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Misincorporation of dAMP opposite 2-hydroxyadenine, an oxidative form of adenine

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ABSTRACT

Nucleotide incorporation opposite an oxidative form of adenine, 2-hydroxyadenine (2-OH-Ade) was investigated. When a primed template with 2-OH-Ade was treated with an exonuclease-deficient Klenow fragment of *Escherichia coli* DNA polymerase I (KF^{exo-}), recombinant rat DNA polymerase β (pol β) or calf thymus DNA polymerase α (pol α), incorporation of dTMP and dAMP was observed. In addition, KF^{exo-} inserted dGMP as well. A steady-state kinetic study indicated that the insertion of dAMP and dTMP opposite the DNA lesion occurred with similar frequency with KF^{exo-} and pol β. Insertion of dTMP opposite 2-OH-Ade was favored to that of dAMP by pol α. Chain extension from the A·2-OH-Ade pair is less favored than that from the T·2-OH-Ade pair by all three DNA polymerases. Analysis of full-length products of *in vitro* DNA synthesis showed that dTMP and dAMP were incorporated by DNA polymerases and that exonuclease-proficient and -deficient Klenow fragments also inserted dGMP opposite 2-OH-Ade. These results suggest that formation of 2-OH-Ade from A in DNA will induce A→T and A→C transversions in cells.

INTRODUCTION

A variety of chemicals, irradiation by X- or γ-rays and ultraviolet light produce oxygen radicals (active oxygens) which modify base and sugar moieties in nucleic acids. Endogenous oxidative processes also produce oxygen radicals. One of the base modifications produced by oxidative stress is 8-hydroxyguanine (8-OH-Gua, 7,8-dihydro-8-oxoguanine) (1). This DNA lesion has been proved to be mutagenic in *in vitro* (2,3), prokaryotic (4,5) and mammalian systems (6–9).

We recently found that 2-hydroxyadenine (2-OH-Ade, 1,2-dihydro-2-oxoadenine or isoguanine) is produced when dA, dATP and DNA are treated with an oxygen radical-forming system, Fe^{2+}–EDTA (H. Kamiya and H. Kasai, unpublished results). Its yield in monomers (dA and dATP) was 70–80 times higher than that in DNA. The yield of 2-hydroxydeoxyadenosine from dA was three to four times less than that of 8-hydroxydeoxyguanosine from dG under the same reaction conditions. The formation of 2-OH-Ade in DNA by a metal–H_2O_2 mixture (10), in chromatin of human cancerous tissues (11) and in hepatic chromatin of mice irradiated with γ-rays (12) has been reported. Switzer *et al.* reported that the Klenow fragment of *Escherichia coli* DNA polymerase I and other DNA polymerases incorporate 2′-deoxy-2-hydroxyadenosine 5′-triphosphate (2-OH-dATP, 'd-iso-GTP' in their papers) opposite T in a DNA template (13,14). Furthermore, we recently found that a replicative DNA polymerase in mammalian cells, pol α, incorporates 2-OH-dATP in *in vitro* DNA synthesis (H. Kamiya and H. Kasai, unpublished results). Therefore, 2-OH-Ade residues are formed in DNA both by incorporation of 2-OH-dATP by DNA polymerases and by direct conversion from A residues in DNA.

As a first approach to determine the mutagenic potential of 2-OH-Ade, we synthesized an oligonucleotide template with the modified base and treated the primed template with DNA polymerases. In addition to dTMP, dAMP was incorporated opposite 2-OH-Ade by the DNA polymerases used. Insertion of dGMP opposite 2-OH-Ade was observed when the Klenow fragment was used in *in vitro* DNA synthesis. Our results suggest that formation of 2-OH-Ade will be mutagenic and induce A→T and A→C transversions in cells.

