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Energetic Coupling Between Clustered Lesions Modulated by Intervening Triplet Repeat Bulge Loops: Allosteric Implications for DNA Repair and Triplet Repeat Expansion

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Abstract

Clusters of closely spaced oxidative DNA lesions present challenges to the cellular repair machinery. When located in opposing strands, base excision repair (BER) of such lesions can lead to double strand DNA breaks (DSB). Activation of BER and DSB repair pathways has been implicated in inducing enhanced expansion of triplet repeat sequences. We show here that energy coupling between distal lesions (8oxodG and/or abasic sites) in opposing DNA strands can be modulated by a triplet repeat bulge loop located between the lesion sites. We find this modulation to be dependent on the identity of the lesions (8oxodG versus abasic site) and the positions of the lesions (upstream versus downstream) relative to the intervening bulge loop domain. We discuss how such bulge loop-mediated lesion cross talk might influence repair processes, while favoring DNA expansion, the genotype of triplet repeat diseases.

Keywords

Abasic site; 8oxodG; Base Excision Repair (BER); Double Strand Break Repair (DSB); triplet repeat expansion; Differential Scanning Calorimetry (DSC); Nucleic Acid Thermodynamics

Introduction

The spontaneous expansion of (CNG)_n triplet repeat sequences leads to the development of a number of debilitating neurological disorders commonly referred to as DNA expansion diseases¹⁻⁵. It has been suggested that DNA expansion involves the formation, perhaps transiently, within triplet repeat domains of slipped DNA bulged loop secondary structures. Such non-canonical regions may be incorrectly processed by the DNA replication and repair machinery during *de novo* DNA synthesis.⁶⁻¹⁶ Recently it has been shown in mouse models of Huntingtons disease that oxidative damage to DNA and its repair by the base excision repair pathway (BER) can trigger expansion of CAG repeat sequences¹⁷⁻¹⁹. Repair of oxidative damage may lead to DNA expansion because the abasic site, the universal repair

intermediate of BER, favors formation of slipped DNA secondary structures, as we have previously shown²⁰.

Oxidation of guanine can form the 8oxodG base, a frequent product of oxidative damage to DNA due to ionizing radiation and/or exogenous or endogenous chemical reagents²¹. Such 8oxodG lesions generally are repaired by the base excision repair pathway (BER)²²⁻²⁵. Oxidative damage to DNA also can lead to the formation of clusters of lesions that are closely spaced in sequence and structure space²⁶⁻²⁸. Such closely spaced lesions present special challenges to the cellular DNA repair machinery in that potential lesion crosstalk may influence the local conformation and energetics of the DNA substrate for the repair machinery²⁹. In addition, binding and processing of one lesion by the various BER repair proteins may influence the ability of the repair machinery subsequently to recognize, bind, and process the second proximate damage site³⁰⁻³³.

In BER, a glycosylase excises the damaged base by N-glycosidic bond cleavage, forming an abasic site intermediate^{34,35}. Excision of the abasic site by either the lyase activity of the glycosylase itself and/or an APE nuclease (apurinic/apyrimidinic endonuclease) results in the formation of a single stranded nick or gap that is subsequently filled in by specialized repair polymerases such as pol β .³⁶⁻³⁹ Because a single strand nick or gap is a critical intermediate in BER, the presence of two or more closely spaced lesions in opposite strands can lead to double strand DNA breaks, if the intervening base paired region is thermodynamically unstable. The resulting double strand break (DSB) will require repair by the much more error prone DSB repair pathways through either homologous or nonhomologous end joining. DSB repair is known to occasionally lead to small insertions and deletions at and near the repair sites, especially when the break occurs within direct repeat sequences.⁴⁰⁻⁴³ This feature makes repair of double strand breaks at or near triplet repeat sequences a likely contributor to repeat DNA expansion and its potentiating of DNA triplet repeat expansion diseases, as has been long advocated.⁴⁴⁻⁴⁸

