisms in DNA through many investigations relying on increasingly refined models.[4−6] Among others,[7−18] our contribution to this problem has consisted of coupling refined DNA-embedded models and hybrid quantum mechanical (QM) calculations to check Löwdin's hypothesis.[10] Indeed, computational chemistry stands as an effective approach to overcome experimental limitations and to capture the actual role that rare tautomers play in the origin of spontaneous mutations. Accurate levels of theory reveal that spontaneous does not imply random mutations, since important alterations in the genetic code are essentially located in the GC base pair. The most recent developments in this field have focused on understanding and quantifying the influence of external agents on the tautomeric equilibrium, as presented in this Account.

2. RARE TAUTOMERS AND SPONTANEOUS MUTATIONS

Rare tautomers are hardly detectable by the biological machinery since they differ from the canonical structure only in the location of one (SPT) or two (DPT) protons in the middle of the double helix strands. The resulting mutations therefore easily escape the biological verification tests and might subsequently induce an accumulation of damage as shown in Figure 2.

In a now seminal paper, Topal and Fresco[19] estimated that the frequency with which rare tautomers appear in DNA lies in the range between 10^{-8} and 10^{-10}. This *a priori* trifling frequency can be nevertheless significant at the human level: since our genome contains roughly 3×10^9 base pairs,[20] 3−300 errors are expected to naturally occur prior replication of DNA. Of course, there are other sources of natural mutation besides PT reactions, such as formation of wobble base pairs and geometric discrimination, and fortunately most mistakes are corrected through the advanced proofreading mechanisms present in cells which ensure the fidelity of the original genetic information.[21] The multiple sources of mutations and their different repair rates prevent, in fact, a fair comparison between theoretical predictions and the observed mutation frequency. The formation rate of rare tautomers remains therefore unknown, but at the very least, calculations should provide an estimate below the total 10^{-8}−10^{-10} threshold.

The first theoretical study on PT reactions in DNA was conducted by Ladik[22] just one year after Löwdin's paper, using the only accessible semiempirical methods at the time. With the passing of time, *ab initio* methods, including density functional theory (DFT), have become available and increasingly powerful, thus facilitating the possibility of using much more refined models.[23−28] In this framework, crucial advances in simulating PT reactions were brought in by Florián and coworkers[23,24] by computing second-order Møller−Plesset (MP2) energies on Hartree−Fock (HF) geometries to explore the energetic profiles of the SPT and DPT reactions in both AT and GC base pairs. As a result, they concluded that Löwdin's mutational mechanism is energetically accessible in the GC base pair but is beyond reach in its AT counterpart. More specifically, they proposed a stepwise mechanism in which the H1 proton is initially transferred from guanine to cytosine, achieving the ion-pair G^-C^+ form, and the H4 proton later migrates back, in a second step, to yield the G*T* tautomer (Figure 1).[24] In Florian's model, the predicted importance of G*T* in unperturbed DNA is small (10^{-6}−10^{-9}) but still exceeds the observable frequency of spontaneous mutation. Leszczynski et al.[25] explored later the tautomeric equilibria of isolated AT and GC in both gas phase and solution, using a more advanced theoretical strategy that combined DFT and MP2 calculations. They finally obtained a GC ↔ G*C* equilibrium constant of 10^{-6}, which still exceeds the experimental reference. These pioneering studies demonstrated that a high level of theory is not enough to ensure reliable

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biological predictions; a suitable chemical model is also mandatory.

Motivated by this challenge, our groups conducted a series of work to assess the role of surrounding effects on the spontaneous tautomeric mutation in DNA.[8−10] More specifically, we modeled first the structure and impact of the H-bonds formed between the solvent-exposed heteroatoms of DNA base pairs and the surrounding water molecules, with the latter being accounted for as active partners playing a direct role in the PT reactions.[8,9] These simulations demonstrated that the carbonyl groups participating in the interbase H-bonds are simultaneously bound to solvent. Indeed, we identified the explicit water molecules that orientate their protons toward the oxygen atom (see Figure 3) and simultaneously weaken the N−H6···O (in AT) and O···H4−N (in GC) interbase H-bonds, eventually impeding the exchange of both protons and hence blocking the second step of DPT. Remarkably, however, it turned out that the same water molecules that close the door to direct DPT, open an alternative water-assisted DPT path in which the H6′ and H4′ protons are exchanged in AT and GC, respectively, through a solvent loop, as shown in Figure 3. This is a striking outcome, revealing that, on the one hand, water molecules act as critical players in maintaining the double helix DNA architecture, as previously evidenced by Kavelac and Hobza,[29] but, on the other, they may catalyze the spontaneous mutation by accepting and donating protons with DNA. Our theoretical studies also confirmed that the SPT zwitterionic product acts only as a transient species and that, in AT, the equilibrium is clearly shifted to the canonical form regardless of whether the mechanism is direct or water-assisted. Consequently, DPT can potentially induce mutations only in GC. The mechanism is, moreover, asynchronous and concerted, since no stable intermediate is found, and DPT is first initiated by H1 and subsequently followed by H4′.[9]

