Calculation of the stabilization energy when the oxidative guanine damages pair with guanine

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Abstract : The genome DNA is constantly exposed to endogenous and exogenous oxidative stresses. Damaged DNA can cause mutations, which may increase the risk of developing cancer, aging, and other diseases. Various oxidative stresses lead to G:C–T:A and G:C–C:G transversions, because guanine is highly susceptible to oxidative stress in the DNA, owing to the lowest oxidation potential among four nucleobases. One typical lesion product of guanine is 8-oxo-7,8-dihydroguanine (8oxoG), and DNA polymerases incorporate adenine but not guanine opposite 8oxoG lesions. More specifically, 8oxoG:A base pairs cause G:C–T:A transversions, and then the other oxidative guanine damages seem to cause G:C–C:G transversions.

2,5-diamino-4*H*-imidazol-4-one (Iz) and 2,2,4-triamino-5(2*H*)-oxazolone (Oz) are the oxidative guanine damages, which can pair with guanine, and several DNA polymerases incorporate guanine opposite these lesions *in vitro*. We have previously reported that the calculated stabilization energy of Iz:G base pair is similar to that of C:G base pair, and that Oz:G base pair is planar and has two hydrogen bonds.

Guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp) are the oxidation products of 8oxoG. It was shown that Klenow fragment incorporated adenine and guanine opposite these lesions. Our previous study proposed Gh:G and Sp:G base pairs, however, the stabilization energy of these base pairs have not been calculated. Therefore, We estimated the stabilization energy of these base pairs by *ab initio* molecular orbital calculations.

Keywords : hydrogen bond, oxidation product, base pair

Introduction

Cellular DNA is constantly assaulted by various endogenous and exogenous oxidative stresses. When the damage is not repaired, it can cause mutations, which can contribute to carcinogenesis, aging, neurological syndromes, and developing other diseases. Guanine has high sensitivity to the oxidative stresses such as one-electron oxidation, singlet oxygen and peroxynitrite in the DNA, due to the lowest oxidation potential than the other nucleobases. G:C–T:A and G:C–C:G transversions is observed *in vivo*; For example, G:C–T:A and G:C–C:G transversions are detected high-frequently in codons 12 and 13 of *K-ras* gene, which are caused by passive smoking.[1] Namely, various oxidative stresses lead to G:C–T:A and G:C–C:G transversions, owing to the high responsivity to the oxidative stresses of guanine as described above. Thus, to investigate the causes of G:C–T:A and G:C–C:G transversions is important in understanding the molecular mechanisms of DNA damage and repair.

8-oxo-7,8-dihydroguanine (8oxoG), a representative oxidation product of guanine, is formed under various oxidative conditions. Eukaryotic DNA polymerases incorporate adenine but not guanine opposite 8oxoG lesions. More specifically, 8oxoG:A base pairs cause G:C–T:A transversions,[2] and the other oxidative guanine damages seem to cause G:C–C:G transversions.

2,5-diamino-4*H*-imidazol-4-one (Iz) and its hydrolysis product 2,2,4-triamino-5(2*H*)-oxazolone (Oz) are oxidation products of guanine, which were reported by Cadet *et al*. in 1994.[3] Iz has donor and acceptor abilities of H-bonding similar to cytosine. We previously calculated the stabilization energy of Iz:G base pair, and that of Iz:G base pair is similar to that of C:G base pair.[4] Thus, these results correspond to the result that guanine is specifically incorporated opposite Iz by DNA polymerases I *in vitro*.[5] On the other hand, primer extensions by several DNA polymerases showed that either guanine or adenine, or both, are incorporated opposite Oz. We previously calculated the stabilization energy of Oz:G and Oz:A base pairs. It was shown that the calculated stabilization energy of Oz:G is larger than that of Oz:A, and that Oz:G base pair is planar and has two hydrogen bonds.[6]

Guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp) are oxidation products of 8oxoG (Fig. 1). Gh is preferentially formed under acidic conditions,[7] and iminoallantoin (Ia) is another equilibrating isomer of Gh,[8] while Sp is formed under basic conditions. It was shown that adenine and guanine are inserted opposite these lesions by Klenow

fragment.[9] The efficiencies of guanine incorporation opposite Gh/Ia are higher than that of adenine incorporation, whereas that of guanine and adenine insertion opposite Sp are similar. Ia and Sp, but not Gh, have donor and acceptor abilities of H-bonding similar to Iz. Namely, in addition to Iz and Oz, Gh/Ia and Sp can explain G:C–C:G transversions. Our previous study proposed Ia:G and Sp:G base pairs,[10] but the stabilization energy of these base pairs have not been calculated. Therefore, we estimated the stabilization energy of these base pairs by *ab initio* molecular orbital calculations, and compared these results with that of C:G base pair.

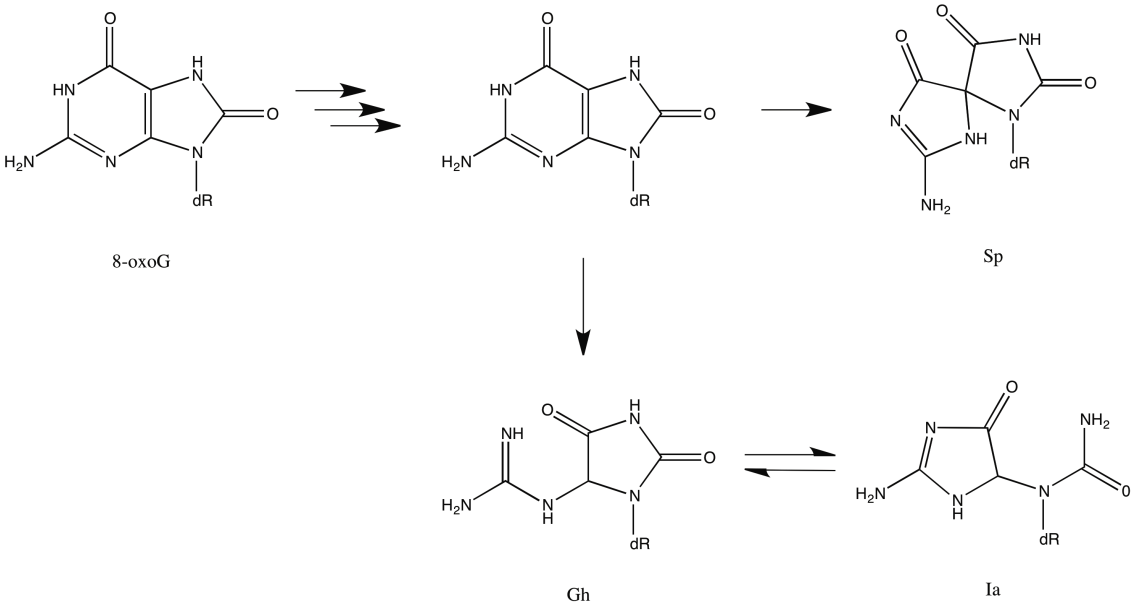


Fig. 1 Oxidation product of 8oxoG.

Methods

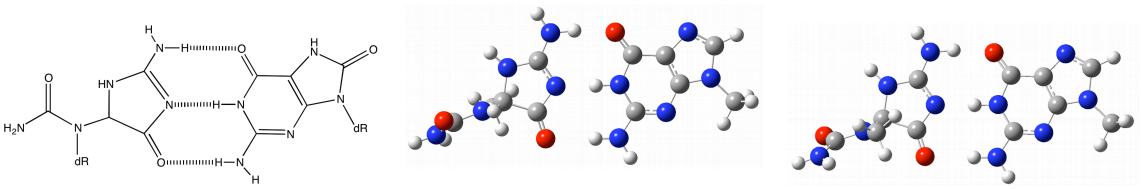
The most stable duplex structures containing Gh:G, Ia:G and Sp:G base pairs , and all atoms were removed except for the bases of these lesions, the complementary base, the 2-deoxyribose C1' carbon and C1' H. Two H atoms were then attached to the C1' methine to complete the N-methylated nucleobases. The Gh:G, Ia:G and Sp:G base pairs were optimized by SCF calculation using Gaussian 03 (HF/3-21G). The optimized structures were visualized with GaussView in Figs. 2-5.