MATERIALS AND METHODS

Materials

T4 polynucleotide kinase was purchased from TOYOBO Co. Calf thymus DNA polymerase α (pol α) was obtained from Molecular Biology Resources Inc. Klenow fragments of DNA polymerase I with and without exonuclease activity (KF^{exo+} and KF^{exo-} respectively) were from Life Technologies Inc. and US Biochemical Co. respectively. Recombinant rat DNA polymerase β (pol β) was purified as described (15). Nuclease P1 and bacterial alkaline phosphatase, type III, were from Yamasa Co. and Sigma Chemical Co. respectively. FPLC-grade 2′-deoxynucleoside 5′-triphosphates (dNTPs) were from Pharmacia.

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Oligonucleotide synthesis

A phosphoramidite derivative of 2-OH-Ade, 9-[2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-6-[(dimethylamino)methylidene]-9*H*-isoguanine 3′-[(2-cyanoethyl)*N*,*N*-diisopropylphosphoramidite], was prepared as described previously (16). An oligonucleotide with 2-OH-Ade and other oligonucleotides were synthesized by the phosphoramidite method in an Applied Biosystems model 394 DNA/RNA synthesizer (Perkin-Elmer). Oligonucleotides were cleaved from the support with concentrated ammonia at room temperature for 60 min and then deprotected by heating the ammonia solution at 55°C for 6 h. We found that deprotection of an oligonucleotide with 2-OH-Ade proceeded under the same conditions as those of unmodified oligonucleotides. Oligonucleotides were purified by reverse-phase HPLC using an Inertsil ODS-2 column (10 × 250 mm; GL Sciences Inc.) with a linear gradient of acetonitrile in 50 mM triethylammonium acetate. This purification step was carried out before and after deprotection of the 5′-dimethoxytrityl group of oligonucleotides with 80% aqueous acetic acid. The oligonuleotides were further purified by anion-exchange HPLC using a TSK-GEL DEAE-2SW column (4.6 × 250 mm; Tosoh Co.) with a linear gradient of ammonium formate in 20% aqueous acetonitrile. Ammonium formate was removed by reverse-phase chromatography using Preparative C_{18} 125-Å (Millipore Co.).

An oligonucleotide with 2-OH-Ade (0.1 A_{260} units) was digested with nuclease P1 (50 μg) in 110 μl 20 mM sodium acetate (pH 4.5) at 37°C for 1 h and then with alkaline phosphatase (1 U) in 130 μl 0.1 M Tris–HCl (pH 7.5) at 37°C for 2 h. The sample was centrifuged at 15 000 r.p.m. for 5 min at room temperature and the supernatant was analyzed by reverse-phase HPLC using an Ultrasphere ODS 5μ column (4.6 × 250 mm; Beckman) with an isocratic system consisting of 10 mM NaH_2PO_4 and 8.0% methanol.

In vitro DNA synthesis

Primers (10 pmol) were labeled at the 5′-ends by T4 polynucleotide kinase (10 U) in the presence of [γ-^{33}P]ATP (0.74 MBq; New England Nuclear) in a buffer solution of 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$ and 10 mM 2-mercaptoethanol in a total volume of 10 μl. After incubation at 37°C for 60 min, unincorporated ATP was removed with NENSORB 20 (Du Pont).

Reactions catalyzed by KF^{exo+} and KF^{exo-} were carried out in a buffer solution containing oligonucleotide templates annealed with primers (0.05 μM), 50 mM Tris–HCl (pH 8.0), 8 mM $MgCl_2$, 5 mM 2-mercaptoethanol, various amounts of dNTP and enzyme in a total volume of 10 μl at 20°C. Experiments with pol α were conducted at 25°C in 10 μl of a reaction mixture containing oligonucleotide templates annealed with primers (0.05 μM), 20 mM Tris–HCl (pH 8.0), 5 mM $MgCl_2$, 3.3 mM 2-mercaptoethanol, 0.2 mg/ml bovine serum albumin and various amounts of dNTP and enzyme. Studies with pol β were carried out at 25°C under similar conditions. Reactions were stopped by addition of termination solution (95% formamide, 0.1% bromophenol blue and 0.1% xylene cyanol). Samples were heated at 95°C for 3 min and then applied to 20% polyacrylamide gels in the presence of 7 M urea. Autoradiograms were obtained with a Fujix BAS 2000 Bio Image Analyzer.

