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Tautomeric equilibria in 8-oxopurines: Implications for mutagenicity

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Summary

Free-radical-induced DNA damage by ionizing radiation leads to a number of oxidized purines, of which 7H-8-oxoguanine (8OG) and 7H-8-oxoadenine (8OA) are predominant and known to cause an appreciable amount of cellular damage. A detailed quantum mechanical study at various levels of theory in both the gas phase and in an aqueous solution has been carried out in order to assess the tautomeric preferences of the bases. The calculated energies of various plausible tautomers suggest that at higher levels of ab initio theory with inclusion of electron correlation, the 8-keto-6-enolic form of 8-oxoguanine (8OG2) would predominate over the 6,8-diketo form (8OG1) in the gas phase whereas the 6-amino-8-keto form (8OA1) predominates over the other possible tautomers of 8-oxoadenine. Aqueous solvation, however, changes the gas-phase order for 8-oxoguanine, 8OG1 turning out to be the major tautomeric species in an aqueous medium. The estimated free energies of hydration by polarized continuum models are indicative that the mutagenically significant amounts of minor tautomeric forms of 8-oxoguanine and 8-oxoadenine exist in the aqueous phase and might be held responsible for inducing transversional as well as transitional mutations.

Introduction

Oxidative DNA damage caused by a wide variety of reactive oxygen species generated by in vivo oxidative metabolism or by exogenous agents such as ionizing radiation and chemical oxidants has been widely implicated for leading to C^8 modification of nucleobases [1–4]. There has been much interest in the properties of these oxidized bases due to a very high incidence of cellular damage by the oxidative agents [5,6]. It has been estimated that the genome of a human cell receives roughly 10^4 oxidative hits per day [7]. Owing to such high incidence, it is largely believed that C^8-oxidation may play a key role in both the aging process and various age-related degenerative diseases, including cancer [8–11]. It has also been shown that oxygen-derived free radicals or cellular metabolism generate apurinic/apyrimidinic lesions which may also have mutagenic and carcinogenic effects [12].

Oxidative modification of DNA bases leads to about 20 pyrimidine and purine base modifications, out of which 8-oxoguanine (8OG), 8-oxoadenine (8OA) and ring-opened formamido-pyrimidine (Fapy) bases are predominant products [13]. The contribution of these species to mutagenesis is currently the subject of a great deal of interest [14–16].

In their monomeric form, these modified bases can adopt several tautomeric structures. Figures 1 and 2 show various possible tautomers of 8OG and 8OA, respectively. Under physiological conditions, the 6,8-dioxo species (8OG1 in Figure 1) is reported to predominate over other possible tautomers [17]. However, NMR spectral data by Kouchakdjian et al. [15] indicate the presence of about 15% of minor tautomers in the case of 8OG. Therefore, the significance of minor tautomers, particularly the enol forms, in base mispairing and inducing mutations cannot be ruled out. The reported ^{15}N NMR studies on 8OA suggested that while the 8-keto form (8OA1) predominates under physiological conditions, the minor 8-enolate tautomer (8OA2) is also identified to exist at higher pK_a values [18]. However, it is not clearly known whether a minor tautomer of 8-oxoadenine would play a role in

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base mispairing. The only available gas-phase ab initio study of 8-oxoguanines at the minimal 3–21G basis set indicated that the 6,8-dioxo form (8OG1) predominates in 8OG [19]. However, there has been no study on the tautomeric energies of these C^8-oxidized products in an aqueous physiological medium although it is generally believed that the tautomeric preferences of bases would be significantly influenced by an aqueous environment. Since some of the minor forms of 8OG and 8OA might well participate in base mispairing, a detailed evaluation of the possible effects of an aqueous environment on the tautomeric equilibrium would lead to a better understanding of their involvement in mutagenesis.

Recent studies on the template properties of 8OG, originally detected in γ-irradiated DNA, have shown this species to be mutagenic both in vivo [20, 21] and in vitro [22,23]. These studies have also established that translesional synthesis can proceed past 8-oxo-7H-dG in primed template reactions catalyzed by DNA polymerase, in which case dA and/or dC is inserted opposite the lesion. The 8-oxo-7H-dG:dA pair is readily extended by DNA polymerase and does not appear to be subject to the editing function of this enzyme [23]. However, the base mispairing and mutagenic specificity of 8OdG is not clearly known. The in vivo study by Cheng et al. [3] of complementary bacteriophage plaque color assays using 8OGTP and DNA polymerase illustrated the mutagenic replication of 8OG as a template causing G→T substitutions and misincorporation of 8OG as substrate causing A→C substitutions, both caused by 8OG·A mispairs.

