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N7 Methylation Alters Hydrogen-Bonding Patterns of Guanine in Duplex DNA

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Supporting Information

ABSTRACT: N7-Alkyl-2′-deoxyguanosines are major adducts in DNA that are generated by various alkylating mutagens and drugs. However, the effect of the N7 alkylation on the hydrogen-bonding patterns of the guanine remains poorly understood. We prepared N7-methyl-2′-deoxyguanosine (N7mdG)-containing DNA using a transition-state destabilization strategy, developed a novel polβ-host−guest complex system, and determined eight crystal structures of N7mdG or dG paired with dC, dT, dG, and dA. The structures of N7mdG:dC and N7mdG:dG are very similar to those of dG:dC and dG:dG, respectively, indicating the involvement of the keto tautomeric form of N7mdG in the base pairings with dC and dG. On the other hand, the structure of N7mdG:dT shows that the mispair forms three hydrogen bonds and adopts a Watson−Crick-like geometry rather than a wobble geometry, suggesting that the enol tautomeric form of N7mdG involves in its base pairing with dT. In addition, N7mdG:dA adopts a novel shifted *anti*:*syn* base pair presumably via the enol tautomeric form of N7mdG. The polβ-host−guest complex structures reveal that guanine-N7 methylation changes the hydrogen-bonding patterns of the guanine when paired with dT or dA and suggest that N7 alkylation may alter the base pairing patterns of guanine by promoting the formation of the rare enol tautomeric form of guanine.

A large number of alkylating anticancer agents and mutagens such as the nitrogen mustards, azinomycins, leinamycin, styrene oxide and aflatoxin B_1 attack the N7 of guanine, the most nucleophilic atom within DNA, to primarily generate N7-alkyl-2′-deoxyguanosines (N7-alkyl-dG).[1,2] The positively charged N7-alkyl-dG has a half-life of several hours to days in duplex DNA and can undergo spontaneous depurination to produce abasic sites, which can induce G to T transversion mutations and interstrand cross-links.[3−5] In addition, N7-alkyl-dG can undergo imidazole ring opening to give alkyl-formamidopyrimidine lesions, which are highly mutagenic.[6,7] Although N7-alkyl-dG has an unmodified Watson−Crick edge, it could affect the base pairing properties of guanine via its electronic and steric effects, thereby inducing mutagenesis. For example, the N7-dG adducts of the acridine half-mustard ICR-191 and aflatoxin B1 have been shown to induce G to A and G to T mutations, respectively.[8−11] Currently, the base pairing properties of N7-alkyl-dG are largely unknown except for aflatoxin B1-N7-dG adducts.[12]

N7-Methyl-2′-deoxyguanosine (N7mdG) is the smallest N7-alkyl-dG and is the major adduct that is produced by endogenous and exogenous methylating agents (e.g., *S*-adenosylmethionine).[2] The formal positive charge at N7 of N7mdG has been shown to lower the pK_a of N1 of guanine by ∼2 units (pK_a of N1 in N7mdG is ∼7).[13,14] The decreased pK_a can facilitate the formation of the enolate or enol tautomeric form of N7mdG at physiological pH (Figure 1A). N7mdG has a half-life of several days in duplex DNA and is removed by alkyladenine DNA

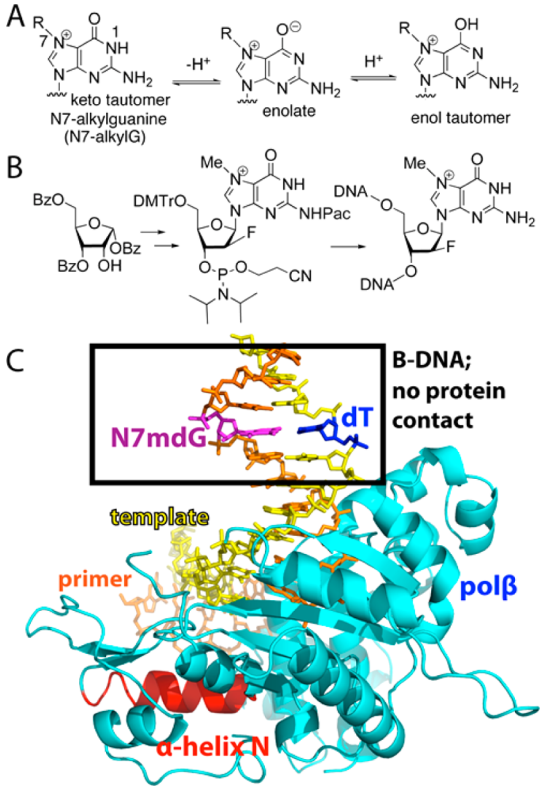

Figure 1. Structure determination of N7mdG-containing DNA using a transition-state destabilization strategy and polβ HGC system. (A) The keto and enol tautomeric forms of N7-alkyl-dG. (B) Preparation of N7mdG-containing DNA using a 2′-fluorine-mediated transition-state destabilization strategy. (C) Overall structure of N7mdG:dT-containing DNA that is determined by polβ HGC system. The base pairs at the 5′ end of the upstream primer are devoid of protein contact and adopt the B-DNA conformation.