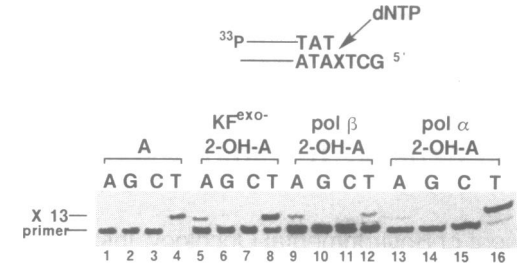

Figure 1. Incorporation of nucleotides in the presence of a single dNTP. Template 1 or template 2 was annealed with ^{33}P-labeled primer 2. Template–primer complex (0.05 μM) was treated with KF^{exo-} (0.01 U), pol α (0.14 U) or pol β (1 or 10 U) in the presence of a single dNTP at 10 μM in a total volume of 10 μl under the conditions described in Materials and Methods. The reaction mixtures were incubated at 20°C (KF^{exo-}) or 25°C (pol α or pol β) for 60 min and were processed as described in Materials and Methods. The data with template 1 (unmodified oligonucleotide) were those obtained with pol α. Lanes 1, 5, 9 and 13, in the presence of dATP; lanes 2, 6, 10 and 14, in the presence of dGTP; lanes 3, 7, 11 and 15, in the presence of dCTP; lanes 4, 8, 12 and 16, in the presence of dTTP. Lanes 1–4, template 1, pol α; lanes 5–8, template 2, KF^{exo-}; lanes 9–12, template 2, pol β (10 U), lanes 13–16, template 2, pol α. The result with 1 U pol β is not shown here.

Steady-state kinetics

Experiments with KF^{exo-} were done under the conditions described above using 0.05–1000 μM dNTP and 0.004–0.2 U enzyme at 20°C for 1–4 min. Reactions with pol α were measured under the conditions described above using 0.25–1000 μM dNTP and 0.14–1.4 U enzyme at 25°C for 1–30 min. Studies with pol β were conducted under the conditions described above using 3–1000 μM dNTP and 0.5–5 U enzyme at 25°C for 1–2 min. The Michaelis constant (K_m) and the maximum velocity of the reaction (V_{max}) were obtained from Lineweaver–Burk plots of the kinetic data (17). Insertion (F_{ins}) and extension (F_{ext}) frequencies were determined relative to T·A according to the equations developed by Mendelman *et al.* (18,19). $F = (V_{max}/K_m$ of wrong pair)/(V_{max}/K_m of right pair), with 'wrong pair' defined as a mismatched base pair or any base pair with 2-OH-Ade. All reaction rates were linear during the course of the reaction in which <20% of the primer was extended (20).

RESULTS

Incorporation of a single dNMP opposite 2-OH-Ade

First, we determined the nucleotides incorporated opposite 2-OH-Ade in the presence of a single dNTP. The extensions of primer 2 annealed with template 2 (Table 1) by KF^{exo-}, pol β and pol α were analyzed by 7 M urea–20% polyacrylamide gel electrophoresis (PAGE). All three DNA polymerases incorporated dAMP in addition to dTMP opposite 2-OH-Ade (Fig. 1, lanes 5, 9 and 13). KF^{exo-} also inserted dGMP opposite the lesion (Fig. 1, lane 6).

Time course of *in vitro* DNA synthesis

Next we studied the time course of *in vitro* DNA synthesis in the presence of 50 μM concentrations of the four dNTPs. The extensions of primer 1 annealed with templates 1 and 2 (Table 1) by KF^{exo-}, pol α and pol β were analyzed by 7 M urea–20%

Table 1. Sequences of oligonucleotides

5' dATGACGGAATA 3'	primer-1 (11mer)
5' dATGACGGAATAT 3'	primer-2 (12mer)
5' dATGACGGAATATN 3'	primer-3 (13mer)
3' dTACTGCCTTATAATCG 5'	template-1 (16mer)
3' dTACTGCCTTATAA*TCG 5'	template-2 (16mer)

A* = 2-OH-Ade; N = A, G or T.