The above considerations suggest that clusters of oxidative lesions at or near triplet repeat domains may be particularly prone to inducing DNA expansion, either through misdirecting the BER repair process or through double strand DNA breaks that occur as a consequence of BER repair at multiple sites. To assess potential energetic origins of such coupled biological events, we have evaluated the impact of such proximal pairs of lesions/repair intermediates on the thermodynamic properties of triplet repeat DNAs. To this end, we have site specifically incorporated 8oxodG (O) and/or tetrahydrofuran (F), a stable abasic site analogue, in place of guanine residues located in opposite strands within oligonucleotide models of slipped DNA structures we previously have described^{20,49,50}. Our choice of 8oxodG is dictated by the observation that deletion of OGG1, the main glycosylase involved in 8oxodG lesion repair, significantly reduces the likelihood of DNA expansion in mouse models of Huntingtons disease¹⁷. Tetrahydrofuran (F) is a stable analogue for the unstable/reactive abasic site that is a universal intermediate in BER repair^{35,51,52}. Here we focus on lesions located in opposite strands upstream and downstream from the bulge loop domain, and we compare our results to those of the corresponding duplex absent the triplet repeat bulge loop. The model systems we have studied are outlined in scheme 1.

Based on the similarity to the Greek letter Ω when represented in two dimensions, we refer to the bulge loop containing constructs in scheme 1 as Ω -DNAs. We refer to an *upstream* lesion as one that is located in the duplex domain five base pairs 5' to the triplet repeat region, and in the same strand as the triplet repeat (Scheme 1 A). A lesion located in the duplex domain five base pairs 3' to the triplet repeat region, and in the opposite strand to the loop domain we will designate as *downstream* lesion. By analogy, and for clarity, we will maintain the same nomenclature of upstream/downstream lesion position for the 22mer

control duplex, even though in this construct the loop domain as reference point is absent (Scheme 1 B). Unless otherwise specified, we will henceforth adhere to the convention of listing the lesions in order *upstream/downstream* for all dual lesion containing constructs.

Assuming B-DNA like parameters, the upstream lesion is topologically on the opposite side of the duplex relative to the loop, whereas the downstream lesion is topologically on the same side of the duplex relative to the loop. Both lesions are separated by roughly one full helical turn, and topologically they are found on opposing sides of the duplex. These design features likely influence the physical properties of our constructs, and may play important roles in modulating recognition and binding of these substrate targets by DNA repair enzymes.

In this paper, we demonstrate that clustered lesions upstream and downstream of triplet repeat bulge loops are characterized by distinct energetic signatures, reflective of properties that likely impact recognition and repair of such lesions. Our data reveal that the triplet repeat bulge loop causes the same lesion to have a different thermodynamic impact depending on whether it is located upstream or downstream of the loop domain. We show that the presence of a bulge loop between lesion sites allows for energy coupling between distal lesions, with this coupling being mediated by the loop. Such energy coupling between distal lesions is absent in conventional duplex DNA. Thus distal lesions that behave as isolated entities in duplex DNA become energetically linked as a consequence of insertion of a bulge loop between the lesions. The presence of the bulge loop and its impact on lesion thermodynamics imposes an asymmetry/ directionality on lesion repair that is absent in conventional duplex DNA. Such thermodynamic coupling between distal lesions modulated by bulge loops may impact BER protein recognition, binding, and processing, even absent steric clashes between proteins and binding induced distortions in the DNA. Our results suggest that the order by which BER repair enzymes initially recognize, bind, and process clustered 8oxodG lesions may be critical for the outcome of the repair process if lesions are found upstream/downstream of a repeat loop domain.

Materials and Methods

Materials

Oligonucleotides were synthesized on a 10 μ mole scale by standard phosphoramidite chemistry using an Äkta DNA synthesizer, and were purified by repeated DMT on/ DMT off reverse phase HPLC, as previously described^{53,54}. To prevent loss of 8oxodG during deprotection, oligonucleotides containing 8oxodG were deprotected in 0.1 M β -mercaptoethanol/ conc. NH_4OH following the protocols of Johnson et al.^{55,56}. The purities of the oligonucleotides were assessed by analytical HPLC and ion spray mass spectroscopy, and were found to be better than 98% by mass spectroscopy. Purified oligonucleotides were dialyzed using dispo-dialyzers with MWCO 500 da (Spectrum, CA) against at least two changes of buffer containing 10 mM Cacodylic acid/Na-Cacodylate, and 0.1 mM Na_2EDTA and sufficient NaCl to yield a final concentration of 100 mM in Na^+ ions. DNA extinction coefficients of the unmodified parent sequences were determined by phosphate assay under denaturing conditions (90°C)^{57,58} and were found to be:

$\epsilon_{\text{X(CAG)6Y}}$ (260nm, 90°C) = 368400 $\text{M}^{-1} \text{cm}^{-1}$; $\epsilon_{\text{Y'(CTG)6X'}}$ (260nm, 90°C) = 342900 $\text{M}^{-1} \text{cm}^{-1}$;
 ϵ_{XY} (260nm, 90°C) = 190400 $\text{M}^{-1} \text{cm}^{-1}$; $\epsilon_{\text{Y'X'}}$ (260nm, 90°C) = 186200 $\text{M}^{-1} \text{cm}^{-1}$. For 8oxodG (O) or abasic site (F) containing oligonucleotides, extinction coefficients were determined from continuous variation titrations (Job plots)⁵⁹ with the complementary parent oligonucleotides, and were found to be $\epsilon_{\text{X(CAG)6Y-F}}$ (260nm, 90°C) = 368400 $\text{M}^{-1} \text{cm}^{-1}$;
 $\epsilon_{\text{XY-F}}$ (260nm, 90°C) = 180000 $\text{M}^{-1} \text{cm}^{-1}$; $\epsilon_{\text{Y'X'-F}}$ (260nm, 90°C) = 176000 $\text{M}^{-1} \text{cm}^{-1}$;
 $\epsilon_{\text{X(CAG)6Y-O}}$ (260nm, 90°C) = 368400 $\text{M}^{-1} \text{cm}^{-1}$; $\epsilon_{\text{XY-O}}$ (260nm, 90°C) = 190400 $\text{M}^{-1} \text{cm}^{-1}$;
 $\epsilon_{\text{Y'X'-O}}$ (260nm, 90°C) = 186200 $\text{M}^{-1} \text{cm}^{-1}$. As expected, for the 40mers, the impact of a

single 8oxodG or abasic site in place of guanine is independent of lesion position and too small to result in a measurable change in extinction coefficient compared to the X(CAG)₆Y parent 40mer.

DSC studies

DSC studies were conducted using a NanoDSCII differential scanning calorimeter (Calorimetry Science Corporation, Utah) with a nominal cell volume of 0.3 ml⁶⁰. Oligonucleotides, at a concentration of 50 μM in strand, were repeatedly scanned between 0°C and 90/95 °C with a constant heating rate of 1°C /min, while continuously recording the excess power required to maintain sample and reference cells at the same temperature. After conversion of the measured excess power values to heat capacity units and subtractions of buffer/buffer scans, the raw DSC traces were normalized for DNA concentration and analyzed using Origin software. The calorimetric enthalpy (ΔH_{cal}) was derived by integration of the excess heat capacity curve, and ΔC_p was derived from the difference in the linearly extrapolated pre- and post-transition baselines at T_m. ΔS was derived by ΔH/T_m, assuming “pseudomonomolecular” behavior in which propagation dominates initiation.⁶¹ Although our constructs formally are bimolecular complexes, their concentration dependent denaturation deviates from a molecularity of two, as generally is the case for complexes of this size⁶². The theoretical entropy correction for a strictly bimolecular reaction of 21 cal mol⁻¹ K⁻¹ at Ct = 50 μM falls within the uncertainties of our entropy values, and is the same for all our constructs. As a result, inclusion of such a molecularity contribution simply scales the magnitudes, while not altering the relative differences in ΔS and ΔG between our constructs. ΔG at the reference temperature was calculated using standard equations taking into account the nonzero heat capacity changes. The T_m is defined as the temperature at the mid point of the integrated excess heat capacity curve for a given conformational transition. At this temperature, for a process that exhibits pseudomonomolecular behavior, the sample is 50% denatured.