While most of these studies have shown the possibility of only G to T type transversional mutations, the in vivo studies on the hot spots of *c-Ha-ras* genes by Kamiya et al. [24] raised the possibility of other types of mutations too. The *ras* genes with 8OG were transfected into NIH3T3 cells, and an analysis of the mutations revealed the G to T type induction in the first positions of codons 12 and 61. On the other hand, the DNA lesion at the second position of codon 12 induced a G to A transition in addition to a G to T transversion. This clearly shows that there is a fair possibility of transitional mutations in addition to rather well-studied targeted transversions. Now, the question arises as to which species of 8OG might be responsible for these G to A type transitional mutations. While it may be argued that 8OG can itself mispair with thymine in its native form to lead to such mutations, the possible involvement of minor enol tautomers in stabilizing such mispairs cannot be ruled out. Figure 3 illustrates some of the possible base-mispairing schemes which involve minor enolic forms of 8-oxoguanine and 8-oxoadenine.

In contrast to 8OG, 8OA is not particularly mutagenic and is found to be at least an order of magnitude less mutagenic than 8-oxoguanine [25]. The structural studies by Evans and co-workers [17,18] have shown that 8OG and 8OA have very strong structural similarities and have shown that both lesions predominate at the physiological pH in the native form and appear to adopt the *syn* conformation around the glycosyl bond. So far, there have been no detailed studies on the tautomeric preferences of 8OA although 6-amino-8-oxoadenine is normally assumed to be the predominant form in vivo. A detailed study on the energetic preferences of various structural forms of 8OA is certainly warranted in order to understand the mutagenic properties of these bases.

The present work, utilizing the semi-empirical and ab initio molecular orbital models, is aimed at understanding the tautomeric preferences of 8OG and 8OA in both the aqueous and gaseous phase to gain an insight into the stability of minor tautomers and at attempting to seek the influence of solvent-induced stabilization of minor tautomers and their possible role in tautomerism-induced mutagenesis. It complements our recent theoretical studies on structures and properties of various classical and non-classical nucleic acid bases and their tautomers [26–28].

Methods

The ab initio LCAO-MO [29] method is used in the present study of tautomers of 8-oxoguanine and 8-oxoadenine. The full geometry optimizations were performed without imposing any symmetry constraints at the HF/6-31G(d,p) [30a] and MP2/6-31G(d,p) [30b] levels of theory. All optimized geometries at HF/6-31G(d,p) were found to be true minima by analysis of respective harmonic vibrational frequencies obtained from diagonalization of force constant matrices with the corresponding Hessian eigenvalues being positive. We have also used the MIDI! basis set which was introduced recently by Evan et al. [31]. Although this basis set is much smaller than 6-31G(d), it has been shown to be capable of yielding more accurate geometries and charges than MP2/cc-pVDZ calculations. In order to evaluate the effect of diffused functions and electron correlation effects, we have carried out single point calculations at the

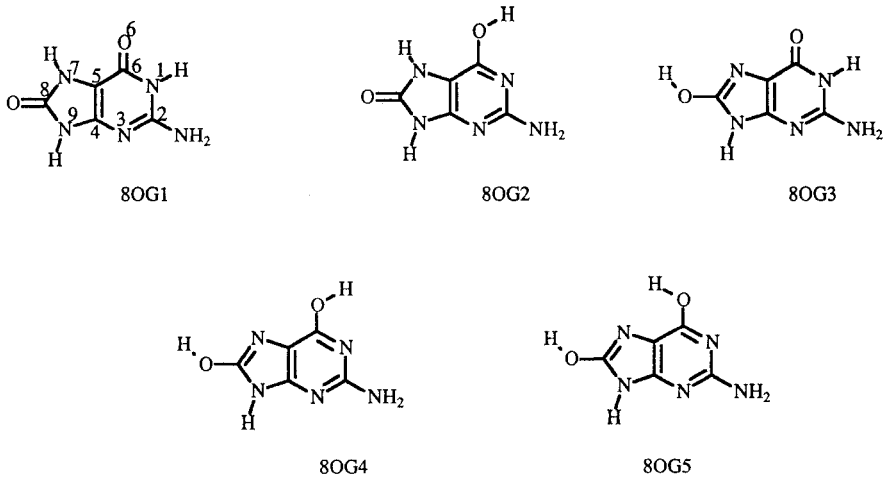

Figure 1. Five tautomers of 8-oxoguanine with numbering system shown on the 8OG1 tautomer.

