On the Enhanced Stability of the Guanine−Cytosine Base-Pair Radical Cation

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Abstract: *Ab initio* (UHF/6-31G*) and density functional (Becke3LYP/D95*) calculations have been used to investigate the structures and stabilities of the radical cations of the DNA bases and base pairs. The calculated structures of the base pairs show excellent agreement with crystallographic data. The most easily oxidizable base, guanine, forms a particularly stable radical cation base pair with cytosine, so that the calculated adiabatic ionization potential for the guanine−cytosine hydrogen-bonded complex is about 0.75 eV lower than that of guanine itself. UBecke3LYP/D95*//UHF/6-31G* calculations show that the shift of the central hydrogen-bonded proton at N1 of guanine to N3 of cytosine is only slightly endothermic (+1.6 kcal mol^{-1}). The product of the corresponding proton shift in the adenine−thymine system is unfavorable by +14.1 kcal mol^{-1}. These results suggest that the guanine−cytosine radical cation represents even more of a thermodynamic sink in oxidized DNA than might be concluded from the ionization potentials of the individual bases, and that it enjoys about 7.3 kcal mol^{-1} extra stabilization from the central low-barrier hydrogen bond.

Introduction

Oxidized pyrimidines and purines have been implicated as key early intermediates in damage of DNA by ionizing radiation[1] and by the MX class of mutagens thought to undergo electron transfer after intercalation in DNA.[2] Experimentalists have, however, long been puzzled by the absence of an equal distribution of adenine (A), thymine (T), guanine (G), and cytosine (C) radicals in irradiated DNA.[1] Positive charge migration in dry DNA is also limited to about 25 nucleotide units,[3ab] although the stacked base pairs ought to be well set up for fast electron transfer.[4] These anomalies have led to the suggestion that fast electron transfer along the DNA chain is interrupted by proton transfer between bases in a base-pair radical cation.[1] Experimental ionization potentials[5ab] reveal that guanine and adenine are the two most easily oxidized bases. Previous theoretical studies[6ab] are in agreement with these measurements. Geometrical relaxation of the base radical cations is, however, significant[7] and also influences both the adiabatic ionization potentials and the rate of interpair electron transfer[4] significantly. We now report *ab initio* and density functional calculations on the ionized DNA bases and base pairs. Our results shed new light on the thermodynamic aspects of base oxidation in DNA.

Method

All calculations used the GAUSSIAN 92 series[8] of programs. We have used *ab initio* (UHF/6-31G*)[9,10] molecular orbital theory to optimize the structures of the base and base-pair radical cations. Minima and transition states were verified by frequency calculations at the same level of theory. Density functional (Becke3LYP/D95*//(UHF/6-31G*))[11,12] theory was used for energy calculations in order to determine vertical and adiabatic ionization potentials. Since there is excellent agreement between experiment and density functional theory for the ionization potentials of single bases, we conclude that this level of theory is also appropriate for the base pairs. The reliable calculation of ionization potentials of base pairs is an important goal because there are as yet no experimental data.

Results and Discussion

The calculated total and zero-point energies of the bases and their tautomeric forms, the base pairs, and the corresponding radical cations are shown in a table in the supporting information. The neutral single bases are known to be nonplanar.[13,14] The corresponding radical cations, however, show a strong flattening of the amino groups and are nearly planar. The N(1)H−N(7)H tautomer of guanine (Figure 1), which has been suggested to be the major tautomer in isolated enviroments,[15] was calculated to be 0.6 kcal mol^{-1} more stable than the

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Table 1. Vertical and Adiabatic Ionization Potentials[a]

ionization potential	guanine	N(1)H−N(7)H guanine	adenine	cytosine	amino−hydroxy cytosine	thymine
vertical (exptl)[b]	8.24	8.24	8.44	8.94	8.94	9.14
vertical (calcd)[c]	7.90	8.05	8.24	8.60	8.58	8.90
Δ (exptl − calcd)	0.34	0.19	0.20	0.34	0.36	0.24
vertical (evaluated)[d]	8.21	8.36	8.54	8.88	8.86	9.16
adiabatic (exptl)[e]	7.77	7.77	8.26	8.68	8.68	8.87
adiabatic (calcd)[f]	7.44	7.57	7.93	8.41	8.33	8.57
Δ (exptl − calcd)	0.33	0.20	0.33	0.27	0.35	0.20
adiabatic (evaluated)[d]	7.78	7.90	8.24	8.70	8.62	8.85

[a] Calculations at Becke3LYP/D95*//(UHF/6-31G*). Ionization potentials in eV. [b] Taken from ref 5a. [c] 00 excitation. [d] Using eq **1**. [e] Taken from ref 5b. [f] Including zero-point energy correction calculated at UHF/6-31G*.

