Looking for Waldo: A Potential Thermodynamic Signature to DNA Damage

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CONSPECTUS: DNA in its simplest form is an ensemble of nucleic acids, water, and ions, and the conformation of DNA is dependent on the relative proportions of all three components. When DNA is covalently damaged by endogenous or exogenous reactive species, including those produced by some anticancer drugs, the ensemble undergoes localized changes that affect nucleic acid structure, thermodynamic stability, and the qualitative and quantitative arrangement of associated cations and water molecules. Fortunately, the biological effects of low levels of DNA damage are successfully mitigated by a large number of proteins that efficiently recognize and repair DNA damage in the midst of a vast excess of canonical DNA. In this Account, we explore the impact of DNA modifications on the high resolution and dynamic structure of DNA, DNA stability, and the uptake of ions and water and explore how these changes may be sensed by proteins whose function is to initially locate DNA lesions. We discuss modifications on the nucleobases that are located in the major and minor grooves of DNA and include lesions that are observed in vivo, including oxidized bases, as well as some synthetic nucleobases that allow us to probe how the location and nature of different substituents affect the thermodynamics and structure of the DNA ensemble. It is demonstrated that disruption of a cation binding site in the major groove by modification of the N7-position on the purines, which is the major site for DNA alkylation, is enthalpically destabilizing. Accordingly, tethering a cationic charge in the major groove is enthalpically stabilizing. The combined structural and thermodynamic studies provide a detailed picture of how different DNA lesions affect the dynamics of DNA and how modified bases interact with their environment. Our work supports the hypothesis that there is a “thermodynamic signature” to DNA lesions that can be exploited in the initial search that requires differentiation between canonical DNA and DNA with a lesion. The differentiation between a lesion and a cognate lesion that is a substrate for a particular enzyme involves another layer of thermodynamic and kinetic factors.

INTRODUCTION

DNA is structurally a promiscuous molecule that can adopt a wide variety of conformations based on its environment. 1 In a complementary manner, subtle modifications to the nucleobases generated by endogenous and exogenous agents can induce localized concomitant changes in the structure and stability of the ensemble. As will be discussed below, these subtle changes can potentially be sensed by biomolecules that are involved in DNA metabolism.

Chemically, the billions of nucleotides in genomic DNA of mammalian cells are relatively stable under physiological conditions (i.e., neutral pH, 37 °C). Considering that there are more than 10^9 nucleotides in the human genome, even extremely inefficient chemical reactions represent a threat, because there is a premium on preserving genetic stability. Accordingly, pathways to repair the effects of deamination, alkylation, and oxidation developed early in evolution. 2

A major repair pathway for damaged bases is base excision repair (BER), which involves a coordinated multistep mechanism involving a number of toxic and mutagenic intermediates (Figure 1). 3,4 The initial step involves a specific DNA glycosylase finding its substrate lesion via one- and three-dimensional diffusion in the midst of a vast excess of canonical bases that in many instances are structurally similar to the damaged base (Figure 2). We will return to the question concerning the initial recognition event below. When the glycosylase finds a cognate lesion, the lesion is extruded into the glycosylase’s active site. This step requires rotation of the base out of the stack and significant distortion of the DNA backbone. Once inside the active site, the modified base is hydrolyzed off the deoxyribose ring to yield an abasic site where a noninformative hydroxyl group replaces the deleted base. Subsequent enzymatic processing of the abasic site by AP endonuclease (APE1), polymerase β (Polβ), and DNA ligase, in combination with numerous accessory proteins, restores the

Received: February 12, 2014
Published: April 4, 2014

dx.doi.org/10.1021/ar500061p | Acc. Chem. Res. 2014, 47, 1446–1454
initially mentioned a potential relationship between DNA lesions and DNA repair involving a “thermodynamic signature” in a study of DNA with an N7,N2-propanoG-C pair, which cannot form a Watson–Crick (W–C) base pair, and with an 8-oG-C pair, which can. The proposal is that thermodynamics can provide a mechanism to distinguish between canonical and noncanonical regions of DNA but not necessarily between damaged DNA and cognate lesions, which is a process that can involve additional thermodynamic or kinetic discrimination. Equally insightful was their comment that “isostructural/isoconformational does not necessarily imply isoenergetic.” This raised the interesting hypothesis that repair enzymes could sense a potential lesion based on its local thermodynamic effect even if the structures of the DNA without and with a lesion were indistinguishable based on low temperature NMR or crystal structures. More recently, others have also suggested, without detailed thermodynamic quantification, that base unstacking and deformability of DNA due to lesions may be important in the initial recognition by repair proteins. The thermodynamic and structural results presented below on base modifications that affect the environment in the major or minor grooves are consistent with the “thermodynamic signature” hypothesis and provide some insights into the origin of the instability when G-C or A-T base pairs are structurally modified by alkylation, deamination, or oxidation. It is of course possible that measurements made in vitro do not reflect how DNA may behave in vivo.

■ MAJOR GROOVE MODIFICATIONS

7-Deazaguanine (c7G)

The N7-position on G is the predominant site for DNA alkylation by a wide variety of chemicals, including many antineoplastic drugs. Alkylation at this position removes the electronegative atom that faces into the major groove, replaces it with an electropositive hydrophobic alkyl group, and creates a cationic charge on the purine. It is important to note that alkylation at N7-G occurs where diffusible cations are observed in high resolution crystal structures of DNA so an N7G substitution would be expected to disrupt major groove cation binding. Because N7-alkyG readily depurinates to an abasic site, c7G (see Figure 3 for structures), which is stable, was introduced into DNA to determine how elimination of the cation binding site in the major groove would affect DNA stability and structure. The c7G was incorporated into the well-studied self-complementary dodecamer, 5′-d[CGCGAATTC-
The methods of analysis applied to the c7G stranded random coil. By measurement of the thermal stability TΔH° by DSC experiments demonstrated that the c7G substituted DNAs release less water and cations than the corresponding canonical sequences. This is a theme repeated with all of the destabilizing substituted DNA that we have studied and reflects the “chicken and egg” relationship between the constituents of the ensemble.