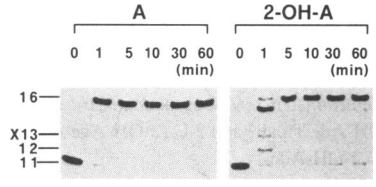

Figure 2. Time course of *in vitro* DNA synthesis catalyzed by KF^{exo-}. Template 1 or template 2 was annealed with ^{33}P-labeled primer 1. Template–primer complex (0.05 μM) was treated with KF^{exo-} (0.01 U) in the presence of the four dNTPs at 50 μM in a total volume of 10 μl under the conditions described in Materials and Methods. Reactions were carried out at 20°C and 1.5 μl samples of the reaction mixtures were taken 1, 5, 10, 30 and 60 min after addition of the enzyme.

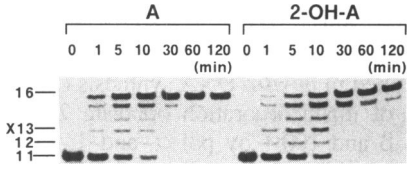

Figure 3. Time course of *in vitro* DNA synthesis catalyzed by pol α. Template–primer complex (template 1 or 2 and primer 1, 0.05 μM) was treated with pol α (0.14 U) at 25°C as described in Materials and Methods. The concentration of dNTPs were 50 μM each. Samples of 1.5 μl of the reaction mixtures were taken 1, 5, 10, 30, 60 and 120 min after addition of the enzyme.

PAGE. KF^{exo-} and pol α read through the site of 2-OH-Ade with reduced reaction rates (Figs 2 and 3), although pol β paused at position 12, the position before 2-OH-Ade (Fig. 4A and B). The retardation was overcome by the use of a large amount of the enzyme (Fig. 4C) or by addition of 500 μM concentrations of dNTPs (data not shown). When unmodified template 1 and pol β were used, a transient pause at position 12 was observed (Fig. 4A, 1 min). The reason for this is unknown, but this position may be a pol β pause site. However, the pause at position 12 was marked in the case of the template with 2-OH-Ade (Fig. 4B).

Analysis of full-length products

Template 2 annealed with primer 1 (Table 1) was treated with KF^{exo+}, KF^{exo-}, pol α and pol β and the full-length products produced were analyzed by denaturing 20% PAGE. Oligonucleotides containing A, T, G (Fig. 5) and C (data not shown) opposite the 2-OH-Ade position can be distinguished by their relative mobilities in the gel. The products of pol β showed the same mobilities as those of standards T and A (Fig. 5). This means that pol β inserted dAMP in addition to dTMP opposite 2-OH-Ade. The ratio of dTMP to dAMP incorporated was 3.5:1. The major

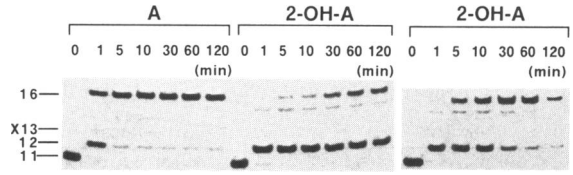

Figure 4. Time course of *in vitro* DNA synthesis catalyzed by pol β. Template–primer complex (template 1 or 2 and primer 1, 0.05 μM) was treated with pol β (10 or 50 U) at 25°C as described in the legend to Figure 3. (**A**) Template 1, 10 U pol β. (**B**) Template 2, 10 U pol β. (**C**) Template 2, 50 U pol β.

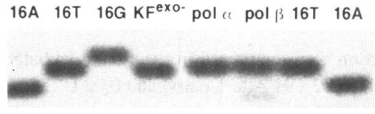

Figure 5. Analysis of full-length products synthesized by DNA polymerases *in vitro*. Template 1 or template 2 was annealed with ^{33}P-labeled primer 1. Template–primer complex (0.05 μM) was treated with KF^{exo-} (0.02 U), pol α (0.7 U) and pol β (50 U) in the presence of the four dNTPs at 100 μM in a total volume of 10 μl under the conditions described in Materials and Methods. Reactions were carried out at 20°C for 30 min (KF^{exo-}) or at 25°C for 60 min (pol α and pol β). After incubation reactions were terminated by addition of formamide–dye solution and the products applied to denaturing 20% polyacrylamide gels (30 × 80 cm, 0.5 mm thick). 16A, 16T and 16G are standard oligonucleotides with the indicated base at position 13 (corresponding to the 2-OH-Ade site). Standard 16C showed greater mobility than 16A (data not shown).