Results and Discussion

We have previously proposed that Ia can pair with guanine and Ia:G can have three hydrogen bonds.[10] In this study, the stabilization energies of this proposed Ia:G base

pair were calculated; the stabilization energy of Ia1:G (Fig. 2) was 39.5 kcal/mol. This result has almost no difference in the calculated stabilization energy of Watson-Crick C:G base pair (39.8 kcal/mol). Since the number of hydrogen bonds of Ia:G base pair are the same as that of C:G base pair, it appears that the stabilization energy depends on the number of hydrogen bonds. In addition, Ia2:G was as stable as Ia1:G, and configurations of the parts not containing hydrogen bonds with guanine have little effect on the stabilization energies.

a) b)

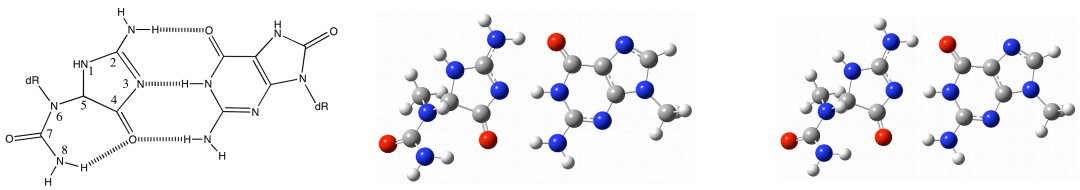


Ia1:G Ia2:G

Fig. 2 The Ia:G base pairs. a) The proposed Ia:G base pair. b) The geometries of Ia1:G and Ia2:G optimized by *ab initio* calculation.

Furthermore, Ia has isomers containing intramolecularly hydrogen bond (O4-H8) (Fig. 3). The stabilization energy of Ia3:G was 38.4 kcal/mol, and that of Ia4:G was 39.1 kcal/mol. Both the base pairs are destabilized as compared to Ia1:G and Ia2:G. It seems likely that the stabilization energy of pairing with guanine is reduced by the decline in electron density contributing to pairing with guanine, due to electron density delocalization of O4.

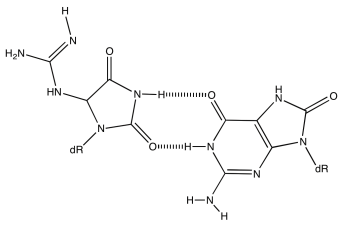
a) b)



Ia3:G Ia4:G

Fig. 3 The Ia:G base pairs containing hydrogen bond in Ia. a) The proposed Ia:G base pair. This numbering was shown in the reference 8. b) The geometries of Ia3:G and Ia4:G optimized by *ab initio* calculation.

a)



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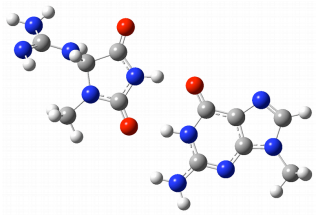
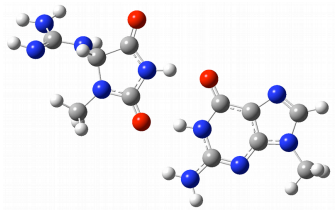
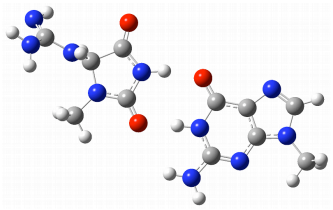