MP2/6-311++G(d,p) [32] level at the HF/6-31G(d,p) and MP2/6-31G(d,p) reference geometries. The ZPE corrections were made as the sum of the zero-point energies (estimated at the HF/6-31G(d,p) level) for all normal-mode vibrations scaled by a recommended factor of 0.9. The gas-phase free energies were calculated by using standard formulae $\Delta G = \Delta H - T\Delta S$; the thermodynamic quantities were evaluated at 298.5 K.

All ab initio calculations were carried out using the GAUSSIAN 94 package [33] while the semi-empirical calculations in aqueous phase were done using the AMSOL 4.5.1 [34] suite of programs. The semi-empirical AM1-SM2 [35] method has been used in this study to evaluate the free energies of hydration of the various tautomers. The AM1-SM2 method was shown to be more reliable than the other semi-empirical methods in representing the solvation effects [36,37]. Besides this method, we have also used the ab initio self-consistent isodensity polarizable continuum model as developed by Tomasi and co-workers [38] and incorporated into GAUSSIAN 94 as an SCI-PCM model [38] to evaluate the electrostatic free energies of hydration. We used a relative permittivity constant of 78.4 to model an aqueous medium and a default 0.001e isodensity surface. The free energy of tautomerization in aqueous solution was subsequently determined according to Equation 1:

$$\Delta G^{aq}_{A\rightarrow B} = \Delta G^{gas}_{A\rightarrow B} + \Delta G^{hyd}_{B} - \Delta G^{hyd}_{A} = \Delta G^{gas}_{A\rightarrow B} + \Delta\Delta G^{hyd}_{A\rightarrow B} \quad (1)$$

Relative free energies of hydration ($\Delta\Delta G_{A\rightarrow B}$) are computed at the AM1-SM2 and HF/SCRF level from the absolute free energies of hydration (ΔG^{hyd}_{A} and ΔG^{hyd}_{B}).

Results and Discussion

Gas-phase free energies

Five tautomers of 8-oxoguanine were considered in the present study and their energies were computed at the ab initio level using a wide range of basis sets and including electron correlation at the MP2 level. The estimated gas-phase energies are tabulated in Table 1. From the data shown, it is evident that the reliable prediction of relative energies is possible only when larger basis sets with inclusion of electron correlation are used. It can be immediately seen from the data that the relative ordering and magnitude of the energy difference among 8-oxoguanine tautomers significantly changes as one increases the size of the basis set. However, at the lower level 3-21G basis set, 8OG1 appears to be more stable than 8OG2 as the level of basis set increases; this ordering reverses, and at the best basis set considered in the present study, 6-311++G(d,p) with inclusion of electron correlation at the MP2 level, the 8-keto-6-enol form (8OG2) turns out to be the predominant form over the other four tautomers. The 8OG2 tautomer is found to be 1 kcal/mol more stable than 8OG1 in the gas phase. The previously reported ab initio gas-phase study at the 3-21G level by Aida and Nishimura [19] indicates that the 8-keto-6-enol tautomer is 2.33 kcal/mol less stable than that of 6,8-diketo guanine. It may be observed that 8OG3 predominates very mar-

ginally over 8OG4 at the MP2/6-31G(d,p) level; the ordering reverses upon inclusion of diffuse functions and extension of double-zeta to triple-zeta values onto the 6-311G(d,p) basis set. It is also worth mentioning that despite its minimal size, the MIDI! basis set shows very good promise in producing reasonably accurate trends of relative stabilities. The single point MP2/6-31G(d,p) energies at MIDI! optimized geometries reproduce an accurate trend in the quality of MP2/6-311++G(d,p)//HF/6-31G(d,p), thereby suggesting that MIDI! geometries might be a reliable and computationally efficient substitute to rather larger basis sets such as 6-31G(d,p).

One remarkable observation is that the inclusion of the 8-oxo group at the C^8 position reduces the energy difference between the keto and enolic forms of 8-oxoguanine when compared to normal guanine. In the latter case, the energy difference between the keto and enolic forms has been estimated to be about 0.14 kcal/mol at MP2/6-311++G(2d,2p)//MP2/6-31G(d,p), with the keto form being the most stable one [28d]. In the case of 8-oxoguanine, the appearance of the 8-oxo group reverses the energetic preference between the two most stable tautomers.