products of pol α and KF^{exo-} (Fig. 5) and of KF^{exo+} (data not shown) had the same mobility as standard T. However, the incorporation of small amounts of dAMP was also detected. With KF^{exo+} and KF^{exo-}, dGMP incorporation was observed. The ratio of dTMP to dAMP incorporated by pol α was 100:1. The ratios of dTMP to dAMP and dGMP inserted by KF^{exo+} and KF^{exo-} were 100:1:3 and 50:1:1 respectively. These results indicate that 2-OH-Ade residues present in DNA strands are miscoding. No incorporation of dAMP or dGMP was observed when the unmodified template (template 1) was used (data not shown).

Kinetics of insertion and extension

We then measured kinetic parameters during *in vitro* DNA synthesis. Insertion of dNMP opposite A and 2-OH-Ade and incorporation of dAMP opposite T on the 5'-flanking side of A and 2-OH-Ade (chain extension) were studied. Table 2 shows the parameters of reactions catalyzed by KF^{exo-}. The frequency of insertion (F_{ins}) of dATP opposite 2-OH-Ade (4.6×10^{-4}) was twice that of dTTP (2.3×10^{-4}). The F_{ins} of dGTP opposite 2-OH-Ade (2.4×10^{-5}) was nine times less than that of dTTP. The frequency of extension (F_{ext}) of T·2-OH-Ade (5.5×10^{-2}) was 4.5 times that of A·2-OH-Ade (1.2×10^{-2}) and 2.5 times higher than that of G·2-OH-Ade (2.3×10^{-2}).

In reactions catalyzed by pol α (Table 3), the F_{ins} of dTTP opposite 2-OH-Ade (5.7×10^{-2}) was eight times that of dATP (6.9×10^{-3}). The F_{ext} of T·2-OH-Ade (7.8×10^{-2}) was 90 times that of A·2-OH-Ade (8.9×10^{-4}).

In reactions catalyzed by pol β (Table 4), The F_{ins} of dATP opposite 2-OH-Ade (0.14) was 3.5 times that of dTTP (3.9×10^{-2}) and was only seven times less than that of dTTP opposite

Table 2. Kinetic parameters of nucleotide insertion and chain extension reactions catalyzed by exonuclease-deficient Klenow fragment[a]

dNTP —T / —AXTCG 5'	K_m (μM)	V_{max} (% min^{-1})	F_{ins}	dATP —TN / —AXTCG 5'	K_m (μM)	V_{max} (% min^{-1})	F_{ext}
A/T	0.15	17	1.0	T/AT	0.51	6.1	1.0
A/A	71	11	1.4×10^{-3}	A/AT	38	8.9	2.0×10^{-2}
A/G	5.3×10^{2}	14	2.2×10^{-4}	G/AT	ND[b]	ND	ND
A*/T	2.8×10^{2}	7.5	2.3×10^{-4}	T/A*T	16	11	5.5×10^{-2}
A*/A	1.3×10^{2}	6.8	4.6×10^{-4}	A/A*T	65	9.3	1.2×10^{-2}
A*/G	6.4×10^{2}	1.8	2.4×10^{-5}	G/A*T	39	11	2.3×10^{-2}

[a]Kinetics of insertion were measured in reactions incubated for 1 or 2 min with 0.004 U (A·T and 2-OH-Ade·T pairs) and 0.02 U (2-OH-Ade·A pair) of exonuclease-deficient Klenow fragment, for 2 or 4 min with 0.02 U (A·A pair) and 0.1 U (A·G pair) of the polymerase and for 1.5 or 3 min with 0.02 U (2-OH-Ade·.G pair) of the enzyme in 10 μl of buffer solution containing templates 1 or 2 primed with ^{33}P-labeled primer 2 (0.05 μM), as described in Materials and Methods. Kinetics of chain extension were determined in reactions incubated for 1 or 2 min with 0.004 U (A·T and 2-OH-Ade·T pairs), 0.02 U (2-OH-Ade·G pair) and 0.2 U (A·A and 2-OH-Ade·A pairs) of Klenow fragment in 10 μl of buffer solution containing templates 1 or 2 primed with ^{33}P-labeled primer 3 (0.05 μM), as described in Materials and Methods. X = A or 2-OH-Ade; A* = 2-OH-Ade.

