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The Acidity and Proton Affinity of the Damaged Base 1,N^6-Ethenoadenine in the Gas Phase versus in Solution: Intrinsic Reactivity and Biological Implications

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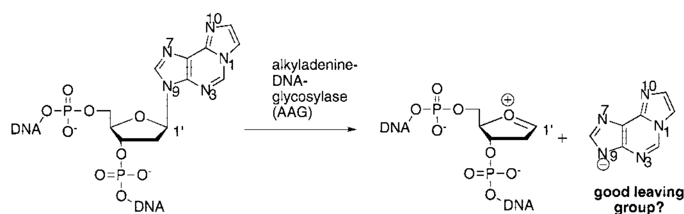

1,N^6-Ethenoadenine (ϵA) is a highly mutagenic lesion that is excised from human DNA by the enzyme alkyladenine DNA glycosylase (AAG). In an effort to understand the intrinsic properties of 1,N^6-ethenoadenine, we examined its gas phase acidity and proton affinity using quantum mechanical calculations and mass spectrometric experimental methods. We measure two acidities for ϵA: a more acidic site (ΔH_{acid} = 332 kcal mol^{-1}; ΔG_{acid} = 325 kcal mol^{-1}) and a less acidic site (ΔH_{acid} = 367 kcal mol^{-1}; ΔG_{acid} = 360 kcal mol^{-1}). We also find that the proton affinity of the most basic site of 1,N^6-ethenoadenine is 232−233 kcal mol^{-1} (GB = 224 kcal mol^{-1}). These measurements, when compared to calculations, establish that, under our experimental conditions, we have only the canonical tautomer of 1,N^6-ethenoadenine present. We also compare the gas phase acidic properties of ϵA with that of the normal bases adenine and guanine and find that ϵA is the most acidic. This supports the theory that AAG and other related enzymes may cleave damaged bases as anions. Furthermore, comparison of the gas phase and aqueous acidities indicates that the nonpolar environment of the enzyme enhances the acidity differences of ϵA versus adenine and guanine.

Introduction

1,N^6-Ethenoadenine (ϵA, **1**) is a highly mutagenic DNA lesion linked to cytotoxicity and carcinogenesis.[1–7] Exogenous sources leading to ϵA formation include vinyl chloride, ethyl carbamate, crotonaldehyde, *N*-nitrosopyrrolidine, chloroethylene oxide, and chloroacetaldehyde.[1,8–12] The ϵA adduct may also be produced in vivo via lipid peroxidation.[8–10]

Because damaged DNA bases differ in structure and properties from normal nucleobases, they intervene with gene replication and expression and must be repaired.[7,13,14] The ϵA lesion is repaired in humans by the base excision repair (BER) pathway, which involves DNA glycosylase enzymes.[12–15] The glycosylase responsible for ϵA excision in humans is alkyladenine DNA glycosylase (AAG). AAG excises a wide variety of damaged nucleobases from double-stranded DNA, including ϵA, hypoxanthine (**2**), 3-methyladenine (**3**), and 7-methylguanine (**4**).[13,14,16–24] An outstanding mechanistic question is how AAG

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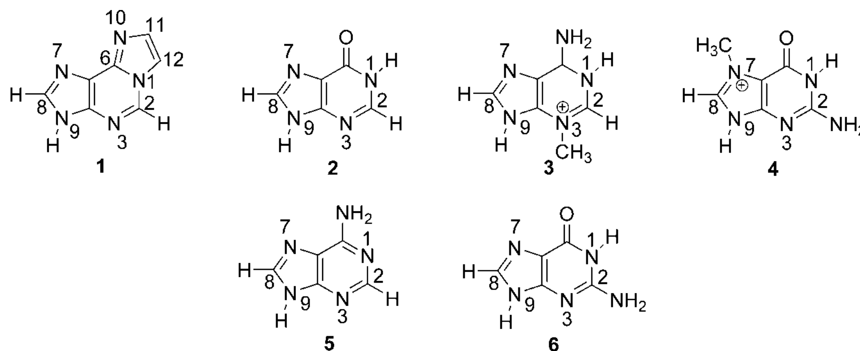
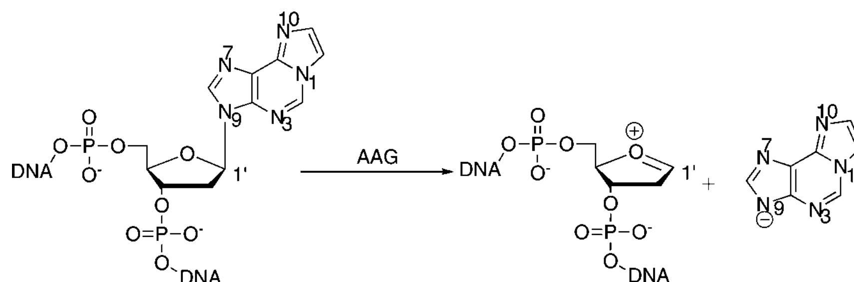


SCHEME 1



cleaves such a broad range of bases, yet leaves normal bases adenine (**5**) and guanine (**6**) untouched.[13,14,19–23,25–28]

Possible mechanisms of depurination include cleavage via the departure of deprotonated 1,N^6-ethenoadenine or cleavage via departure of neutral 1,N^6-ethenoadenine (which requires preprotonation of the damaged nucleobase).[13,14,20,22,29–35]

We have set forth the mechanistic proposal that AAG cleaves mutated purine substrates such as εA as anions (Scheme 1).[29,31–34] Such a mechanism has also been proposed for a related enzyme, thymine DNA glycosylase (TDG), which cleaves pyrimidine nucleobases.[30] We postulate that AAG differentiates among substrates by cleaving those nucleobases which are the most facile to remove. For the mechanism shown in Scheme 1, the ease of excision should be related to how good of a leaving group the anionic, deprotonated nucleobase is. Our theory is that deprotonated damaged bases are better leaving groups than deprotonated normal nucleobases. Furthermore, we propose that the differences among the leaving group abilities of various anionic, deprotonated nucleobases are enhanced in a nonpolar environment; by providing such an environment, AAG is able to execute its broad specificity.[19–21,29–31]

