Population of 6-Enol Form is Higher in 8-Oxoguanine than in Guanine

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We investigate the relative stabilities of tautomers of 8-oxoguanine (8OG) and guanine (G) comprehensively at various levels of theory up to MP4 and CCSD(T) in the gas phase. We find that the population of the enol form at C6 position in 8OG is higher than that in G at any level of theory which we used. This may partly account for the high mutagenicity of 8OG.

8-Oxoguanine (8OG) is one of the oxidative DNA damage products and is produced by the oxidation at C8 position of guanine (G). Its mutagenic potential has been studied widely in vivo and in vitro,[1,2] and it is known that 8OG causes transversion as well as transition mutation. The oxidation brings about the loss of the fidelity as a carrier of the genetic information.

To elucidate the origin of the mutagenicity of 8OG, the relative stability between the 8OG tautomers has been a subject of interest.[3] This problem is not solved yet, because the computational results depend strongly on the level of theory used.[4–7] In this letter, we show comprehensively the relative stabilities of all kinds of tautomers of 8OG as well as G at various levels of theory. We present the comparison of the relative population of the 6-enol form to 6-keto form between 8OG and G, which sheds light on the origin of the mutagenicity of 8OG.

There are 96 possible tautomeric forms for 8OG and 36 for G. The tautomeric forms were carefully screened with the following procedure. First, we optimized the geometries of all possible tautomeric forms at the levels of HF/6-31G(d) and MP2/6-31G(d) with C_s or with C_1 symmetries followed by the vibrational analyses. We obtained 79 tautomers for 8OG and 35 for G at both levels. We confirmed that each of the tautomers corresponds to the energy minimum. Secondly, we selected the 10 most stable tautomers at the MP2/6-31G(d) level for both 8OG and G, and we optimized the geometries and performed the vibrational analyses at the MP2/aug-cc-pVDZ level. The complete list of the total energies and the relative energies are shown in the Supporting Information (Tables S1–S6).[20] All calculations in this letter were carried out without frozen-core approximations, i.e. all electrons were included in the correlation calculations.

Among the selected 10 tautomers of 8OG, the two types of tautomers (6,8-diketo and 6-enol-8-keto; Figure 1a) are exclusively dominant (Table S5).[20] The relative energy of the third stable tautomer compared to these two tautomers is around 6 kcal/mol. The energy difference is so large that the further inclusion of higher-order electron correlation or using a larger basis set may not change the relative stability of this form. Thus, we have selected these two most stable tautomers for 8OG and performed very comprehensive calculations with various levels of theory (Table 1). The complete list is given in the supporting information (Table S8).[20]

As shown in Table 1, both of MP2 and CCSD(T) methods

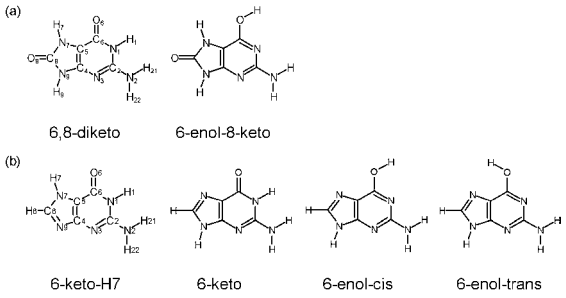

Figure 1. Schematic representation and atom numbering of major tautomers of (a) 8-oxoguanine and (b) guanine.

show that the 6-enol-8-keto form is more stable than the 6,8-diketo form when a larger basis set is used, while MP4 shows that the 6,8-diketo form is stable. The energy difference between the two forms is very small at any computational level. There has been no experimental data in literatures concerning the detection of tautomers of 8OG in the gas phase or in the matrix-isolated state. Taking account of the very small energy difference between the two forms, the relative stability must be affected by the surroundings. We urge experimental researchers to investigate the 8OG tautomeric forms in various circumstances.