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glycosylase (AAG) in humans and AlkA in *E. coli*.[15,16] It has been proposed that N7mdG could promote mutagenic replication by forming Watson−Crick-like base pair with dT,[13] but the three-dimensional structure of such base pairs has not been reported so far. Systematic investigation of base pairing properties of N7-alkyl-dG has been hampered due in part to the technical difficulty of site-specific incorporation of N7-alkyl-dG. In addition, the stability of N7-alkyl-dG is not suitable for the crystallographic experiments. We recently utilized a 2′-fluorine-mediated transition-state destabilization strategy (Figure 1B) to determine the structure of N7mdG:dCTP in the active site of human DNA polymerase β (polβ),[17] which showed the formation of Watson−Crick N7mdG:dCTP base pair under the influence of the protein. As an initial step toward elucidating potential N7-alkyl-dG-mediated mutagenesis, we report herein the base pairing properties of N7mdG and the effect of N7mdG on the stability of duplex DNA.

To elucidate the base pairing properties of N7mdG in the absence of protein contacts, we have developed a novel polβ host−guest complex (HGC) system,[18] where the base pair of interest is in B-DNA and does not engage in any contacts with protein (Figure 1C). We determined eight crystal structures of guanine or N7mdG base-paired with dC, dT, dG, or dA using the polβ HGC system at pH 7.5 (Figure 2, see Table S1 for refinement statistics).

The N7mdG:dC structure indicates that N7mdG forms three hydrogen bonds with dC, suggesting that N1−H of N7mdG engages in hydrogen bondings (Figure 2B). Hydrogen-bond distances in the N7mdG:dC base pair are 2.3, 2.8, and 3.3 Å, indicating that N7 methylation moderately alters the base pair geometry of dG:dC. By contrast, published structure of polβ with incoming dCTP base paired with templating N7mdG in the catalytic pocket showed that the distance for all three hydrogen bonds in the N7mdG:dCTP base pair is 2.9 Å.[17] The difference in hydrogen-bond distances of the N7mdG:dC and the published N7mdG:dCTP base pairs suggests that the N7mdG:dCTP with an ideal Watson−Crick geometry is induced by a protein contact. The presence of N7mdG:dC in DNA triggers a local conformational change near the lesion base pair. In particular, N7mdG:dC and dG:dC base pairs have considerably different parameter values for buckle (10.7° vs −0.3°), propeller twist (−15.6° vs −1.5°), and opening (14.1° vs −0.5°) distortions (see Table S2).

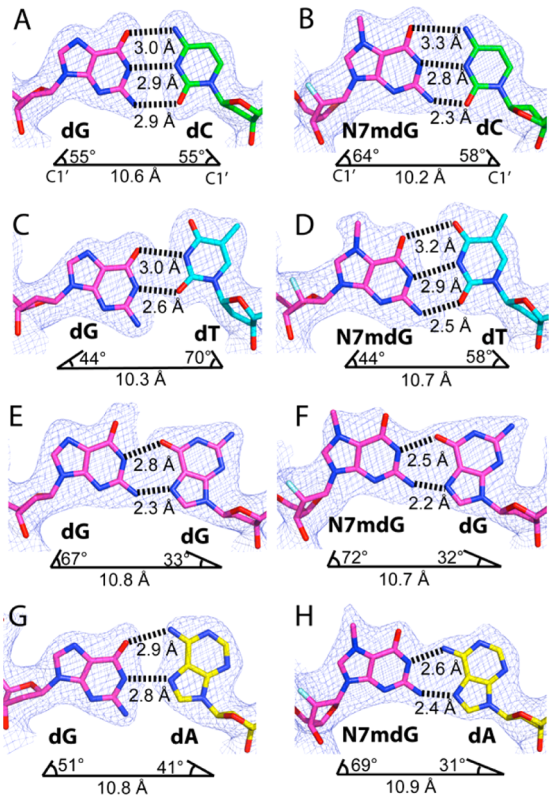

Figure 2. Effect of guanine N7 methylation on base pairing properties of guanine. Hydrogen-bonding patterns of: (A) dG:dC, (B) N7mdG:dC, (C) dG:dT, (D) N7mdG:dT, (E) dG:dG, (F) N7mdG:dG, (G) dG:dA, and (H) N7mdG:dA. $2F_0-F_c$ electron density maps contoured at 1σ around the base pairs in polβ-HGC complexes. The base pair geometry including the C1′-C1′ distances and λ angles is shown.

