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Looking for Waldo: A Potential Thermodynamic Signature to DNA Damage

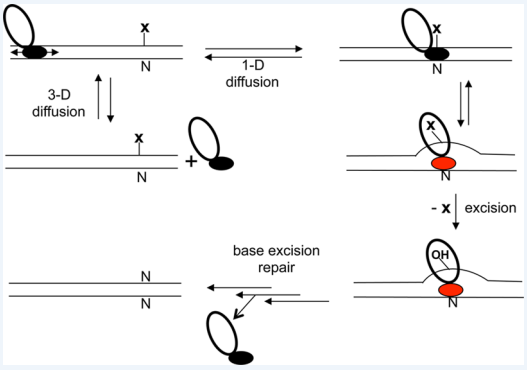
Barry Gold,*[,†] Michael P. Stone,[§] and Luis A. Marky[#]

[†]Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, United States

[§]Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235, United States

[#]Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, Nebraska 68198, United States

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 quantative 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).[2,3] 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

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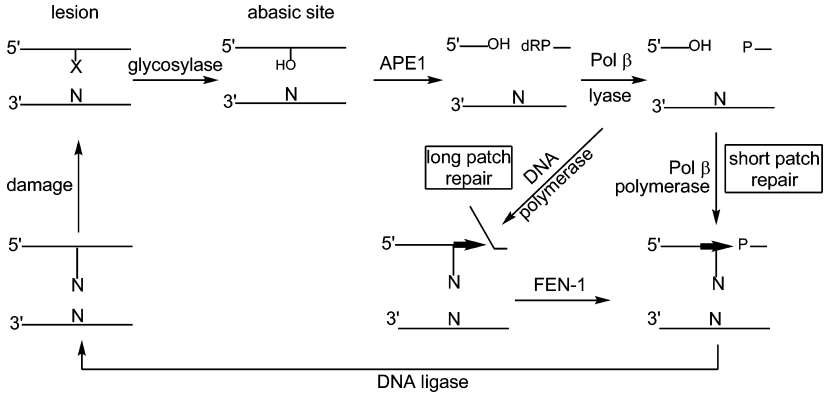

Figure 1. Outline of mammalian base excision repair (N = 2′-deoxynucleoside; X = modified 2′-deoxynucleotide) via short and long patch repair pathways.

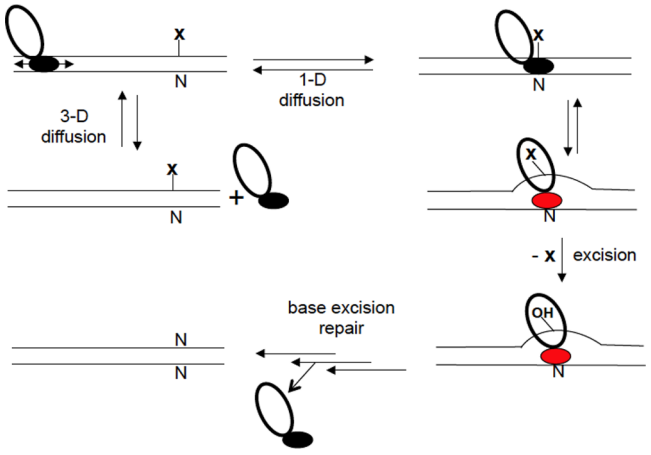

Figure 2. Scheme for the initial recognition of a substrate in DNA via a "thermodynamic signature" mechanism. The depiction of X is not meant to imply that is maintained in an extrahelical conformation. Black oval is glycosylase protein associated with DNA by nonspecific electrostatic interactions. Red oval indicates protein at a potential substrate in DNA where favorable enthalpic interactions can occur: it requires distortion of the DNA backbone so the potential substrate lesion can enter protein's active site. At a cognate lesion, there will be stabilizing enthalpic interactions between glycosylase and the lesion base and eventual excision of the lesion off the DNA backbone. At noncognate lesions, the lack of enthalpic stabilization will allow the complex to dissociate. Note that the glycosylase may stay associated with the abasic site product after the excision step.