[b]ND, not determined.

Table 3. Kinetic parameters of nucleotide insertion and chain extension reactions catalyzed by DNA polymerase α[a]

dNTP —T / —AXTCG 5'	K_m (μM)	V_{max} (% min^{-1})	F_{ins}	dATP —TN / —AXTCG 5'	K_m (μM)	V_{max} (% min^{-1})	F_{ext}
A/T	0.47	2.9	1.0	T/AT	1.3	2.1	1.0
A/A	6.8×10^{2}	6.0	1.4×10^{-3}	A/AT	1.3×10^{2}	0.20	9.1×10^{-4}
A*/T	8.9	3.2	5.7×10^{-2}	T/A*T	44	5.7	7.8×10^{-2}
A*/A	33	1.4	6.9×10^{-3}	A/A*T	2.6×10^{2}	0.38	8.9×10^{-4}

[a]Base insertion was measured in reactions incubated for 3 or 6 min with 0.14 U pol α (A·T pair), for 5 or 10 min with 0.14 U (2-OH-Ade·T pair) and 0.7 U (2-OH-Ade·A pair) of the polymerase and for 1, 2 or 3 min with 0.7 U of the enzyme (A·A pair), as described in Materials and Methods. Kinetics of chain extension were determined in reactions incubated for 3 or 6 min (A·T pair) or for 5 or 10 min (2-OH-Ade·T pair) with 0.14 U pol α and for 10, 20 or 30 min with 1.4 U of the enzyme (all other pairs). X = A or 2-OH-Ade; A* = 2-OH-Ade.

A. However, the F_{ext} of A·2-OH-Ade (1.1×10^{-2}) is half that of T·2-OH-Ade (2.5×10^{-2}).

The F_{ins} of dATP and dTTP opposite 2-OH-Ade in reactions with pol α were 15–250 times those with KF^{exo-}. The F_{ins} of dTTP and dATP opposite 2-OH-Ade in reactions with pol β were 1.5 times lower and 20 times higher respectively than those with pol α. The F_{ext} of base pairs involving 2-OH-Ade were in the magnitude of 10^{-2} in all the reactions catalyzed by the three polymerases except that of A·2-OH-Ade with pol α. It appears that extension from the A·2-OH-Ade pair is lower than that from T·2-OH-Ade in the polymerase reactions studied.

Table 4. Kinetic parameters of nucleotide insertion and chain extension reactions catalyzed by DNA polymerase β[a]

dNTP —T / —AXTCG 5'	K_m (μM)	V_{max} (% min^{-1})	F_{ins}	dATP —TN / —AXTCG 5'	K_m (μM)	V_{max} (% min^{-1})	F_{ext}
A/T	74	12	1.0	T/AT	13	15	1.0
A*/T	1.1×10^{3}	6.9	3.9×10^{-2}	T/A*T	3.5×10^{2}	9.9	2.5×10^{-2}
A*/A	5.2×10^{2}	11	0.14	A/A*T	1.9×10^{2}	2.4	1.1×10^{-2}

[a]Base insertion was measured in reactions incubated for 1 or 2 min with 1 U (A·T pair) and 5 U (2-OH-Ade·T pair) of pol β for 1 min with 1 U polymerase (2-OH-Ade·A pair), as described in Materials and Methods. Kinetics of chain extension were determined in reactions incubated for 1 or 2 min with 1U (A·T pair), 0.5 U (2-OH-Ade·T pair) and 2 U (2-OH-Ade·A pair) pol β. X = A or 2-OH-Ade; A* = 2-OH-Ade.

