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Structure of oxidatively damaged nucleic acid adducts. 3. Tautomerism, ionization and protonation of 8-hydroxyadenosine studied by ^{15}N NMR spectroscopy

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ABSTRACT

Natural abundance ^{15}N NMR spectroscopy and ancillary spectroscopic techniques have been employed to study the solution structure of 8-hydroxyadenosine. 8-Hydroxyadenosine is a naturally occurring oxidized nucleic acid adduct that is generally implied to have an 8-hydroxy tautomeric structure. ^{15}N NMR chemical shifts and coupling constants, however, indicate that the modified base exists as an 8-keto tautomer. The pH dependence of ^{15}N NMR and UV spectra showed the presence of two pK_a's, at 2.9 and 8.7, corresponding to protonation at N1 and ionization at N7, respectively. The latter results in the formation of an 8-enolate structure. Unusual upfield shifts of the ^1H and ^{15}N resonances of the NH_2 group, and a reduction in the one-bond coupling constant $^1J_{N6-H6}$, is indicative of an unfavorable steric or electronic interaction between the NH_2 group and the adjacent N7-H proton. This interaction results in a subtle change in the structure of the NH_2 group. In addition to being a possible mechanism for alteration of hydrogen bonding in oxidized DNA, this type of interaction gives a better understanding into N7-N9 tautomerism of adenine. Furthermore, the structure of 8-hydroxyadenosine has been related to possible mechanisms for mutations.

INTRODUCTION

The C8-oxidation of the purine DNA bases, guanine and adenine, is one of the major forms of oxidative base damage, which can result from the action of ionizing radiation (1) and other oxygen radical generating systems (2). These chemical modifications are thought to have important biological implications in aging and cancer (3,4). We have previously characterized the structure of the mutagenic adduct 8-oxo-2'-deoxyguanosine and its ribosyl analog 8-oxoguanosine (5,6). Another C8-oxidized purine base is 8-hydroxyadenine (7), which has been detected in neoplastic liver of fish (8), as well as in urine samples of ferrets and humans (9). Recently, Guy *et al.* (10) prepared and characterized several oligodeoxynucleotides containing 8-hydroxyadenine moieties and showed (11) that a modified oligomer was three times more susceptible to DNA chain ruptures when treated with γ-irradiation and heated in the presence of piperidine. Immunological (12), high performance liquid chromatography-electrochemical (HPLC-EC) (13), and gas chromatography-mass spectral (GC-MS) (8,9) assays have been developed for this modified base. Despite the potential biological significance, structural information about this important lesion and its derivatives, as well as possible structure function relationships, is limited. Previous infrared spectral analyses have tentatively assigned the structure of 8-hydroxy-2'-deoxyadenosine in the solid state as the 8-keto form (10). In addition, 8-hydroxyadenosine has been reported to exist in the *syn* conformation about the glycosyl bond (14,15).

The detailed analysis of tautomerism, protonation, ionization and conformation in solution of this lesion is an important consideration in determining the forms available for base pairing in macromolecular structures. There are at least four possible tautomeric forms of the neutral species involving the N1, C6, N7 and C8 positions (**Ia-Id**) as well as two alternative molecular conformations about the glycosyl bond, *syn* (**Ie**) and *anti* (**If**). All potential structures could affect base pairing and conformation of nucleic acids. In aqueous solution, the situation is further complicated by the presence of additional pH-dependent tautomeric and ionic species. In order to study further the structure of oxidatively damaged nucleic acid adducts, we wish to report the characterization of 8-hydroxyadenosine (8-OH-A, **I**) in solution. By conducting an analysis of the pH dependence of ^{15}N NMR spectra, detailed structural information on the tautomerism, ionization, and conformation of 8-OH-A has been obtained. These data are discussed in terms of possible mechanisms for altered biological function.