Finally, we evaluated the gas-phase free energies of the 8-oxoguanine tautomers at the highest basis set considered in the present study with ZPE corrections. The corresponding values of free energies are included in Table 1. At the MP2/6-311++G(d,p)//MP2/6-31G(d,p) level, the 8OG2 form is estimated to be about 1.5 kcal/mol more stable than 8OG1. Thus, it may be concluded that in the gas phase, the 8-keto-6-enolic form of 8-oxoguanine predominates over the other tautomeric species considered. A stability order for the 8-oxoguanine tautomers may be established as follows in the gas phase: 8OG2 > 8OG1 > 8OG4 > 8OG3 > 8OG5.

We now turn to a discussion about 8-oxoadenine tautomerism. Five tautomers of 8-oxoadenine are considered in the present study. Unlike the case of 8-oxoguanine, the mutagenic character of 8-oxoadenine is relatively less known. We would like to study the prototropic changes in 8-oxoadenine as it is interesting to know how much the appearance of the 8-oxo group alters the tautomeric equilibria in this class of purines. As discussed above, we have evaluated the energies of various 8-oxoadenine tautomers using the same basis sets as in the case of 8-oxoguanine, and the results are tabulated in Table 2. At all levels of theory, the 8-oxo-6-amino form is predicted to be the most stable one. Although the relative energies of other tautomers depend on the size and quality of the basis set employed, one can observe a reasonable convergence in energies at the MP2/6-311++G(d,p) level using the MP2/6-31G(d,p) geometries. At this basis set, 8OA1 is predicted to be about 7.1 kcal/mol more stable than 8OA2 while the energies of the remaining three tautomers are significantly higher than that of 8OA1. It should be mentioned that the energy difference between normal adenine and 6-imino-adenine (anti to the N^1 position) was estimated to be about 12.3 kcal/mol by previous studies at the MP2/6-31G(d,p) level [39]. The appearance of the 8-oxo group tends to destabilize the corresponding tautomer 8OA3 (see Figure 2) while the corresponding energy difference between the keto form (8OA1) and the imino form (8OA3) is estimated to be about 15 kcal/mol. This is in contrast to the case of 8-oxoguanine where the presence of the 8-oxo group tends to stabilize the enolic form (8OG2, compare Figures 1 and 2).

When the ZPE and thermal corrections are included to estimate the gas-phase free energies, 8OA2 turns out to be 7.8 kcal/mol less stable than the major tautomer, 8OA1. Thus, we can conclude that 8OA1 might be observed as the only single tautomeric species that predominates in the gas phase.

Influence of aqueous solvation

Solvation has been known to greatly influence the tautomeric preferences of various heterocyclic species [40–42]. Since the biological relevance of relative populations of various tautomeric structures of 8-oxopurine bases in the aqueous phase is of considerable importance in understanding spontaneous mutations in vivo, an estimation of the free energies of hydration is solicited in the present study. In order to assess the influence of the solvent on the relative energetics of the various tautomers, the solvation model AM1-SM2 at the semi-empirical level and the ab initio SCI-PCM models at the HF/6-31G(d,p) and MIDI! geometries have been used to evaluate the free energy changes in an aqueous medium. The estimated values of the free energies of hydration are shown in Table 3.

It is encouraging to see a good general correlation between the AM1-SM2 solvation model and the ab initio SCI-PCM models. However, the AM1-SM2 values seem to be systematically lower than the corresponding SCI-PCM values estimated at both the HF/6-31G(d,p) and MIDI! geometries. The hydration energies estimated by the SCI-PCM/MIDI! model are closer to the AM1-SM2 values than the SCI-PCM/6-31G(d,p) values. Despite the methodological differ-

Table 1. Gas-phase energies of 8-oxoguanine tautomers (kcal/mol)