Among the selected 10 tautomers of G, the 4 types (Figure 1b) are found to be the most stable (Table S6).[20] We have selected these four tautomers for G and performed the same calculations as for 8OG. The list for the fifth most stable tautomer is shown in Table S8.[20]

The relative energies between the 4 dominant forms of G depend on the method used. The order at MP4 and CCSD(T) is 6-keto-H7 < 6-keto < 6-enol-cis < 6-enol-trans. This is similar to the previous report.[8] The existence of both keto and

Table 1. Relative energies of 8OG tautomers in kcal/mol

level	6,8-diketo[a]	6-enol-8-keto[a]
MP2/6-31G(d)	0.00 (0.00)	1.17 (0.93)
MP2/6-311G(d,p)	0.00	−0.82
MP2/6-311+G(d,p)	0.00	−0.97
MP2/6-311++G(d,p)	0.00	−0.99
MP2/6-311+G(2d,2p)	0.00	−0.64
MP2/6-311+G(2df,2pd)	0.00	−0.87
MP2/6-311+G(df,pd)	0.00	−2.02
MP2/cc-pVDZ	0.00 (0.00)	−0.49 (−0.61)
MP2/cc-pVTZ	0.00	−0.97
MP2/aug-cc-pVDZ	0.00 (0.00)	−0.40 (−0.62)
MP2/aug-cc-pVTZ	0.00	−0.45
MP4(SDTQ)/cc-pVDZ[b]	0.00	0.51
MP4(SDTQ)/aug-cc-pVDZ[c]	0.00	0.52
CCSD(T)/cc-pVDZ[d]	0.00	−0.49
CCSD(T)/aug-cc-pVDZ[d]	0.00	−0.46
CCSD(T)/aug-cc-pVDZ[c]	0.00	−0.41

[a]The zero-point energy corrected values are in parentheses. [b]Geometry at MP4(SDQ)/cc-pVDZ. [c]Geometry at MP4(SDQ)/aug-cc-pVDZ. [d]Geometry at CCSD/cc-pVDZ.

Table 2. Relative energies of G tautomers in kcal/mol

level	6-keto-H7[a]	6-keto[a]	6-enol-cis[a]	6-enol-trans[a]
MP2/6-31G(d)	−0.11 (−0.02)	0.00 (0.00)	2.42 (2.30)	3.09 (2.97)
MP2/6-311G(d,p)	0.19	0.00	0.61	1.18
MP2/6-311+G(d,p)	−0.28	0.00	0.12	0.71
MP2/6-311++G(d,p)	−0.29	0.00	0.11	0.68
MP2/6-311+G(2d,2p)	−0.70	0.00	0.18	0.39
MP2/6-311+G(2df,2pd)	−0.68	0.00	−0.09	0.05
MP2/6-311+G(df,pd)	−0.26	0.00	−0.94	−0.44
MP2/cc-pVDZ	0.22 (0.32)	0.00 (0.00)	0.88 (0.91)	1.39 (1.46)
MP2/cc-pVTZ	−0.53	0.00	−0.07	0.16
MP2/aug-cc-pVDZ	−0.73 (−0.54)	0.00 (0.00)	0.35 (0.29)	0.60 (0.56)
MP2/aug-cc-pVTZ	−0.78	0.00	0.19	0.36
MP4(SDTQ)/cc-pVDZ[b]	0.17	0.00	1.72	2.27
MP4(SDTQ)/aug-cc-pVDZ[c]	−0.80	0.00	1.14	1.47
CCSD(T)/cc-pVDZ[d]	0.16	0.00	0.61	1.21
CCSD(T)/aug-cc-pVDZ[d]	−0.70	0.00	0.15	0.52
CCSD(T)/aug-cc-pVDZ[c]	−0.75	0.00	0.17	0.54

[a]The zero-point energy corrected values are in parentheses. [b]Geometry at MP4(SDQ)/cc-pVDZ. [c]Geometry at MP4(SDQ)/aug-cc-pVDZ. [d]Geometry at CCSD/cc-pVDZ.