correct canonical base in place of the lesion. While there are 11 human DNA glycosylases that either repair a specific lesion or repair many different lesions,[2,3] once the abasic site is generated, the BER process is independent of the structure of the original lesion. Therefore, the specificity in finding and removing a lesion is a property of the DNA glycosylase. High-resolution structures provide clear insights into the late stages of the selectivity of the different glycosylases for different lesions and the enzymatic steps in base excision.[4,5] However, they do not provide information on how the glycosylases can initially rapidly search and identify damaged bases, and eventually substrates, in the presence of a large excess of canonical DNA base pairs. A number of laboratories have explored this question, and the conclusions vary on the basis of the nature of the experimental system and the glycosylase studied.[3−6] What happens when the glycosylase arrives at a potential lesion is less well understood. Plum and Breslauer initially mentioned a potential relationship between DNA lesions and DNA repair involving a "thermodynamic signature" in a study of DNA with an N^1,N^2-propanoG·C pair, which cannot form a Watson−Crick (W−C) base pair, and with an 8-oG·C pair, which can.[7] 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.[3−6,8] 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[7] 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.[9] 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[10] so an N7G substitution would be expected to disrupt major groove cation binding. Because N7-alkylG readily depurinates to an abasic site,[9] 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-

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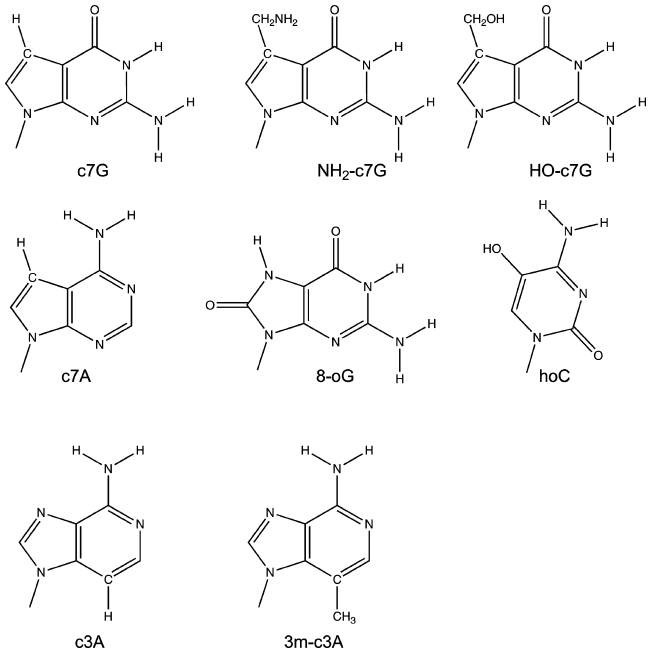

Figure 3. Structures of modified bases and lesions: 7-deazaguanine (c7G); 7-aminomethyl-7-deaza-guanine (NH_2-c7G); 7-hydroxymethyl-7-deazaguanine (HO-c7G); 7-deazaadenine (c7A); 8-oxoguanine (8-oG); 5-hydroxycytosine (hoC); 3-deazaadenine (c3A); 3-methyl-3-deazaadenine (3m-c3A).

c7G-CG]-3′.[11] The advantages of using this easy to crystallize sequence is offset by its propensity to form an intramolecular hairpin.[12] To avoid this complication, c7G was introduced into 5′-d[GCGAATTC-c7G-C] and 5′-d[GAGAGCGCTCTC], (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 T_M, except in the 5′-A-c7G-C sequence (Table 1, only 10 mM NaCl data are shown).[11] 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 $T\Delta S$ (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 Δn_{H_2O} and Δn_{Na^+} 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.[13] 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(triisopropylsilyl) substituted derivatives of c7-dG and dG and analyzing their interaction with the similarly derivatized dC by NMR in $CDCl_3$, which eliminates stacking interactions.[14] 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[CGCGAATTC-(c7G)-CG] were solved (Figure 4).[11] Both the NMR and crystal structures of DNA without and with the c7G substitution are virtually identical, including base pairing and stacking at the c7G·C region, with one exception.[11] In the crystal structure, a highly conserved Mg^{2+} 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-hydroperoxytetrahydrofuran, which selectively reacts with C in ss-DNA or within noncanonical bp's.[15] 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,[16] but interestingly the 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.[10] 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.