Analysis of experimental heat capacity curves

The experimental excess heat capacity curves of our Ω-DNA's were fit to the following model modified from that originally described by Wyman and Gill for n independent, two-state transitions.^{63,64}

$$C_p(T) = \sum_{i=1}^n C_p(T)_i + \frac{\tau^2}{R} * \sum_{i=1}^n \frac{(\Delta H(T)_i)^2 * \exp[-\Delta H(T)_i * (\tau - \tau_{mi}) / R]}{\{1 + \exp[-\Delta H(T)_i * (\tau - \tau_{mi}) / R]\}^2} \quad (\text{eq.1})$$

Equation 1 is modified from equation (5.36) given in Wyman and Gill to account for the temperature dependence of the enthalpy (ΔH(T)_i) and to take account of the contributions of the differing native and denatured heat capacity of each of the sub-transitions. The impact of strand dissociation is not considered in this model, because concentration dependent denaturation studies show the denaturation process for our Ω-DNA's to behave in a pseudomonomolecular manner. Nevertheless, we note, that strand separation may slightly impact the shape of the melting curves at high temperature.

In equation 1, $\tau = \frac{1}{T}$ is the inverse temperature at any point along the curve and $\tau_{mi} = \frac{1}{T_{mi}}$ is the inverse melting temperature of the i th component; ΔH(T)_i is the enthalpy change associated with unfolding of the i th component at temperature T determined from the enthalpy change at T_{mi} according to the standard relations:

$$\Delta H(T)_i = \Delta H(T_{mi})_i - \Delta C_{p_i} (T_{mi} - T) \quad (\text{eq.2a})$$

and

$$\Delta C p_i = C p_i^D - C p_i^N \quad (\text{eq.2b})$$

where D and N indicate denatured and native respectively.

We assume that each transition's contribution to the overall heat capacity change is proportional to its contribution to the overall enthalpy change; specifically

$$\Delta C p_i = \frac{\Delta H(T_m)_i}{\sum_{i=1}^n \Delta H(T_m)_i} \Delta C p(T^*)$$

where $\Delta C p(T^*)$ is the heat capacity change for the overall denaturation process at the

$$\text{weighted average transition temperature } T^* = \frac{\sum_{i=1}^n T_{mi} \Delta H_i}{\sum_{i=1}^n \Delta H_i}.$$

The native and denatured state heat capacities of the i th component are assumed to change linearly with temperature and are described by equation 3:

$$C p(T)_i^N = m^N T + C p(0)^N \quad (\text{eq.3a})$$

The heat capacity of the denatured state differs from that of the native state by $\Delta C p_i$ at T^* , that is $C p(T^*)_i^D = C p(T^*)_i^N + \Delta C p_i$ so

$$C p(T)_i^D = m^D (T - T^*) + C p(T^*)_i^N + \Delta C p_i \quad (\text{eq.3b})$$

The imposition of linear models for the temperature dependences of the native and denatured heat capacities with the pre- or post-transition baselines of each transition sharing a common slope m^N or m^D may appear somewhat arbitrary, however, we note that the overall experimental excess heat capacity curves outside the melting domain are best described by linear changes in native and denatured heat capacities.

From the temperature dependence of the native and denatured state heat capacities the contribution of the i th component to the heat capacity baseline, $C p(T)_i$ is calculated according to the following relation:

$$C p(T)_i = \alpha(T)_i C p(T)_i^N + (1 - \alpha(T)_i) C p(T)_i^D \quad (\text{eq.4})$$

where $\alpha(T)_i$ represents the fraction of the i th component that remains native at temperature T and is calculated according to equation 5.

$$\alpha(T)_i = \frac{\Delta H(T)_i - \left\langle \frac{\Delta H(T)_i^* \exp[-\Delta H(T)_i^* (\tau - \tau_{mi})/R]}{1 + \exp[-\Delta H(T)_i^* (\tau - \tau_{mi})/R]} \right\rangle}{\Delta H(T)_i} \quad (\text{eq.5})$$

Heat capacity curves were fit using the model described above and the Solver function in Microsoft Excel. The 2n+4 adjustable parameters in the fits were T_{mi} ; $\Delta H(T_m)_i$, $\Delta C p(T^*)$, $C p(0)^N$, m^D and m^N .