The possibility that electronic changes in c7G affected its H-bonding properties was addressed by preparing the 3′,5′-bis(trisopropylsilyl) substituted derivatives of c7-dG and dG and analyzing their interaction with the similarly derivatized dC by NMR in CDCl3, which eliminates stacking interactions. The results demonstrate that dG and c7G have very similar intrinsic H-bonding properties. To probe whether the c7G modification altered the structure of DNA, the X-ray and NMR (at 15 °C) structures of 5′-d[CCGCCAATTC-(c7G)-CG] were solved (Figure 4). Both the NMR and crystal structures of DNA without and with the c7G substitution are virtually identical, including base pairing and spacing at the c7G-C region, with one exception. In the crystal structure, a highly conserved Mg2+ near C9/G10 is not observed (Figure 5). The temperature-dependent exchange of the imino protons did shed some light on the differences in enthalpic stabilization observed in the DSC experiments. The major change was not at the c7G-C pair but at the adjacent 3′-G-C pair that was almost completely broadened at 35 °C, while the counterpart in the unmodified duplex is observable at 45 °C. To confirm the apparent increased dynamics near the c7G residue, a DNA with c7G substitutions was chemically footprinted using 2-hydroxytetrahydrofuran, which selectively reacts with C in ssDNA or within noncanonical bp’s. The footprinting studies show cleavage at the C paired with c7G, which is not observed in the unmodified duplex.

The binding of cations to the polyanionic phosphate backbone of DNA has been extensively studied, but interestingly only cations normally observed in crystal structures of DNA are near the major groove edge of G (Figure 6) and in the narrow minor groove at A/T rich sequences. By disturbing major groove cation binding, it appears that we have enthalpically destabilized DNA. This illustrates the important role of major groove cations in DNA stability and structure and raises the possibility that disruption of major groove cation binding can be a general destabilizing feature of some DNA lesions.

![Diagram](https://example.com/diagram.png)

**Figure 3.** Structures of modified bases and lesions: 7-deazaguanine (c7G); 7-aminoethyl-7-deaza-guanine (NH2-c7G); 7-hydroxymethyl-7-deazaguanine (HO-c7G); 7-deazaadenine (c7A); 8-oxoguanine (O8G); 5-hydroxymethylcytosine (5OHMC); 3-deazaadenine (c3A); 3-methyl-3-deazaadenine (3m-c3A).

c7G-[CG]-3′. The advantages of using this easy to crystallize sequence is offset by its propensity to form an intramolecular hairpin. To avoid this complication, c7G was introduced into 5′-d[GCGCGAATTC-c7G-C] and 5′-d[GAGAGCGCTTC], (c7G at 3′ or 5′). The methods of analysis applied to the c7G substitution are representative of the approaches used to characterize the other modifications discussed in this Account.

UV based thermal stability experiments at pH 7.0 in 10 mM NaCl showed that substitution of 7cG did not significantly affect the Tm except in the 5′-A-c7G-C sequence (Table 1, only 10 mM NaCl data are shown). However, differential scanning calorimetry (DSC) experiments demonstrated that the presence of c7G lowered the thermodynamic stability by 0.8-2.5 kcal mol−1 due to a 10-22 kcal mol−1 reduction in the ΔH term that was only partially compensated by an increase in TΔS (Table 1). Duplex formation is enthalpy driven, and there is a net uptake of water molecules and cations vs the single stranded random coil. By measurement of the thermal stability as a function of the log of water activity and cation concentration, the ΔnH2O and ΔnNa+ values can be derived based upon the assumption that the random coils of the modified and canonical sequence have similar levels of hydration and cation binding. As expected with the unfolding of less stable duplexes, the c7G substituted DNAs release less water and cations vs the corresponding canonical sequences. This is a theme repeated with all of the destabilizing substituted DNA that we have studied and reflects the “chicken and egg” relationship between the constituents of the ensemble.

The possibility that electronic changes in c7G affected its H-bonding properties was addressed by preparing the 3′,5′-bis(trisopropylsilyl) substituted derivatives of c7-dG and dG and analyzing their interaction with the similarly derivatized dC by NMR in CDCl3, which eliminates stacking interactions. The results demonstrate that dG and c7G have very similar intrinsic H-bonding properties. To probe whether the c7G modification altered the structure of DNA, the X-ray and NMR (at 15 °C) structures of 5′-d[CCGCCAATTC-(c7G)-CG] were solved (Figure 4). Both the NMR and crystal structures of DNA without and with the c7G substitution are virtually identical, including base pairing and spacing at the c7G-C region, with one exception. In the crystal structure, a highly conserved Mg2+ near C9/G10 is not observed (Figure 5). The temperature-dependent exchange of the imino protons did shed some light on the differences in enthalpic stabilization observed in the DSC experiments. The major change was not at the c7G-C pair but at the adjacent 3′-G-C pair that was almost completely broadened at 35 °C, while the counterpart in the unmodified duplex is observable at 45 °C. To confirm the apparent increased dynamics near the c7G residue, a DNA with c7G substitutions was chemically footprinted using 2-hydroxytetrahydrofuran, which selectively reacts with C in ssDNA or within noncanonical bp’s. The footprinting studies show cleavage at the C paired with c7G, which is not observed in the unmodified duplex.

The binding of cations to the polyanionic phosphate backbone of DNA has been extensively studied, but interestingly only cations normally observed in crystal structures of DNA are near the major groove edge of G (Figure 6) and in the narrow minor groove at A/T rich sequences. By disturbing major groove cation binding, it appears that we have enthalpically destabilized DNA. This illustrates the important role of major groove cations in DNA stability and structure and raises the possibility that disruption of major groove cation binding can be a general destabilizing feature of some DNA lesions.