MATERIALS AND METHODS

NMR spectra were recorded in the ^{15}N, ^{13}C and ^1H configurations on a Bruker AM500 spectrometer utilizing 10-, 10-, and 5-mm probes, respectively. The ^1H NMR (500 MHz) chemical shifts are reported in ppm by assigning the DMSO-$_{d6}$

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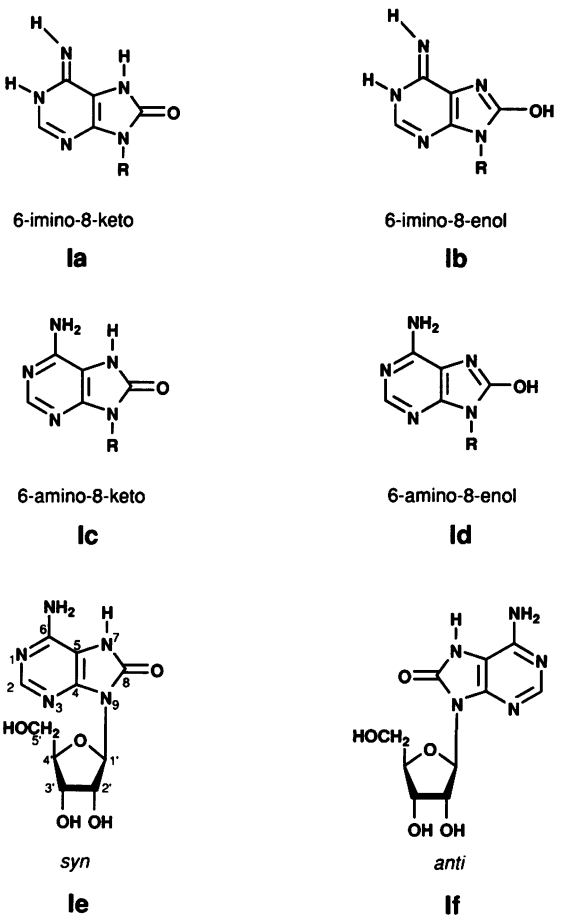

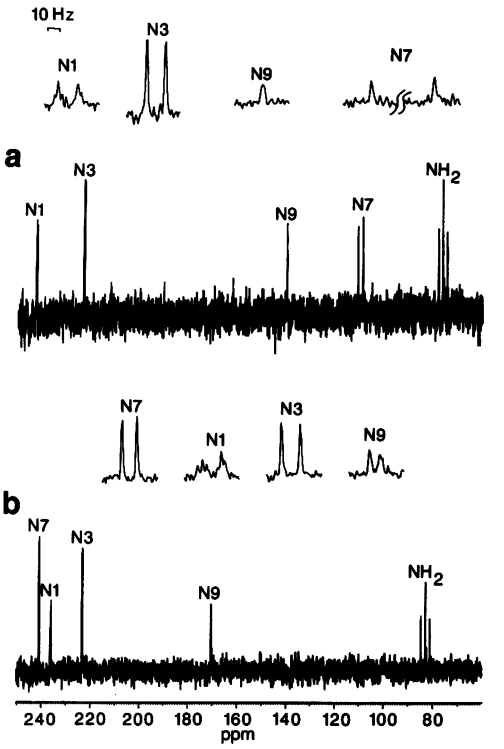

Figure 1. Natural abundance 50.7 MHz ^{15}N NMR spectra in DMSO are shown for (a) 8-OH-A (0.4 M) and (b) adenosine (1.0 M) in DMSO. Small couplings are expanded in the upper insets. The NH_2 resonance exhibited no fine structure.

signal to 2.50 ppm. The chemical shifts of the signals in the 126 MHz ^{13}C NMR spectra were obtained with the DMSO-$_{d6}$ signal assigned as 39.5 ppm. The natural abundance ^{15}N NMR spectra were obtained at a frequency of 50.7 MHz, and chemical shifts were reported in ppm downfield from external ^{15}N-enriched $NaNO_3$ in D_2O by assigned the resonance to 376.5 ppm. Typical conditions for ^{15}N NMR spectra were as follows: flip angle, 40°; data size, 64K; sweep width, 30 kHz; recycle delay, 2s. Further details about the conditions for NMR spectroscopic measurements have been described previously (6). UV spectra were recorded on a Varian DMS 80 UV-visible spectrophotometer.

The synthesis of 8-OH-A (I) was attempted by using a procedure similar to that described for the preparation of 8-oxoguanosine derivatives (16); i.e., C8-benzyloxylation of 8-bromoadenosine followed by catalytic hydrogenation. It turned out to be a single step reaction, since the second step (hydrogenation) was not necessary due to the labile nature of the 8-benzyloxyl group in the acidic work-up conditions. A similar demethylation at the C8-position was observed in the preparation of the 3',5'-*O*-disilyl derivative of 8-oxoguanosine from 8-bromoguanosine (6).