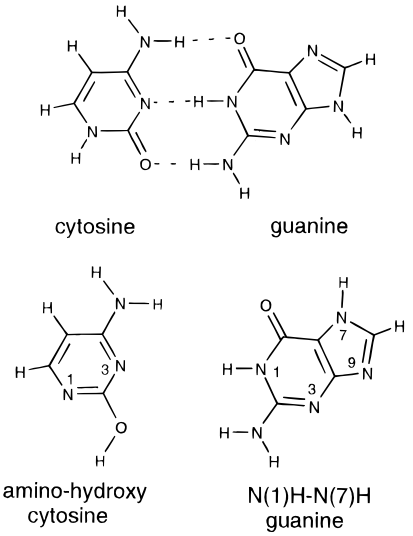

Figure 1. Numbering of guanine and cytosine.

Watson−Crick tautomer, although the radical cation is found to be 2.4 kcal mol^{-1} less stable. The calculated adiabatic ionization potentials of both tautomers, however, lie very close together (guanine 7.44 eV vs N(1)−N(7)H guanine 7.57 eV). Cytosine has also been shown to exist as a non Watson−Crick tautomeric form in matrix isolation studies.[16] Our calculations suggest that this amino−hydroxy tautomer is only 0.1 kcal mol^{-1} more stable than cytosine while the corresponding radical cations are identical in energy. The calculated ionization potentials of the single bases correlate well with experimental data (see Table 1), so we conclude that the calculational method used is suitable for the calculation of ionization potentials for the Watson−Crick base pairs AT and GC.

The calculations show that the adiabatic ionization potentials of guanine and its N(1)−N(7) tautomer are significantly lower than those of the other bases, despite the fact that the experimental vertical IPs of guanine and adenine differ by only 0.2 eV (see Table 1). The large (9.6 kcal mol^{-1}) geometrical relaxation energy for the guanine radical cation makes it particularly stable, both thermodynamically and kinetically. The calculated vertical to adiabatic relaxation energies (G, 0.46 eV; N(1)−N(7) G, 0.48 eV; A, 0.31 eV; C, 0.19 eV; amino−hydroxy C, 0.25 eV; T, 0.33 eV) correlate only moderately well with the differences between experimental vertical and adiabatic ionization potentials (G, 0.47 eV; A, 0.18 eV; C, 0.26 eV; T, 0.27 eV). Because, however, the experimental data are the differences between two different measurements, our calculated data are probably more reliable. It is not possible to determine which of the guanine and cytosine tautomers is the preferred radical cation in the gas phase from the correlation between calculated and experimental ionization potentials.

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Table 2. Complexation Energies upon Base-Pair Formation (without BSSE) and Ionization Potentials at Becke3LYP/D95*//(UHF/6-31G*)[a]

base pair	complexation energy[b]	IP calcd vertical[c]	IP calcd adiabatic[b]	IP evaluated[d] vertical	IP evaluated[d] adiabatic
GC	−27.5	7.16	6.71	7.51	7.08
AT	−12.3	7.74	7.45	8.06	7.79

[a] Energies in kcal/mol, ionization potentials in eV. [b] Including zero-point energy correction calculated at UHF/6-31G*. [c] 00 excitation. [d] Corrected ionization values are ±0.010 eV (two standard deviations of the linear regression for the base monomers).

Complexation of the complementary bases, however, also affects the ionization potentials. The GC and AT neutral and positively charged base pairs were therefore optimized at HF/6-31G* and the geometries[17] used for single-point Becke3LYP/D95* calculations to determine accurate ionization potentials. The results are shown in Table 2.