enol forms has been experimentally observed in the matrix-isolated infrared spectroscopy,[9,10] and also in the supersonic jet utilizing R2PI spectroscopy.[11–14]

The keto–enol tautomerization has been one of the research subjects in chemistry. In molecules with aromatic rings, the computational relative stability between keto and enol tautomers may depend on the theory and the basis set used.[15–17] 8OG and G show the same tendency here. As shown in Tables 1 and 2, the inclusion of a diffuse function has a large effect on the relative stability of tautomers, while adding another diffuse function has little effect. After the comprehensive computations, we found that the basis set balance is important to elucidate the relative stabilities of tautomers. Especially, the balanced use of the polarization functions between the heavy atoms and hydrogen atoms is crucial. The more polarization functions are put on hydrogen atoms, the more the enol type gets stable. On the other hand, the polarization functions on the heavy atoms bring about the reversed effects (See Table S8).[20] Thus, the over-polarization of hydrogen atoms compared to heavy atoms makes enol type more stable than keto type. It seems that the Dunning's correlation consistent basis sets are well balanced.[18]

As shown above, the 6-keto and 6-enol forms are dominant for both 8OG and G. The relative population of the two forms can be estimated from the relative energy of the enol to the keto forms. In Table 3, the relative population of the 6-enol form of 8OG is shown together with those of G. The list of all the computational data is given in Table S9.[20] Although the stability between the enol and the keto forms at C6 position may depend on the theoretical method used and is still controversial, the population of the 6-enol form of 8OG is higher than G in the gas phase, regardless of the theoretical method or the basis set used. The 6-enol form of 8OG can pair with thymine and could cause G:C to A:T transition mutation in DNA. The possibility of higher population of 6-enol in 8OG than in G must be taken into account when experimental data are analyzed concerning the mutagenicity of 8OG.

The calculations were carried out using the Gaussian 03 program[19] on the PC cluster system at QuL*i*S and the NEC SX7 at the Research Center for Computational Science. This study was partly supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Table 3. Relative population of 6-enol form to 6-keto form

	8OG	G	
level	$K^{a,d}$	$K^{b,d}$	$K^{c,d}$
MP2/6-31G(d)	0.14 (0.21)	0.02 (0.02)	0.01 (0.01)
MP2/6-311G(d,p)	4.00	0.35	0.13
MP2/6-311+G(d,p)	5.15	0.82	0.30
MP2/6-311++G(d,p)	5.35	0.83	0.32
MP2/6-311+G(2d,2p)	2.93	0.74	0.52
MP2/6-311+G(2df,2pd)	4.34	1.17	0.92
MP2/6-311+G(df,pd)	30.46	4.93	2.11
MP2/cc-pVDZ	2.30 (2.83)	0.23 (0.21)	0.10 (0.08)
MP2/cc-pVTZ	5.21	1.13	0.77
MP2/aug-cc-pVDZ	1.97 (2.87)	0.55 (0.61)	0.36 (0.39)
MP2/aug-cc-pVTZ	2.14	0.72	0.55
MP4(SDTQ)/cc-pVDZ[e]	0.42	0.05	0.02
MP4(SDTQ)/aug-cc-pVDZ[e]	0.41	0.14	0.08
CCSD(T)/cc-pVDZ[e]	2.29	0.36	0.13
CCSD(T)/aug-cc-pVDZ[e]	2.18	0.77	0.41
CCSD(T)/aug-cc-pVDZ[e]	1.99	0.75	0.40

[a][6-enol-8-keto]/[6,8-diketo]. [b][6-enol-cis]/[6-keto]. [c][6-enol-trans]/[6-keto]. [d]The values in parentheses correspond to those using the zero-point energy corrected energies. [e]See the corresponding footnotes of Table 1 or Table 2.

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