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Structural and electronic properties of guanine and guanosine

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Abstract

The structural and electronic properties of guanine and guanosine have been investigated theoretically by performing semi-empirical and ab initio molecular orbital theory calculations. The geometry of the systems have been optimized considering the semi-empirical molecular orbital theory at the level of Austin model 1, and the electronic properties of the systems have been calculated by ab initio restricted Hartree–Fock with including full MP2 correlation correction in their ground state. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Intracellular generation of reactive oxygen species (ROS) causes oxidative DNA damage and leads to the formation of DNA bases such as 8-oxoguanine (8OG) (8-hydroxyguanine) via hydroxyl radical attack at the C8-position of deoxyguanosine or thymine glycol. A direct measurement of ROS production in target organs is not currently possible in vivo. Therefore, theoretical approaches are considered in the elucidation of mechanism(s) and assessment of potential risks. Biologically speaking, the most significant product of oxidative DNA damage is 8OG (a C8 modified nucleobase) [1]. The level of 8-hydroxy-deoxyguanosine (8OHdG) is measured as a product of oxidatively damaged DNA; it is employed as a biomarker in tissues or body fluid [2]. Current estimates of steady-state levels are less than 100–1000 8OHdG residues in normal cells [3]. The contribution of oxidatively modified DNA bases, with the predominant products of 8OG, 8-oxoadenine (8OA) and ring-opened formamido-pyrimidine (Fapy), to mutagenesis is currently the subject of a great deal of interest.

The genome of a human cell has been estimated to receive roughly 10^4 oxidative hits per cell per day from endogenous oxidants by Ames et al.[1] This promutagenic damage is effectively but not perfectly repaired; the normal steady-state level of just 8OHdG (one of about 20 known oxidative DNA adducts) in rat DNA has been measured as 1/130,000 bases or about 47,000 per cell.

Owing to such high incidence, it is largely believed that oxidative DNA damage through C8-oxidation may play a key role in aging process, mutagenesis, reproductive cell death and various age-related degenerative diseases, including cancer [4]; it was found that 8-keto-6-enolic form was the most stable

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[1] http://socrates.berkeley.edu/mutagen/ames.PNASI.html

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tautomer of 8OG in the gas phase [5]. In the case of 8OA, the 8-keto-6-amino tautomer was predicted to be the most stable species. The appearance of the 8-oxo group had a significant effect in stabilizing the enolic and imino tautomers. The addition of free energies of hydration to the gas phase energies shifts the 8,6-diketo form of guanine (8OG1) to be the more stable than all other tautomeric species considered in the study. Their estimations suggested that both 8OG and 8OA might be of mutagenic significance since the equilibrium constants of tautomerization fell well within the replication frequency of DNA. They attributed the existence of such minor tautomeric forms of 8-oxopurines to possible $A \rightarrow C$ type and $G \rightarrow T$ type transitional mutations.

Kohda et al. [6] have shown 8-methyl-2′-deoxyguanosine as a result of methyl radical attack at the C8-position of 2′-deoxyguanosine in duplex DNA to create a mutagenic lesion, generating $G \rightarrow C$ and $G \rightarrow T$ transversions and deletion. Yang et al. [7] have shown that lead acetate induces DNA damage through strand breakage and 8OHdG adduct formation and that singlet oxygen is the principal species involved. DNA damage prevention by scavengers was also investigated. Singlet oxygen scavengers were found to be more effective than hydroxyl radical scavengers in protection from lead/H_2O_2-induced 8OHdG adducts.

Numerous experimental studies on the biological implications of C8-oxidized purines as products of oxidative DNA damage have been carried out. Under physiological conditions the 6,8-dioxo species of guanine tautomer predominate over the other modified bases. The DNA template properties of 6,8-dioxo tautomer indicate in vivo and in vitro mutagenic properties. The base-mispairing and mutagenic specificity of this guanine derivative is not clearly known. The dioxo tautomer showed a clear preference to pair with cytosine in the normal Watson–Crick fashion more favorable than the normal G/C pair [8].

As oxidative DNA damage is considered to be a pathogenic event in the induction of many cancers, a reduction in the rate of such damage by catechins (the polyphenols of green tea) acting as antioxidants may lead to a reduced risk of cancer. It is known that catechins are efficient scavengers of ROS per se and inhibit the formation of 8-oxodeoxyguanosine (8-oxo-dG). A reduction in the rate of oxidative DNA damage in humans, in terms of this marker, has also been observed following the ingestion of foods which contain high amounts of glucosinolates, but similar studies have not been done with foods containing flavonoids. A mechanism of electron transfer (or H-atom transfer) from catechins to ROS-induced radical sites on the DNA as an antioxidant role for catechins has been proposed [9].