8-Hydroxyadenosine (8-OH-A)

A solution of 8-bromoadenosine (4.85 g, 0.014 mole, Sigma, St. Louis, MO) in 40 mL of dry DMSO was added to a mixture of sodium (1 g) dissolved in 35 mL of benzyl alcohol and 100 mL of DMSO. This mixture was heated at 65°C for 24 hr. and then cooled to room temperature. The reaction mixture was acidified with glacial acetic acid and then poured into 1 L of anhydrous ether. The resulting precipitate was collected, dissolved in methanol, and then mixed with 5 g of silica. The solvent was evaporated and the silica residue was applied to a gravity silica column that was eluted with $CHCl_3$:MeOH (4:1). The appropriate fractions were collected, evaporated to dryness and recrystallized from water to yield 0.8 g (20%) of pure product as white crystals. m.p. (Thomas-Hoover) 236–238°C [lit.(17) 237–238°C], EIMS (Finnigan 4023 mass spectrometer, 70 ev) m/z 283 (M^+, 2%), 151 (Base+H, 100%). CIMS (methane) m/z 284 (M+H, 100%), 152 (Base+2H, 70%). HPLC (Beckman 5μ ODS Ultrasphere, 250×10 mm i.d., a 30 min. linear gradient with 5–10% MeOH:H_2O, 3 mL/min) retention time, 11.45 min.

RESULTS AND DISCUSSION

NMR resonance assignments

The ^{15}N resonance assignments of 8-OH-A in DMSO were made on the basis of ^{15}N-^1H coupling constants and by comparisons to that of adenosine (18–21) and 8-oxoguanosine (6). The proton coupled ^{15}N spectrum exhibited five clearly defined resonances (Figure 1a). The prominent triplet at 76.2 ppm ($^1J_{N6\text{-}H6}$ = −89.3 Hz) was readily assigned to the exocyclic amine at C6. The resonances at 242.5 and 223.2 ppm were assigned to N1 and N3, respectively. This is based on the fact that while both nitrogens show two-bond couplings with H2 ($^2J_{N3\text{-}H2}$ = 15.3 Hz and $^2J_{N1\text{-}H2}$ = 15.7 Hz), that of N1 is further coupled with the exocyclic NH_2 ($^3J_{N1\text{-}NH2}$ = ca. 3 Hz). The doublet at 109.7 ppm was attributed to N7 from its large one-bond N-H coupling constant ($^1J_{N7\text{-}H7}$ = −98.7 Hz) and the large upfield shift (131.2 ppm) from that of adenosine (Figure 1b). The assignment was confirmed by selective decoupling of the

Table I. Influence of 1 molar equivalent trifluoroacetic acid on ^{15}N NMR chemical shifts and coupling constants of adenosine (1.0 M) and 8-hydroxyadenosine (0.4 M) in DMSO.

						adenosine				
molar equiv. of acid	chemical shift (ppm)					coupling constant (Hz)[a]				
	N1	N3	N7	N9	NH_2	$^1J_{N6-H6}$	$^2J_{N3-H2}$	$^2J_{N7-H8}$	$^2J_{N9-H8}$	$^2J_{N1-H2}$
0	236.1	223.2	240.9	170.3	82.7	−89.9	15.0	12.3	8.5	15.3
1	173.0	224.8	243.8	177.2	90.0	−91.8	13.6	12.0	7.6	ND[b]

						8-hydroxyadenosine			
	chemical shift (ppm)					coupling constant (Hz)[a]			
	N1	N3	N7	N9	NH_2	$^1J_{N6-H6}$	$^1J_{N7-H7}$	$^2J_{N3-H2}$	$^2J_{N1-H2}$
0	242.5	223.2	109.7	139.9	76.2	−89.3	−98.7	15.2	15.7
1	197.5	222.9	112.3	143.8	80.4	ND[b]	−99[c]	14.1	ND[b]

[a] The coupling constants are expressed as absolute values with the exception of one-bond N-H couplings, which are assumed to be negative. The digital resolution was 0.45 Hz/point.
[b] Not detected due to resonance broadening.
[c] An approximate value due to broadening.

N7-H proton at 10.37 ppm. The extent of the upfield shifts observed for the N7 and N9 resonances (131.2 and 30.4 ppm) of 8-OH-A is in close agreement with those (138.4 and 28.6 ppm) observed for a similar transformation of guanosine to 8-oxoguanosine (6). The remaining singlet at 139.9 ppm was assigned to N9, which was apparently broadened by unresolved couplings with H7 and sugar protons. The ^{15}N NMR data of 8-OH-A along with those of the parent compound adenosine in DMSO at ambient temperature are summarized in Table I.