| sequence | Tm,b (°C) | ΔG° (kcal/mol) | ΔH° (kcal/mol) | TΔS° (kcal/mol·K) | ΔnH2O,c | ΔnNa+,d |
|----------|-----------|---------------|---------------|------------------|---------|---------|
| 5′-CCCGAACCGTGGC | 33.3 | −7.0 | −116.0 | −109.0 | −2.3 ± 0.1 | −38.0 ± 2.0 |
| 5′-CCCGAACCGTGGC-c7G-CG | 35.7 | −6.1 | −106.0 | −99.9 | −1.7 ± 0.1 | −22.0 ± 2.0 |
| 5′-CCCGAACCGTGGC | 29.5 | −5.6 | −80.1 | −74.5 | −2.2 ± 0.1 | −30.0 ± 4.0 |
| 5′-CCCGAACCGTGGC-c7G-C | 28.5 | −4.6 | −68.4 | −63.8 | −1.8 ± 0.1 | −21.0 ± 2.0 |
| 5′-CCCGAACCGTGGC | 68.4 | −4.4 | −31.0 | −26.6 | −0.26 ± 0.02 | −18.0 ± 2.0 |
| 5′-CCCGAACCGTGGC-c7G-C | 63.7 | −3.5 | −27.0 | −23.5 | −0.21 ± 0.02 | −15.0 ± 1.5 |
| 5′-GGAGGAGCTCTC | 48.7 | −6.9 | −78.2 | −71.3 | −3.3 ± 0.2 | −41 ± 3 |
| 5′-GGAGGAGCTCTC-c7G-CG | 44.7 | −4.4 | −56.3 | −51.9 | −2.1 ± 0.1 | −25 ± 2 |
| 5′-GA-(c7G)-AGGGCTCTC | 47.2 | −6.1 | −72.0 | −65.9 | −2.4 ± 0.1 | −31 ± 3 |

All parameters are measured from UV (Tm,b) and DSC melting curves in 10 mM sodium phosphate buffer (pH 7.0) using 10 μM DNA. The experimental uncertainties are as follows: Tm,b (±0.5 °C), ΔH° (±3%), ΔG° (±5%), TΔS° (±3%). In kcal-mol−1. Per mole of DNA.
To determine the significance of cation binding to DNA stability, we designed a modification that stably placed a cation at a position in the major groove similar to that of diffusible cations observed in the crystal structures (Figure 6). The 7-aminomethyl-c7G (NH2-c7G)\(^{18}\) (Figure 3) was incorporated into different sequence contexts, and the oligomers were thermodynamically and structurally characterized vs c7G substituted and unmodified DNA.\(^{17}\) As an isosteric control, 7-hydroxymethyl-c7G (HO-c7G) was synthesized\(^{18}\) and introduced into the same oligomers. The thermodynamic parameters for the unfolding of the canonical, c7G, NH2-c7G, and HO-c7G are shown in Table 2.\(^{17}\) The NH2-c7G modification locally stabilizes DNA vs the same unmodified sequence (\(\Delta \Delta G^\circ = -2.2\) kcal mol\(^{-1}\)). The increased stability was shown by differential scanning calorimetry (DSC) to be due to the enthalpy term (\(\Delta \Delta H^\circ = -14.7\) kcal mol\(^{-1}\), Figure 7). The central role of the cationic charge in stabilization was demonstrated by thermodynamic characterization of the same DNA sequence with a neutral isosteric HO-c7G residue. DNA with HO-c7G is significantly less stable (\(\Delta \Delta G^\circ = +4.5\) kcal mol\(^{-1}\)) than DNA with the NH2-c7G substitution and less stable (\(\Delta \Delta G^\circ = +2.3\) kcal mol\(^{-1}\)) than unmodified DNA. The local change in stabilization induced by NH2-c7G was verified by temperature-dependent imino 1H NMR studies that show that the equilibrium constants (\(K_{eq}\)) for bp opening (in the absence and presence of NH3 base catalyst) of the two 5′-bp’s was reduced vs the canonical sequence (Figure 8).\(^{19}\) In the NMR structure, the tethered amino group points out into solvent and does not make contact with the phosphate backbone or atoms on the flanking bases (Figure 9).\(^{19}\) Based upon all of these data, we conclude that the presence of the electrostatic charge due to the cationic amine in the major groove is enthalpically stabilizing. Similar thermodynamic and structural results were observed for DNA with 7-deazaadenine substitutions.\(^{20}\)

### Table 2. Thermodynamic Profiles for the Formation of DNA with c7G, NH2-c7G, and HO-c7G in 10 mM NaCl at 20 °C\(^{11,17}a\)

| sequence               | \(T_m^b\) | \(\Delta G^c\) | \(\Delta H^c\) | \(\Delta T\Delta S^c\) | \(\Delta H_m^d\) | \(\Delta H_{NH3}^d\) | \(\Delta H_{NH2}^d\) |
|------------------------|-----------|---------------|---------------|----------------------|-----------------|---------------------|---------------------|
| S′-GAGGCCGCTCTC        | 47.8      | -6.9          | -78.2         | -71.3                | -3.3 ± 0.2      | -41 ± 1             |
| S′-GAGA-(c7G)-CGCTCTC  | 44.7      | -4.4          | -56.3         | -51.9                | -2.1 ± 0.1      | -25 ± 2             |
| S′-GAGA-(NH2-c7G)-CGCTCTC | 52.0    | -8.1          | -92.9         | -83.8                | -2.8 ± 0.1      | -38 ± 4             |
| S′-GA-(c7G)-AGGGCTCTC  | 47.2      | -4.6          | -54.5         | -49.9                | -1.6 ± 0.1      | -18 ± 2             |
| S′-GA-(NH2-c7G)-AGGGCTCTC | 47.2   | -6.1          | -72.0         | -65.9                | -2.4 ± 0.1      | -31 ± 3             |
| S′-GA-(HO-c7G)-AGGGCTCTC | 54.4    | -7.9          | -75.5         | -67.6                | -2.4 ± 0.1      | -26 ± 2             |
| S′-GA-(HO-c7G)-AGGGCTCTC | 47.5    | -3.3          | -37.9         | -4.6                  | -1.5 ± 0.1      | -8 ± 1              |
| S′-GGCCGGCCGCTC        | 62.1      | -12.3         | -98.1         | -85.8                | -1.9 ± 0.2      | -34 ± 3             |
| S′-GAGC-(NH2-c7G)-CGGGCTC | 61.5    | -10.2         | -82.4         | -72.2                | -1.5 ± 0.1      | -33 ± 3             |
| S′-GAGTGCGCACTC        | 50.0      | -8.4          | -90.7         | -82.3                | -2.5 ± 0.2      | -48 ± 5             |
| S′-GAGT-(NH2-c7G)-CGCACTC | 52.7    | -9.3          | -93.1         | -83.8                | -2.4 ± 0.2      | -27 ± 3             |
| S′-GAGGGCCGGCCTC       | 56.5      | -11.9         | -108.0        | -96.0                | -2.9 ± 0.2      | -52 ± 4             |
| S′-GAGG-(NH2-c7G)-CGGCCCTC | 69.0   | -13.7         | -95.5         | -81.8                | -1.9 ± 0.2      | -41 ± 4             |