It is now widely accepted theory that cancer arises as the result of a series of somatic cell mutations. Normally damage to DNA is efficiently repaired, but if damage accumulates beyond the repair capacity of a cell, then the mutation frequency increases, as does the likelihood of carcinogenesis. Synthetic oligonucleotides containing 8-hydroxyguanine in a specific position were used as a template for DNA synthesis and shown to be misread both at the modified base and at adjacent pyrimidine bases; indicating 8-hydroxyguanine completely lacking specific base-pairing [10]. In addition, at the adjacent pyrimidine bases, the insertion of incorrect bases was observed.

8OHdG, the sensitive indicator of oxidative DNA damage and a major product of this damage is produced by enzymatic cleavage after 8-hydroxylation of guanine. Since 8OHdG is an unstable molecule, it is further hydrolyzed to 8-hydroxyguanine and both molecules are released into systemic circulation and secreted in urine. Measurement of plasma 8OG may provide a more accurate and stable assay for oxidative DNA damage and assessing exposure levels of environmental mutagens, because 8OG is not affected by the work-up procedure. 8OHdG has also been found in target organs of animals or human cells [4,11,12].

Diabetic status is also associated with increased production of ROS and this condition in turn has been suggested as one of the pathological mechanisms of diabetic complications, including diabetic vascular complications. Park et al. [13] found 8OG to be a useful biomarker of oxidative DNA damage in diabetic patients, concluding that the plasma 8OG level significantly correlated with tissue 8OHdG level.

Proteggente et al. [12] reported significant gender differences in levels of DNA bases such as Fapy, 8OA, 5-hydroxycytosine (5OC). Collins et al. [14] have measured blood levels of dietary antioxidants (such as vitamin C, vitamin E and carotenoids) and

Table 1
Some of the molecular properties of the guanine (8OG) and guanosine (8OHdG) molecules in their ground state (ab initio results)

Quantity	Guanine	Guanosine
Number of electrons	86	140
Number of doubly occupied levels	43	70
Number of total orbitals	65	108
Number of primitive Gaussians	195	324
Multiplicity	Singlet	Singlet
Molecular point group	C_1	C_1

8-oxo-dG concentrations in lymphocyte DNA, in healthy men and women from five European countries. Their results showed that 8-oxo-dG levels in lymphocyte DNA vary significantly according to sex and country. Oxidative DNA damage was not significantly affected by carotenoid supplementation; nor was there any association with mean baseline levels of antioxidants, which were generally similar in the five countries.

van Zeeland et al. [11] found an inverse relationship between 8OHdG in peripheral leukocytes and cigarette smoking, with subjects who usually smoked more than 20 cigarettes/day having the lowest levels of the adduct.

The very high incidence of cellular damage by oxidative agents necessitates the study of the properties of these oxidized bases. The detailed mechanism of 8-oxopurine induced mutagenesis has yet to be elucidated.

Aida and Nishimura [10] have studied theoretically the tautomeric forms of guanine and they found that 6,8-diketo form of 8-hydroxyguanine was the most stable structure. Recently Meyer et al. [15] have investigated the guanine and uracil quartets and guanine quartet metal/ion complexes theoretically. Reynisson and Steenken [16] have investigated the interaction of guanine with water theoretically. Zhanpeisov et al. [17] have investigated the tautomeric rearrangements in the 6,8-dithioguanine theoretically. Gu and Leszcynski [18] have investigated the influence of the oxygen on C8-oxidative guanine theoretically. Cysewski et al. [19] have studied the tautomerization of Fapy–guanine theoretically. Cysewski [20] has investigated the tautomeric and coding properties of 8OG theoretically. Along this line, we have investigated the structural and electronic properties of isolated guanine and guanosine molecules theoretically by performing semi-empirical molecular orbital and ab initio calculations because of their biological and medical importance.