DISCUSSION

Hydroxylation of the C-2 position of A produces 2-OH-Ade. We expect that modification in the base pairing region will induce incorporation of incorrect dNMP, such as O^6-methylguanine, hypoxanthine and xanthine (21–23). In the presence of a single dNTP, DNA polymerases incorporated dAMP in addition to dTMP opposite 2-OH-Ade (Fig. 1). KF^{exo-} also inserted dGMP. These findings were confirmed by analysis of full-length products obtained in *in vitro* DNA synthesis (Fig. 5).

The ratios of misincorporation opposite 2-OH-Ade (T:A = 3.5:1 by pol β and 100:1 by pol α, and T:A:G = 100:1:3 by KF^{exo+}) are lower than those opposite 8-OH-Gua in a different sequence context (C:A = 4:1 by pol β and 1:200 by pol α and 7:1 by KF^{exo+}) (2), except pol β. Although misincorporation opposite a modified base is sequence-dependent (24), these results may indicate that 2-OH-Ade is less mutagenic than 8-OH-Gua. However, it is unclear whether mutagenicity of 2-OH-Ade in cells is lower than 8-OH-Gua, because repair efficiency for 2-OH-Ade is unknown.

Kinetic data obtained in this study (Tables 2–4) show that the F_{ins} of dATP opposite 2-OH-Ade was higher than the F_{ins} of dTTP opposite 2-OH-Ade with KF^{exo-} and pol β (by factors of 2.0 and 3.5 respectively). In reactions catalyzed by pol α, the F_{ins} of dTTP was eight times that of dATP. The F_{ext} of A·2-OH-Ade was less than that of T·2-OH-Ade in reactions catalyzed by the three DNA polymerases. In the experiments with KF^{exo-}, the F_{ins} and F_{ext} of G·2-OH-Ade were nine and two times lower than those of T·2-OH-Ade.

In aqueous solution, major tautomers (calculated as 90%) of the 9-methyl and ribosyl derivatives of 2-OH-Ade are in the N(1)H, 2-keto form (25). However, the equilibrium shifts in the direction of the enol form with decreasing solvent polarity (25). A DNA molecule is surrounded by a DNA polymerase molecule when DNA synthesis occurs. The polarity of the active site of a DNA polymerase is difficult to estimate, but is less than that of water. Thus the amount of the enol (2-hydroxy) form of 2-OH-Ade in DNA to which a DNA polymerase binds is probably higher than that in water, as suggested by Switzer *et al.* (14). Accordingly, the contribution of the enol form must be important when considering the miscoding properties of 2-OH-Ade. Postulated base pairs involving 2-OH-Ade are shown in Figure 6. With T, the enol