\(^a\)All parameters are measured from UV (\(T_m^b\)) and DSC melting curves in 10 mM sodium phosphate buffer (pH 7.0) using 10 μM DNA. The experimental uncertainties are as follows: \(T_m^b\) (±0.5 °C); \(\Delta H_m^d\) (±3%); \(\Delta G^\circ(20)\) (±5%); \(\Delta T\Delta S^c\) (±3%). \(^b\)In °C. \(^c\)In kcal mol\(^{-1}\). \(^d\)Per mole of DNA.
Thermodynamic destabilization is also observed with 8-oxoguanine (8-oG). The results from these studies demonstrate the importance of major groove cations in the enthalpic stabilization of DNA and that perturbation at a major groove cation binding site can directly reduce local DNA stabilization, albeit in a sequence dependent fashion. 

8-Oxoguanine (8-oG)

Thermodynamic destabilization is also observed with 8-oxoguanine (8-oG), the predominant DNA lesion produced by oxidizing agents (Table 3). Based on NMR and crystal structures, the W—C H-bonding characteristics are preserved despite the fact that the atoms that line the major groove are significantly altered. As observed for c7G, the destabilization arises from a reduced enthalpy term that is not fully compensated by an increase in entropy, and there is a large reduction in hydration and cation binding (Table 3). The temperature-dependent imino $^1$H NMR spectrum for 5'-d[GAGA-(8-oG)-CGCTCTC] further confirms that the destabilization is localized at the 8-oG and the adjacent 3' T. Experiments with other sequences indicate a significant sequence dependency to the destabilizing effect of 8-oG.

5-Hydroxycytosine (5-hoC)

5-Hydroxycytosine, another oxidized lesion produced by reactive oxygen species, places a hydroxyl group into the major groove with the electrostatic H on the hydroxyl group pointing out into solvent. This change in the groove environment would be expected to exert a sequence dependent effect on cation binding, especially when there is a flanking G. A complete thermodynamic analysis of this lesion in 5'-d[GAGACGCTT-(5-ho-C)-TC] and 5'-d[GCGGAATTT-(5-ho-C)-GCG] was performed (Table 3). The lesion, which is capable of W—C pairing with G was highly destabilizing vs the unmodified sequences due to a reduced enthalpic term. There is a significant decrease in the negative band at 240 nm in the CD that is consistent with reduced base stacking, Temperature-dependent NMR studies of 5'-d[GCGGAATTT-(5-hoC)-GCG] reveal the local instability of the 5-ho-dC bp, which is almost completely exchanged at 5 °C. This is usually the most stable bp in the canonical sequence. Conversely, the central A/T core of the duplex becomes the most stable region in the 5-hoC substituted DNA indicating that the instability is selectively transmitted toward the 3'-terminus, similar to that observed for c7G and 8-oG.

The instability of the 5-hoC-G bp approaches that of a C-C mismatch (Table 3), which is thermally the most unstable bp mismatch. The magnitude of the effect, even within a T—(5-hoC)—T sequence, suggested that cation displacement cannot completely account for the instability. The possibility that 5-ho-dC may populate tautomeric structures has been previously investigated. NMR does not indicate the presence of an imino tautomer, but UV resonance Raman spectroscopy indicated that the imino tautomer increased 100-fold vs dC. However, the imino tautomer was still less than 0.1% of the amino tautomer, so it cannot account for the colligative effect on bp thermodynamics. Another possibility involves ionization of the 5-hydroxy group. The pKₐ of the hydroxyl group in 5-ho-dC is 7.37. The thermal stabilities ($T_{m}$) and CD spectra of the 5-hoC and unmodified DNA were monitored at pH ranging from 5 to 8.5. There was no significant difference suggesting that the ionization of 5-hoC was not a factor in the destabilization. Dipole−dipole interactions between bp’s play an important role in bp stability and stacking. Prior calculations of the dipole moment for 5-ho-dC indicate a decrease from 6.1−7.6 D for dC to 4.6−4.9 D for the amino−keto tautomer of 5-ho-dC. The poor base stacking of 5-hoC in duplex DNA has also been observed in the structures and pre-steady-state kinetics of dNTP insertion opposite the lesion.

Despite the clear instability of the 5-hoC-G bp, the crystal structure is indistinguishable from the canonical sequence (Figure 10). A result that confirms that "isostructural/ isoconformational does not necessarily imply isoenergetic."
**Table 3. Thermodynamic Profiles for the Formation of DNA with 8-oG and 5-hoC in 10 mM NaCl at 20 °C**

| sequence | $T_M$ | $\Delta G^\circ$ | $\Delta H^\circ$ | $T\Delta S^\circ$ | $\Delta n_{\text{O}2\text{H}}$ | $\Delta n_{\text{HO}}$ |
|----------|-------|------------------|------------------|-----------------|-----------------|-----------------|
| 5'-GAGAGCGGCTTC | 48.7 | -6.9 | -78.2 | -71.3 | -3.3 ± 0.2 | -41 ± 3 |
| 5'-GAG-(8-oG)-CGGCTTC | 39.4 | -3.2 | -39.3 | -36.1 | -1.0 ± 0.1 | -14 ± 2 |
| 5'-GCGAATTCCG | 29.5 | -5.6 | -80.1 | -74.5 | -2.2 ± 0.2 | -30 ± 4 |
| 5'-GCGAATTC-(8-oG)-C | 23.2 | -2.3 | -44.4 | -42.1 | -1.7 ± 0.1 | -15 ± 1 |
| 5'-CGCGTTTTCGGG | 68.4 | -4.4 | -31.0 | -26.6 | -0.3 ± 0.1 | -18 ± 2 |
| 5'-CGCGTTTTC-(8-oG)-CG | 56.5 | -1.8 | -16.6 | -14.8 | -0.3 ± 0.1 | -7 ± 1 |
| 5'-GAGCGCGGCGCTTC | 62.1 | -12.3 | -98.1 | -85.8 | -1.9 ± 0.2 | -34 ± 3 |
| 5'-GAGAGCGG-(5-hoC)-TC | 15.0 | +0.2 | -31.1 | -31.3 | e | e |
| 5'-GA_CAGGCGTTCCTC | 8.5 | -8.4 | -90.7 | -82.3 | -2.5 ± 0.2 | -48 ± 5 |
| 5'-CGCGAATTCGGG | 33.3 | -6.9 | -116.0 | -109.0 | -2.3 ± 0.2 | -38 ± 2 |
| 5'-CGCGAAATT-(5-hoC)-GCG | 31.5 | -2.8 | 74.3 | -71.5 | -10.0 ± 0.1 | -21 ± 3 |