Method	8OG1	8OG2	8OG3	8OG4	8OG5
HF/3-21G(d)	0.00	2.26	20.92	24.61	25.81
HF/6-31G(d)	0.00	0.00	14.33	14.81	16.44
HF/6-31G(d,p)	0.00	−1.60	12.77	11.48	13.05
MP2/6-31G(d,p)	0.00	−0.20	10.65	10.78	12.18
MP2/6-31G(d,p)//MIDI!	0.00	−0.56	10.76	10.31	11.59
MP2/6-311++G(d,p)//HF/6-31G(d,p)	0.00	−1.26	9.58	8.52	9.82
MP2/6-311++G(d,p)//MP2/6-31G(d,p)	0.00	−1.03	9.38	8.49	9.82
Free-energy (ΔG_f, 298.7) MP2/6-311++G(d,p)//HF/6-31G(d,p)	0.00	−1.25	10.00	9.99	10.34
MP2/6-311++G(d,p)//MP2/6-31G(d,p)	0.00	−1.47	9.87	9.11	10.32

Table 2. Gas-phase energies of 8-oxoadenine tautomers (kcal/mol)

Method	8OA1	8OA2	8OA3	8OA4	8OA5
HF/3-21G(d)	0.00	6.62	18.17	27.99	33.55
HF/6-31G(d)	0.00	10.71	16.84	18.23	26.19
HF/6-31G(d,p)	0.00	8.85	16.71	18.16	24.49
MP2/6-31G(d,p)	0.00	8.05	15.06	15.59	21.21
MP2/6-31G(d,p)//MIDI!	0.00	7.32	15.40	16.17	21.53
MP2/6-311++G(d,p)//HF/6-31G(d,p)	0.00	7.16	15.66	16.43	21.01
MP2/6-311++G(d,p)//MP2/6-31G(d,p)	0.00	7.05	15.09	15.69	20.17
Free-energy (ΔG_f, 298.7) MP2/6-311++G(d,p)//HF/6-31G(d,p)	0.00	8.27	15.21	16.56	21.69
MP2/6-311++G(d,p)//MP2/6-31G(d,p)	0.00	7.79	15.55	16.44	21.74

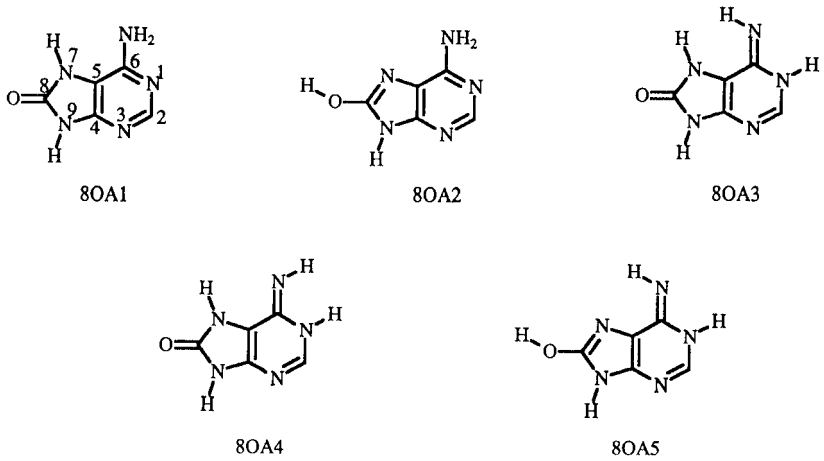

Figure 2. Five tautomers of 8-oxoadenine with numbering system shown on the 8OA1 tautomer.

Table 3. Calculated relative free energies of hydration by the AM1-SM2 and SCI-PCM methods, and the dipole moments with relative dipole moments with respect to 8OG1 or 8OA1 in parentheses.

Tautomer	AM1-SM2 (kcal/mol)	SCI-PCM[a] (kcal/mol)	SCI-PCM [b] (kcal/mol)	Dipole moment[c] (D)
8OG1	0.00	0.00	0.00	7.48 (0.00)
8OG2	3.87	5.52	4.56	4.38 (−0.31)
8OG3	−0.91	−0.41	−0.83	5.85 (1.47)
8OG4	1.56	5.37	3.89	1.99 (−3.86)
8OG5	1.88	5.07	3.62	3.63 (1.64)
8OA1	0.00	0.00	0.00	3.14 (0.00)
8OA2	−3.38	2.12	0.94	2.84 (−0.30)
8OA3	−4.05	−2.23	−2.76	3.22 (0.38)
8OA4	−6.33	−3.22	−3.66	6.16 (2.92)
8OA5	−5.53	−0.05	−1.08	3.03 (−3.13)

[a] Estimated at the HF/6-31G(d,p) geometry
[b] Estimated at the HF/MIDI! geometry
[c] Gas-phase values estimated at the MP2/6-311++G(d,p)//MP2/6-31G(d,p) level.