Due to the strong deviation from two-state behavior in longer duplex DNA's, only Ω -DNA heat capacity curves were analyzed using this model. We fit our experimental data to this

model for $n=1$, $n=2$ and $n=3$, and find that we can obtain good agreement between the experimental curves and the fitted curves when $n=2$ for all Ω -DNA constructs: $n=3$ does not give a statistically significant improvement in fit of the experimental parameters, whereas $n=1$ does not result in a reasonable fit to the experimental data. The results of our analysis are listed in the supplementary material in Table S1.

Results and Discussion

Global properties of double lesions in bulge loop and duplex DNA Structures: Similarities and differences

The primary focus of our current studies is the elucidation of the energy landscape of triplet repeat bulge loops and duplex DNAs containing clustered oxidative lesions that are targets for BER repair. The aim of these investigations is to define the energy states/ landscapes of the DNA substrate(s) targeted by the BER machinery. In addition to the intrinsic value of such characterizations, this “baseline” information is required for subsequent binding studies with purified BER repair enzymes. We have chosen to study the 8oxodG lesion as a frequent product of oxidative damage, and the abasic site as the universal intermediate formed during BER repair. Within our constructs, four combinations of two lesions in opposing strands with 8oxodG, F, and 8oxodG plus F lesions respectively are possible. These can be considered models for the initial damage (8oxodG/8oxodG), intermediates that can form as a consequence of BER glycosylase activity at one of the damage sites (8oxodG/F, F/8oxodG), and the products of the initial BER glycosylase activity at both damage sites (F/F). Figure 1 shows the excess heat capacity curves we have measured for all combinations of 8oxodG/ F double lesions in our Ω -DNAs, and for the 22mer control duplexes. Also shown in black are the excess heat capacity curves for the unmodified Ω -DNA and the 22mer parent molecules. The corresponding thermodynamic data are listed in Table 1 (Ω -DNA's) and Table 2 (22mers). Also included in these tables are the thermal and thermodynamic data for Ω -DNA's and 22mers with only a single 8oxodG or abasic site located either upstream or downstream. While we already have published some of the data on single lesions (single abasic site in Ω -DNA) in a different context,²⁰ we restate them here, as these results are needed to dissect and understand the impact of double lesions.

Inspection of Figure 1 reveals that the double lesions exert a significant impact on the overall thermodynamics of both the 22mer and Ω -DNA constructs. In general the presence of the lesions causes a decrease in T_m and a change in shape of the melting curve indicating a lesion induced alteration of the enthalpy change. The magnitude of the changes in T_m and enthalpy depend on the particular lesion pair and the DNA construct containing the lesion pair. As might be expected, we find that an abasic site in general exerts a much stronger impact on T_m and ΔH than an 8oxodG lesion^{51,62,65}. The absence of a base is more disruptive to DNA energetically (and structurally) than a chemical modification that does not significantly disrupt the ability of the affected base to pair with its opposing base and remain stacked within the helix. The most significant lesion induced change in melting curve occurs for the 8oxodG/F Ω -DNA lesion pair, where the single transition melting curve of the unmodified parent DNA splits into two well separated domains, with very different melting temperatures (Figure 1 A, red curve). Neither the melting curve of the reverse F/ 8oxodG lesion pair nor any of the other melting curves show a similar split into two separate melting domains for either Ω -DNA or 22mer constructs. Clearly, the 8oxodG/F Ω -DNA lesion pair is unusual and its importance will be discussed below. In general, our observations suggest that DNA conformation/topology, sequence and lesion order all contribute to the thermodynamic impact of a given lesion pair. Our results also show that lesions in Ω -DNA constructs differ from lesions in conventional duplex DNA in a number of important ways. In the following sections we will elaborate on the commonalities and

differences in the thermodynamic impact of the different lesion pairs on either the duplex or bulge loop constructs.