All parameters are measured from UV ($T_M$) and DSC melting curves in 10 mM sodium phosphate buffer (pH 7.0) using 10 μM DNA. The experimental uncertainties are as follows: $T_M$ (±0.5 °C); $\Delta H_{\text{ad}}$ (±3%); $\Delta G_{\text{ad}}$ (±5%); $T\Delta S_{\text{ad}}$ (±3%). In °C. In kcal·mol⁻¹. Per mole of DNA. Not determined.

**Figure 10.** Crystal structure (1.4 Å resolution) of 5'-d[CGCGAA-(hoC)-GCG]; only the 5'-A-(hoC)-G base pairs are shown [PDB 4F3U].

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**MINOR GROOVE MODIFICATION**

3-Dezaadenine (c3A) and 3-Methyl-3-dezaadenine (3m-c3A)

The minor groove of DNA at A/T rich sequences is narrow, which places the phosphates on the complementary strands in close proximity. As a result, there is an ordered stretch of water and cations that serve to insulate the repulsive phosphate–phosphate interactions. Accordingly, we tested how the replacement of the N3 atom with a C–H would affect DNA stability and structure. The thermodynamics for the unfolding of 5'-d[GAG-(c3A)-GCGCTTC] and 5'-d[CGCGA-(c3A)-TTCGCG] vs the unsubstituted self-complementary sequences was measured (Table 4). The c3A destabilizes DNA by 2.4 and 7.9 kcal·mol⁻¹, respectively, and the $\Delta H^\circ$ and $T\Delta S^\circ$ parameters suggested the formation of an intramolecular hairpin with a CGCG stem (see Table 3 for thermodynamic parameters for 5'-d[CGCGTTTTCGGG]). The relatively small effect in the 5'-d[GAG-c3A-GCGCTTC] sequence was unexpected, but it is possible that its minor groove is not as narrow and that a structured hydration pattern does not exist.

To further perturb the electrostatic environment in the minor groove, a 3-methyl-3-deazaadenine (3m-c3A) was introduced into the same positions as that discussed for the c3A dodecamers. The 3m-c3A is a stable isostere of N3-methyladenine, which is a major adduct formed by DNA methylating agents. The introduction of a hydrophobic methyl group into the minor groove exerts a significant destabilizing effect with a $\Delta\Delta G^\circ$ of +4.5 kcal·mol⁻¹ for 5'-d[GAG-(3m-c3A)-GCGCTTC] (Table 4). As observed for other lesions, the destabilization arises from reduction in enthalpic stabilization (>-39 kcal·mol⁻¹). The release of water and cations upon duplex unfolding are also significantly reduced by approximately 50%.

**DNA HYDRATION**

How lesions qualitatively and quantitatively affect DNA hydration provides additional information on how a lesion affects the DNA ensemble. Depending on where water molecules interact with DNA, the structure and role of water varies. The formation of ds-DNA results in extensive immobilization of two distinct types of water: structural/ hydrophobic water and electrostricted water. Electrostricted water is associated with hydration of the charged phosphate.

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**Table 4. Standard Thermodynamic Profiles for the Formation of DNA with c3A and 3m-c3A in 10 mM NaCl at 20 °C**

| sequence | $T_M$ | $\Delta G^\circ$ | $\Delta H^\circ$ | $T\Delta S^\circ$ | $\Delta n_{\text{O}2\text{H}}$ | $\Delta n_{\text{HO}}$ |
|----------|-------|------------------|------------------|-----------------|-----------------|-----------------|
| 5'-GAGAGCGGCTTC | 48.7 | -6.9 | -78.2 | -71.3 | -3.4 ± 0.2 | -41 ± 3 |
| 5'-GAG-(3m-c3A)-CGGCTTC | 45.2 | -6.0 | -75.8 | -69.8 | -3.0 ± 0.1 | -35 ± 3 |
| 5'-GAG-(3m-c3A)-CGGCTTC | 38.8 | -2.4 | -39.0 | -36.4 | -1 ± 0.1 | -24 ± 2 |
| 5'-CGCGAATTCGGG | 63.6 | -6.9 | -116.0 | -109.0 | e | e |
| 5'-CGCGA-(3m-c3A)-TTCGCG | 70.0 | -5.3 | -36.7 | -31.4 | e | e |
| 5'-CGCGA-(3m-c3A)-TTCGCG | 68.9 | -4.7 | -32.9 | -28.2 | e | e |

All parameters are measured from UV ($T_M$) and DSC melting curves in 10 mM sodium phosphate buffer (pH 7.0) using 10 μM DNA. The experimental uncertainties are as follows: $T_M$ (±0.5 °C); $\Delta H_{\text{ad}}$ (±3%); $\Delta G_{\text{ad}}$ (±5%); $T\Delta S_{\text{ad}}$ (±3%). In °C. In kcal·mol⁻¹. Per mole of DNA. Not determined.
backbone. Structural water lines the hydrophobic surfaces of DNA and is more ordered than electrostricted water and has longer interaction lifetimes with DNA. It is the structural water that is sensitive to local DNA stability changes that occur due to DNA lesions. Because of the intimate and dynamic interaction between the DNA ensemble, it is difficult to distinguish between lesions decreasing the thermodynamic stability due to their direct effect on the local interaction with water vs the region around lesions being poorly hydrated due to the instability induced by the lesion. Regardless, for all the lesions that we have studied, thermodynamic destabilization is mirrored by lower hydration of the DNA.

