

The Mechanism of Mutation Initiated by One-Electron Oxidation

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Introduction

The aqueous redox chemistry of the nucleosides and nucleotides has been extensively investigated for the last 40 years using pulse radiolysis, laser photolysis, electron spin resonance, and other time resolved and steady state techniques.¹ More recently, theoretical methods have been employed in the study of redox damage of DNA.¹ This intensive interest in the components of DNA is understandable since it carries our genetic code and, if damaged, can lead to mutations possibly resulting in cancer.^{2,3} Furthermore, oxidative damage of DNA is implicated in aging⁴ and drug resistance of bacteria.⁵ It is now understood that DNA damage initiated by ionising radiation elicits a complicated set of events engaging various signalling pathways in cells.⁶

Deprotonation Alters Hydrogen Bonding Capabilities

Interestingly, it has been found that when organic molecules are one-electron oxidized in the aqueous phase, a rapid deprotonation occurs from hydrogen bond donors, undoubtedly driven by the massive solvation energy of the proton ($\Delta G_{\text{aq}} = -263.9$ kcal/mol).⁷⁻⁹ Thus, the pK_{a} of cytosine (C) is lowered from 12.15 to lie between 2 and 4 when C is one-electron oxidised.^{8,10,11} With respect to DNA, guanine (G) is its most easily oxidized component¹² and when double stranded DNA's π -stack loses an electron, the positive charge migrates to G-C rich areas in the double strand.¹³⁻¹⁶ The pK_{a} of G is lowered significantly from 9.4 to 3.9 at the nitrogen-1 atom (N1) as depicted in Fig. 1.^{10,17,18}

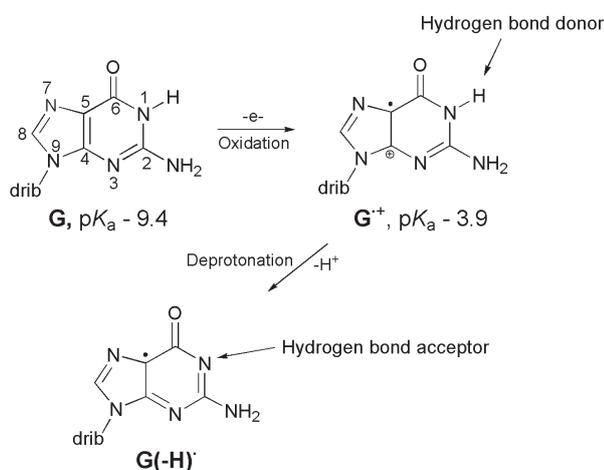


Fig. 1. Oxidation and deprotonation of guanine (G) and its radical cation ($\text{G}^{\bullet+}$): The pK_{a} of G is drastically lowered upon one-electron oxidation and the subsequent deprotonation of N1-H changes it from a hydrogen bond donor to a hydrogen bond acceptor; the numbers of the atoms constituting G are shown and drib is a 2'-deoxyribose moiety.

After departure of the proton from the N1-site, it becomes a hydrogen bond acceptor instead of a hydrogen bond donor. The question emerged as to whether this event leads to a change in the pairing ability of the G moiety with other bases.¹⁹ In fact, it is a common view that ligand hydrophobicity gives affinity, whereas hydrogen bonding gives specificity for interactions in biochemical systems.²⁰ Simulating one-electron oxidation and the consequent deprotonation of the central N1-proton for G-C using density functional theory (DFT),²¹ a new slipped configuration of the base pair was formed as depicted in Fig. 2.¹⁹ This slipped configuration, G(-H)-C, was later independently derived by Bera *et al.* using a systematic search for all possible hydrogen bonding configurations between G(-H) \cdot^+ and C.²² The predicted base pairing energy (BPE) is -18.2 kcal/mol for G(-H)-C.^{19,23} It lies between the BPE's of the adenine-thymine base pair (A-T) at -13.0 kcal/mol and of G-C of -21.0 kcal/mol.^{24,25}

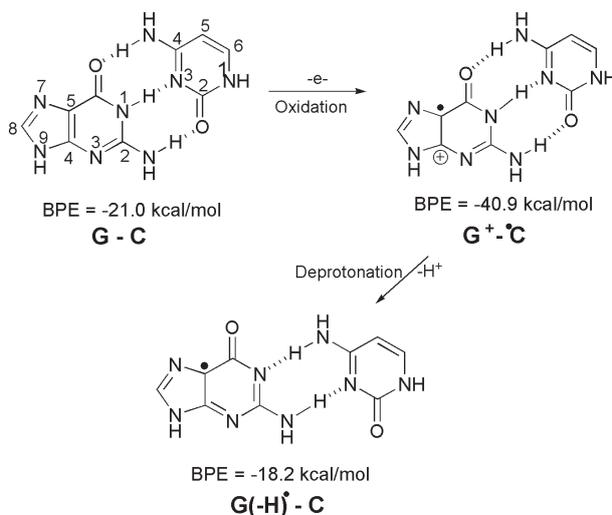


Fig. 2. Deprotonation-induced structural change of the G-C base pair initiated by one-electron oxidation leading to the shifted base pair G(-H)-C; BPE = Base Pairing Energy.