The impact of the lesions on the overall heat capacity change associated with the global denaturation of duplex and bulge loop DNA's is minimal

Despite differences in T_m and shape of the melting curves for the single and double lesion containing duplexes and Ω -DNA bulge loop structures studied here, there also are a number of features these constructs share in common. Most notable among those is the apparent invariance of the heat capacity change associated with global denaturation within the Ω -DNA and duplex DNA families. We find, on average, the apparent heat capacity change for the Ω -DNA constructs¹ to be $\Delta C_p = 1600 \pm 170 \text{ cal mol}^{-1} \text{ K}^{-1}$, whereas the apparent heat capacity change associated with the global melting of the corresponding 22mers is only $\Delta C_p = 800 \pm 80 \text{ cal mol}^{-1} \text{ K}^{-1}$. The latter result compares favorably with previous estimates of heat capacity changes associated conventional duplex DNA oligonucleotides⁶⁶⁻⁷¹. We also observe a more pronounced temperature dependence in the apparent native state heat capacity (C_p^{Native} apparent) of the bulge loop construct relative to the 22mer. While the presence of the bulge loop causes a doubling of the magnitude of the ΔC_p value, single base lesion type, position, or number of lesions appears to have little impact on the magnitude of the ΔC_p value in either the Ω -DNA or 22mer construct. We interpret the bulge loop induced increase in ΔC_p and stronger temperature dependence in C_p^{Native} apparent as being reflective of increased conformational flexibility/ reduced structural rigidity/ increased distribution of populated microstates in the Ω -DNA's relative to the 22mer duplexes⁷²⁻⁷⁴. The apparent lack of an appreciable impact of single base lesions on ΔC_p likely is due to the resultant heat capacity signal being too small to be measured reliably as it is expected to be on the order of the ΔC_p contribution of an average base pair ($\Delta C_p = 36 \text{ to } 70 \text{ cal mol}^{-1} \text{ K}^{-1} \text{ bp}$). We do, however, find that abasic sites cause a shift in the apparent heat capacity of the native state to less negative values in both the 22mer and Ω -DNA construct, an observation that we previously made for single abasic sites in Ω -DNA's²⁰. We propose the latter observation reflects a combination of increased conformational flexibility, reduced structural rigidity, and increased distribution of populated microstates caused by the perturbation of the DNA due to the abasic site⁷⁵. The 8oxodG lesion, which is less disruptive and maintains base pairing and stacking, does not result in such a measurable shift in native state heat capacity. Such loop and/or lesion induced differences in native state flexibility may play important roles in repair protein recognition, binding and processing of such lesions. In the following section we dissect the impact of DNA conformation on lesion thermodynamics.

The lesion induced thermal destabilization has different enthalpic and entropic origins in the 22mer duplex compared to the Ω -DNA constructs

We can isolate the impact of lesions within different DNA constructs through a two step analysis. We first relate the thermodynamic impact of the lesion within a given family of DNA constructs to the corresponding unmodified parent structure. We then use the resulting data to compare across different structures. Table 3 lists such differences in thermal and thermodynamic parameters determined at a common reference temperature of 55°C for all single and double lesions studied here. We selected 55°C as suitable reference temperature as it is close to the T_m of the most unstable 22mer construct (F/F 22mer) and near the middle of the range of T_m 's spanned by our Ω -DNA constructs. The choice of 55°C as reference temperature minimizes the need for lengthy and error prone extrapolations. Excluded from this comparison are the Ω -DNA's with an abasic site located downstream from the loop

¹Excluded in the average is the value for Ω -DNA's with a single F lesion located downstream, as the double peak and broad temperature range of the melting transition makes determination of a reliable ΔC_p value difficult.

domain, as the double peak observed for these Ω -DNA's compromises a comparison to the corresponding lesions in the 22mer duplex.

Inspection of the data listed in Table 3 reveals some clear and intriguing patterns. Note that the same lesion or pair of lesions in Ω -DNA and 22mer duplex cause roughly similar shifts in T_m (ΔT_m) and free energy ($\Delta\Delta G$) relative to their respective parent constructs. However, the thermodynamic origins of these similar T_m and ΔG shifts are quite different in the different DNA structures. Specifically, we find the enthalpic impact ($\Delta\Delta H$) for a given lesion/ lesion pair to be between two to three times larger when the lesion is located in the Ω -DNA construct compared to the corresponding lesion in the 22mer duplex. This enthalpic impact is largely compensated by a corresponding two to threefold entropy change ($\Delta\Delta S$), causing the observed similarities in ΔT_m and $\Delta\Delta G$. The difference in $\Delta\Delta H$ and $\Delta\Delta S$ for the same lesion or lesion pair in the duplex and the duplex arms of our bulge loop construct suggests propagated lesion-induced effects as opposed to localized (nearest neighbor) lesion induced perturbations⁷⁶. In other words the lesions induced perturbations are not confined to the site where the lesion is located, and reflect thermodynamic coupling between the lesion and the loop domain 5 bases upstream or downstream from the lesion site⁷⁷. Such energy coupling or allostery between a triplet repeat loop domain and lesions located upstream and/or downstream of the loop is likely to impact lesion recognition and processing by the various repair enzymes even absent direct contact between the repair enzymes and the loop domain.