What type of water is affected by lesions? Calorimetric, osmotic stress, density, and ultrasound and volume change experiments can provide differential information on the types of water associated with DNA as a result of introducing a lesion. Comparative analysis of the signs of $\Delta\Delta G$ (i.e., $\Delta\Delta H - \Delta T\Delta S$) and $\Delta\Delta V$ provides information on the type of water released during duplex unfolding. When $\Delta\Delta G$ and $\Delta\Delta V$ have similar signs, participation of electrostricted water is indicated, while opposite signs indicate structural water. This analysis is based upon the release of heat in the immobilization of electrostricted water where the water dipoles are compressed. In contrast, the energetic contribution for the release of structural water is close to nil due to improved packing around hydrophobic groups that eliminates void spaces.

For example, the data (not shown) indicate that the c3A modification results in the participation of electrostricted water, while the placement of a hydrophobic methyl group in 3m-c3A results in a decrease in release of electrostricted water.

For 8-oG, upon unfolding of the ds-DNA, there is a change in net hydration exchange involving interconversion of structural to electrostricted water molecules.

### BASE STABILITY AND RECOGNITION BY DNA GLYCOSYLASES

As detailed above, many of the lesions that are substrates for glycosylases appear to be able to maintain normal W-C base pairing and stacking arrangements and differ only in their thermodynamic properties and resultant change in hydration and cation binding. Although a great deal is known about the enzymology of DNA glycosylases, the exact details that allow the glycosylase to initially differentiate between an undamaged base, a damaged base, and a cognate substrate are not fully understood. However, early events include deformation of the DNA backbone and insertion of amino acid side chains into the DNA to fill and stabilize the void left in the base stack when the modified base rotates into the active site of the glycosylase. In the case of UNG, the rate of rotation of U out of the bp stack is not altered by the glycosylase; UNG traps the extrahelical conformation through stabilizing active site interactions with U. Fortunately, the opening rate constants for U and T opposite A were compared using NMR, and the bp opening rate is 6-fold faster for the U-A pair vs T-A pair in the same sequence. The authors argue that “the enhanced intrinsic opening rates of destabilized base pairs allow the bound glycosylase to sample dynamic extrahelical excursions of thymidine and uracil bases as the first step in recognition.” In unpublished work (Marky and Khutishvili), a sequence-dependent decrease in enthalpic stabilization of DNA was observed ranging from +3.5 kcal/mol for 5′-[CCCGAAT-(U)CGCC] to +11 kcal/mol for 5′-[GGCGAA-(U)-TCCGG] vs DNA with an A-T base pair. However, due to compensation by the entropy term, the $\Delta G$ differences are relatively small. Still, the $\Delta\Delta H$ indicates reduced base stacking in dA-dT vs dA-dT is qualitatively consistent with the difference in opening rates. For other glycosylases, for example, OGG, a more complex early recognition strategy is used. The reduced enthalpic stabilization that we routinely observe for DNA lesions will lower the energy barrier for deformation of the backbone and base extrusion, regardless of how the different glycosylases distort the DNA and flip the potential lesion into the active site. As mentioned above, discrimination of a noncognate vs cognate lesion by the glycosylase involves additional thermodynamic and kinetic processes.

A remarkable example of how thermodynamics can be used to find a cognate lesion is the bacterial glycosylase AlkD, which selectively removes N3- and N7-alkylpurine lesions. AlkD does not have a catalytic pocket. Based on crystal studies with a 3m-c3A-T bp, the enzyme distorts DNA and traps thermodynamically unstable N-alkylpurines in a solvent exposed conformation through electrostatic interactions with phosphates on the strand opposite the lesion. For a hydrolytically unstable base, for example, N3-methyladenine, this solvent exposure increases the rate of nonenzymatic depurination: the hydrolytic stability of N3-methyladenine in ss-DNA is 40-fold lower than that in ds-DNA.

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**Notes**

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**ACKNOWLEDGMENTS**

The authors thank the students and staff who performed the research discussed and the financial support from NIH and NSF.

**REFERENCES**

1. (a) Saenger, W. Principles of Nucleic Acid Structure; Springer-Verlag: New York, 1983. (b) Bloomfield, V. A.; Crothers, D. M.; Tinoco, L., Jr. Nucleic Acids: Structures, Properties, and Functions; University Science Books: Sausalito, CA, 1999.

2. Friedberg, E. C.; Walker, G. C.; Siede, W.; Wood, R. D.; Schultz, R. A.; Ellenberger, T. DNA Repair and Mutagenesis, 2nd ed.; ASM Press: Washington, DC, 2006.

3. (a) Fromme, J. C.; Verdine, G. L. Base excision repair. *Adv. Protein Chem.* 2004, 69, 1–41. (b) Fu, D.; Calvo, J. A.; Samson, L. D. Balancing repair and tolerance of DNA damage caused by alkylating agents. *Nat. Rev. Cancer* 2012, 12, 104–120. (c) Friedman, J. I.; Stivers, J. T. Detection of damaged DNA bases by DNA glycosylase enzymes. *Biochemistry* 2010, 49, 4957–4967.

4. (a) Hosfield, D. J.; Daniels, D. S.; Mol, C. D.; Putnam, C. M.; Parikh, S. S.; Tainer, J. A. DNA damage recognition and repair pathway coordination revealed by the structural biochemistry of DNA repair enzymes. *Prog. Nucleic Acid Res. Mol. Biol.* 2001, 68, 315–347. (b) Yang, W. Poor base stacking at DNA lesions may initiate recognition by many repair proteins. *DNA Repair* 2006, 5, 654–666.

5. (a) Banerjee, A.; Santos, W. L.; Verdine, G. L. Structure of a DNA glycosylase searching for lesions. *Science* 2006, 311, 1153–1157. (b) Barrett, T. E.; Savva, R.; Panayotou, G.; Barlow, T.; Brown, T.; Jiricny, J.; Pearl, L. H. Crystal structure of a G:T/U mismatch-specific DNA glycosylase: Mismatch recognition by complementary-strand interactions. *Cell* 1998, 92, 117–129. (c) Barrett, T. E.; Schäfer, O. D.; Savva, R.; Brown, T.; Jiricny, J.; Verdin, G. L.; Pearl, L. H. Crystal structure of a thwarted mismatch glycosylase DNA repair complex. *EMBO J.* 1999, 18, 6599–6609.