Comparison of the calculated and experimental ionization potentials for the single bases (eight values: A, T, G, C, both vertical and adiabatic) gives the following regression equation (in eV):

$$IP_{exptl} = (0.951 \pm 0.041)IP_{calcd} + 0.701 \pm 0.336 \quad (1)$$

This equation gives best estimate vertical and adiabatic ionization potentials of 7.51 and 7.08 for GC and 8.06 and 7.79 for AT, all with error limits of ±0.010 eV (twice the standard deviation of the regression equation (eq 1)).

The GC base pair has a particularly low (6.71 eV calculated directly, 7.08 eV corrected) adiabatic ionization potential, 0.73 eV lower than that of guanine alone. Similarly, the vertical ionization potential of GC (7.51 eV) is calculated to be 0.74 eV lower than that of isolated G. The lowerings of the AT ionization potentials compared to A (0.50 eV vertical, 0.48 eV adiabatic) are still significant, but lower than those found for GC. Thus, the adiabatic ionization potential of GC is calculated to be 0.71 eV lower than that of AT.

Parts a and b of Figure 2 show superimposed GC/GC$^{\bullet+}$ and AT/AT$^{\bullet+}$ structures optimized at HF/6-31G*. AT$^{\bullet+}$ shows a strong distortion away from the structure of neutral AT, but this is largely the result of a shortening of one hydrogen bond, a lengthening of the other, and the resulting swiveling movement around the short bond. GC shows far less obvious changes on ionization, but they are energetically more significant (see below).

Compared to the crystal structures of DNA fragments,[18] which were used as experimental references in previous studies,[7,13]

(17) Geometries were optimized in planar C_s symmetry. These were shown to be minima by frequency calculations at the same level as the optimization.

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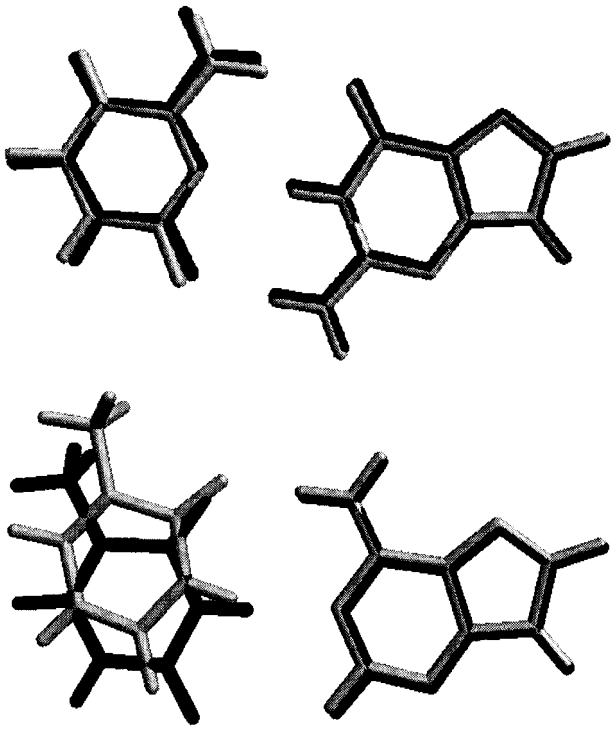


Figure 2. (a, top) Overlayed structures of the calculated guanine−cytosine base pairs: black, neutral; gray, radical cation. (b, bottom) Overlayed structures of the calculated adenine−thymine base pairs: black, neutral; gray, radical cation.

Table 3. Changes in the Length of the Hydrogen Bonds between Neutral and Radical Cationic States[a]

base pair	atoms	neutral	radical cation
guanine−cytosine	O−HN	1.921 (1.008)	2.174 (0.999)
	NH−N	(1.008) 2.036	(1.022) 1.941
	NH−O	(1.002) 2.017	(1.018) 1.768
adenine−thymine	NH−O	(1.000) 2.089	(1.036) 1.685
	N−HN	1.990 (1.013)	2.230 (1.002)

[a] Bond lengths of covalently bonded hydrogens in parentheses. All lengths in angstrøms.

the calculated distance between the two nitrogens involved in N-glycosidal bonding to the DNA backbone (N1 at cytosine and N9 at guanine) is slightly longer (9.049 Å compared with 9.036 Å) in the neutral GC base pair. In the radical cation there is a significant shortening to 8.915 Å. The adenine−thymine base pair also shows a slightly long N9 at adenine to N1 at thymine distance (8.963 Å experimental, 9.001 Å calculated). The lengths of all hydrogen bonds are shown in Table 3.