Table 1. Standard Thermodynamic Profiles for the Formation of DNA with c7G in 10 mM NaCl at 20 °C[11a]

sequence	T_M[b]	ΔG°[c]	ΔH_{cal}[c]	$T\Delta S_{cal}$[c]	Δn_{Na^+}[d]	Δn_{H_2O}[d]
5′-CGCGAATTCGCG	33.3	−7.0	−116.0	−109.0	−2.3 ± 0.15	−38.0 ± 2.0
5′-CGCGAATTC-(c7G)-CG	35.7	−6.1	−106.0	−99.9	−1.7 ± 0.13	−22.0 ± 2.0
5′-GCGAATTCGC	29.5	−5.6	−80.1	−74.5	−2.2 ± 0.15	−30.0 ± 4.0
5′-GCGAATTC-(c7G)-C	28.5	−4.6	−68.4	−63.8	−1.8 ± 0.15	−21.0 ± 2.0
5′-CGCGTTTTCGCG	68.4	−4.4	−31.0	−26.6	−0.26 ± 0.02	−18.0 ± 2.0
5′-CGCGTTTTC-(c7G)-CG	63.7	−3.5	−27.0	−23.5	−0.21 ± 0.02	−15.0 ± 1.5
5′-GAGAGCGCTCTC	48.7	−6.9	−78.2	−71.3	−3.3 ± 0.2	−41 ± 3
5′-GAGA-(c7G)-CGCTCTC	44.7	−4.4	−56.3	−51.9	−2.1 ± 0.1	−25 ± 2
5′-GA-(c7G)-AGCGCTCTC	47.2	−6.1	−72.0	−65.9	−2.4 ± 0.1	−31 ± 3

[a]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), ΔH_{cal} (±3%), $\Delta G^{\circ}_{(20)}$ (±5%), $T\Delta S_{cal}$ (±3%). [b]In °C. [c]In kcal·mol^{-1}. [d]Per mole of DNA.

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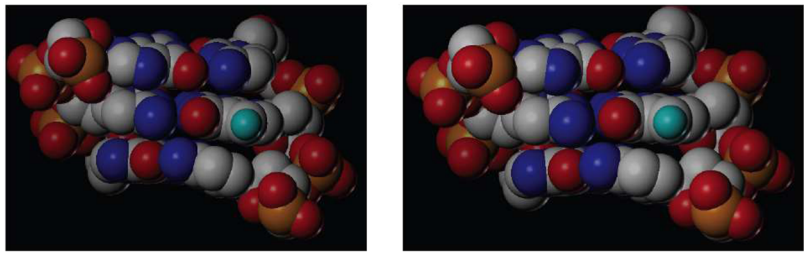

Figure 4. Comparison of the crystal (PDB 2QEG) (left) and NMR (PDB 2QEF) (right) structures of c7G in 5′-C-(c7G)-C sequence.[11b]

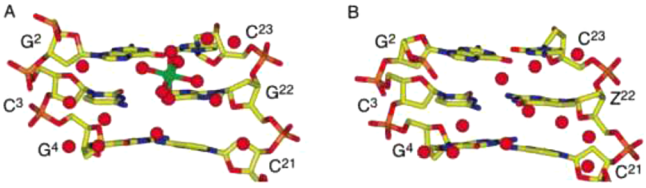

Figure 5. Crystal structures of (A) 5′-d[CGCGAATTCGCG] and (B) 5-d[CGCGAATTC-(c7G)-CG]. Reproduced from ref 11b. Copyright 2008 American Chemical Society.