6. (a) Cao, C.; Jiang, Y. L.; Stivers, J. T.; Song, F. Dynamic opening of DNA during the enzymatic search for a damaged base. *Nat. Struct. Biol.* 2004, 11, 1230–1236. (b) Baldwin, M. R.; O'Brien, P. J. Nonspecific DNA binding and coordination of the first two steps of base excision repair. *Biochemistry* 2010, 49, 7879–7891. (c) Schonhoff, J. D.; Stivers, J. T. Timing facilitated site transfer of an enzyme on DNA. *Nat. Chem. Biol.* 2012, 8, 205–210. (d) Qi, Y.; Spong, M. C.; Nam, K.; Banerjee, A.; Jiricny, J.; Pearl, L. H. Encounter and extrusion of an intrahelical lesion by a DNA repair enzyme. *Nature* 2009, 462, 762–766. (e) Blaine, P. C.; van Oijen, A. M.; Banerjee, A.; Verdin, G. L.; Xie, X. S. A base-excision DNA repair protein finds intrahelical lesion bases by fast sliding in contact with DNA. *Proc. Natl. Acad. Sci. U.S.A.* 2006, 103, 5752–5757.

7. (a) Cao, C.; Jiang, Y. L.; Stivers, J. T.; Song, F. Dynamic opening of DNA during the enzymatic search for a damaged base. *Nat. Struct. Biol.* 2004, 11, 1230–1236. (b) Baldwin, M. R.; O'Brien, P. J. Nonspecific DNA binding and coordination of the first two steps of base excision repair. *Biochemistry* 2010, 49, 7879–7891. (c) Schonhoff, J. D.; Stivers, J. T. Timing facilitated site transfer of an enzyme on DNA. *Nat. Chem. Biol.* 2012, 8, 205–210. (d) Qi, Y.; Spong, M. C.; Nam, K.; Banerjee, A.; Jiricny, J.; Pearl, L. H. Encounter and extrusion of an intrahelical lesion by a DNA repair enzyme. *Nature* 2009, 462, 762–766. (e) Blaine, P. C.; van Oijen, A. M.; Banerjee, A.; Verdin, G. L.; Xie, X. S. A base-excision DNA repair protein finds intrahelical lesion bases by fast sliding in contact with DNA. *Proc. Natl. Acad. Sci. U.S.A.* 2006, 103, 5752–5757.

8. (a) Cao, C.; Jiang, Y. L.; Stivers, J. T.; Song, F. Dynamic opening of DNA during the enzymatic search for a damaged base. *Nat. Struct. Biol.* 2004, 11, 1230–1236. (b) Baldwin, M. R.; O’Brien, P. J. Nonspecific DNA binding and coordination of the first two steps of base excision repair. *Biochemistry* 2010, 49, 7879–7891. (c) Schonhoff, J. D.; Stivers, J. T. Timing facilitated site transfer of an enzyme on DNA. *Nat. Chem. Biol.* 2012, 8, 205–210. (d) Qi, Y.; Spong, M. C.; Nam, K.; Banerjee, A.; Jiricny, J.; Pearl, L. H. Encounter and extrusion of an intrahelical lesion by a DNA repair enzyme. *Nature* 2009, 462, 762–766. (e) Blaine, P. C.; van Oijen, A. M.; Banerjee, A.; Verdin, G. L.; Xie, X. S. A base-excision DNA repair protein finds intrahelical lesion bases by fast sliding in contact with DNA. *Proc. Natl. Acad. Sci. U.S.A.* 2006, 103, 5752–5757.

9. (a) Saenger, W. Principles of Nucleic Acid Structure; Springer-Verlag: New York, 1983. (b) Bloomfield, V. A.; Crothers, D. M.; Tinoco, L., Jr. Nucleic Acids: Structures, Properties, and Functions; University Science Books: Sausalito, CA, 1999.

10. (a) Saenger, W. Principles of Nucleic Acid Structure; Springer-Verlag: New York, 1983. (b) Bloomfield, V. A.; Crothers, D. M.; Tinoco, L., Jr. Nucleic Acids: Structures, Properties, and Functions; University Science Books: Sausalito, CA, 1999.

11. (a) Saenger, W. Principles of Nucleic Acid Structure; Springer-Verlag: New York, 1983. (b) Bloomfield, V. A.; Crothers, D. M.; Tinoco, L., Jr. Nucleic Acids: Structures, Properties, and Functions; University Science Books: Sausalito, CA, 1999. (c) Banerjee, A.; Santos, W. L.; Verdine, G. L. Structure of a DNA glycosylase searching for lesions. *Biochemistry* 2010, 49, 4957–4967.

12. (a) Fromme, J. C.; Verdine, G. L. Base excision repair. *Adv. Protein Chem.* 2004, 69, 1–41. (b) Fu, D.; Calvo, J. A.; Samson, L. D. Balancing repair and tolerance of DNA damage caused by alkylating agents. *Nat. Rev. Cancer* 2012, 12, 104–120. (c) Friedman, J. I.; Stivers, J. T. Detection of damaged DNA bases by DNA glycosylase enzymes. *Biochemistry* 2010, 49, 4957–4967.

13. (a) Saenger, W. Principles of Nucleic Acid Structure; Springer-Verlag: New York, 1983. (b) Bloomfield, V. A.; Crothers, D. M.; Tinoco, L., Jr. Nucleic Acids: Structures, Properties, and Functions; University Science Books: Sausalito, CA, 1999. (c) Banerjee, A.; Santos, W. L.; Verdine, G. L. Structure of a DNA glycosylase searching for lesions. *Biochemistry* 2010, 49, 4957–4967.