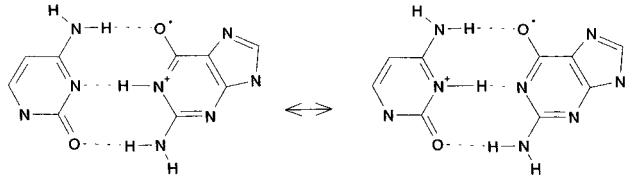
The calculated interaction energy upon base-pair formation given in Table 2 for GC is in slightly better agreement with experimental data[19] (−27.5 kcal mol^{-1} (calculated) vs 21.0 kcal mol^{-1} (experimental)) than the value found at MP2//DZP/HF/6-31G* (−29.6 kcal mol^{-1}).[13] However Gould and Kollman[13] have shown that BSSE lowers the calculated complexation energy up to 10 kcal mol^{-1} with the 6-31G* basis set. For the Watson−Crick AT base pair no experimental complexation energy is available.

Compared to a recent study[14] which also used density functional approaches, our calculated hydrogen bond lengths are consistently longer by about 0.3 Å (LSD) and 0.2 Å (NLSD). Gould and Kollman have also reported partial details of HF/6-31G* base-pair geometries.[13] If one considers the usual differences between crystal structures and the gas phase of about

Table 4. Energies[a] of the Calculated Structures Involved in the Shift of the Central Proton in the Guanine−Cytosine Radical Cation

theory level	**1**: GC$^{\bullet+}$	TS	**2**: G(+H)C(−H)$^{\bullet+}$
UHF/6-31G*	±0.0	+12.4	+1.7
UBecke3LYP/D95*//(UHF/6-31G*)	±0.0	+3.8	+1.2
ZPE (UHF/6-31G*)	148.0	145.2	148.3
UHF/6-31G* + ZPE	±0.0	+9.5	+2.0
UBecke3LYP/D95*//(UHF/6-31G*) + ZPE	±0.0	+0.9	+1.6

[a] In kcal mol^{-1}.



1 **2**

Figure 3. Proton shift in the guanine−cytosine radical cation can be regarded as two resonance structures.

0.01 Å, the agreement between the calculated structures at the Hartree−Fock level and the experimental data[18] is excellent.

In the radical cation of AT the distance between the N1 and N9 atoms increases to 9.414 Å because of a strong shortening of the OHN hydrogen bond. As in the neutral base pair, this may be the result of the lack of a DNA backbone in the calculations but nevertheless demonstrates the effect of the hydrogen bonding. The same trend was found at HF/3-21G for GC$^{\bullet+}$, although the structure of AT$^{\bullet+}$ was less perturbed.[7]

In order to explain the stabilization one must take subsequent reaction steps of the radical cation into account. The shortening of the central hydrogen bridge (see Table 3) is a hint at a possible proton shift toward cytosine.[1] We have optimized the geometry of the product of this proton shift at the same level of theory as for the other base pairs and also performed a transition state search. Energies for these stationary points were calculated at Becke3LYP/D95* and zero-point energy corrected (see Table 4).

The product of the proton shift from N1 of guanine to N3 of cytosine along the central hydrogen bridge is only 1.6 kcal mol^{-1} less stable than the original guanine−cytosine radical cation. A previous study[7] found 1.2 kcal mol^{-1} at 6-31+G(d)//3-21G. The corresponding proton shift in AT$^{\bullet+}$ from N3 of cytosine to N1 of adenine is about 14.1 kcal mol^{-1} less stable than the AT$^{\bullet+}$ hydrogen-bonded complex and therefore unlikely to occur. For the activation barrier we found a value of 3.8 kcal mol^{-1} at Becke3LYP/D95*//(UHF/6-31G*). Zero-point energy correction reduces this "barrier" to 0.9 kcal mol^{-1}, making the energy profile for the proton shift monotonically increase. The actual "real" free energy profile for this process may have either a central minimum or a shallow double-minimum shape.