In elucidating the DPT mechanism that promotes spontaneous mutations, a number of groups, including ours, have found a significantly twisted propeller-like conformation for G*C* in single GC base pair studies.[9,24,25] It came to our attention then that the confinement brought about by the DNA double helix, which obviously contributes to planarity, might impede the formation of such twisted tautomers, thus affecting the DPT mechanism. Hence, we refined our computational protocol[10] by sandwiching the hydrated GC base pair in the center of a triad of base pairs which, according to simulations by Chen et al. of the PT reaction in the GC radical anion,[30] is the smallest DNA motif able to account for stacking interactions. Such a model combines both hydration and stacking effects (see Figure 4) while remaining computationally tractable under the ONIOM approach, which allows us to apply different levels of theory to specific chemical regions or *layers*. In particular, we used DFT with advanced exchange-correlation functionals (e.g., Truhlar's M06-2X)[31] to treat the layer of interest encompassing both the central and border base pairs and a semiempirical approach to describe the lateral backbone region, which has a limited impact on the PT process. This refined model leaves the same qualitative conclusion unaltered, that is, the most favorable mechanism for spontaneous mutation through the PT process continues to be the water-assisted exchange of H1 and H4′ protons in GC. However, in contrast with isolated models that overshoot, often grossly, the total amount of the G*C* rare tautomers, the equilibrium constant computed for the water catalyzed mutation drops to 10^{-11}, a value that is now compatible with the observed rates.

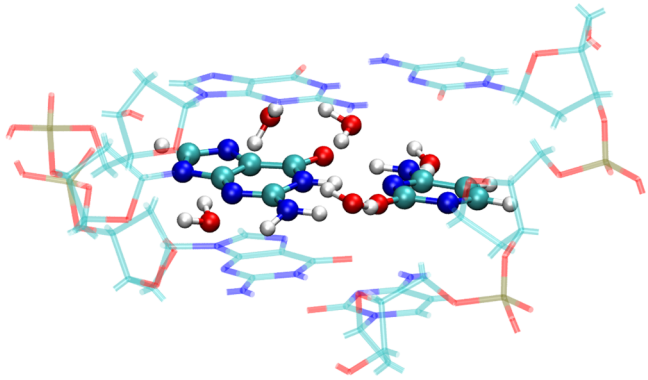

Figure 4. Hybrid QM/MM layer definition for the microhydrated DNA fragment $(DG:dC)_3$. The DNA-embedded GC base pair is highlighted as balls-and-sticks, while the rest of the sequence is represented in wireframe.

It is now recognized that forthcoming theoretical works in the field should be performed using realistic models that include both hydration and stacking. However, there is not yet any clear-cut quantitative estimate of the role played by rare tautomers inside the cell due to the complexity of the biological processes involved. Further work is therefore necessary to better establish the mechanism of action of the rare tautomers on living organisms. In this context, the application of precise molecular dynamics (MD) is certainly adequate and timely to provide a dynamic picture of the PT reactions beyond the static energetic profile reported to date.[26,27] Some first steps in that direction have already been taken by Liang and co-workers by exploring the proton motions using *ab initio* MD.[28]

3. TAUTOMERIC EQUILIBRIA IN ATTACKED DNA

Spontaneous PT reactions in DNA and their resulting tautomeric forms can be altered by both reactive chemicals (e.g., metals and free radicals) and physical perturbations (e.g., high-energy radiation and electric fields). Indeed, as recently demonstrated by Wang, Schaefer, and co-workers,[32] an induced change of the electronic structure of the base pairs will probably modify the tautomeric equilibria. Although genotoxic agents can therefore amplify and worsen the damaging role of spontaneous mutations, its action may also be positively driven by controlling their influence in malignant cells as we summarize below.