Our goal is therefore to ascertain whether the deprotonated forms of the damaged bases excised by AAG are in fact better leaving groups than the normal bases. We assess leaving group ability by ascertaining the acidity of the damaged bases: the more acidic the base at N9, the better a leaving group its conjugate base should be. Furthermore, we assess the acidity in the gas phase, which is the "ultimate" nonpolar environment;

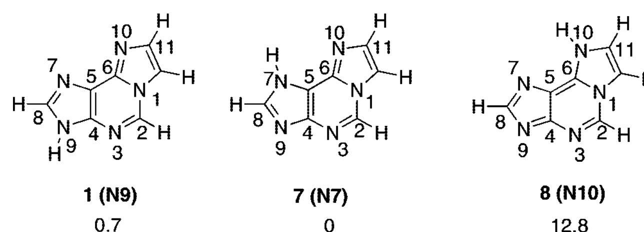


FIGURE 1. Relative enthalpies (ΔH in kcal mol^{-1}) of the three most stable tautomers of 1,N^6-ethenoadenine, calculated at B3LYP/6-31+G* (298 K).

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(33) Previous work indicates that glycosidic bond hydrolyses proceed via a highly dissociative S_N2 or a stepwise S_N1 mechanism; to simplify Scheme 1, we show the mechanism as S_N1. Furthermore, the mechanism for enzyme cleavage also involves a "base-flipping" step, whereby the nucleobase is "flipped" into the enzyme active site; this step may also differ for different substrates. It is not known whether base flipping is a relatively fast or slow step in AAG, and herein we focus on the steps involving actual nucleobase excision. See refs 13, 14, and 22.
(34) Some damaged nucleobases are positively charged (such as 7-methyl guanine); these nucleobases may be cleaved as neutrals. See refs 20 and 22.
(35) Another prevalent mechanism is that the damaged nucleobase is protonated before cleavage. See refs 20 and 22. Among εA, adenine, and guanine, εA is the most basic. One might argue that therefore, under such a mechanism, εA would be most easily cleaved (since it would be most easily protonated). However, we conducted calculations to ascertain leaving group ability of neutral εA versus adenine versus guanine, and although εA is most easily protonated, it is the poorest neutral leaving group (which is consistent with its higher PA).

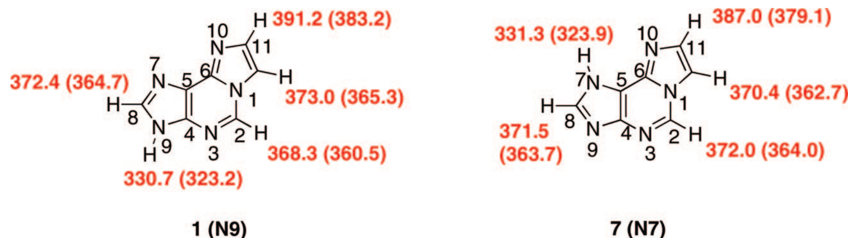

FIGURE 2. Calculated acidities (ΔH_{acid}; ΔG_{acid} in parentheses; all values in kcal mol^{-1}) of the two most stable tautomers of 1,N^6-ethenoadenine at B3LYP/6-31+G* (298 K).

our theory is that any differences in acidity between 1,N^6-ethenoadenine and normal bases should be greater in the gas phase than in solution. To our knowledge, the gas phase acidity of 1,N^6-ethenoadenine is unreported. In this paper, we describe a study of the acidic and basic properties of 1,N^6-ethenoadenine in an effort to both characterize this damaged base and also to probe how it might be recognized by AAG.

Results and Discussion

Computational Results. Tautomers: As is common with nucleobases, 1,N^6-ethenoadenine has several possible tautomers. We calculated the relative enthalpies of all eight possible tautomers of 1,N^6-ethenoadenine (see Supporting Information) at B3LYP/6-31+G*. The most stable tautomer in the gas phase is calculated to be the "N7" tautomer **7** (proton resides on the N7). The canonical structure **1**, which is the biologically relevant "N9" tautomer, is calculated to be less stable than the N7 tautomer by 0.7 kcal mol^{-1}. The next most stable "N10" tautomer **8** (proton resides on the N10) is 12.8 kcal mol^{-1} less stable than the N7 tautomer. Therefore, our calculations indicate that most likely under our gas phase conditions only the N7 and/or N9 tautomers will be present (Figure 1).

Acidity: The acidities of the eight possible tautomers were calculated (see Supporting Information), but herein we only discuss the most stable tautomers **1** and **7** (Figure 2). The most acidic site of the N9 tautomer **1** is N9−H (ΔH_{acid} = 330.7 kcal mol^{-1}; ΔG_{acid} = 323.2 kcal mol^{-1}). The remaining protons are all C−H protons that are much less acidic. For the N7 tautomer **7**, the most acidic N7−H proton has a computed acidity of 331.3 (ΔH_{acid})/323.9 (ΔG_{acid}) kcal mol^{-1}, which is quite close to the acidity of the N9−H site of the N9 tautomer **1**. The remaining less acidic sites are all C−H protons (Figure 2).

Proton Affinity: To provide a more complete picture of ϵA reactivity, we also calculated the proton affinities (PA; ΔH) and gas phase basicities (GB; ΔG) of the N9 tautomer **1** and the N7 tautomer **7** (Figure 3). For the N9 tautomer **1**, the most basic site is the N10 (PA = 232.6 kcal mol^{-1}; GB = 224.9 kcal mol^{-1}); the next most basic site is the N7 (PA = 223.7 kcal mol^{-1}; GB = 216.1 kcal mol^{-1}), and the third most basic site is the N3 (PA = 207.1 kcal mol^{-1}; GB = 199.8 kcal mol^{-1}). The least basic sites are N1 and N9. For the N7 tautomer **7**, the most basic N9 site is calculated to have a proton affinity of 223.0 kcal mol^{-1} (GB = 215.4 kcal mol^{-1}), which is much less basic than the most basic N10 site of the N9 tautomer **1**. The N10 site of the N7 tautomer is comparable in basicity to the N9 site. The remaining PAs/GBs on the N7 tautomer are shown in Figure 3.

