Effect of Guanine Stacking on the Oxidation of 8-Oxoguanine in B-DNA

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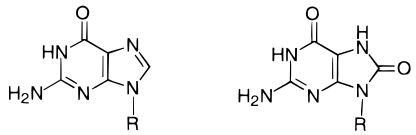
Dihydro-8-oxoguanine (8G), often called 8-oxoguanine, is a modified base formed from guanine (G) by a variety of oxidative reagents.[1] The guanine bases in DNA are not oxidized randomly to 8G. Saito et al. demonstrated experimentally that G residues located 5′ to a second G are the most easily oxidized.[2] Calculations showed that the GG interaction is unique and that the highest occupied molecular orbital (HOMO) of a GG stack is especially high in energy and concentrated on the 5′G.[3] As a consequence, one-electron oxidation and electrophilic attack on this G are favored. If electron transfer along DNA is facile,[4] GG stacks can act as thermodynamic sinks, and the hole caused by an oxidizing agent would eventually migrate to this location. 8G is mutagenic and must be eliminated from the organism for survival.[5,6] Elimination of this modified base can be accomplished by base excision DNA repair (BER) enzymes.[7] Further oxidation of 8G generates an alkali-labile site.[8] Either mechanism can result in removal of the damaged bases and repair.[7] Do the same factors that cause the 5′G of a GG stack to be oxidized easily also influence the ease of oxidation of 8G? Does the position of 8G determine whether it is further oxidized? We report here (1) the prediction that 8G in a 5′-(8G)G-3′ stack is particularly prone to oxidation and (2) a simple model to explain why GG and (8G)G stacks are uniquely oxidizable.

Free 8G in solution is 2 orders of magnitude more reactive toward singlet oxygen ($^1O_2$) and is more easily oxidized than G.[9−12] However, very little is known about its chemical behavior in DNA. It has been demonstrated that both radical[1,13] and singlet oxygen[14] photooxidations can efficiently generate 8G in DNA. For the singlet oxygen mediated mechanism, formation of 8G is a major pathway.[12] For the radical mediated mechanism, it is still uncertain whether the formation of 8G from the guanine radical cation is a minor or a major pathway.[15,16] In both cases, the HOMO energy of the base reflects the ease of oxidation or reactivity toward electrophilic oxidants.

Scheme 1



G 8G

Table 1. IPs (in eV)[a] of G, 8G, and B-Form Stacked Contiguous G and 8G

5′-base(s)-3′	RHF/6-31G*	B3LYP/6-31G*
G	7.72 (7.14)[b]	7.31
GG	7.34 (6.68)	6.64
8G	7.54 (7.12)	6.93
(8G)G	7.06 (6.54)	6.38
G(8G)	7.37 (6.64)	6.51

[a] IPs estimated by Koopmans' theorem (RHF) or vertically (B3LYP). [b] (IP calculated for the parent molecule with a paired *N*-methylated cytidine).

Although theoretical calculations of DNA bases have been extensive, only a few ab initio calculations on stacked nucleobases have been reported.[3,17] For monomeric DNA nucleobases, the HOMO energies of the molecules calculated at the RHF level correlate well with the experimental vertical IPs.[18,19] In the current work, double strands of B-form DNA with a GG sequence were built using MACROMODEL[20] and the geometries were optimized with the AMBER force field, which provides good geometries for DNA.[21,22] The geometries obtained were comparable to crystal structures of B-form DNA.[23] For quantum mechanical studies, the dinucleoside backbones were removed from the coordinate file, keeping the positions of all other atoms fixed, and were replaced by standard methyl groups. The energies of the HOMOs were computed at the HF/6-31G* level using GAUSSIAN94[24] and SPARTAN.[25] Vertical IPs were also evaluated using density functional theory (Becke3LYP/6-31G*) by calculating and comparing neutral and radical ion energies.[26] The energies obtained are in good agreement with previously reported work which conducted optimizations and calculated energetics at the HF/6-31+G(d) level for G[19] and at the MP2/6-31G* level for GG.[17,27]

The effect of G stacking is summarized in Table 1. The decrease in IP for the 5′G is ca. 0.4−0.7 eV in 5′-GG-3′, similar to the change reported by Sugiyama et al.[3] We find that the IP of 8G also drops ≈0.5 eV when it is stacked 5′ to G. When the

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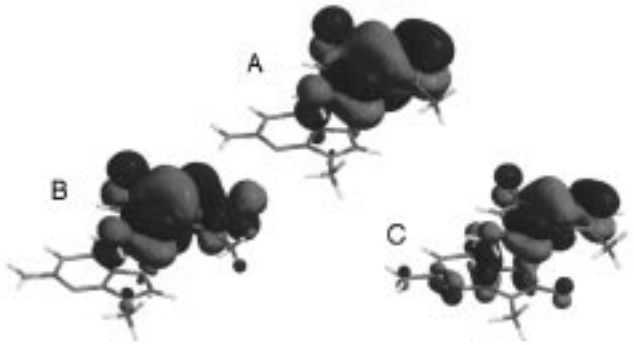

Figure 1. Orbital contour plot of the HOMOs of three B-DNA-type arrangements (HF/6-31G*): A, 5′-GG-3′; B, 5′-(8G)G-3′; C, 5′-G(8G)-3′; 5′, top; 3′, bottom.

hydrogen-bonded cytidine is included, the calculations also show this reduction in ionization energy upon stacking (0.5 eV in 5′-GG-3′, 0.6 eV in 5′-(8G)G-3′). These calculations show that an 8G 5′ to G becomes much easier to oxidize than an isolated 8G and therefore should undergo rapid additional oxidation with formation of an alkali-labile site in DNA.