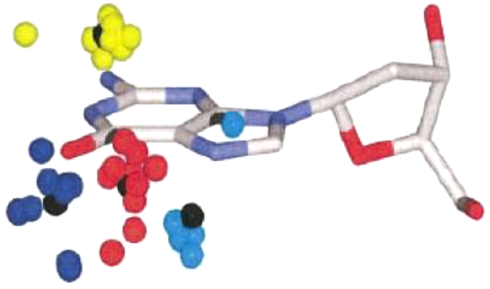

Figure 6. High occupancy binding sites of monovalent cations associated with the major groove of canonical DNA at G·C pairs: dark blue, near O^6-G; light blue, near N7-G; red, between N7-G and O^6-G; yellow, located between stacked G's in 5′-GC sequence; black, average of position at different sites.[10a]

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).[10] The 7-aminomethyl-c7G (NH_2-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.[17] The thermodynamic parameters for the unfolding of the canonical, c7G, NH_2-c7G, and HO-c7G are shown in Table 2.[17] The NH_2-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 NH_2-c7G substitution and less stable ($\Delta\Delta G^\circ$ = +2.3 kcal·mol^{-1}) than unmodified DNA. The local change in stabilization induced by NH_2-c7G was verified by temperature-dependent imino ^{1}H NMR studies that show that the equilibrium constants (K_{eq}) for bp opening (in the absence and presence of NH_3 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 substutitions.[20]

Table 2. Thermodynamic Profiles for the Formation of DNA with c7G, NH_2-c7G, and HO-c7G in 10 mM NaCl at 20 °C[11,17a]

sequence	T_M[b]	ΔG°[c]	ΔH°[c]	$T\Delta S^{\circ}$[c]	Δn_{Na^+}[d]	Δn_{H_2O}[d]
5′-GAGAGCGCTCTC	48.7	−6.9	−78.2	−71.3	−3.3 ± 0.2	−41 ± 3
5′-GAGA-(c7G)-CGCTCTC	44.7	−4.4	−56.3	−51.9	−2.1 ± 0.1	−25 ± 2
5′-GAGA-(NH_2-c7G)-CGCTCTC	52.0	−9.1	−92.9	−83.8	−2.8 ± 0.1	−38 ± 4
5′-GAGA-(HO-c7G)-CGCTCTC	47.2	−4.6	−54.5	−49.9	−1.6 ± 0.1	−18 ± 2
5′-GA-(c7G)-AGCGCTCTC	47.2	−6.1	−72.0	−65.9	−2.4 ± 0.1	−31 ± 3
5′-GA-(NH_2-c7G)-AGCGCTCTC	54.4	−7.9	−75.5	−67.6	−2.4 ± 0.1	−26 ± 2
5′-GA-(HO-c7G)-AGCGCTCTC	47.5	−3.3	−37.9	−4.6	−1.5 ± 0.1	−8 ± 1
5′-GAGCGCGCGCTC	62.1	−12.3	−98.1	−85.8	−1.9 ± 0.2	−34 ± 3
5′-GAGC-(NH_2-c7G)-CGCGCTC	61.5	−10.2	−82.4	−72.2	−1.5 ± 0.1	−33 ± 3
5′-GAGTGCGCACTC	50.0	−8.4	−90.7	−82.3	−2.5 ± 0.2	−48 ± 5
5′-GAGT-(NH_2-c7G)-CGCACTC	52.7	−9.3	−93.1	−83.8	−2.4 ± 0.2	−27 ± 3
5′-GAGGGCGCCCTC	56.5	−11.9	−108.0	−96.0	−2.9 ± 0.2	−52 ± 4
5′-GAGG-(NH_2-c7G)-CGCCCTC	69.0	−13.7	−95.5	−81.8	−1.9 ± 0.2	−41 ± 4

[a]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); ΔH_{cal} (±3%); $\Delta G^{\circ}_{(20)}$ (±5%); $T\Delta S_{cal}$ (±3%). [b]In °C. [c]In kcal·mol^{-1}. [d]Per mole of DNA.