The ^{13}C NMR resonances of 8-OH-A in DMSO were assigned by long-range selective decoupling experiments, and the results are in accord with previously reported assignments (14). Complete ^1H NMR assignments were made by conventional homonuclear decoupling experiments. Both the ^1H and ^{13}C assignments of the adduct in DMSO at ambient temperature are as follows: ^1H NMR δ 10.37 (N7-H), 8.02 (H2), 6.59 (NH_2), 5.67 (H1′), 5.26 (OH2′), 5.18 (OH5′), 5.08 (OH3′), 4.86 (H2′), 4.12 (H3′), 3.86 (H4′), 3.60, 3.46 (H5′, H5′); ^{13}C NMR δ 151.59 (C8), 150.70 (C2), 147.23 (C6), 146.58 (C4), 103.60 (C5), 85.73 (C1′), 85.51 (C4′), 71.00 (C3′), 70.36 (C2′), 62.40 (C5′).

Tautomerism in DMSO

The possible occurrence of 8-enol (**Ib** and **Id**) or 6-imino (**Ia** and **Ib**) tautomers of 8-OH-A was investigated by natural abundance ^{15}N NMR spectroscopy. As shown in Figure 1, the N7 resonance of 8-OH-A in DMSO exhibited a well-resolved one-bond N-H coupling ($^1J_{N7-H7}$ = −98.7 Hz), that is characteristic of protons bound to a trigonally hybridized nitrogen atom (22,23). In addition, this shift lies in the range of urea-type nitrogens, which is consistent with a conversion of the N7 atom from a 'pyridine-like' to a 'pyrrole-like' nitrogen (18,20,24). These observations clearly establish 8-OH-A as the C8-keto form (**Ic**). The possibility of C6-imino tautomeric forms (**Ia** and **Ib**) of 8-OH-A can be ruled out based on the observation that both the N1 and NH_2 resonances of 8-OH-A retain the chemical shift and coupling characteristics of adenosine, which is known to exist in the 6-amino form (25). If the adduct was in the 6-imino form, dramatic chemical shift changes of the N1 and NH_2 resonances would be expected from the above-mentioned 'pyrrole-pyridine type nitrogen' argument. For example, Morelli *et al.* (26) have shown that the N1 and NH_2 nitrogen signals of 1-methyladenosine, which is locked into the 6-imino form, moved 88 ppm upfield and 101 ppm downfield, respectively, as compared to adenosine. The small downfield shift of N1 and upfield NH_2 shift of 8-OH-A compared to adenosine cannot be explained by such a tautomeric shift, as the shifts are in the reverse direction of what would be predicted.

Interaction between the NH_2 group and the N7-H proton

Unusual NMR spectral parameters associated with the NH_2 group indicate a structural perturbation. Compared to the NH_2 moiety of adenosine, the ^{15}N resonance of 8-OH-A is shifted upfield by 6.5 ppm and the ^1H resonance (*vide infra*) is shifted upfield by 0.79 ppm. In addition, the N1 resonance is deshielded by 6.4 ppm, while that of N3 is essentially unchanged. The upfield shift in the ^{15}N spectrum of 8-OH-A is similar to that observed in comparing the spectra of 2,6-dimethylaniline to aniline (5.5 ppm) (27). In 2,6-dimethylaniline, the nitrogen lone pair delocalization into the aromatic ring is perturbed by steric interaction with the ortho methyl groups. In aniline derivatives, there are also parallel correlations between the amino nitrogen chemical shift, the proton chemical shift and the π-electron density (22). The ^1H NMR chemical shift for the amino protons of 8-OH-A (6.59 ppm) are upfield of adenosine (7.38 ppm) and other C8-substituted adenosine derivatives (7.60 and 7.27 ppm for 8-bromo- and 8-azidoadenosine, respectively). In addition, a small decrease of $^1J_{N6-H6}$ (ca. 0.5 Hz) of 8-OH-A is noted (Table I), which indicates an increase in the sp^3 character. These results can be explained, at least in part, by a reduction in the delocalization of the nitrogen lone pair into the pyrimidine ring, resulting in a loss of partial double bond character of the C6-N6 bond of 8-OH-A. Molecular models of 8-OH-A revealed that there is considerable steric interaction between the exocyclic NH_2 group and the N7-H proton. It should also be noted that the ^{15}N and ^1H chemical shifts of the NH_2 group of 8-OH-A are strikingly similar to those of the guanosine NH_2 group (74.2 ppm and 6.56 ppm) (6), suggesting that they have a similar degree of lone pair delocalization into their respective pyrimidine rings.