Table 4. Estimated free energies of tautomerization ($\Delta g^{aq}_{A\rightarrow B}$) of 8-oxoguanine and 8-oxoadenine with respect to 8OG1 and 8OA1 kcal respectively (mol)[a]

Method	8OG2	8OG3	8OG4	8OG5
MP2/6-311++G(d,p)//HF/6-31G(d,p)	3.24	9.91	12.10	14.93
MP2/6-311++G(d,p)//MP2/6-31G(d,p)	2.40	8.96	10.67	12.20
MP2/6-311++G(d,p)//MIDI!	3.31	9.85	11.87	13.47
	8OA2	8OA3	8OA4	8OA5
MP2/6-311++G(d,p)//HF/6-31G(d,p)	4.55	11.41	9.90	16.29
MP2/6-311++G(d,p)//MP2/6-31G(d,p)	4.40	11.50	10.11	16.21
MP2/6-311++G(d,p)//MIDI!	3.94	11.35	9.84	16.00

[a] Estimated according to Equation 1 using AM1-SM2 free energies of hydration.

ences between the AM1-SM2 and ab initio SCI-PCM models, it is remarkable to observe good similarity between the two sets of values although the magnitude of differences is rather large in some cases. This could be attributed to the fact that the SCI-PCM models take into account only the electrostatic polarization effects in the continuum model while the AM1-SM2 model, which takes into account both the electric polarization effects and the CDS terms (cavitation, dispersion and solvent structure), is developed to reproduce the experimental free energies of hydration.

The calculated relative free energies of hydration of the 8-oxoguanine and 8-oxoadenine tautomers with respect to 8OG1 and 8OA1 along with the corresponding gas-phase dipole moments are shown in Table 3. In the case of 8-oxoguanine, both models predict that 8OG3 is the most hydrated one while the 8OG2 tautomer is the least hydrated species, with the corresponding dipole moments of 8OG3 and 8OG2 being 5.85 and 4.38 D, respectively. 8OG3 is estimated to be about 0.91 kcal/mol more hydrated than 8OG1 at the AM1-SM2 level and about 0.83 kcal/mol at the SCI-PCM/MIDI! level. 8OG2 is the least hydrated and is about 3.87 and 4.56 kcal/mol less hydrated than 8OG1 as predicted by the AM1-SM2 and SCI-PCM models, respectively. Similarly in the case of 8-oxoadenine, 8OA4 is predicted to be the most hydrated one with a corresponding dipole moment of 6.16 D. Here too, one can observe qualitatively a similar trend by both AM1-SM2 and SCI-PCM models. 8OA2 is predicted to be about 3.4 kcal/mol more hydrated than 8OA1 at the

AM1-SM2 level while the SCI-PCM method predicts it to be about 0.94 kcal/mol less hydrated than 8OA1.

Our interest in estimating the free energies of hydration is to obtain the free energies of tautomerization of 8-oxoguanine and 8-oxoadenine. Estimated free energies of tautomerization according to Equation 1 are tabulated in Table 4. Usually one obtains the relative free energies of two species in solution by determining the relative free energies in the gas phase and adding the relative free energies of solvation to it. This method has been shown recently to give reasonably good estimates of free energies of the heterocyclic species in an aqueous medium [37,43,44]. With our best estimates of free energy of tautomerization at the MP2/6-311++G(d,p)//MP2/6-31G(d,p) gas-phase energies and the AM1-SM2 free energies of hydration, we predict a free-energy difference of about 2.4 kcal/mol between 8OG1 and 8OA2 in an aqueous solution. It is worth noting that even though in the gas phase the 8-keto-6-enolic form (8OA2) predominates over the other possible tautomers, solvation changes the stability order, the native tautomer 8OG1 turning out to be the predominant one in an aqueous phase. Similarly, in the case of 8-oxoadenine, we predict a free-energy difference of about 4.4 kcal/mol between the two most stable tautomers, 8OA1 and 8OA2. As can be observed, the solvation has a significant influence in altering the gas-phase order of the relative stability of both 8-oxoguanine and 8-oxoadenine. In the case of 8OA, there are no previous theoretical reports on the tautomeric energies. According to our results, the 8-keto-6-amino form (8OA1) is the most stable one. The next stable tautomer is shown to be the 6-amino-8-enol form (8OA2) and is predicted to be 4.4 kcal/mol less stable than the native one in an aqueous phase.