For reasons delineated in the next section, we also calculated the proton affinity of an alkylated derivative of ϵA, 9-methyl-1,N^6-ethenoadenine (**9**, 9-me-ϵA, Figure 4). This methylated derivative is slightly more basic than the parent 1,N^6-ethenoadenine.

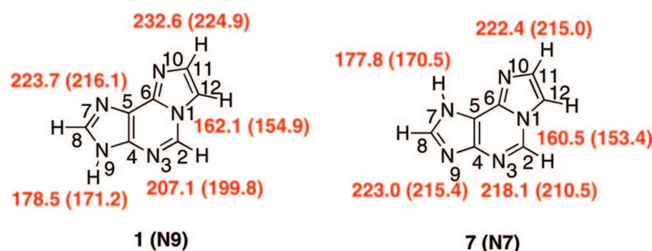

FIGURE 3. Calculated proton affinities (ΔH; gas phase basicity values (ΔG) in parentheses; all values in kcal mol^{-1}) of the two most stable tautomers of 1,N^6-ethenoadenine at B3LYP/6-31+G* (298 K).

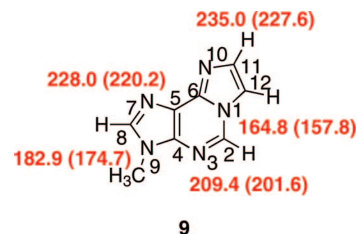

FIGURE 4. Calculated proton affinities (ΔH; gas phase basicity values (ΔG) in parentheses; all values in kcal mol^{-1}) of 9-methyl-1,N^6-ethenoadenine at B3LYP/6-31+G* (298 K).

The proton affinity of the most basic site of 9-methyl-1,N^6-ethenoadenine is calculated to be 235.0 kcal mol^{-1} (GB = 227.6 kcal mol^{-1}), which is just under 3 kcal mol^{-1} more basic than that of ϵA. The other basic sites are about 2−5 kcal mol^{-1} more basic than the corresponding sites on 1,N^6-ethenoadenine.

Experimental Results. Acidity: Calculations indicate that the most acidic site of the N9 tautomer is the N9−H and the most acidic site of the N7 tautomer is the N7−H (Figure 2). The two acidities are not expected to be differentiable experimentally since they differ by less than 1 kcal mol^{-1}. Table 1 summarizes the acidity bracketing results for the most acidic site of 1,N^6-ethenoadenine. We find that deprotonated 1,N^6-ethenoadenine deprotonates perfluoro-*tert*-butyl alcohol ($(CF_3)_3COH$, ΔH_{acid} = 331.6 ± 2.2 kcal mol^{-1}; ΔG_{acid} = 324.0 ± 2.0 kcal mol^{-1}) but not hydrogen chloride (HCl, ΔH_{acid} = 333.4 ± 0.1 kcal mol^{-1}; ΔG_{acid} = 328.1 ± 0.2 kcal mol^{-1}). In the opposite direction, chloride (Cl^-) deprotonates 1,N^6-ethenoadenine but $(CF_3)_3CO^-$ does not. Therefore, we bracket the most acidic site of 1,N^6-ethenoadenine to be ΔH_{acid} = 332 ± 2 kcal mol^{-1} (ΔG_{acid} = 325 ± 3 kcal mol^{-1}). This value is consistent with both the calculated acidity of the N9−H of the N9 tautomer **1** (ΔH_{acid} = 330.7; ΔG_{acid} = 323.2 kcal mol^{-1}) as well as the N7−H site of the N7 tautomer **7** (ΔH_{acid} = 331.3; ΔG_{acid} =323.9 kcal mol^{-1}).

We also bracketed the gas phase acidity of the less acidic site of 1,N^6-ethenoadenine, using a method developed in our

TABLE 1. Summary of Results for Acidity Bracketing of More Acidic Site of 1,N^6-Ethenoadenine

reference compound	ΔH_{acid}[a] (kcal mol^{-1})	ΔG_{acid}[a] (kcal mol^{-1})	proton transfer[b] ref acid	conjugate base
1,1,1-trifluoro-2,4-pentanedione	328.3 ± 2.9	322.0 ± 2.0	+	−
3,5 bis(trifluoromethyl)phenol	329.8 ± 2.1	322.9 ± 2.0	+	−
difluoroacetic acid	331.0 ± 2.2	323.8 ± 2.0	+	−
perfluoro-*tert*-butyl alcohol	331.6 ± 2.2	324.0 ± 2.0	+	−
hydrogen chloride	333.4 ± 0.1	328.1 ± 0.2	−	+
pyruvic acid	333.5 ± 2.9	326.5 ± 2.8	−	+
malononitrile	335.8 ± 2.1	328.1 ± 2.0	−	+
2-bromopropionic acid	336.8 ± 2.1	329.8 ± 2.0	−	+
trifluoro-*m*-cresol	339.3 ± 2.1	332.4 ± 2.0	−	+
acetic acid	348.1 ± 2.2	341.1 ± 2.0	−	+

[a] Acidities are in kcal mol^{-1}.[36] [b] A "+" indicates the occurrence and a "−" indicates the absence of proton transfer.