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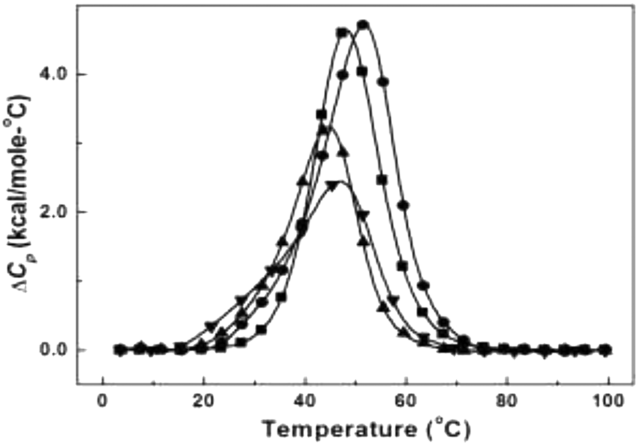

Figure 7. DSC for 5′-d(GAGA-X-CGCTCTC): X = (■) G; (▲) c7G; (●) NH_2-c7G; (▼) HO-c7G. Reproduced from ref 17. Copyright 2009 American Chemical Society.

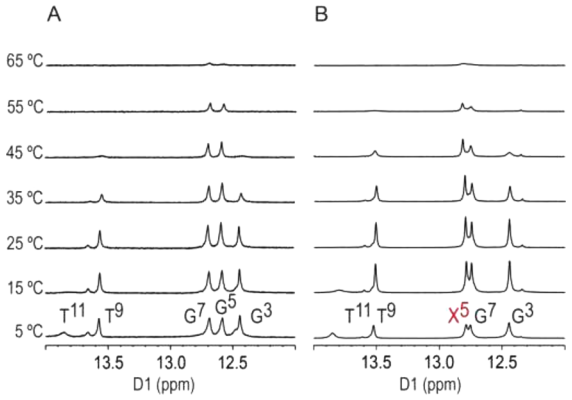

Figure 8. Plots of imino proton exchange rates, k_{ex}, obtained by monitoring magnetization transfer from water as a function of ammonia base catalyst: (a) 5′-d[GAGAGCGCTCTC] and (b) 5′-d[GAGA-(NH_2-c7G)-CGCTCTC]. Reproduced from ref 19. Copyright 2013 American Chemical Society.

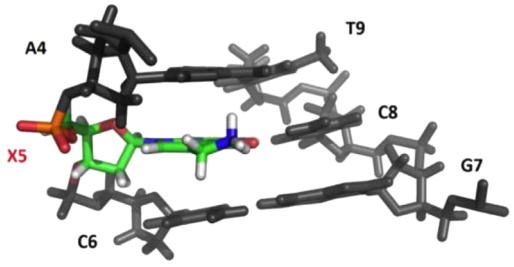

Figure 9. NMR structure of 5′-d(GAGAXCGCTCTC): X = NH_2-c7G (colored by atom type). The base pairing and base stacking is normal, and the amino group points out into solvent. Adapted from ref 19. Copyright 2013 American Chemical Society.

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).[21] Based on NMR[22a] and crystal[22b] 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).[23] 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-9. Experiments with other sequences indicate a significant sequence dependency to the destabilizing effect of 8-oG.[22−24]

5-Hydroxycytosine (5-hoC)

5-Hydroxycytosine, another oxidized lesion produced by reactive oxygen species,[25] places a hydroxyl group into the major groove with the electropositive 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[GAGAGCGCT-(5-ho-C)-TC] and 5′-d[CGCGAATT-(5-hoC)-GCG] was performed (Table 3).[26] 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[CGCGAATT-(5-hoC)-GCG] reveal the local instability of the 5-ho-dC·dG 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[11] and 8-oG.[23]

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.[27] 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.[28] NMR does not indicate the presence of an imino tautomer,[28a] but UV resonance Raman spectroscopy indicated that the imino tautomer increased 100-fold vs dC.[28b] 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 pK_a 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's ranging from 5 to 8.5.[26] 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.[1] 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.[29] 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.[30]