Relevance for biological implications

It has long been speculated that the presence of minor tautomers of nucleic acid bases would play a key role in inducing spontaneous mutations in DNA [45]. Consequently, there have been a number of experimental [46] and theoretical studies [26a, 28a,c, 47, 48] to support this idea. To date, no experimental technique has been able to identify the significant concentrations of minor tautomers in nucleic acid bases to support this hypothesis since most of the experimental techniques are not sufficiently accurate to detect low concentrations of possible minor tautomers of nucleic acid bases. Accurate ab initio quantum chemical methods provide a reasonable substitute to the experimental methods in predicting the tautomeric equilibria in nucleic acid bases. Recent studies on isolated bases and base pairs suggest that tautomeric transition for guanine to the mutagenically significant 6-enolic form might be a thermodynamically feasible process, which could lead to G→A type transitional mutations [49]. MP2/6-31G(d,p)//HF/6-31G(d) calculations on the guanine-cytosine base pair predicted that keto-enolic tautomeric transition in the base pair likely occurs in 1 in 10^6–10^9 base pairs. However, such a possibility was ruled out in the case of the adenine-thymine base pair [50]. Experimental studies suggest that any tautomeric transition, with the corresponding equilibrium constants less than 10^{-6}, is significant for inducing mutations in the DNA duplex [51]. The equilibrium constants of tautomerization for both 8-oxoguanine and 8-oxoadenine are estimated using gas-phase free energies and AM1-SM2 free energies of hydration according to the standard Arrhenius equation, $K = \exp(-\Delta G/RT)$, and the corresponding data is tabulated in Table 5. At the MP2/6-311++G(d,p)//MP2/6-31G(d,p) level, 8OG2 is estimated to be only about 2.4 kcal/mol less stable than the major tautomer 8OG1. The free energies predicted at the MP2/6-311++G(d,p) level using HF/6-31G(d,p) and MIDI! geometries (with AM1-SM2 free energies of hydration) are also close to this energy (3.24 and 3.31 kcal/mol, respectively). From these calculations, a tautomeric equilibrium constant of 1.8×10^{-2} at 298.5 K is predicted at the MP2/6-311++G(d,p)//MP2/6-31G(d,p) level for an 8OG1 to 8OG2 transition and indicates that the 8-keto-6-enolic form of 8-oxoguanine may be expected to exist in a significant population in an aqueous medium, and could be attributed to G→T type transitional mutations.

Also in the case of 8-oxoadenine, we predict an equilibrium constant of 6.0×10^{-4} at the MP2/6-311G(d,p)//MP2/6-31G(d,p) level, indicating that the presence of the minor tautomeric form 8OA2 could be of some mutagenic significance. As shown in Figure 3, the 8-enolic form of 8-oxoadenine (8OA2) is expected to play a key role in inducing A→C type transitional mutations.

Thus, it can be concluded that the tautomeric transitions from the native tautomers of 8-oxoguanine and 8-oxoadenine to the minor enolic forms could be thermodynamically feasible processes and might be of some significance for mutagenic events. Particularly in this case it may be implicated for inducing G→T type transitional mutations in the case of 8-oxoguanine