TABLE 2. Summary of Results for Acidity Bracketing of Less Acidic Site of 1,N^6-Ethenoadenine

reference compound	ΔH_{acid}[a] (kcal mol^{-1})	ΔG_{acid}[a] (kcal mol^{-1})	proton transfer[b] ref acid
formic acid	345.3 ± 2.2	338.3 ± 2.0	+
acetic acid	348.1 ± 2.1	341.1 ± 2.0	+
pyrrole	359.6 ± 2.9	351.8 ± 2.0	+
2-fluoroaniline	362.6 ± 2.2	355.3 ± 2.0	+
N-ethylaniline	364.1 ± 2.1	356.8 ± 2.0	+
aniline	366.4 ± 2.1	359.1 ± 2.0	+
p-toluidine	367.3 ± 2.1	360.1 ± 2.0	−
acetone	369.1 ± 2.1	361.9 ± 2.0	−
3-ethyl-3-pentanol	370.9 ± 2.8	364.3 ± 2.7	−
benzylbromide	372.1 ± 2.1	364.9 ± 2.0	−
3,3-dimethyl-1-butanol	372.5 ± 2.8	365.9 ± 2.7	−
acetonitrile	372.9 ± 2.1	365.2 ± 2.0	−
2-ethyl-1-butanol	373.1 ± 2.0	366.5 ± 2.1	−
4-chlorotoluene	374.0 ± 2.1	366.8 ± 2.0	−
2-butanol	374.1 ± 2.0	367.5 ± 2.1	−
1-propanol	376.0 ± 2.1	369.4 ± 2.0	−
ethanol	378.3 ± 1.0	371.7 ± 1.1	−
methanol	381.7 ± 1.0	375.1 ± 1.1	−

[a] Acidities are in kcal mol^{-1}.[36] [b] A "+" indicates the occurrence and a "−" indicates the absence of proton transfer.

laboratory (Table 2).[31,32,37,38] We find that the conjugate base of 1,N^6-ethenoadenine deprotonates aniline ($C_6H_5NH_2$, ΔH_{acid} = 366.4 ± 2.1; ΔG_{acid} = 359.1 ± 2.0 kcal mol^{-1}) but does not deprotonate *p*-toluidine ($CH_3C_6H_4NH_2$, ΔH_{acid} = 367.3 ± 2.1; ΔG_{acid} = 360.1 ± 2.0 kcal mol^{-1}). We therefore bracket the gas phase acidity of the less acidic site of 1,N^6-ethenoadenine to be ΔH_{acid} = 367 ± 3 kcal mol^{-1}; ΔG_{acid} = 360 ± 3 kcal mol^{-1}.

Therefore, in terms of acidity, we bracket two sites for ϵA ($\Delta H_{acid}/\Delta G_{acid}$): 332/325 and 367/360 kcal mol^{-1}. Because both the N9 and N7 tautomers have acidic sites in the vicinity of both these experimental values (Figure 2), these experiments alone do not reveal which tautomer(s) is (are) present. We therefore embarked on proton affinity experiments to help answer this question.

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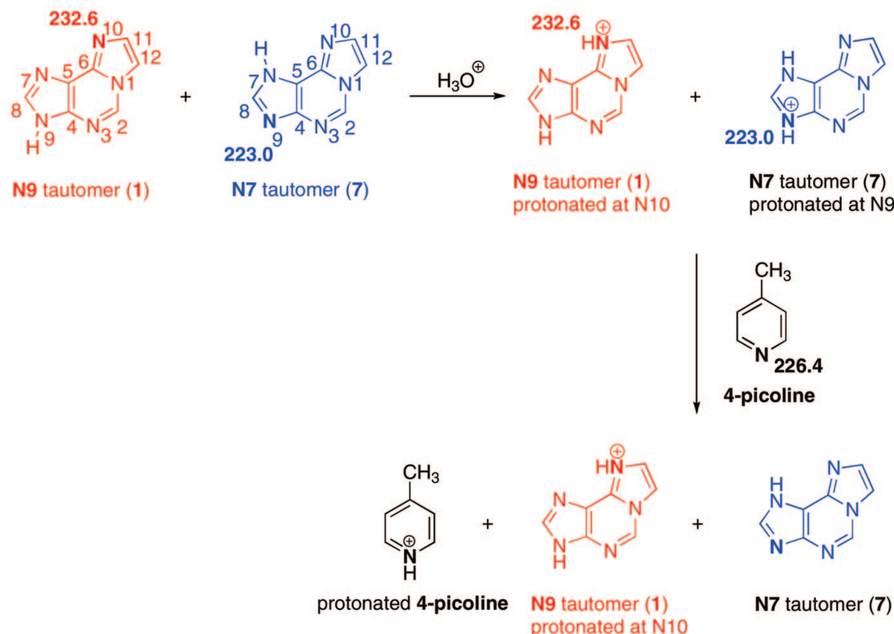
Proton Affinity: As the calculations indicate, the proton affinities (PA = ΔH) of the most basic site of the N9 tautomer **1** and the N7 tautomer **7** are 232.6 and 223.0 kcal mol^{-1}, respectively. On the basis of our computational and acidity bracketing results, particularly the calculation that tautomers **1** and **7** are close in terms of stability ($\Delta\Delta H$ = 0.7 kcal mol^{-1}), we envision three possible scenarios for our PA bracketing experiments: (i) only the N9 tautomer **1** is present; (ii) only the N7 tautomer **7** is present; (iii) both tautomers **1** and **7** are present. If only the N9 canonical tautomer **1** is present, protonation by hydronium will yield the N10-protonated species (calculated PA = 232.6 kcal mol^{-1}). Depending on the accuracy of the calculations, we would expect to bracket a proton affinity around 233 kcal mol^{-1}. The second scenario is that only the N7 tautomer **7** is present. Again, if the calculations are accurate, the bracketing experiments should target a proton affinity of about 222−223 kcal mol^{-1} (corresponding to the proton at N9 and/or N10 on **7**, Figure 3). If both the N9 and N7 tautomers **1** and **7** are present under our gas phase conditions, then the bracketing experiments should yield more complex results since the N9 and N7 tautomers have such different PAs.[39] Usually when bracketing experiments are conducted, there is a clear "crossover point" as is seen in our acidity experiments, between HCl and perfluoro-*tert*-butyl alcohol (Table 1). However, if both the N9 tautomer, with a PA of about 233, and the N7 tautomer, with a PA of about 222, are present, then reactions with reference bases in the 222−233 range will yield intriguing results, without a clean crossover point. For example, let us assume that in fact both the N9 and N7 tautomers are present, and that the former has a PA of 233 and the latter a PA of 222. What would happen if we utilized a reference base between the range of 222 and 233, such as 4-picoline (PA = 226.4 ± 2.0 kcal mol^{-1})? The expected reactions of protonated ϵA with 4-picoline and protonated 4-picoline with ϵA are shown in Scheme 2.