3.1. Chemical Agents

Cisplatin $[Pt(NH_3)_2Cl_2]$ has been one of the most potent drugs used in cancer therapy since its discovery by Rosenberg in 1965.[33] Its activity arises basically from an attack of the metallic center on two adjacent guanine basis at the N7 site (circled in Figure 1), which eventually causes a cross-link lesion.[34] The excess of positive charge introduced in guanines also results in more acidic H1 and H2 protons, which is known to promote the formation of rare tautomers.[35] Stimulated by these findings, we adapted our theoretical model to appraise the tautomeric equilibria under cisplatin influence by coupling MD simulations to quantum mechanics/molecular mechanics simulations (QM/MM) in a dAGGC double helix fragment as shown in Figure 5 (top).[36]

Our results showed that the Pt−DNA adduct promotes the SPT of H1 from guanine to cytosine. A sequence effect was also detected: although cisplatin is bound to two GC base pairs, the tautomerization process only occurs in the guanine base that

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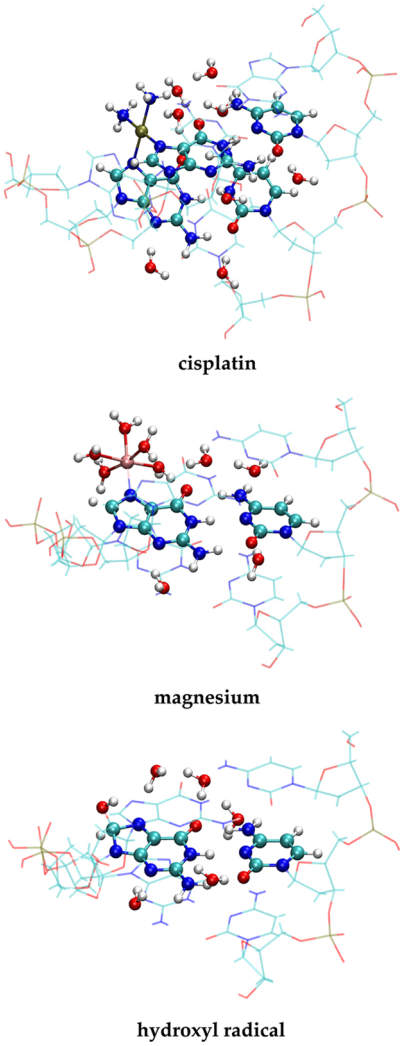

Figure 5. Model systems designed for simulating the impact of external chemical agents.

stacks with an adenine base. Indeed, this corresponding base pair conserves the nearly planar structure better, which in turn facilities the proton transfer reaction.[36] Interestingly, the calculated relative energy for the induced rare tautomer of about 8 kcal/mol indicates that cisplatin induces permanent tautomeric errors at a 10^{-6} rate, significantly larger than that for undamaged DNA. PT reactions are therefore expected to further contribute to the biological activity of cisplatin as a source of sequence-specific mutations. It will be interesting to compare these results with those for other approved platinum-based drugs, including carboplatin and oxalaplatin, to elucidate the effect of metal substituents.

While the natural occurrence of platinum in living bodies is low, there are a number of metal ions naturally present in cells, like Na^+, K^+, Ca^{2+}, and Mg^{2+}, which also deserve proper attention.[37−39] Among them, monovalent cations are usually employed to neutralize the phosphate groups of the lateral DNA backbone, whereas divalent cations, particularly Mg^{2+}, perform more essential biological functions implying direct reactions with nucleic acids.[40]

Although essential for many biological reactions, the Mg^{2+} cation also displays an important mutagenic effect when its concentration exceeds a threshold, causing strong alterations of the DNA structure. Oliva and Cavallo have recently demonstrated that Mg^{2+} also interacts with DNA through the N7 of guanine,[41] so we have consequently investigated the PT reactions in the Mg^{2+}−GC adduct using the model system shown in Figure 5 (center).[42,43] The QM/QM′ calculations performed on this hydrated Mg^{2+}−three base pair DNA sequence reveal an activation of the single H1 proton, with a SPT equilibrium constant of 10^{-3}, which is 3 orders of magnitude higher than that for cisplatin. However, these magnesium-induced rare tautomers have a smaller mutagenic impact because they rapidly reverse to the canonical structure. Calculations performed on both cisplatin- and magnesium-attacked DNA show first that planarity plays a pivotal role in the PT reactions and second that the harmful effects are strongly dependent on the rare tautomer's lifetime, which should exceed the time for the base pair opening during the cell replication (10^{-10} s) to be significant at a biological level.[24]