The steric/electronic interaction between the NH_2 group and the N7-H proton of 8-OH-A is worthy of comment relative to the factors effecting the N7-H or N9-H tautomeric equilibrium

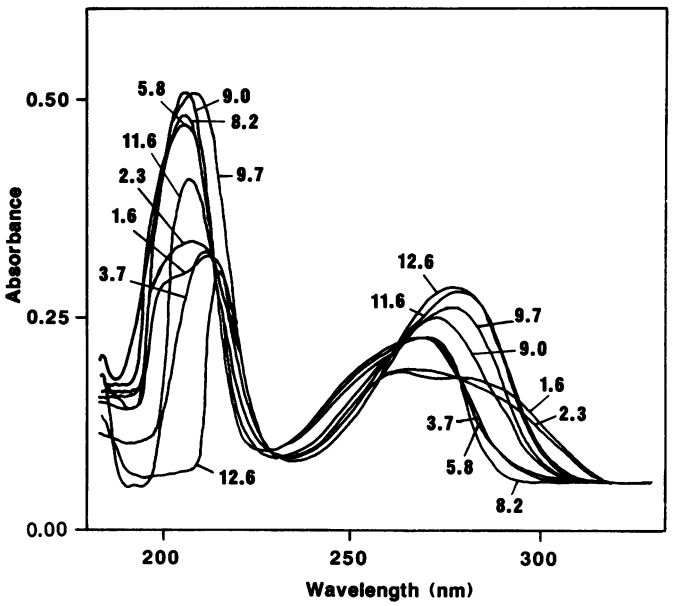

Figure 2. UV spectra of 8-OH-A shown as a function of pH from pH 1.6 to 12.6. The pH values are indicated for each spectrum.

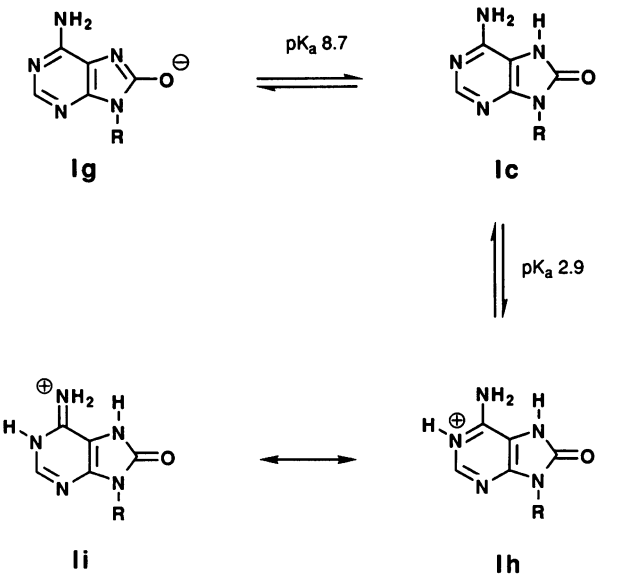

Table II. ^{15}N Chemical shifts of 8-hydroxyadenosine in aqueous solution.

pH	conc. (M)	N1	N3	N7	N9	NH_2
1.6	0.14	162.7	220.6	114.5	145.7	80.3
6.7	0.05	232.2	217.0	112.2	ND[a]	73.6
11.0[b]	0.23	227.7	216.3	164.8	143.6	66.6

[a] Not detected due to low signal to noise ratio.
[b] $^2J_{N1\text{-}H2} = {}^2J_{N3\text{-}H2} = 14.1$ Hz.

of adenine. This is a subject that has received much theoretical and experimental attention (28–32). If the afore-mentioned interaction is significant, it would be expected to destabilize the N7-H tautomer of adenine in favor of the N9-H tautomer. In fact, all the previous studies clearly demonstrated the N9-H tautomer of adenine is the most abundant (29–32). Unfavorable charge separation and possible repulsions between the unshared electrons (N3 vs. N9) encountered in the N7-H tautomer have been suggested to explain this phenomenon (33). We note that the population of the N7-H tautomer reported (29) for purine (40%) and 6-methoxypurine (32%) is significantly higher than that of adenine (15%). This is evidence of a destabilizing interaction between the NH_2 group and the N7-H proton in adenine. A steric argument has been used to explain a dramatic change in tautomeric equilibrium toward the N9-H tautomer in the case of N,N-dialkyl substituted adenine derivatives (33). These results on purine derivatives support our proposal for a weak destabilizing interaction in 8-OH-A.