The Situation in Double Stranded DNA

Under what circumstances can $\text{G}^{\bullet+}-\text{C}$ in the DNA stack lose the central N1 proton making up one of the Watson-Crick hydrogen bonds? It does not have access to the aqueous phase since it is the central hydrogen bond and it is flanked by base pairs on either side in the double stranded DNA helix. It is imperative that N1-H comes into contact with the water phase (water acting as a proton acceptor), *i.e.* within G-C the G(N1-H)-C(N3) Watson-Crick hydrogen bond has to be broken for the N1 proton to be lost (see Fig. 2). The hydrogen bonds between the base pairs may be broken in any of three situations. First is the *swing-out* of the bases by concerted thermal motions of the DNA strand.^{26,27} This mechanism

is unlikely since it takes place on the milli-to-micro second time scale and it is in competition with further charge migration in the DNA helix and/or with water addition to C8 of G^{+} . The rates of these processes are estimated as $5 \times 10^7/s$ and $6 \times 10^4/s$, respectively, *i.e.* in the micro–nano-second timescale.^{16,28} Furthermore, the BPE of $G^{+}-C$ is increased to -40.9 kcal/mol compared to -21.0 kcal/mol of its parent pair inhibiting the frequency of the breathing motions of the base pair.^{24,29,30} Second, when duplication of DNA occurs, the DNA strand is untwisted and the hydrogen bonds between the bases are broken to allow the duplication of the strand. Third, the H-bonds may be broken during DNA transcription to messenger RNA as this proceeds in a manner similar to the duplication of DNA. Also, it has been suggested that deprotonation occurs from the exocyclic amine group of C in $G^{+}-C$, based on pulse-radiolysis and kinetic isotope experiments.³¹⁻³³ The proposed deprotonation mechanism is shown in Fig. 3. This reaction cascade can lead to the $G(-H)^{\bullet}-C$ slipped configuration.³⁴

Deprotonated to the aqueous phase

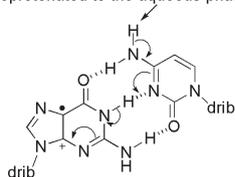


Fig. 3. Possible mechanism involving the *exocyclic* amine moiety of C as proton donor of the one-electron oxidized base pair; the initial charge sits on G in the complementary strand; spin-charge separation between G and C plays a crucial role in the reaction cascade and the depicted deprotonation can lead to the formation of $G(-H)^{\bullet}-C$ –see ref. 34.

Base Pairing of the Deprotonated Guanine Radical

A related question emerged as to whether it is possible to pair T, A and G itself to $G(-H)^{\bullet}$. This was investigated using the DFT method and the results are presented in Fig. 4.¹⁹

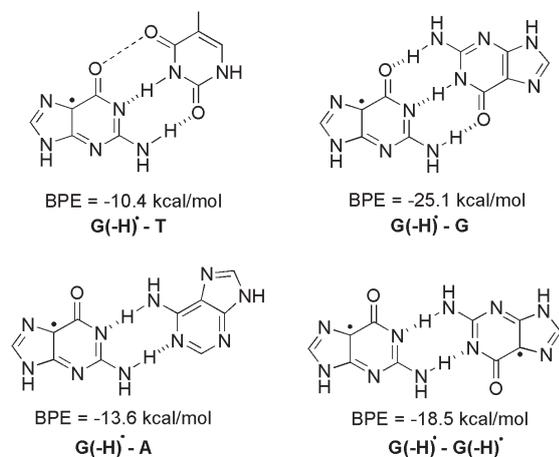


Fig. 4. The unnatural base pairs between $G(-H)^{\bullet}$ and the other bases – see ref. 19 The substantial base pairing energy (BPE) for the non-classical complexes depicted leads to the conclusion that $G(-H)^{\bullet}$ does not have any specificity for C.

Armed with the knowledge of the $G(-H)^{\bullet}-C$ base pair with only two hydrogen bonds, $G(-H)^{\bullet}$ was paired to T and structurally optimized. The BPE was calculated to

be -10.4 kcal/mol for $G(-H)^{\bullet}-T$, which is comparable to the A–T base pairing energy (-13.0 kcal/mol).^{24,25,29,35} The relatively low energy can be explained in terms of the non-planarity of the bases with respect to each other. From the calculations, they appear to be *ca.* 25° out of plane, measured at their carbonyl groups O^6 (G) and O^4 (T). The distance between these oxygen atoms is 3.5 Å, a proximity which leads to Coulombic repulsion and hence the non-planar conformation. The calculated hydrogen bonding energy of $G(-H)^{\bullet}-A$ base pair is -13.6 kcal/mol, as shown in Fig. 4. This binding is somewhat stronger than for the natural A–T pairing (-13.0 kcal/mol).^{24,25,29,35}