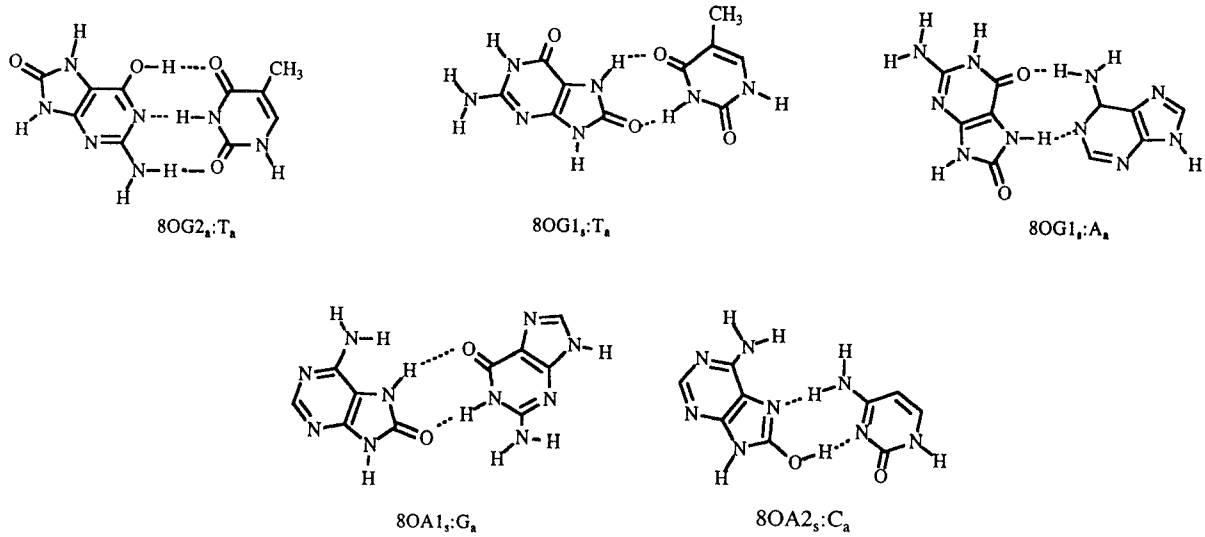

Figure 3. Schematic representation of some plausible base-mispairing schemes involving both minor and major tautomeric forms of 8-oxoguanine and 8-oxoadenine.

Table 5. Estimated equilibrium constants for tautomerization ($K^{TAU}_{A\rightarrow B}$, at 298.5 K) in an aqueous medium[a]

Tautomeric transition	MP2/6-311++G(d,p)// HF/6-31G(d,p)	MP2/6-311++G(d,p)// MP2/6-31G(d,p)
8OG1 → 8OG2	0.4×10^{-2}	1.8×10^{-2}
8OG1 → 8OG3	0.6×10^{-7}	2.8×10^{-7}
8OG1 → 8OG4	0.1×10^{-8}	1.6×10^{-8}
8OG1 → 8OG5	0.1×10^{-10}	1.2×10^{-9}
8OA1 → 8OA2	4.6×10^{-4}	6.0×10^{-4}
8OA1 → 8OA3	4.5×10^{-9}	3.9×10^{-9}
8OA1 → 8OA4	5.7×10^{-8}	4.0×10^{-8}
8OA1 → 8OA5	1.2×10^{-12}	1.4×10^{-12}

– [a]AM1-SM2 free energies of hydration are used in evaluating the free energies of tautomerization ($K^{TAU}_{A\rightarrow B}$).

and A→C type transitional mutations in the case of 8-oxoadenine besides the experimentally established possible G→A type and A→G type transversional mutations induced by major tautomeric forms of both 8OG and 8OA as shown in Figure 3.

Conclusions

The tautomerism in C^8-oxidized purines, 8-oxoguanine (8OG) and 8-oxoadenine (8OA), has been studied in the gas and aqueous phase at various levels of ab initio theory in order to assess the tautomeric stability and the possibility of base-induced mutations by minor tautomeric forms of various 8-oxo products. Some important observations from the present work may be delineated as follows:

(1) In the gas phase, the most stable tautomer of 8-oxoguanine is shown to be the 8-keto-6-enolic form while in the case of 8-oxoadenine the 8-keto-6-amino tautomer is predicted to be the most stable species. At the best MP2/6-311++G(d,p)//MP2/6-31G(d,p) level considered in the present study, a relative stability order of 8OG2>8OG1>8OG4>8OG3>8OG5 for 8-oxoguanine and 8OA1>8OA2>8OA3>8OA4>8OA5 for 8-oxoadenine is established in the gas phase. The appearance of the 8-oxo group has a significant effect in stabilizing the enolic and imino tautomers. While in the case of 8-oxoguanine it stabilizes the 6-enolic form, it has a destabilizing effect on the stability of the 6-imino tautomer of 8-oxoadenine.

(2) The addition of free energies of hydration to the gas-phase energies shifts the 8,6-diketo form of guanine (8OG1) to be more stable than all other tautomeric species considered in the present study. However, in both the gas phase and aqueous phase, the 8-keto-6-amino form of 8-oxoadenine appears to be the most stable tautomeric species at the best basis set used in the present calculations. Our estimations of equilibrium constants of tautomerization suggest that both 8-oxoguanine and 8-oxoadenine might be of much mutagenic significance with the respective equilibrium constants of 1.8×10^{-2} and 6.0×10^{-4} which fall well within the replication frequency of DNA. We

attribute the existence of such minor tautomeric forms of 8-oxopurines to possible A→C type and G→T type transitional mutations.

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