Recently, Koizume et al.[28] synthesized oligonucleotides with 8G in different positions. When 8G is not stacked, oxidation followed by piperidine treatment leads to preferential cleavage (69%) at this position. When 8G is located contiguously 5′ to G, the selectivity is slightly greater (76%), as would be expected from the increased ease of oxidation predicted by these calculations. More significant is the photooxidation of the "unnatural" oligonucleotide 5′-G(8G)-3′, because there is a competition between G being 5′ to 8G, while 8G has the inherently lower IP. Strikingly, the oligonucleotide is cleaved preferentially at the G position in ca. 1.5:1 ratio even though an isolated 8G is more easily oxidized.[28] Our calculations successfully predict that G should be more easily oxidized in 5′-G(8G)-3′. A GG or 5′-(8G)G-3′ stack has the HOMO largely localized on the 5′ residue (Figure 1 A,B), and oxidation will be essentially exclusive at this site. In the G(8G) stack, however, the localization is only slightly in favor of the 5′G. We can also explain the results obtained by Cullis et al.,[15] who claim that 8G is not a precursor to alkali-labile sites. Their oligonucleotide contains a 5′-GGG(8G)-3′ sequence. According to our calculations, the HOMO of this sequence will be completely localized on the 5′ guanines and, therefore, no oxidation of 8G is expected: the distribution of the HOMO for 5′-GGG(8G)-3′, calculated at the HF/6-31G* level, is 5′-100:50:9:1-3′. Recently, Gasper and Schuster[29] oxidized DNA double strands containing both 5′-(8G)G-3′ and 5′-GG-3′. Cleavage is observed on the 8G of 5′-(8G)G-3′ but not on the distal 5′-GG-3′, in agreement with our results.

What is the origin of the IP alterations caused by base stacking? Both the HOMO and the second HOMO of GG have similar shapes (i.e., the signs and relative magnitudes of the coefficients are unaltered) to that of the HOMO of isolated G. The HOMO of GG is mostly located on the 5′G, and the SHOMO is mostly located on the 3′G. Similar behavior is found for the LUMO and SLUMO. Although the role of orbital mixing was emphasized in the work of Sugiyama et al.,[3] the fact that the shape of the orbitals does not change upon stacking suggests that electrostatic interactions are responsible for the increase in energy of the HOMO, rather than orbital mixing. Furthermore, HOMO−HOMO mixing should be maximized when the bases are stacked in perfect alignment because of maximum overlap between the orbitals of the two bases, while Sugiyama found instead that misaligned stacks in B- and A-form DNA give maximum HOMO energy and localization.[3]

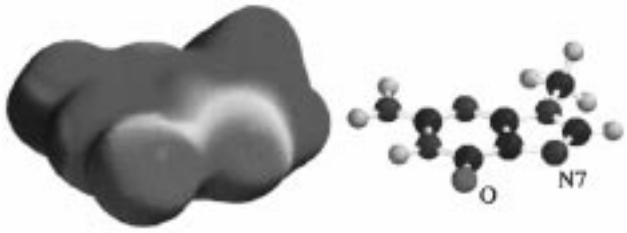

Figure 2. Electrostatic potential map of G, calculated at the HF/6-31G* level. Red indicates negative electrostatic potential, and blue represents positive.

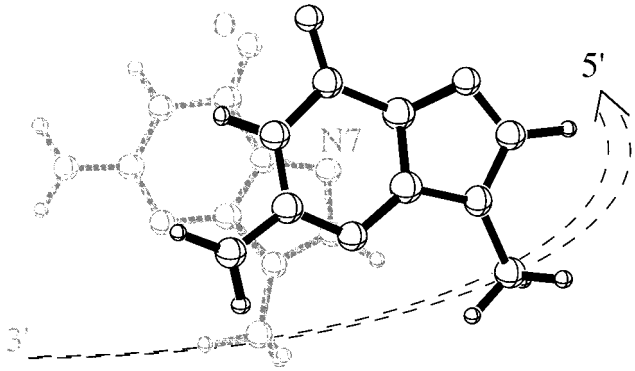

Figure 3. Top view of a B-DNA-type arrangement of two stacked guanines: 5′ (black, top), 3′ (gray, bottom).

The electrostatic potential map of G (Figure 2) shows significant concentration of negative charge on O and N7. Since N7 of the 3′G is located just below the six-membered ring of the 5′G in B-form DNA (Figure 3), the negative charge stabilizes a cation located in a 5′G of a GG stack. The negative O is also expected to be influential. To test this possibility, the 3′G was rotated and translated to optimize the position of the electron-rich N7 and O relative to the 5′G. As long as the atoms are in the vicinity of the other G, HF/6-31G* calculations show that the HOMO is almost entirely localized on the 5′ base. Other arrangements with N7 and O further from the second G cause the HOMO to be more evenly distributed between the two bases.[3]

This electrostatic model has far-reaching consequences, since it provides a simple way to predict how different sequences and geometries alter reactivities in intact DNA. The dominance of heteroatom$-\pi$ interactions is reminiscent of experimental structural studies which led to the postulate that the strength of base stacking is related to heteroatom$-\pi$ interactions.[30,31] Several computational investigations have shown that electrostatic effects dominate the strength of interaction between stacked nucleobases.[17,27,32] GG stacks in B-form DNA provide the strongest stacking interactions,[17,27] and this causes the greatest IP alteration to the 5′G as well. The consequences of these electrostatic effects on oxidation of DNA bases in different forms of DNA are being investigated.

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