The stabilization of GC$^{\bullet+}$ can thus be considered a specific effect, rather than a general solvation of the positive charge. This specific stabilization mechanism is best understood as a significant contribution from a proton-shifted resonance structure (Figure 3).

Alternatively, GC$^{\bullet+}$ can be considered as a good candidate for stabilization by a strong hydrogen bond, although the extra stabilization is not as high as has been proposed for enzyme systems.[20] Isodesmic reactions show that the extra stabilization enjoyed by the base-pair radical cation relative to the uncomplexed base radical cation is largest for GC$^{\bullet+}$:

(19) Yanson, I.; Teplitsky, A.; Sukhodub, L. *Biopolymers* **1979**, *18*, 1149.

(20) Cleland, W. W.; Kreevoy, M. M. *Science* **1994**, *264*, 1887.

$$A^{\bullet+} + AT \rightarrow AT^{\bullet+} + A \qquad -10.2 \text{ kcal mol}^{-1}$$

$$G^{\bullet+} + GC \rightarrow GC^{\bullet+} + G \qquad -17.4 \text{ kcal mol}^{-1}$$

If $AT^{\bullet+}$ is taken as a standard, this extra stabilization is about 0.31 eV (7.3 kcal mol^{-1}).

Probable Effects of Adjacent Base Pairs. Individual GC sites thus provide the most stable centers for a positive hole in DNA. The stacking effect of the adjacent base pairs, however, should provide extra "solvation" of the hole and result in differences in stability between oxidized GC pairs in different environments. Both theoretical studies on one- and three-electron bonding[21] and experimental work on complexation between aromatic molecules and radical cations in the gas phase[22] have shown that symmetrical odd-electron bonding between identical partners is most favorable and that complexation energies fall off exponentially with increasing difference between the ionization potentials of the partners. This means that $GC^{\bullet+}$ is most effectively "solvated" by GC, so that the central base pair of a GC−GC−GC triplet stack should represent the global minimum position for a positive hole in ionized DNA. Notably, the MX class of mutagens, which are thought to function by one-electron oxidation,[23] cause most damage at exactly this position.[24,25] The selectivity of such reagents may therefore be a purely thermodynamic phenomenon, rather than the result of site-specific binding. The number and location of GC−GC−GC triplet stacks may also influence the genetic susceptibility of DNA to oxidative mutation. Semiempirical MO calculations on DNA-triplet radical cations with the cationic center localized on the central base pair[26] suggest that GC−$GC^{\bullet+}$−GC does indeed enjoy a larger stabilization than other $GC^{\bullet+}$-centered triplets. This effect appears to be fairly independent of the twist angle between the stacked base pairs, but is being investigated at higher levels of theory.

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Conclusions

The slight and constant deviation of the calculated ionization potentials from experimental data shows that the chosen level of theory−DFT single-point energies with Becke3LYP/D95* at fully optimized geometries using UHF/6-31G*—is adequate for a good description of single nucleic acid bases and hydrogen-bonded base pairs. The calculations confirm the sequence of the experimental ionization potentials from guanine to thymine and show that the guanine−cytosine base pair is more easily oxidizable than the corresponding adenine−thymine pair. The geometrical changes involved on one-electron oxidation are more apparent for the adenine−thymine system, but the guanine−cytosine pair is even more of a thermodynamic sink than expected on the basis of the individual base ionization potentials. $GC^{\bullet+}$ can undergo a facile proton shift along its central hydrogen bond. This leads to about 7.3 kcal mol^{-1} specific extra stabilization of $GC^{\bullet+}$ relative to $AT^{\bullet+}$ because of the special character of this hydrogen bond.

All of these electronic effects taken together result in the remarkable stabilization of $GC^{\bullet+}$ compared to $AT^{\bullet+}$ and explain the distribution of nucleic acid base radical cations in one-electron-oxidized DNA.

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Supporting Information Available: Total and zero-point energies for the species reported (1 page). See any current masthead page for ordering and Internet access instructions. Total and zero-point energies for the species reported plus GAUSSIAN archive entries for the Becke3LYP calculations are available as an ASCII file on the Internet.

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