2. Method of calculation

In the present study, the guanine and guanosine molecules have been considered theoretically by performing both semi-empirical molecular orbital theory and ab initio calculations. The closed formula of the guanine (8-oxoguanine, hereafter referred to as 8OG) is in the form $C_5N_5O_2H_5$, and the closed formula of the guanosine (8-hydroxydeoxyguanosine, hereafter referred to as 8OHdG) is in the form $C_{10}N_5O_4H_{13}$. Preoptimization has been performed by applying the molecular-mechanics method [21] using MM + force field [22]; this makes easier to perform full optimization by extended methods. The Austin model 1 (AM1) semi-empirical method [23] within the restricted Hartree–Fock (RHF) [24] formalism has been considered to optimize fully the geometry of the systems considered.

Geometry optimization is carried out by using a conjugate gradient method (Polak–Ribiere algorithm [25]), then the electronic structure of the system has been calculated by applying the ab initio RHF with including full MP2 correlation correction [26] in the ground state. The minimum basis set (STO-3G) [27] has been used in the calculations, which may give qualitative but reliable information about the systems considered. In a resent work on small biological molecules [28], it was obtained that the results of STO-3G basis and extended basis sets were close to each other. Therefore, in this work we have preferred small basis set, namely STO-3G basis set, for isolated guanine and guanosine molecules or guanine and guanosine molecules in the gas phase. The SCF convergency is set to 0.001 kcal/mol in the geometry optimization within AM1 method and 0.00001 kcal/mol in the electronic calculation within ab initio method. We have performed all the calculations by using the HyperChem-5.1 packet.

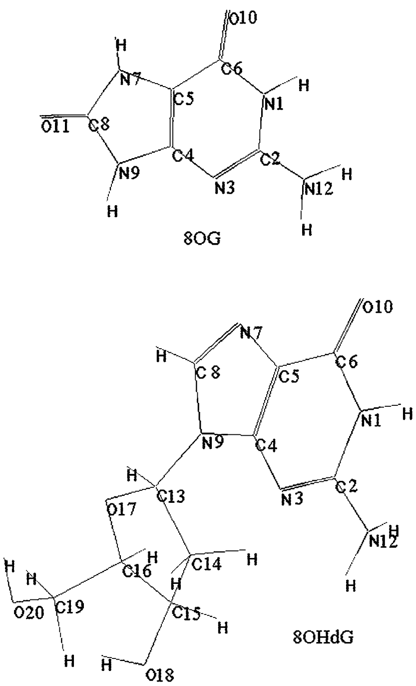

Fig. 1. The optimized structures of guanine (8OG) and guanosine (8OHdG) molecules in their ground state; AM1 results.

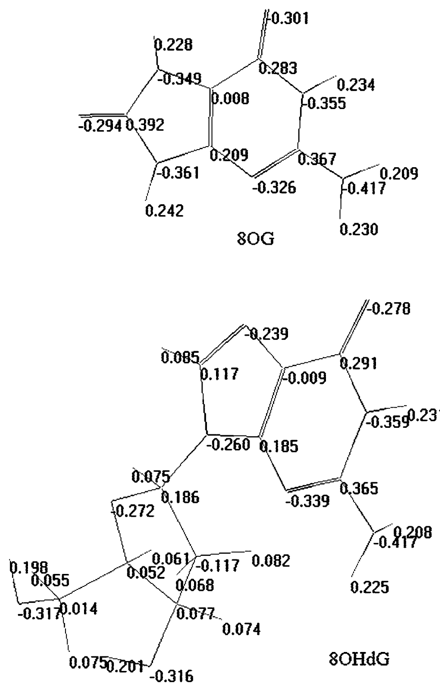

Fig. 2. Excess charge on the atoms of guanine (8OG) and guanosine (8OHdG) molecules in their ground state; ab initio results.

3. Results and discussion

Some of the molecular properties of the systems considered are given in Table 1. The AM1 method gives almost a planar structure as more stable for isolated guanine molecule and a non-planar structure as more stable for guanosine molecule. The optimized structures of the molecules are shown in Fig. 1 with numbered atomic labels. In the guanosine molecule, the guanine part almost keeps its structure as its isolated form, however, the additional pentagonal ring, the sugar based pentagonal ring, in the guanosine is not planar with guanine part, which is almost perpendicular to guanine part. The non-planar structure of the guanosine increases the molecular volume of the system a lot. The structural properties of the guanine molecule in the present work agree with the previous predictions [10,15,20].