The N7mdG:dT structure, refined to 2.2 Å resolution, shows the formation of a novel Watson−Crick-like N7mdG:dT base pair with an interbase hydrogen-bond distance range of 2.5−3.2 Å (Figure 2D). The hydrogen-bonding pattern of N7mdG:dT significantly differs from that of wobble dG:dT (Figure 2C). While the dG:dT base pair forms two hydrogen bonds between N1 and O6 of dG and O2 and N3 of dT, respectively, the N7mdG:dT base pair forms three hydrogen bonds between N1, N2, and O6 of N7mdG and N3, O2, and O4 of dT, respectively. The base pair geometry of N7mdG:dT including the C1′-C1′ distance and λ angles is very similar to that of a correct base pair (e.g., dG:dC). Published studies show that mismatches with Watson−Crick-like base pair geometry can occur in the presence of protein contacts[19−22] but occur only transiently in the absence of protein contacts.[23] Watson−Crick-like N7mdG:dT formation in the absence of protein contacts indicates that N7 methylation greatly increases the population of the Watson−Crick-like mispair that typically exists in low abundance. The Watson−Crick-like N7mdG:dT with three hydrogen bonds appears to arise through the enol tautomer of N7mdG rather than the keto tautomer that is involved in the N7mdG:dC base pair (Figures 3C and 2B). The conformation of the N7mdG:dT-containing DNA is essentially identical to that of the dG:dC-containing DNA (RMSD = 0.21 Å, Figure 3A). Taken together, the results imply that, during DNA replication, templating N7mdG may favorably base pair with both incoming dCTP and dTTP via its dual coding potential, which involves the keto and enol tautomers of N7mdG.

The N7mdG:dG structure shows that N7mdG adopts an *anti* conformation and forms two hydrogen bonds with *syn*-dG (Figure 2F), which is similarly observed in the dG:dG base pair (Figure 2E). The base pair geometry of N7mdG:dG is essentially identical to that of dG:dG, which indicates that the keto tautomeric form of N7mdG participates in the base pairing.

The N7mdG:dA structure indicates that the guanine N7 methylation significantly alters the conformation of dG:dA base pair (Figure 2H). In the dG:dA structure, O6 and N1 of dG are hydrogen bonded to N6 and N7 of dA, respectively (Figure 2G). By contrast, in the N7mdG:dA structure, N1 and N2 of N7mdG are hydrogen bonded to N6 and N7 of dA, respectively. The N7mdG in N7mdG:dA shifts ∼2 Å toward the major groove relative to dG in dG:dA (Figure 3E). This shifted *anti*-N7mdG:*syn*-dA base pairing has not been observed before and presumably occurs through the enol tautoermic form of N7mdG (Figure 3F). The shifted *anti*-N7mdG:*syn*-dA induces a relatively large distortion of neighboring base pairs (Figure 3D).

To evaluate the effect of guanine-N7 methylation on the stability of duplex DNA, we determined melting temperatures

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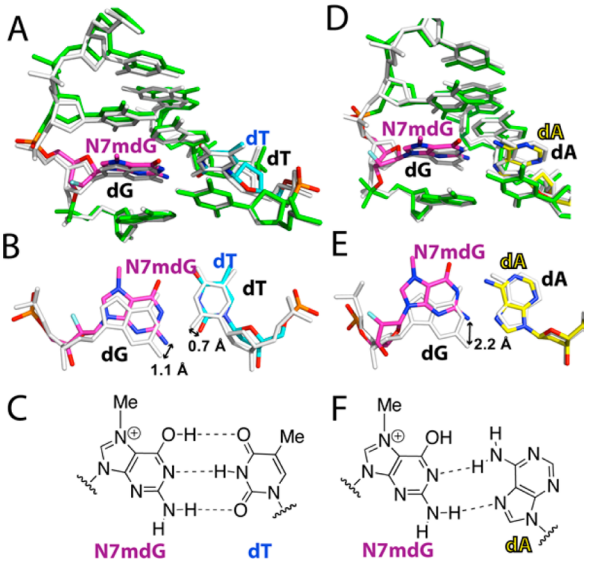

Figure 3. Effect of guanine N7 methylation on the conformation of dG:dT- or dG:dA-containing DNA. (A) Comparison of the dG:dT- and N7mdG:dT-containing DNA. (B) Comparison of base pair conformation of dG:dT and N7mdG:dT. (C) Formation of Watson−Crick-like N7mdG:dT pair via the enol tautomeric form of N7mdG. (D) Comparison of dG:dA- and N7mdG:dA-containing DNA. (E) Comparison of base pair conformation of dG:dA and N7mdG:dA. (F) Formation of the shifted N7mdG:dA base pair via the enol tautomeric form of N7mdG.