In Reaction A, 4-picoline is basic enough to deprotonate the protonated N7 tautomer but not basic enough to deprotonate the protonated N9 tautomer.[39] One would call this reaction a "+" since one would see proton transfer, even though only one tautomer, the N7 tautomer, is reacting. In the opposite direction (Reaction B), the N7 tautomer cannot deprotonate protonated 4-picoline but the N9 tautomer can. Again, one would call this reaction a "+" even though now only the N9 tautomer is reacting. The mass spectrometer cannot differentiate protonated N9 tautomer from protonated N7 tautomer; the *m*/*z* ratio is the same. Therefore, the reaction with 4-picoline would be marked as occurring in both directions. The interesting feature is that *any* reference base between the PAs of the two tautomers will appear as a "+,+" since in one direction the N7 tautomer will react and in the other the N9 tautomer will. Thus, rather than a clean crossover point, one would see a range (if calculations are accurate, between about 222 and 233 kcal mol^{-1}) where proton transfer occurs in both directions! Therefore, if a mixture of the N7 and N9 tautomers is present, the bracketing experiments will reveal this via a large crossover range. If only the N7 or the N9 tautomer is present, then the crossover point will be clean, and we can bracket a corresponding PA for whichever tautomer prevails.

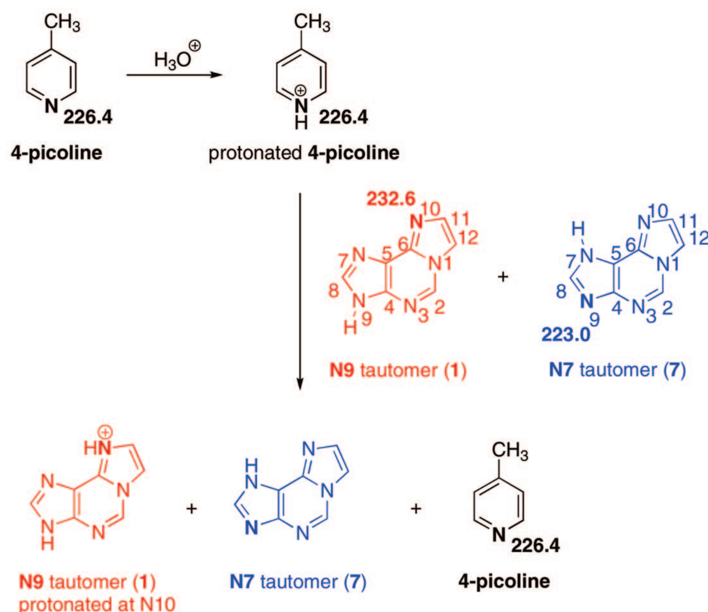
The experimental PA bracketing results are summarized in Table 3. The protonated 1,N^6-ethenoadenine protonates the first four reference bases: 1-pyrrolidino-1-cyclopentene, tributylamine, *N*,*N*-dimethylcyclohexylamine, and triethylamine. The

SCHEME 2

Reaction A



Reaction B



conjugate acids of these four reference bases do not protonate neutral 1,N^6-ethenoadenine. These results indicate that 1,N^6-ethenoadenine is less basic than these particular reference bases. We find that proton transfer occurs in both directions for 1-methyl piperidine and 1-methyl pyrrolidine. For piperidine and weaker bases, protonated 1,N^6-ethenoadenine does not react with the neutral bases, but 1,N^6-ethenoadenine can deprotonate protonated piperidine and weaker bases. Therefore, we bracket the gas phase proton affinity of the most basic site of 1,N^6-ethenoadenine to be PA $= 232 \pm 4$ kcal mol^{-1} (GB $= 224 \pm 4$ kcal mol^{-1}). Comparison of this experimental result with our calculations (Figure 3) implies that we have bracketed the N10 site on the N9 tautomer **1**.

In order to mitigate the ambiguity associated with the possibility of more than one tautomer of 1,N^6-ethenoadenine present, we also examined the 9-methyl-1,N^6-ethenoadenine derivative **9**.[40,41] This compound is a "frozen" form of the N9

(39) It is also interesting to note that the ion formed from N7 protonation of the N9 tautomer **1** is the same ion as that formed from N9 protonation of the N7 tautomer **7**. Deprotonation of this ion can therefore yield either **1** (deprotonation of the N7 proton) or **7** (deprotonation of the N9 proton). Therefore, in the FTMS proton affinity bracketing experiments, there is a possibility that some of the N7 tautomer **7** gets protonated by hydronium ion, then deprotonated by neutral **1** to form another molecule of **1**. We have no way of being certain that this "isomerization" is not occurring. Regardless of whether this isomerization occurs, our bracketing experiments appear to measure only the canonical tautomer **1**.