The excess charge on the atoms of the systems considered are shown on the atoms in Fig. 2. As one expects, the excess charge accumulation on the atoms are different for each system. In the guanine molecule, oxygen atoms have -0.301 and -0.294 unit of electron charge; these atoms play an important role of attractive centers within the molecule. On the other hand, in the guanosine molecule oxygen atoms have -0.278, -0.272, -0.317 and -0.316 unit of electron charge. The charge of O10 reduces from -0.301 in guanine to -0.278 in guanosine. The oxygen atom O11 in guanine does not appear in guanosine.

The three-dimensional maps of total charge densities and electrostatic potentials of the systems studied are shown in Figs. 3 and 4, respectively. The oxygen atoms in guanine gave a negative character to the whole molecule, hence guanine has a great influence upon its surroundings. The electrostatic force plays a key role in base–base stacking interactions in the DNA double helix. However, the addition of sugar base pentagonal ring to guanine entirely changes the electrostatic potential of the molecule. Although the negative domains are located near the oxygen atoms O10, O11 and nitrogen atom N3 on the guanine molecule, the negative domains on

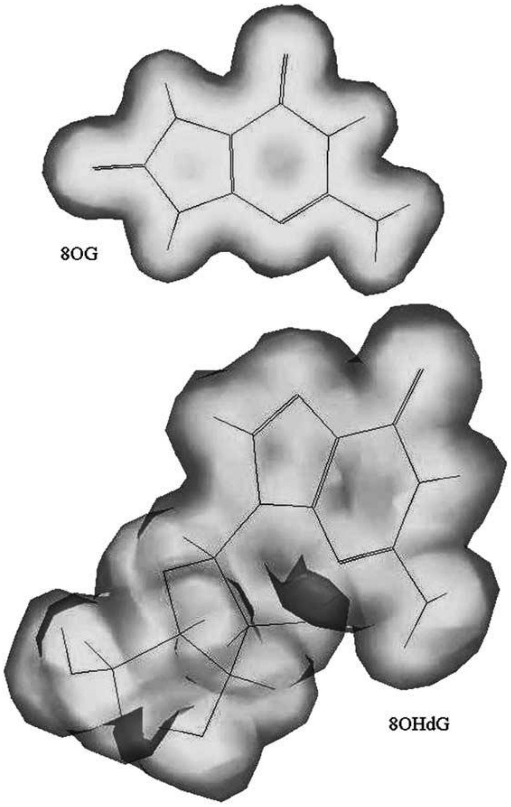

Fig. 3. Three-dimensional charge density maps of guanine (8OG) and guanosine (8OHdG) molecules in their ground state; ab initio results.

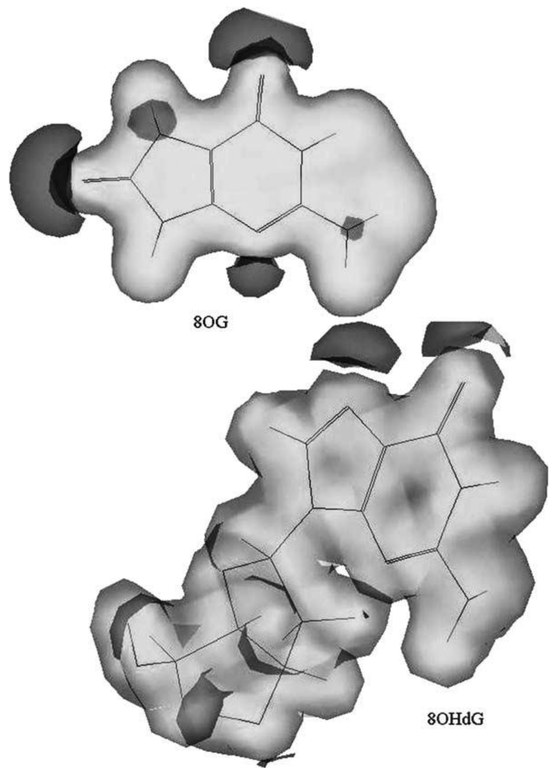

Fig. 4. Three-dimensional electrostatic potential maps of guanine (8OG) and guanosine (8OHdG) molecules in their ground state; ab initio results.

the guanosine molecule are located near the oxygen atom O10 and nitrogen atom N7. Similar electrostatic potential characteristics were also obtained on Fapy–guanine tautomers [19] and 8-hydroxyguanine [10]. The location of the fields corresponding to the negative or positive values of the electrostatic potential is important when one considers the formation of intermolecular complexes.