(T_m) for dG:dN-, N7mdG:dN-, and 2′-fluorine-2′-deoxyguanosine (FdG)-containing 16-mer duplex DNA using fluorescence measurement that involves the use of a double-stranded DNA-specific dye SYBR Green I.[24] Control experiments with FdG-containing DNA show that the effect of fluorine atom on the melting temperature of duplex DNA is negligible (Table 1).[25]

Table 1. Effect of Guanine N7 Methylation on the Melting Temperatures (T_m) of dG:dN-Containing DNA

dN	dG:dN	T_m (°C) FdG:dN	N7mdG:dN
dC	65.4 (±0.04)[a]	65.3 (±0.03)	64.3 (±0.06)
dT	60.6 (±0.06)	60.7 (±0.04)	61.1 (±0.03)
dG	61.3 (±0.03)	61.2 (±0.03)	58.8 (±0.04)
dA	60.8 (±0.05)	60.2 (±0.03)	55.7 (±0.04)

[a]Standard deviations were determined based on three independent experiments.

The N7mdG:dC- and N7mdG:dG-containing DNA are slightly less stable than the corresponding dG:dN-containing DNA, whereas the N7mdG:dA-containing DNA is much less stable than the dG:dA-containing DNA. The lower melting temperature of N7mdG:dC-containing DNA as compared to the corresponding dG:dC-containing DNA is consistent with the considerably different parameter values for buckle, propeller twist, and opening distortions (Table S2). The large destabilization by N7mdG:dA is consistent with the observed large distortion in DNA conformation (Figure 3D,E). On the other hand, N7mdG:dT-containing DNA is slightly more stable than dG:dT-containing DNA, which is consistent with the observation of Watson−Crick-like N7mdG:dT base pair and minimal distortion in the neighboring base pairs (Figure 3A). This suggests that the presence of N7mdG:dT does not significantly affect the stability of duplex DNA.

The differences between the hydrogen-bonding patterns of the N7mdG:dN and dG:dN base pairs suggest that N7 methylation affects dG′s base pairing properties by increasing the population of dG′s enol tautomer, which is calculated to be ∼ million-fold less abundant than the keto tautomer.[26] While the hydrogen-bond-donor/-acceptor properties of the Watson−Crick edge in dG do not vary among the dG:dN base pairs, those in N7mdG vary, in an opposite-base dependent manner, among the N7mdG:dN base pairs. When paired with dT or dA, the enol tautomer of N7mdG involves in hydrogen bonds, whereas the keto tautomer involves when paired with dC or dG. The N7mdG′s abilty to induce both Watson−Crick N7mdG:dC and Watson−Crick-like N7mdG:dT base pairs is reminiscent of the dual coding potential of the mutagenic lesion 2′-deoxy-8-oxoguanosine (8-oxodG), which can adopt both Watson−Crick 8-oxodG:dC and Hoogsteen 8-oxodG:dA base pairs.[27] As 8-oxodG uses its *anti* or *syn* conformers to assume 8-oxodG:dC and 8-oxodG:dA with a normal base pair geometry, N7mdG uses its enol or keto tautomers to produce N7mdG:dC and N7mdG:dT with a normal base pair geometry. The dual coding properties of N7mdG probably result from N7-methylation-mediated stabilization of the enol tautomer of N7mdG. Such stabilization can reduce the free energy difference between the keto and enol tautomers of dG,[26] which would enable a facile utilization of the both tautomers in base pairings in a way to form tighter interbase hydrogen bonds.

In summary, the results reported here show that guanine-N7 methylation alters hydrogen-bonding patterns of the guanine and affects the stability of duplex DNA. Our study resulted in the first observation of Watson−Crick-like N7mdG:dT and the shifted N7mdG:dA base pairs, which presumably involve the enol tautomeric form of N7mdG. The formation of a stable Watson−Crick-like N7mdG:dT base pair in duplex DNA suggests that N7mdG, if not repaired, may induce G to A transition mutations. Nonbulky N7-alkyl groups (e.g., ethyl, propyl) are likely to exert a similar effect on hydrogen-bonding patterns of guanine as the N7-methyl group. The predominant G to A mutations that are induced by the N7-dG adducts of acridine half-mustard[8] might involve the enol tautomeric form of the modified guanine. The use of the polβ HGC system in combination with the transition-state destabilization strategy may enable the structure determination of various alkylation adducts that are produced by bulky alkylating mutagens and drugs (e.g., tobacco-specific nitrosamine (NNK), *N*-benzyl *N*-methyl nitrosamine, ptaquiloside, acridine half-mustards, nitrogen mustards), which would further our understanding on N7-alkyl-dG-mediated mutagenesis and facilitate the structure-based rational design of novel alkylating agents. Kinetic and structural studies of various DNA polymerases bypassing N7mdG lesion are in progress in our laboratory, and the results will be reported elsewhere in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b10172.

Figures and refinement statistics (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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