14. (a) Saenger, W. Principles of Nucleic Acid Structure; Springer-Verlag: New York, 1983. (b) Bloomfield, V. A.; Crothers, D. M.; Tinoco, L., Jr. Nucleic Acids: Structures, Properties, and Functions; University Science Books: Sausalito, CA, 1999.
decamer containing 7,8-dihydro-8-oxoguanine. Proc. Natl. Acad. Sci. U.S.A. 1995, 92, 719–723.

(23) Singh, S. K.; Szulik, M. W.; Ganguly, M.; Khutishvili, I.; Stone, M. P.; Marky, L. A.; Gold, B. Characterization of DNA with an 8-oxoguanine modification. Nucleic Acids Res. 2011, 39, 6789–6801.

(24) Yennie, C. J.; Delaney, S. Thermodynamic consequences of the hyper-oxidized guanine lesion guanidinohydantoin in duplex DNA. Chem. Res. Toxicol. 2012, 25, 1732–1739.

(25) (a) Wagner, J. R.; Cadet, J. Oxidation reactions of cytosine DNA components by hydroxyl radical and one-electron oxidants in aerated aqueous solutions. Acc. Chem. Res. 2010, 43, 564–571. (b) Wallace, S. S. Biological consequences of free radical-damaged DNA bases. Free Radical Biol. Med. 2002, 33, 1–14.

(26) Ganguly, M.; Szulik, M. W.; Donahue, P. S.; Clancy, K.; Stone, M. P.; Gold, B. Thermodynamic signature of DNA damage: characterization of DNA with a 5-hydroxy-2′-deoxyctydine•2′- deoxyguanosine base pair. Biochemistry 2012, 51, 2018–2027.

(27) Pan, S.; Sun, X.; Lee, J. K. Stability of complementary and mismatched DNA duplexes: Comparison and contrast in gas versus solution phases. Int. J. Mass Spectrom. 2006, 253, 238–248.

(28) (a) LaFrancois, C. J.; Fujimoto, J.; Sowers, L. C. Synthesis and characterization of isotopically enriched pyrimidine deoxynucleoside oxidation damage products. Chem. Res. Toxicol. 1998, 11, 75–83. (b) Suen, W.; Spiro, T. G.; Sowers, L. C.; Fresco, J. R. Identification by UV resonance Raman spectroscopy of an imino tautomer of 5-hydroxy-2′-deoxycytidine, a powerful base analog transition mutagen with a much higher unfavored tautomer frequency than that of the natural residue 2′-deoxycytidine. Proc. Natl. Acad. Sci. U.S.A. 1999, 96, 4500–4505.

(29) (a) Krauss, M.; Osman, R. Electronic spectra of the H and OH adducts of cytosine. J. Phys. Chem. A 1997, 101, 4117–4120. (b) Cysewski, P. Structure and properties of hydroxyl radical modified nucleic acid components: Tautomerism and miscoding properties of 5-hydroxy-2′-oxocytosine. J. Mol. Struct. 1999, 466, 49–58.

(30) Zahn, K. E.; Averill, A.; Wallace, S. S.; Doublé, S. The miscoding potential of 5-hydroxycytosine arises due to template instability in the replicative polymerase active site. Biochemistry 2011, 50, 10350–10358.

(31) Szulik, M. W.; Nociek, B.; Joachimiak, A.; Stone, M. P. see http://www.rcsb.org/pdb/explore/explore.do?structureId=4F3U.

(32) (a) Drew, H. R.; Wing, R. M.; Takano, T.; Broka, C.; Tanaka, S.; Itakura, K.; Dickerson, R. E. Structure of a B-DNA dodecamer: Conformation and dynamics. Proc. Natl. Acad. Sci. U.S.A. 1981, 78, 2179–2183. (b) Dickerson, R. E.; Goodsell, D. S.; Kopka, M. L.; Pjura, P. E. The effect of crystal packing on oligonucleotide double helix structure. J. Biomol. Struct. Dyn. 1987, 5, 557–579.

(33) (a) Tereshko, V.; Minasov, G.; Egli, M. A “hydrate-ion” spine in a B-DNA minor groove. J. Am. Chem. Soc. 1999, 121, 3590–3595. (b) Westhof, E. Water: An integral part of nucleic acid structure. Annu. Rev. Biophys. Chem. 1988, 17, 125–144.

(34) Ganguly, M.; Wang, R.-W.; Marky, L. A.; Gold, B. Thermodynamic characterization of DNA with 3-dezaadenine and 3-methyl-3-deazaadenine substitutions. J. Phys. Chem. B 2010, 114, 7656–7661.

(35) (a) Marky, L. A.; Kapke, D. W. Enthalpy-entropy compensation in nucleic acids: Contribution of electrostriction and structural hydration. Methods Enzymol. 2000, 323, 419–441. (b) Marky, L. A.; Kapke, D. W. Probing the hydration of the minor groove of A•T synthetic DNA polymers by volume and heat changes. Biochemistry 1989, 28, 9982–9999.

(36) (a) Rayan, G.; Tsamaloukas, A. D.; Macgregor, R. B., Jr.; Heerklotz, H. Helix-coil transition of DNA monitored by pressure perturbation calorimetry. J. Phys. Chem. B 2009, 113, 1738–1742. (b) Dragan, A. I.; Russell, D. J.; Privalov, P. L. DNA hydration studied by pressure perturbation scanning microcalorimetry. Biopolymers 2009, 91, 95–101.

(37) Parker, J. B.; Stivers, J. T. Dynamics of uracil and 5-fluorouracil in DNA. Biochemistry 2011, 50, 612–617.

(38) Rubinson, E. H.; Gowda, A. S. P.; Spratt, T. E.; Gold, B.; Eichman, B. F. An unprecedented nucleic acid capture mechanism for excision of DNA damage. Nature 2010, 468, 406–411.

(39) (a) Margison, G. P.; O’Connor, P. J. Biological implications of the instability of the N-glycosidic bone of 3-methyldeoxyadenosine in DNA. Biochim. Biophys. Acta 1973, 331, 349–356. (b) Fuji, T.; Saito, T.; Nakasaka, T. Purines. XXXIV. 3-Methyladenosine and 3-methyl-2′- deoxyadenosine: Their synthesis, glycosidic hydrolysis, and ring fission. Chem. Pharm. Bull. 1989, 37, 2601–2609.