Tautomerism, ionization and protonation in aqueous solution

Ultraviolet spectra of 8-OH-A were recorded in the range of pH 1.6 to 12.6 (Figure 2) in order to determine pK_a values. Several isosbestic points were obtained indicating the presence of multiple ionic species (34). Analysis of the spectral changes yielded two pK_a's at pH 2.9 and 8.7. It is interesting to note that the basic pK_a of 8-OH-A is 3.0 pK_a units lower than that observed for the isoelectronic N7-H for 8-oxoguanosine, where the negative charge of the first ionization at N1 suppresses the second one (6). The acidic pK_a is also 0.7 units lower than that of adenosine (35), reflecting the electron-withdrawing effect of the C8-keto group and the altered structure of the NH_2 group (*vide supra*). Thus, approximately 7% of the molecule should exist in the C8-enolate (**Ig**) form at physiological pH (7.4).

^{15}N NMR spectroscopy has proven to be particularly useful for probing the sites of ionization and protonation of nitrogens in nucleosides and their derivatives. These sites are important to the biological activities of these molecules (24,36). However, due to time requirements for obtaining ^{15}N spectra in aqueous solution, data for 8-OH-A were recorded at only three pH values. These pH values were selected from the pK_a values to give a neutral species (pH 6.7), a mostly ionized species (pH 11.0) and a mostly protonated species pH (1.6).

Figure 3b shows the natural abundance ^{15}N NMR spectrum of 8-OH-A at pH 6.7 recorded with proton decoupling in aqueous solution. The protonated nitrogens (NH_2 and N7) exhibit intense negative signals while those of the non-protonated nitrogens (N1 and N3) are very low; the N9 atom was not detectable due to low signal to noise ratio. It has been shown that the protonated nitrogens have large negative NOEs (nuclear Overhauser effects), while the non-protonated pyridine-like nitrogens have an unfavorable NOE on the order of −1 (18). We were not able to obtain a ^{15}N spectrum with proton coupling because of lower solubility of 8-OH-A at neutral pH. The resonances for the azine-like nitrogens of 8-OH-A are generally shifted upfield (2.6–10.3 ppm) compared to those obtained in DMSO (Table I), indicating the presence of a solvent effect (18). This also suggested that, like adenosine, the nitrogens of 8-OH-A are subject to strong hydrogen bonds with H_2O, in spite of the structural modification in the imidazole ring.

The proton coupled ^{15}N spectrum at pH 11.0 (Figure 3a) shows a dramatic downfield shift of the N7 signal by 52.6 ppm, while the resonances N1 and NH_2 move slightly upfield and that of N3 remains essentially unchanged. This clearly indicates that the ionization results in the formation of a C8-enolate anion, which unequivocally establishes the locale of the ionization (**Ig**).

^{15}N NMR chemical shifts are known to be very sensitive to protonation. In fact, the protonated nitrogens in pyridine and purine rings come into resonance at fields as much as 100 ppm

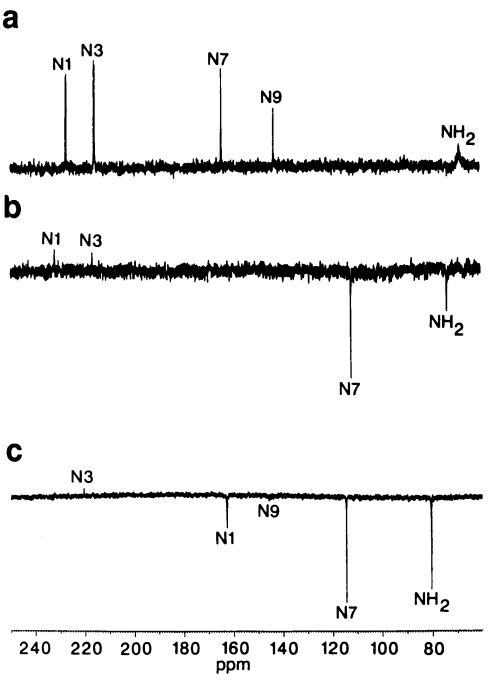

Figure 3. Natural abundance 50.7 MHz ^{15}N NMR spectra of 8-OH-A in H_2O recorded at (a) pH 11 (0.23 M) with no proton decoupling (b) pH 6.7 (0.04 M) with proton decoupling and (c) pH 1.6 (0.13 M) with proton decoupling. Some of the resonances in (b) and (c) are inverted due to the relatively large negative NOEs of the protonated nitrogens.