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TABLE 3. Summary of Results for Proton Affinity Bracketing of More Basic Site of 1,N^6-Ethenoadenine

reference compound	proton affinity[a] (ΔH, kcal mol^{-1})	gas phase basicity[a] (ΔG, kcal mol^{-1})	proton transfer[b] ref base	proton transfer[b] conjugate acid
1-pyrrolidino-1-cyclopentene	243.6 ± 2.0	236.2 ± 2.0	+	−
tributylamine	238.6 ± 2.0	231.3 ± 2.0	+	−
N,*N*-dimethylcyclohexylamine	235.1 ± 2.0	227.7 ± 2.0	+	−
triethylamine	234.7 ± 2.0	227.0 ± 2.0	+	−
1-methyl piperidine	232.1 ± 2.0	224.7 ± 2.0	+	+
1-methyl pyrrolidine	230.8 ± 2.0	223.4 ± 2.0	+	+
piperidine	228.0 ± 2.0	220.0 ± 2.0	−	+
pyrrolidine	226.6 ± 2.0	218.8 ± 2.0	−	+
4-picoline	226.4 ± 2.0	218.8 ± 2.0	−	+
3-picoline	225.5 ± 2.0	217.9 ± 2.0	−	+
cyclohexanamine	223.3 ± 2.0	215.0 ± 2.0	−	+
pyridine	222.0 ± 2.0	214.7 ± 2.0	−	+
N-methyl propionamide	220.0 ± 2.0	212.6 ± 2.0	−	+
aniline	210.9 ± 2.0	203.3 ± 2.0	−	+

[a] Proton affinities and gas phase basicities are in kcal mol^{-1}.[36] [b] A "+" indicates the occurrence and a "−" indicates the absence of proton transfer.

TABLE 4. Summary of Results for Proton Affinity Bracketing of More Basic Site of 9-Methyl-1,N^6-Ethenoadenine

reference compound	proton affinity[a] (ΔH, kcal mol^{-1})	gas phase basicity[a] ΔG, (kcal mol^{-1})	proton transfer[b] ref base	proton transfer[b] conjugate acid
1-pyrrolidino-1-cyclopentene	243.6 ± 2.0	236.2 ± 2.0	+	−
tributylamine	238.6 ± 2.0	231.3 ± 2.0	+	−
N,*N*-diisopropylethylamine	237.6 ± 2.0	230.3 ± 2.0	+	−
2,2,6,6-tetramethylpiperidine	235.9 ± 2.0	228.0 ± 2.0	+	−
N,*N*-dimethylcyclohexylamine	235.1 ± 2.0	227.7 ± 2.0	+	+
triethylamine	234.7 ± 2.0	227.0 ± 2.0	+	+
1-methyl piperidine	232.1 ± 2.0	224.7 ± 2.0	−	+
1-methyl pyrrolidine	230.8 ± 2.0	223.4 ± 2.0	−	+
piperidine	228.0 ± 2.0	220.0 ± 2.0	−	+

[a] Proton affinities and gas phase basicities are in kcal mol^{-1}.[36] [b] A "+" indicates the occurrence and a "−" indicates the absence of proton transfer.

tautomer, with an alkyl group at N9. Proton affinity bracketing studies indicate that proton transfer occurs when protonated 9-methyl-1,N^6-ethenoadenine **9** and 2,2,6,6-tetramethylpiperidine (PA = 235.9 kcal mol^{-1}) are allowed to react; however, proton transfer does not occur in the reverse direction (Table 4). Furthermore, no proton transfer reaction is observed between protonated 9-methyl-1,N^6-ethenoadenine and 1-methyl piperidine (PA = 232.1 kcal mol^{-1}), but proton transfer is observed when 9-methyl-1,N^6-ethenoadenine is allowed to react with protonated 1-methyl piperidine. When the reference bases *N*,*N*-dimethylcyclohexylamine (PA = 235.1 kcal mol^{-1}) and triethylamine (PA = 234.7 kcal mol^{-1}) are used, proton transfer is observed in both directions. Therefore, we bracket the PA of the most basic site in 9-methyl-1,N^6-ethenoadenine to be 235 ± 4 kcal mol^{-1} (GB = 227 ± 4 kcal mol^{-1}). We believe we have bracketed the proton affinity of N10 site of the methylated derivative **9** because our experimental result is consistent with the calculated proton affinity of the N10 site (PA_{calc} = 235.0; GB_{calc} = 227.6 kcal mol^{-1}). Comparison of this experimental result (235 ± 4 kcal mol^{-1}) with our experimental bracketing result for the parent 1,N^6-ethenoadenine (232 ± 4 kcal mol^{-1}) leads us to conclude that the most basic site we bracketed on ϵA is the N10 site of N9 tautomer **1**.

We also measured the proton affinities of 1,N^6-ethenoadenine and 9-methyl-1,N^6-ethenoadenine using the Cooks kinetic method, as a check of our bracketing results. For 1,N^6-ethenoadenine, we used 2,4-lutidine (PA = 230.1 kcal mol^{-1}), 1-methyl pyrrolidine (PA = 230.8 kcal mol^{-1}), 1-methyl piperidine (PA = 232.1 kcal mol^{-1}), triethylamine (PA = 234.7 kcal mol^{-1}), *N*,*N*-dimethylcyclohexanamine (PA = 235.1 kcal mol^{-1}), and 2,2,6,6-tetramethylpiperidine (PA = 235.9 kcal mol^{-1}) as the reference bases, yielding a proton affinity of 233 ± 2 kcal mol^{-1}, which is consistent with the bracketing results (232 ± 4 kcal mol^{-1}). For 9-methyl-1,N^6-ethenoadenine, seven reference bases were used: 1-methyl piperidine, triethylamine, *N*,*N*-dimethylcyclohexanamine, 2,2,6,6-tetramethylpiperidine, tripropylamine (PA = 236.9 kcal mol^{-1}), *N*,*N*-diisopropylethylamine (PA = 237.6 kcal mol^{-1}), and tributylamine (PA = 238.6 kcal mol^{-1}). The proton affinity is measured to be 236 ± 2 kcal mol^{-1}, which is consistent with the bracketing result (235 ± 4 kcal mol^{-1}).