Free radicals are another class of reactive molecules present in the cellular environment that interact with native DNA. A prominent member of this family is the hydroxyl radical ($^{\bullet}OH$), which has been shown to be associated with the bioactivity of metallodrugs.[44] It is not easy to capture the complete picture of the $^{\bullet}OH$ radical attack mechanism because it depends on a number of physiological variables, including the pH of the medium.[45] Nevertheless, the radical attack to the C8 position of the guanine base (Figure 1) is expected to play an important role since it leads to the most stable GC−OH adduct.[46,47] Unlike cations, this adduct is now neutral, although calculations performed using the model depicted in Figure 5 (bottom) demonstrated that the unpaired electron introduced by the hydroxyl radical also activates the SPT of H1.[48] The computed QM/QM′ mutation frequency lies now in the range of 10^{-5}, but its lifetime is too short to lead to a permanent mutation. Accordingly, the GC−OH adduct presumably evolves to other oxidation products without significantly promoting any rare tautomer, specially because of the presence of fast side competing mechanisms.

These three systems clearly illustrate how chemical agents, either naturally present or purposely delivered in cells, modulate PT reactions. We should emphasize also that the GC base pair is always the target of these agents. DNA mutations through PT reactions are therefore initiated in the base pair that most contributes to the double helix stability.[49]

3.2. Toward Selective Mutation with Physical Agents

The success of a chemotherapy drug depends to a large extent on its accumulation in tumoral tissues. Unfortunately, it is not easy to drive a chemical agent toward cancerous cells, due in part to the side reactions that occur during its transport and delivery. An alternative therapeutic approach to *internal* treatment consists of combining chemotherapy with the action of an *external* physical agent.[50] This route has two main advantages: it allows us to treat a specific region while exerting full control of the exposure intensity and time.

In a recent review, Kumar and Sevilla[6] showed that incident radiation promoting the formation of radicals in DNA affects drastically the tautomeric equilibria, with the bases becoming more acidic or basic depending on whether they lose or gain one electron. The resulting induced tautomeric equilibria, based on both proton-coupled electron transfer and proton-coupled hole transfer, have been explored theoretically using increasingly realistic DNA models.[30,51−53] In the case of oxidative damage, the positive hole is transmitted through the double helix until it is stopped by transfer of the H1 proton within the

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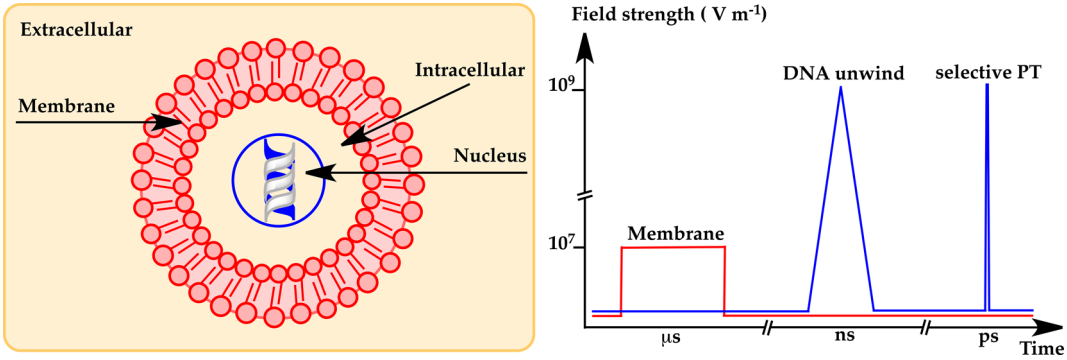

Figure 6. Simplified model for main cellular components (left) and schematic representation of the biological action of electric field pulses depending on the amplitude and duration (right).

one-electron oxidized GC base pair ($G^{+\bullet}C$).[54] As proposed by Guallar et al.[55] and subsequently confirmed by Sobolewski and Domcke,[56] irradiation with UV light activates a charge transfer from the HOMO localized on guanine to the LUMO on cytosine, which leads to a $G^{+}C^{-}$ zwitterionic-like structure (not to be confused with the SPT zwitterionic products). After photoexcitation, the H1 proton is transferred from guanine to cytosine as in the case of the oxidized $G^{+\bullet}C$ base pair.