higher than that of the corresponding unprotonated nitrogens (24). Figure 3c shows a 69.5 ppm upfield shift of N1 at pH 1.6, while the other nitrogen signals are shifted slightly downfield. It should be noted here that the ^{15}N measurements at neutral and acidic pH were made in the proton decoupled mode. Thus, while the neutral spectrum showed essentially two signals corresponding to the proton bearing nitrogens (NH_2 and N7), the spectrum at pH 1.6 produced an additional signal at 162.7 ppm caused by the protonated N1 atom (**Ih**). It was possible to obtain proton coupled ^{15}N spectra due to higher water solubility of the charged species.

The NH_2 resonance of 8-OH-A at acidic pH was deshielded 6.7 ppm relative to that near neutral pH, while it was shielded by a similar magnitude at basic pH. The downfield shift indicates that the partial double bond character of the C6-N6 bond increases when a proton is bound to N1. Thus, the contribution of the resonance structure, **Ii**, becomes more important. It is worth noting the similarity between the resonance form (**Ii**) and the 6-imino form of 1-methyladenosine discussed earlier. Much stronger deshielding effects have been observed for the amino nitrogens of protonated cytidine (37) and 3,5'-cycloadenosine derivative (38) (13 and 28 ppm, respectively), where partial double bond character of the C4-N4 and C6-N6 bonds are caused by delocalization of the positive charge at N3 for both compounds. An X-ray study confirmed the delocalization of the lone pair in protonated cytidine (39). In basic solution, however, this lone pair delocalization is likely to be restricted by the electron-rich purine ring (**Ig**).

Comparisons on the effect of protonation were made between 8-OH-A and A using trifluoroacetic acid as a protonating agent. Table I shows that addition of 1 molar equivalent of trifluoroacetic acid to 8-OH-A in DMSO produces qualitatively similar

Table III. UV Spectral parameters of adenosine, 8-hydroxyadenosine and its derivatives.

Compound	pH	λmax, nm[a]	ϵmax, mM^{-1}
adenosine[b]	1.5	257	14.6
	7.9	260	14.9
8-OH-A	1.6	263 (283)	9.7
	7.1	269	15.0
	12.6	281	17.0
7-Methyl-8-oxo-adenosine[c]	1.0	264 (282)	11.3
	7.0	267	14.9
	11.0	268	15.6
1-Methyl-8-oxo-adenosine[c]	1.0	268 (281)	15.8
	7.0	279	18.3
	11.0	283	13.8

[a] The parentheses indicates a shoulder or point of inflection.
[b] Taken from reference 40.
[c] Taken from reference 41.

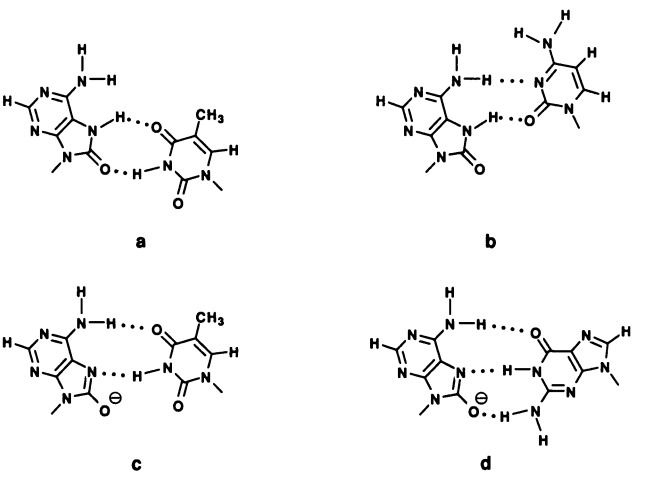

Figure 4. Possible base pairings and mispairings of 8-OH-A depending on the ionization and conformation about the glycosyl bond are shown: (a) *syn*-8-keto-A:T pair; (b) *syn*-8-keto-A:C mispair; (c) *syn*-8-enolate-A:T pair; and (d) *syn*-8-enolate-A:G mispair.

variations of chemical shifts and N-H couplings as those of adenosine, which is known to protonate exclusively at the N1 nitrogen (33). Thus, the dramatic upfield shift of the N1 nitrogen resonance, while the other resonances remain relatively unaffected, indicates that both compounds protonate at this position. The magnitude (45.0 ppm) of the upfield shift observed for the N1 resonance of 8-OH-A is noticeably smaller than that (63.1 ppm) of adenosine under the same conditions. This may occur because the C8-keto group on the imidazole ring induces the lone pair of the N1 atom to delocalize into the electron deficient imidazole ring, producing an overall reduction of basicity of the N1 atom. This argument is further substantiated by the lower acidic pK_a of 8-OH-A as compared to adenosine (*vide supra*). Small decreases (1.1 Hz) of $^2J_{N3\text{-}H2}$ in 8-OH-A (Table I) were additional evidence of N1 protonation.