Although the volume of the guanosine molecule is relatively larger than that of the guanine molecule, the charge distribution on the guanine molecule generates larger dipole moment than that of the guanosine molecule; this makes guanine molecule more reactive and attractive than guanosine molecule for their surrounding. From the three-dimensional potential map one can conclude that the additional pentagonal part, the sugar base pentagonal ring, in the guanosine molecule looks more attractive with respect to the guanine part within the guanosine molecule. The molecular orbital energy (eigenvalue) spectra of the systems studied are shown in Fig. 5. Interestingly both systems have similar eigenvalue spectrum.

The calculated energy values (by both AM1 and ab

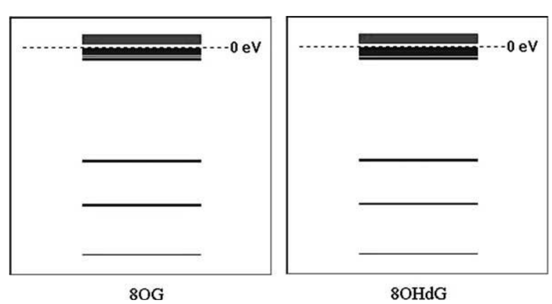

Fig. 5. Eigenvalue spectra of guanine (8OG) and guanosine (8OHdG) molecules in their ground state; ab initio results.

Table 2
Some of the calculated energy values and dipole moments μ of the guanine (8OG) and guanosine (8OHdG) molecules in their ground state with singlet symmetry

Quantity	Guanine	Guanosine
AM1		
TE (*)	−54,962.775	−87,052.128
BE (*)	−1800.251	−3277.327
IAE (*)	−53,162.523	−83,774.802
EE (*)	−251,385.171	−541,977.561
CCI (*)	196,422.396	454,925.433
HoF (*)	−1.173	−87.865
Ab initio		
TE (*)	−380,864.729	−594,053.682
MP2 (*)	−414.908	−620.979
eKeeeN (*)	−807,034.791	−1,537,485.897
NRE (*)	426,584.970	944,053.194
LLE (+)	−549.929	−551.828
HOMO (+)	−4.937	−5.471
LUMO (+)	5.757	5.831
ΔE (+)	10.794	11.302
HLE (+)	29.171	29.400
μ_x	0.464	−1.149
μ_y	5.458	−1.198
μ_z	1.809	−3.771
μ	5.769	4.121

TE, total energy; BE, binding energy; IAE, isolated atomic energy; EE, electronic energy; CCI, core–core interaction; HoF, heat of formation; MP2, MP2 correlation contribution; eKeeeN, eK, ee and eN energy; NRE, nuclear repulsion energy; LLE, lowest level energy; ΔE, HOMO–LUMO gap; HLE, highest level energy. Energies marked with (*) are in kcal/mol, marked with (+) are in eV, the dipole moments are in Debyes.

initio methods) of the systems studied are given in Table 2. The highest occupied and the lowest unoccupied molecular orbital energies (HOMO and LUMO, respectively) and the interfrontier molecular orbital energy gap (LUMO–HOMO energy difference, ΔE) with the lowest and highest level energy values are also given in Table 2. The calculated dipole moment values of the systems considered are also given in Table 2.

According to AM1 calculation, binding energy of the guanine and guanosine molecules are about −1800 and −3277 kcal/mol, respectively. Heat of formation of the same molecules are about −1 and −88 kcal/mol, respectively, and they are exothermic. The ratio of the heat of formation values of both molecules is much larger than that of their binding energies. This indicates that the guanine molecule is more reactive and fragments easily with respect to the guanosine molecule and has high pairing ability. Similar results were also obtained for 8OG [20] and 8-oxo-purines [8].

According to ab initio calculation HOMO–LUMO gap of the guanine and guanosine molecules are about 11 eV. ΔE values of the guanine molecule is slightly less than that of the guanosine molecule. The additional pentagonal ring on guanine does not change ΔE considerably. Furthermore, both HOMO and LUMO are mainly localized on the guanine part in the guanosine molecule. The guanine molecule has relatively larger dipole moment, about 6 Debyes, however, guanosine molecule has a dipole moment value of about 4 Debyes. One may conclude that guanine is a highly polar molecule, therefore it may interact with its surrounding, especially with other polar molecules in the cell more strongly; this makes the guanine molecule as a potential source of damage in the cells.

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