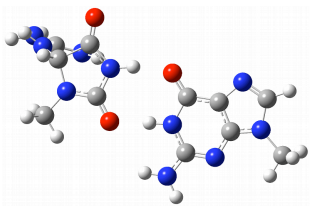
Gh1:G



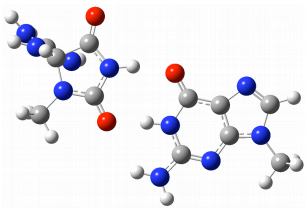
Gh2:G



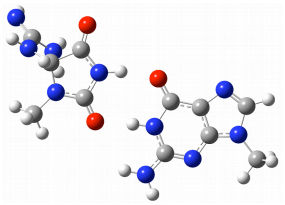
Gh3:G



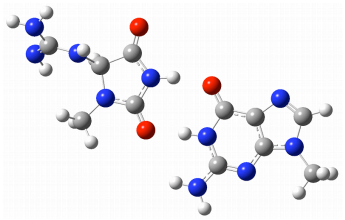
Gh4:G



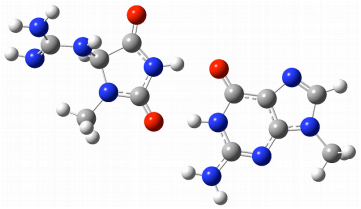
Gh5:G



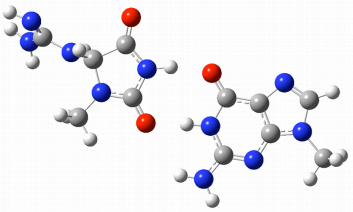
Gh6:G



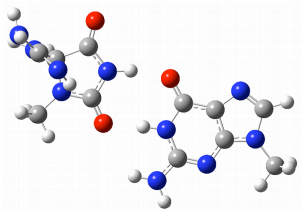
Gh7:G



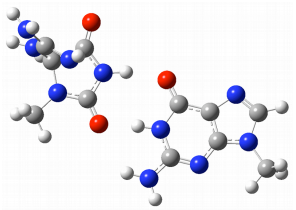
Gh8:G



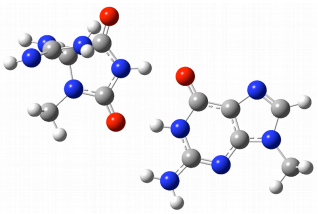
Gh9:G



Gh10:G



Gh11:G



Gh12:G

Fig. 4 The Gh:G base pairs. a) The proposed Gh:G base pair. b) The geometries of Gh1:G-Gh12:G optimized by *ab initio* calculations. The stabilization energies were shown in Table 1.

In contrast to our proposed Ia:G base pairs, Beckman *et al.* proposed that Gh:G has two hydrogen bonds.[11] Gh has many structural conformations, and the stabilization energies of Gh isomer1-12 (Gh1-12) were calculated and compared (Fig. 4). Both stabilization enegies of Gh2:G and Gh8:G were 27.8 kcal/mol; Gh2:G and Gh8:G were more stable than base-pairing with other isomers, although the reason is unknown. The stabilization energy of Gh2:G was 11.7 kcal/mol lower than that of Ia1:G and Ia2:G. The stabilization energy is considered to depend on the number of hydrogen bonds. Therefore, Gh may tautomerize to Ia when guanine is incorporated opposite Gh/Ia.