Comparative ultraviolet absorption spectral analysis

Results from ultraviolet absorption spectra provide supporting evidence for the ionization and tautomerism of 8-OH-A in aqueous solution. Table III compares the ultraviolet absorption

spectra of 8-OH-A with adenosine (40) and its methyl derivatives (41) at various pH values. The λmax in the ultraviolet spectrum of 7-methyl-8-oxoadenosine at pH 11 corresponds with the spectra recorded for 8-OH-A at pH 1.6 and 7.1. At pH 12.6, the absorption spectrum for 8-OH-A shows similar wavelength maxima to that of 1-methyl-8-oxoadenosine at pH 11, but not to that of the 7-methyl derivative. In addition, the ultraviolet spectrum of 8-OH-A at pH 12.6 shows a 12 nm bathochromic shift, which is consistent with a complete aromatization of the purine ring as would be expected for conversion to a C8-enolate (**Ig**).

Conformation about the glycosyl bond

The conformation of the adduct has been determined by NMR spectroscopy. From ^{1}H and ^{13}C NMR spectra, we observed a characteristic downfield shift of H2′ (0.28 ppm) and an upfield shift of C2′ (3.44 ppm) for 8-OH-A compared to adenosine in DMSO, which is in general agreement with previous studies (14,15). In addition, we found that the vicinal C-H coupling constant $^{3}J_{C4\text{-}H1'}$ for 8-OH-A (5.3 Hz) is larger than that of adenosine (3.6 Hz) and comparable to that of purine nucleotides that are known to be in the *syn* form (42, 43). These results demonstrate that the adduct exists preferentially in the *syn* conformation.

Biological Implications

Physicochemical investigations of the properties of nucleic acid monomers have provided considerable insight into structure-function relationships of nucleic acids in general. The present report describes an investigation of the structure, tautomerism, ionization and protonation of 8-OH-A, which reveals several important biological consequences for base mispairing and mutation. As the 8-keto tautomer in the *syn* conformation, 8-OH-A could pair with thymine in a non Watson–Crick manner (Hoogsteen–Wobble like) or mispair with cytosine (Figure 4a and 4b). Furthermore, the minor C8-enolate form could pair with thymine and guanine, resulting in a normal base insertion or an adenine to guanine transition (Figure 4c and 4d). The possibility of normal Watson–Crick base pairing with thymine also arises from the equilibrium between the *syn* and *anti* conformers or from the formation of Z-DNA. The *syn* conformation for 8-OH-A is significant in view of its ability to distort the DNA molecule into a position that is unsuitable for normal base pairing in the A or B forms. Alternatively, it could promote the formation of the Z-form of nucleic acids. Thus it is possible that the *syn* conformation of oxidative damage may favor formation of the Z-form of nucleic acids (44).

Of potential significance is the steric/electronic interaction between the NH_2 group and the N7-H proton of 8-OH-A, which distorts the NH_2 group from the conformation found in the adenine base. The possible mispairings shown in Figure 4 were based on electronic considerations. Although the 'electronic complementarity' is a major factor for specific base-base interactions, the minor deviation from the standard bonding geometry also could be an important consequence for this oxidized adduct. In this connection, it is worth noting the reduced nucleophilicity of the N1 atom of 8-OH-A compared to that of adenosine. This implies reduced electron density at N1, which would be less favorable for the formation of Watson–Crick base pairs. Subtle changes in the dipolar properties are also of considerable interest in terms of the potential for altering stacking interactions in the double helical structure of DNA.

On the basis of these results, we propose to use the prefix '8-oxo' instead of '8-hydroxy' (e.g., '8-oxoadenosine' in place of '8-hydroxyadenosine') in naming this class of compounds. Although the latter has the advantage of systematic correctness, it tends to be misleading regarding the actual structure and its biological implications.

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