Practical application of ionizing or UV radiation for selectively mutating cells under *in vivo* conditions is rather limited. Since DNA is located in the nucleus of the cell, highly energetic radiation will initiate a cascade of chemical reactions with other components, such as the membrane, prior to interaction with nucleic acids (Figure 6). Which external agent could then be used to selectively damage DNA, while sparing nontarget biomolecules? Recent work by the Datta and Matta groups[57,58] performed on small DNA models points in the direction of potential activation of PT reactions by application of intense external electric fields.

We have checked the use of intense fields for enhancing the PT exchange in the GC base pair.[59] MD and QM/MM calculations thus revealed that very intense strengths (in the range of 10^9 V m^{-1}) applied for periods longer than 10 ps cause a permanent strand unwinding, rather than promotion of PT.[60] However, shorter pulses induce an increase of the H1 proton acidity in GC, which energetically favors the zwitterionic-SPT form (see Figure 1) in comparison with the canonical form.[61] As explained by Schoenbach and co-workers,[62] the cell can be seen as a tiny electrical circuit in which microsecond pulses affect the external membrane while sub-microsecond pulses enter the intracellular region when the applied voltage is around 10^7 V m^{-1}. Accordingly, ultrashort intense electric field pulses might potentially fulfill the duration prerequisite to reach DNA without being blocked by other components and thus induce a selective DNA damage as shown schematically in Figure 6. Interestingly, electric fields act as directionally specific mutagenic sources, since the PT reaction is activated only for those guanine−cytosine base pairs that orientate their central N−H1···N H-bond toward the direction of the field.[60] Although further investigation is required to describe with higher accuracy the cellular response to these intense electric fields,[63] specially to appraise the effect of such perturbation in biomolecules that surrounds DNA in the cellular environment, a first estimation of the E_{ext} upper limit to which cells can be exposed without promoting the random distortion of the double helix structure is now available.[60,61]

4. CONCLUDING REMARKS

Fifty years after Löwdin's hypothesis, the *in vivo* detection of rare tautomeric forms in DNA remains beyond reach. However, the development of novel algorithms for quantum calculations, along with the exponential increase of computational resources, has enabled several groups to explore proton transfer reactions in DNA. This *in silico* approach has been used recently to investigate possible alterations of natural tautomeric equilibria induced by external chemical and physical agents. In this context, the GC base pair emerges as the cornerstone of DNA replication fidelity: on the one hand, it contributes decisively to keep the double strands bound, and on the other, it turns out to be the DNA component that is most prone to promote mutations through PT reactions induced by either chemical or physical agents.

In this Account, we have illustrated how external agents may affect the GC base pair's electronic structure and eventually favor the formation of noncanonical structures. Among the agents that can be used for promoting mutations in a controlled fashion, electric fields emerge as one of the most promising candidates since the perturbation can be straightforwardly modulated by tuning intensity and time. As we continue to explore the proton transfer reactions occurring in DNA base pairs, progress in the control of the spontaneous mutation mechanism is expected that will eventually allow us to program malignant cell suicide.

AUTHOR INFORMATION

Corresponding Author

*E-mail: jpceron@um.es.

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Notes

The authors declare no competing financial interest.

Biographies

Denis Jacquemin (1974) obtained his Ph.D. at the University of Namur (1998). After a postdoctoral experience at the University of Florida, he came back to Europe, where he was appointed research associate of the Belgian FNRS (2003) and next Professor at the University of Nantes in France (2010) and at the Institut Universitaire de France (2012). His research focuses mainly on modeling electronically excited states.

José Zuñiga (1961) teaches Physical Chemistry at the University of Murcia (Spain), where he received his Ph.D. in 1985. After a postdoctoral stay at Emory University in Atlanta, he rejoined the Department of Physical Chemistry of the University of Murcia where he became Associate Professor in 1989 and Professor in 2004. His current research focuses on the theoretical study of molecules of biological interest.

Alberto Requena (1948) is a Professor and leader of the Quantum Chemistry group at the University of Murcia. He gained his Ph.D. from the University of Murcia in 1975 and had postdoctoral stays at the Paris-Sud University in Orsay (France) and the Tel Aviv University (Israel). He has authored almost 150 scientific papers in the Quantum Chemistry and Molecular Spectroscopy areas and directed a number of research projects.

José Pedro Cerón-Carrasco (1980) earned his Ph.D. at the University of Murcia in Spain. He joined the University of Namur in Belgium (2009) and the University of Nantes in France (2011), during his postdoctoral training supported by Fundación Séneca. He is currently a Marie Curie researcher in the Quantum Chemistry group of Murcia. His main research interests are the applications of theoretical methods to the study of biosystems including DNA and metallodrugs.

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