It therefore appears that, under our conditions, only the N9 tautomer **1** of 1,N^6-ethenoadenine is present. Our acidity and proton affinity experiments are consistent with this conclusion.[39]

One interesting difference in the bracketing versus the Cooks method experiment is that in the former the neutral ϵA is added via the heatable solids probe, whereas in the latter the proton-bound dimers are prepared in solution and then electrosprayed. It therefore appears from our acidity and proton affinity experiments that gas phase ϵA, whether vaporized from the solid form or from solution, under our conditions, exists as the canonical tautomer **1**.

Biological Implications

Adenine alkyl glycosylase (AAG) is a broadly specific enzyme that cleaves several mutated purine bases, including 1,N^6-ethenoadenine, hypoxanthine, 3-methyladenine, and 7-methylguanine (**1**−**4**) from double-stranded DNA.[19,20,22] The exact mechanism by which AAG recognizes and excises the damaged bases, but leaves adenine (**5**) and guanine (**6**) untouched, is unknown. Our previous studies with hypoxanthine and 3-methyladenine have led us to propose a mechanism involving anionic cleavage (Scheme 1).[13,14,19,20,22,29–33] This mechanism is also proposed for a related enzyme, thymine DNA glycosylase (TDG), which cleaves mutated *pyrimidine* bases from DNA.[30] In this mechanism, a nucleophile such as an activated water attacks the C1′ and the damaged nucleobase leaves in its deprotonated form. We postulate that AAG discriminates the damaged bases from the normal bases because the deprotonated damaged bases are better leaving groups than the deprotonated normal bases. Furthermore, our hypothesis is that the differences in leaving group ability of the damaged versus the normal bases are enhanced in the gas phase. We recently found this to be true with hypoxanthine; hypoxanthine is more acidic than adenine and guanine (thus the conjugate base of hypoxanthine would be the best leaving group), *and* the differences in acidity are even greater in the gas phase than in aqueous solution. We therefore propose that AAG provides a hydrophobic environment that allows it to easily differentiate damaged bases from normal bases.

We sought to ascertain whether ϵA fits into this picture. Is ϵA more acidic than adenine and guanine? How do those acidities differ in the gas phase versus in solution? The ΔH_{acid}

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of the N9−H of 1,N^6-ethenoadenine (N9 tautomer **1**) is calculated to be 330.7 kcal mol^{-1}, which is 4.1 kcal mol^{-1} more acidic than adenine (calculated ΔH_{acid} = 334.8 kcal mol^{-1}) and 3.6 kcal mol^{-1} more acidic than guanine (calculated ΔH_{acid} = 334.3 kcal mol^{-1}) in the gas phase.[29] The error bars on known experimental values of adenine and guanine acidity render comparison to ϵA difficult (ΔH_{acid} (ϵA) = 332 ± 2 kcal mol^{-1}; ΔH_{acid} (adenine) = 332−333 ± 2−3 kcal mol^{-1}; ΔH_{acid} (guanine) = 331−332 ± 3−4 kcal mol^{-1}).[31,42] Therefore, we conducted Cooks kinetic experiments on the ϵA•A and ϵA•G deprotonated dimers and find that ϵA is 3.42 ± 0.11 kcal mol^{-1} more acidic than adenine and 2.54 ± 0.01 kcal mol^{-1} more acidic than guanine. These results indicate that, if the damaged base leaves in a deprotonated anionic form when the enzyme catalyzed excision occurs, deprotonated 1,N^6-ethenoadenine is the best leaving group among adenine, guanine, and 1,N^6-ethenoadenine. The next question is, what are the relative acidities of ϵA versus adenine and guanine in the gas phase versus in solution? Enhanced acidity differences in the gas phase would be consistent with our theory that AAG provides a hydrophobic active site to discriminate damaged from normal bases.

We measured the aqueous pK_a of ϵA to be 9.9, which is comparable in acidity to both adenine (9.8) and guanine (10.0).[43–45] We also conducted solvent dielectric calculations to ascertain how the relative acidities change in a more polar environment.[46,47] The calculated acidity of the N9−H of 1,N^6-ethenoadenine (N9 tautomer **1**) in water is 294.1 kcal mol^{-1}, which is comparable to that of adenine (294.8 kcal mol^{-1}) and guanine (294.6 kcal mol^{-1}). The differences in acidity in solution among ϵA, adenine, and guanine are thus significantly lower than the differences found in the gas phase. This result, coupled with our similar result for hypoxanthine, implies that the nonpolar active site in AAG could contribute to specificity by enhancing the differences in acidity among adenine, guanine, and damaged bases.[29,35,48]

Conclusions

Our experimental and computational study of 1,N^6-ethenoadenine indicates that, although both the N9 tautomer **1** and the N7 tautomer **7** may be close in gas phase stability, we measure the acidities and proton affinity of only the N9 tautomer **1**. Comparison of the acidic properties of 1,N^6-ethenoadenine to those of the normal nucleobases adenine and guanine, both in the gas phase and in solution, supports our theory that AAG cleaves damaged nucleobases as anions and that the active site may take advantage of a nonpolar environment to favor deprotonated damaged bases such as 1,N^6-ethenoadenine as a leaving group versus deprotonated adenine or guanine. Our earlier studies on hypoxanthine are consistent with this theory as well. Future studies of the gas phase properties of other damaged nucleobases that serve as substrates for AAG are underway.

Methods

All chemicals except 9-methyl-1,N^6-ethenoadenine are commercially available and were used as received. 9-Methyl-1,N^6-ethenoadenine was synthesized following literature procedure.[40,41]

Acidity and proton affinity experiments were conducted using a Fourier transform ion cyclotron resonance mass spectrometer (FTMS) with a dual cell setup, which has been described previously.[31,37] In our FTMS, two adjoining 1 in. cubic cells are positioned collinearly with the magnetic field produced by a 3.3 T superconducting magnet. The pressure of the dual cell is pumped down to less than 1×10^{-9} Torr. The solid nucleobases are introduced to the cells via a heatable solids probe. Ions are generated via reaction with H_3O^+ or OH^- ions. Ions can be transferred from one cell to the second cell via a 2 mm hole in the center of the central trapping plate. Transferred ions are cooled by a pulse of argon that raises the cell pressure to 10^{-5} Torr.[49] Experiments are conducted at ambient temperature.