Despite the clear instability of the 5-hoC·G bp, the crystal structure is indistinguishable from the canonical sequence (Figure 10),[31,32] a result that confirms that "isostructural/isoconformational does not necessarily imply isoenergetic."[7]

Table 3. Thermodynamic Profiles for the Formation of DNA with 8-oG and 5-hoC in 10 mM NaCl at 20 °C[23,26a]

sequence	T_M[b]	ΔG°[c]	ΔH°[c]	$T\Delta S^{\circ}$[c]	Δn_{Na^+}[d]	Δn_{H_2O}[d]
5′-GAGAGCGCTCTC	48.7	−6.9	−78.2	−71.3	−3.3 ± 0.2	−41 ± 3
5′-GAGA-(8-oG)-CGCTCTC	39.4	−3.2	−39.3	−36.1	−1.0 ± 0.1	−14 ± 2
5′-GCGAATTCGC	29.5	−5.6	−80.1	−74.5	−2.2 ± 0.2	−30 ± 4
5′-GCGAATC-(8-oG)-C	23.2	−2.3	−44.4	−42.1	−1.7 ± 0.1	−15 ± 1
5′-CGCGTTTTCGCG	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′-GAGCGCGCGCTC	62.1	−12.3	−98.1	−85.8	−1.9 ± 0.2	−34 ± 3
5′-GAGAGCGCG-(5-hoC)-TC	15.0	+0.2	−31.1	−31.3	e	e
5′- GA-C-AGCGCTCTC	8.5	−8.4	−90.7	−82.3	−2.5 ± 0.2	−48 ± 5
5′-CGCGAATTCGCG	33.3	−6.9	−116.0	−109.0	−2.3 ± 0.2	−38 ± 2
5′-CGCGAATT-(5-hoC)-GCG	31.5	−2.8	74.3	−71.5	−1.0 ± 0.1	−21 ± 3

[a]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); ΔH_{cal} (±3%); $\Delta G^{\circ}_{(20)}$ (±5%); $T\Delta S_{cal}$ (±3%). [b]In °C. [c]In kcal·mol^{-1}. [d]Per mole of DNA. [e]Not determined.

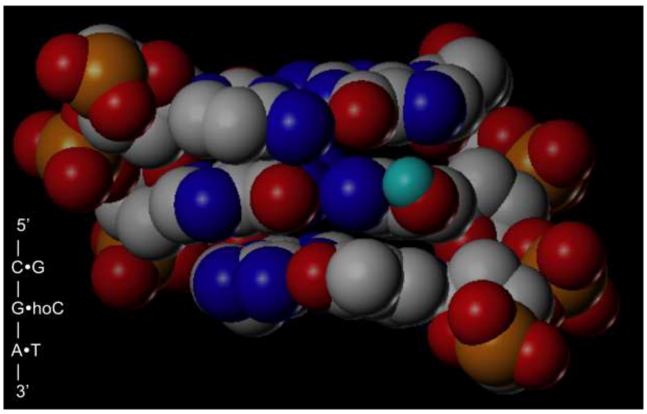

Figure 10. Crystal structure (1.4 Å resolution) of 5′-d[CGCGAA-(hoC)-GCG]: only the 5′-A-(hoC)-G base pairs are shown [PDB 4F3U].[31]

MINOR GROOVE MODIFICATION

3-Deazaadenine (c3A) and 3-Methyl-3-deazaadenine (3m-c3A)

The minor groove of DNA at A/T rich sequences is narrow,[32] 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.[32,33] 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)-GCGCTCTC] and 5′-d[CGCGA-(c3A)-TTCGCG] vs the unsubstituted self-complementary sequences was measured (Table 4).[34] The c3A destabilizes DNA by 2.4 and 7.9 kcal·mol^{-1}, respectively, and the ΔH° 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[CGCGTTTTCGCG]). The relatively small effect in the 5′-d[GAG-c3A-GCGCTCTC] 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.[34] The 3m-c3A is a stable isostere of N3-methyladenine, which is a major adduct formed by DNA methylating agents.[9] 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)-GCGCTCTC] (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