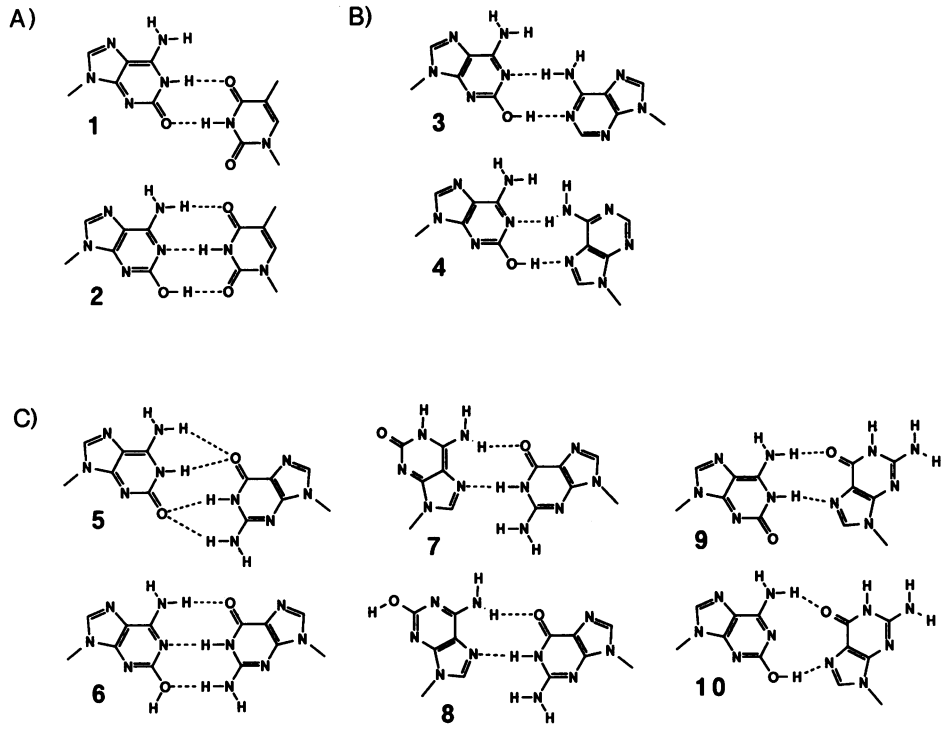

Figure 6. Postulated base pairs involving 2-OH-Ade. (**A**) 2-OH-Ade·T pair. (**B**) 2-OH-Ade·A pair. (**C**) 2-OH-Ade·G pair.

tautomer of 2-OH-Ade can form a pair in a Watson–Crick manner (**2**, Fig. 6A). In this putative pair the two bases pair with three hydrogen bonds as G·C. Also, the keto tautomer can form a pair in wobble alignment (**1**, Fig. 6A). Base-pairing of 2-OH-Ade with A is of particular interest, because incorporation of dAMP opposite the modified base was observed. We propose a base pair of the enol tautomer of 2-OH-Ade·A (*anti–anti*, **3**, Fig. 6B). The keto and enol tautomers can form a pair with G in an *anti–anti* manner (**5** and **6**, Fig. 6C). Also, both tautomers can form a Hoogsteen pair with G when 2-OH-Ade adopts the *syn* conformation (**7** and **8**, Fig. 6C). An *N*-protected derivative of 2′-deoxy-2-hydroxyadenosine adopts both *syn* (60%) and *anti* (40%) conformations (16). Moreover, by calculating the molecular mechanics of 2′-deoxy-2-hydroxyadenosine 5′-monophosphate, we found that the *syn* conformers of both tautomers were as stable as the *anti* conformers (H. Kamiya and H. Kasai, unpublished data). 2-OH-Ade residues in DNA may adopt the *syn* conformation and pair with G in the Hoogsteen manner. Alternatively, 2-OH-Ade (*anti*) can pair with A or G (*syn*) (**4**, **9** and **10** in Fig. 6B and C). Although it is improbable that incoming dATP or dGTP adopts the *syn* conformation, 2-OH-Ade·A and 2-OH-Ade·G (*anti–syn*) pairs may be formed after incorporation of purine nucleotides.

Switzer *et al.* suggested the possibility of dAMP incorporation opposite 2-OH-Ade by KF^{exo+} (14). In this report we have shown that DNA polymerases incorporate dAMP as well as dTMP opposite 2-OH-Ade. Moreover, the Klenow fragment inserted dGMP opposite the DNA lesion. The results of this study predict that A→T and A→C transversions are induced by 2-OH-Ade. Feig and Loeb found that the mutation spectra induced by DNA damage generated by oxygen radicals depend on the kind of DNA polymerases used (26). In their study, A→C substitutions were elicited by pol α and A→T mutations by pol β. We found that pol α and pol β incorporated dAMP in proportions of about 1 and 23% respectively of the total nucleotides incorporated. Thus, A→T transversions induced by pol β, which were observed by Feig and Loeb, may be due to formation of 2-OH-Ade by oxygen radicals. Loeb *et al.* also found that treatment of single-stranded DNA with Fe^{2+} induced A→T and A→C transversions in *E.coli* (27). The fact that the Klenow fragment of DNA polymerase I inserted A and G opposite 2-OH-Ade indicates the possible involvement of the modified base in the mutational process.

In this study we show that dAMP and dGMP are incorporated opposite 2-OH-Ade by DNA polymerases *in vitro*. We are now examining the mutagenicity and mutation spectra of 2-OH-Ade in bacterial and mammalian cells.

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