Acidity and proton affinity (and gas phase basicity) are assessed using bracketing experiments in the FTMS, which have been described previously.[31,37] Briefly, for acidity bracketing, hydroxide ions are generated first by pulsing water into the FTMS cell and sending an electron beam (8 eV, 6 μA, beam time 0.5 s) through the center of the cell. The hydroxide ions deprotonate neutral molecules "M" (either nucleobases or reference bases) to yield the $[M - H]^-$ ions. The $[M - H]^-$ ion is allowed to react with the neutral nucleobase or reference base. The same procedure is used for bracketing proton affinity, where hydronium ions (10 eV, 6 μA, beam time 0.2 s) are used for protonation. The occurrence of proton transfer is regarded as an evidence that the reaction is exothermic ("+" in tables).

In our experiments, we have pseudo-first-order conditions, where the amount of the neutral substrate is in excess relative to the reactant ions. Reading the pressure from an ion gauge is often unreliable, both because of the gauge's remote location as well as varying sensitivity for different substrates.[32,50] We therefore "back out" the neutral pressure from a control reaction; this procedure has been described previously by us.[32] Briefly, we obtain the pseudo-first-order rate constant for the reaction of hydroxide and the neutral substrate. Because hydroxide is very basic, we assume this reaction proceeds at the theoretical collision rate.[51,52] We can then use the calculated collisional rate constant to "back out" the neutral pressure.

We also used the Cooks kinetic method in a quadrupole ion trap (LCQ) mass spectrometer[53–57] to measure the proton affinities of the nucleobases and to conduct a relative acidity study of ϵA, adenine, and guanine. For the proton affinity studies, the proton-bound complex ions are generated by electrospray (ESI).[58] For each experiment, a solution of the nucleobase and reference base is prepared (10^{-3} to 10^{-4} M solutions in methanol; a small amount of acetic acid is also added). An electrospray needle voltage of ∼4.5 kV was used. The flow rate is 25 μL/min. The proton-bound complex ions were isolated and then dissociated by applying collision-induced dissociation (CID); the complexes were activated for about 30 ms. Finally, the dissociation product ions are detected

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to give the ratio of the protonated analyte and protonated reference base. A total of 40 scans was averaged for the product ions.

The Cooks kinetic method involves the formation of a proton-bound complex, or dimer, of the unknown A and a reference base of known proton affinity (eq 1)

$$AH^{\oplus} + B_i \xleftarrow{k_1} [A\cdots H^{\oplus}\cdots B_i] \xrightarrow{k_2} A + B_iH^{\oplus} \quad (1)$$

where A represents 1,N^6-ethenoadenine and B_i denotes a series of reference bases with known proton affinities.[36,53–57] The proton-bound dimer $[A\cdots H^{\oplus}\cdots B_i]$ is dissociated via collision-induced dissociation (CID). The rate constants k_1 and k_2 are for the two different dissociation pathways. The relationship of these rate constants to PA is shown in eq 2:

$$\ln\frac{k_1}{k_2} = \ln\frac{AH^{\oplus}}{B_iH^{\oplus}} = \frac{\Delta G(B_i) - \Delta G(A)}{RT_{eff}} \approx \frac{\Delta H(B_i) - \Delta H(A)}{RT_{eff}} = \frac{PA(A) - PA(B_i)}{RT_{eff}} \quad (2)$$

where R is the gas constant and T_{eff} is the effective temperature[59] of the activated dimer.[53–57] The ratio of the amounts (intensities) of the two protonated products yields the relative proton affinities of the two compounds of interest, assuming the dissociation has no reverse activation energy barrier and that the dissociation transition structure is late and therefore indicative of the stability of the two protonated products. These assumptions are generally true for proton-bound systems.[57,60,61] In order to obtain the proton affinity of compound A, the natural logarithm of relative intensity ratios is plotted versus the proton affinities of a series of reference bases, where the slope is $(-1/RT_{eff})$ and the y-intercept is $(PA(A)/RT_{eff})$. The T_{eff} is obtained from the slope. The proton affinity of compound A, (PA(A)), is calculated from either eq 2 or the y-intercept.

For the ϵA acidity studies, the Cooks kinetic method was used on the deprotonated dimers of ϵA•adenine and ϵA•guanine to assess relative values. The deprotonated ϵA•adenine or ϵA•guanine complex ions are generated by ESI.[58] For each experiment, a solution of ϵA and adenine or ϵA and guanine is prepared (10^{-3} M solutions in water mixed with 20% ethanol; a small amount of formic acid is needed to dissolve guanine). As with the proton affinity measurements, an electrospray needle voltage of ~4.5 kV was used and the flow rate is 25 μL/min. CID activation of the isolated deprotonated dimers is for 30 ms, and a total of 40 scans was averaged for the product ions. A T_{eff} of 434 K, obtained from calibration experiments with guanine, was used.

We used two methods to measure the pK_a of 1,N^6-ethenoadenine. In the first, we simply prepared a solution of a known concentration of ϵA, and then added a 0.5 stoichiometric amount of sodium hydroxide; we then assume that pH = pK_a. In the second method, we utilized changes in absorbance at 240 nM versus pH.[62] We measured the pK_a five times and report the averaged value.

The B3LYP method and the 6-31+G* basis set as implemented in Gaussian03 were used for all the gas phase calculations.[63–68] All the geometries are fully optimized and frequencies are calculated; no scaling factor is applied. Reported values herein are at 298 K.[69] Solvation studies were conducted using the CPCM method (full optimizations at B3LYP/6-31+G*; UAKS cavity as implemented in Gaussian03).[46,47] A dielectric constant of 78.4 was used to simulate an aqueous environment.

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Supporting Information Available: Cartesian coordinates for all calculated species and full citations for references with greater than 16 authors are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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