The hydrogen bond energy of $G(-H)^{\bullet}-G$ (see Fig. 4) is similar to that of $G-C$.³⁶ This is not surprising because three hydrogen bonds are present in both structures. A second type of a G–G base pair is conceivable between two $G(-H)^{\bullet}$ moieties [$G(-H)^{\bullet}-G(-H)^{\bullet}$], as shown in Fig. 4. For this, the hydrogen bond energy is -18.5 kcal/mol, somewhat lower than for $G(-H)^{\bullet}-G$ since it has one less hydrogen bond. Pt(II) electrophile coordinates at N7 of G. This acidifies the N1 proton, akin to what happens during the oxidation of G. With these Pt–G species, structures similar to $G(-H)^{\bullet}-G$ and $G(-H)^{\bullet}-G(-H)^{\bullet}$ were observed by ¹H-NMR and X-ray crystallography,³⁷ thus providing experimental evidence for their existence.

Oxidation During DNA Duplication

Using *in situ* photolysis electron paramagnetic resonance (EPR), Hildenbrand and Schulte-Frohlinde detected a long-lived radical (lifetime ~ 5 s) that was produced only from double stranded DNA when ionised with ≤ 220 nm light in an aqueous solution at pH ~ 7 .³⁸ This radical was assigned to $G(-H)^{\bullet}$. The rate of DNA duplication was measured to lie between 5 and 500 nucleotides per second depending on the cell type, the species and other factors.^{39,40} Considering the long lifetime of $G(-H)^{\bullet}$ in double stranded DNA and the rapid DNA duplication rate, the scenario emerges that in case of one-electron oxidation during mitosis-/meiosis $G(-H)^{\bullet}$ is formed when the two strands unwind. As shown in Fig. 4, base pairs can form with all of the nucleotides with binding energies similar to the classical A–T and G–C Watson-Crick base pairs. This means that $G(-H)^{\bullet}$ does not have specific affinity for C, *i.e.* it is completely promiscuous when it comes to base pairing. Therefore $G(-H)^{\bullet}$ can pair with all of the nucleotides and lead to mispairing. A depiction of this scenario is presented graphically in Fig. 5.

The mechanism presented here is new and provides an alternative to the scenario that mispairing of the DNA bases is mostly caused by oxidative end products such as 7,8-dihydro-8-oxoguanine (8-OG). These products are closed shell, *i.e.* they are not radical species and, therefore, have much longer lifetimes than $G(-H)^{\bullet}$. Redox product 8OG is one of many derived from DNA one-electron oxidation and subsequent water addition to G.^{17,18,41} It can form syn-anti base pairs⁴² with all of the nucleotides and these have base pairing energies of ~ -10 kcal/mol.⁴³ The 8OG–A base pair is depicted in Fig. 6 as an example of *syn-anti* base pairs.

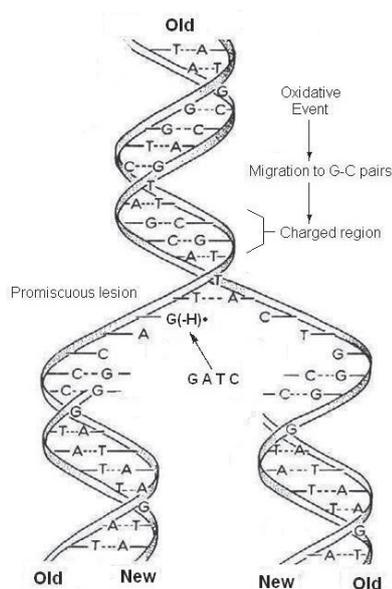


Fig. 5. As the two strands of the double helix unwind, each pairs up with the appropriate bases to form a new double helix. The two new helices are identical to each other and to the original. This process is compromised by one-electron oxidation of the π -DNA stack, deprotonation from G^{+} and the subsequent formation of $G(-H)^{\bullet}$ that is promiscuous in regard to base pairing.

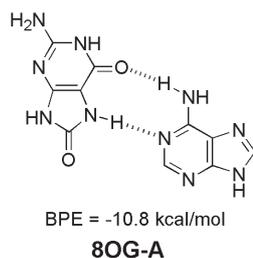


Fig. 6. The *syn-anti* base pair of **8OG-A**; BPE = Base Pairing Energy.

Conclusions

In this review, an alternative mechanism for promiscuous base pairing during DNA duplication initiated by one-electron oxidation is proposed based on theoretical calculations. Some experimental results that support the existence of the non-classical base pairs discussed exists, *i.e.* for the slipped $G(-H)^{\bullet}-C$ and the $G(-H)^{\bullet}-G$ base pairs. Further experimental and theoretical work is needed to corroborate the mechanism proposed. In particular, experiments conducted with time resolved resonance Raman spectroscopy on model DNA duplication systems are pertinent.

References & Notes

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