Table 4. Standard Thermodynamic Profiles for the Formation of DNA with c3A and 3m-c3A in 10 mM NaCl at 20 °C[34a]

sequence	T_M[b]	ΔG°_{cal}[c]	ΔH°[c]	$T\Delta S_{cal}$[c]	Δn_{Na+}[d]	Δn_{H_2O}[d]
5′-GAGAGCGCTCTC	48.7	−6.9	−78.2	−71.3	−3.4 ± 0.2	−41 ± 3
5′-GAG-(c3A)-GCGCTCTC	45.2	−6.0	−75.8	−69.8	−3.0 ± 0.1	−35 ± 3
5′- GAG-(3m-c3A)-GCGCTCTC	38.8	−2.4	−39.0	−36.6	−1.4 ± 0.1	−24 ± 2
5′-CGCGAATTCGCG	63.6	−6.9	−116.0	−109.0	e	e
5′-CGCGA-(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

[a]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); ΔH_{cal} (±3%); $\Delta G^{\circ}_{(20)}$ (±5%); $T\Delta S_{cal}$ (±3%). [b]In °C. [c]In kcal·mol^{-1}. [d]Per mole of DNA. [e]Not determined.

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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.[35,36] 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.[35] 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.[35] 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.[34] 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.[23]

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.[6a] 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.[37] 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 Khutsishvili), a sequence-dependent decrease in enthalpic stabilization of DNA was observed ranging from +3.5 kcal·mol^{-1} for 5′-d[CCGGAAT-(U)CGCC]) to +11 kcal·mol^{-1} for 5′-d[GGCGAA-(U)-TCCGG] vs DNA with an A·T base pair. However, due to compensation by the entropy term, the ΔG differences are relatively small. Still, the $\Delta\Delta H$ indicates reduced base stacking in dA·dU vs dA·dT is qualitatively consistent with the difference in opening rates.[37] For other glycosylases, for example, OGG, a more complex early recognition strategy is used.[4c,6e] 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.[38] 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.[39]

AUTHOR INFORMATION

Corresponding Author

*E-mail: goldbi@pitt.edu.

Notes

The authors declare no competing financial interest.

Biographies

Barry Gold received his A.B. in chemistry from Hunter College (City University of New York) and a Ph.D. in organic chemistry from the University of Nebraska—Lincoln. After completing a postdoctoral fellowship at the University of Toronto, he joined the Eppley Institute for Research in Cancer at the University of Nebraska Medical Center (UNMC) where he served as the Associate Director for Basic Research at the UNMC Eppley Cancer Center. In 2005, he relocated to the University of Pittsburgh as professor and chair of the Department of Pharmaceutical Sciences. He is a Fellow of the American Association for the Advancement of Science. His research interests are focused on the roles of DNA damage and repair in carcinogenesis.

Michael P. Stone received his B.S. in Biochemistry at the University of California, Davis, and a Ph.D. in Chemistry at the University of California, Irvine, with Philip N. Borer. After postdoctoral training at the University of Rochester with Thomas R. Krugh, he joined the faculty at Vanderbilt University, where he is professor and chair of Chemistry. His research interests include the structural consequences of DNA damage and their relationship to DNA replication and repair.

Luis A. Marky received his B.S. in chemistry from Universidad Peruana Cayetano Heredia in Lima, Peru, and a Ph.D. in chemistry from Rutgers University. He carried out his postdoctoral work in the Department of Chemistry at Rutgers University. From 1987 to 1997, he was on the faculty in the Department of Chemistry at New York University. He joined the Department of Pharmaceutical Sciences at the University of Nebraska Medical Center as a professor in 1997. He was a Fulbright Scholar in 1998. His research interests are in the biophysics of nucleic acids including thermodynamics, kinetics,

hydration, and ligand binding. Throughout his career, he has been involved in the mentoring of young scientists. Currently, he is a rotating program director in the Molecular Biophysics Cluster of the Division of Molecular and Cellular Biosciences at the National Science Foundation.

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