The calculated stabilization energies of Sp1:G and Sp2:G were 37.6 kcal/mol (Fig. 5), and these stabilization energies are 1.9 kcal/mol less than that of Ia1:G and Ia2:G base pairs. In fact, although the efficiency of guanine incorporation opposite Gh/Ia were higher than that of adenine incorporation, guanine were incorporated opposite Sp to almost the same efficiency as adenine.[9] Thus, the stabilization energy of Sp:G was lower, which are likely to contribute to increase of the efficiency that adenine is inserted opposite Sp.

a) b)

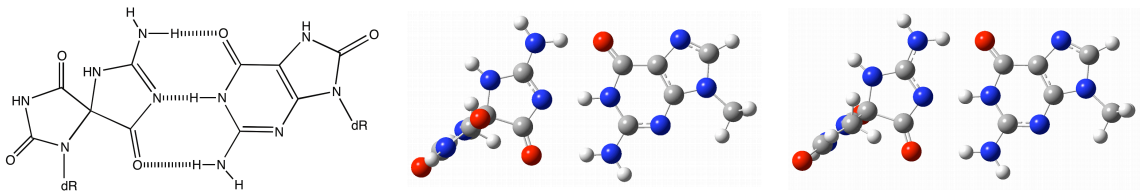


Fig. 5 The Sp:G base pairs. a) The proposed Sp:G base pair. b) The geometries of Sp1:G and Sp2:G optimized by *ab initio* calculation.

As described above, the stabilization energies of Ia:G and Sp:G having three hydrogen bonds were 37.6-39.5 kcal/mol, whereas those of Gh:G having two hydrogen bonds were 26.3-27.8 kcal/mol. These results indicate that stabilization of base pairs depends on the number of hydrogen bonds. Conversely, these results suggest that stabilization of base pair is only modestly influenced by structural configuration of the parts not containing hydrogen bonds with guanine.

In this study, the stabilization energies of oxidative lesions substituted CH_3 instead of 2'-deoxyribose were calculated, and geometrical positioning of 1'-carbons were not fixed. Thus, C1' position in duplex DNA should be considerable. In addition, the

calculated stabilization energy of Watson-Crick C:G base pair was 39.8 kcal/mol using Gaussian 03 (HF/3-21G), whereas the experimental value of stabilization energy of natural Watson-Crick C:G base pair is 21.0 kcal/mol.[12] This calculated energy was 18.8 kcal/mol higher than the experimental value, due to calculation using HF/3-21G. Therefore, it is necessary to calculate Ia:G, Gh:G, and Sp:G using higher level basis set and DFT calculation in water model. Furthermore, for more accurate calculations, correction with zero-point vibrational energy (ZPE), the basis set superposition error (BSSE) and the reorganization energy are also required in future.

Table1. stabilization energies when the oxidative guanine damages pair with guanine.

Base pair	ΔE	Base pair	ΔE
Ia1:G	39.5	Gh6:G	26.3
Ia2:G	39.5	Gh7:G	27.4
Ia3:G	38.4	Gh8:G	27.8
Ia4:G	39.0	Gh9:G	26.8
Gh1:G	27.4	Gh10:G	26.5
Gh2:G	27.8	Gh11:G	26.9
Gh3:G	27.0	Gh12:G	27.3
Gh4:G	26.8	Sp1:G	37.6
Gh5:G	26.9	Sp2:G	37.6

* ΔE, HF/3-21G stabilization energy (kcal/mol).

Conclusions

The stabilization energies of Ia1:G and Ia2:G were 39.5 kcal/mol, which were higher than that of Ia:G containing intramolecularly hydrogen bond. Their calculated stabilization energies were similar to that of Watson-Crick C:G base pair (39.8 kcal/mol). In contrast, the stabilization enegies of Gh:G having two hydrogen bonds were 27.8 kcal/mol, and their stabilization energies were lower compared to Ia:G. The stabilization energies of Sp:G base pairs were 37.6 kcal/mol, which were slightly less than that of Ia1:G and Ia2:G base pairs. Thus, stabilization of base pairs depends on the number of hydrogen bonds. In addition, these results suggest that structural

configuration of the parts not containing hydrogen bonds with guanine have insignificant effect on stabilization of base pair.

Anknowledgements

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