Single lesions vs. double lesions—We have determined the extent to which clustered lesion differ from single lesions and to assess if it is possible to predict the thermal and thermodynamic impact of clustered lesions based solely on knowledge of the contributions of the isolated individual lesions. Figure 2A compares the excess heat capacity curve determined for the Ω -DNA construct containing an abasic site upstream/ 8oxodG downstream (F/8oxodG) to the corresponding excess heat capacity curve obtained for the Ω -DNA's with either a single abasic site upstream or a single 8oxodG lesion downstream. Also shown is the excess heat capacity curve for the unmodified parent Ω -DNA. The corresponding excess heat capacity curves for the 22mer with abasic site/ 8oxodG lesions are shown in Figure 2B. The results for 8oxodG/8oxodG, 8oxodG/F and F/F lesions in Ω -DNA's and 22mers are shown in the supplementary material. To assess the impact of the dual lesions, we calculated the thermodynamic consequences of each single lesion relative to the unmodified parent, and we compare them to the dual lesion impact at a common reference temperature (55°C). The resulting data are presented in Table 4 A and B.

Lesions one helical turn apart in duplex DNA are thermodynamically independent entities

Visual inspection of the excess heat capacity curves in Figure 2B reveals an apparent additive behavior for upstream, downstream and upstream/downstream lesions. The shift in T_m /shape of the melting curve upon substitution of G by 8oxodG in the parent 22mer (Green and Black curves) is quite similar to the shift in T_m /shape observed upon substitution of G by 8oxodG in the abasic lesion containing 22mer (Red and Blue curves). This initial impression is born out by inspection of the data in Table 4A. The thermodynamic impact of any given pair of double lesions in the 22mer can be expressed as the sum of the thermodynamic impact of the corresponding individual lesions. For example, the thermodynamic impact of F upstream/8oxodG downstream ($\Delta\Delta H=-8.3$ kcal mol⁻¹, $\Delta\Delta S=-7.8$ cal mol⁻¹ K⁻¹, $\Delta\Delta G=-5.74$ kcal mol⁻¹) is, within error margins, identical to the sum of thermodynamic impact for F upstream plus 8oxo-G downstream determined for 22mers with a single lesions ($\Delta\Delta H=(-9.6) + (1.6)=-8.0$ kcal mol⁻¹; $\Delta\Delta S=(-14.8)+(6.8)=-8.0$ cal mol⁻¹ K⁻¹; and $\Delta\Delta G=(-4.76)+(-0.62)=-5.38$ kcal mol⁻¹). In the 22mer duplex the lesions act as independent entities and do not interact with each other in thermodynamic terms.

Within the context of duplex DNA our results suggest that the thermodynamic impact of lesions separated by one full helical turn is purely local, an observation that is consistent with nearest neighbor models for duplex DNA⁷⁶. The possible exception to this generalization is the 22mer with 2 abasic sites, where the thermodynamic impact calculated as the sum of the individual abasic site contributions is larger than the measured value, a counterintuitive result. Whether this observation reflects a real reduction in thermodynamic impact due to crosstalk between lesions is currently unclear. We note that the dual abasic site significantly lowers the T_m and impacts the shape of the 22mer melting curve, in addition to causing the melting curve to overlap the unusual low temperature shoulder observed for the 22mer with F downstream.

Triplet repeat bulge loops cause lesions one helical turn apart to be thermodynamically 'aware' of each other

By contrast to the excess heat capacity curves shown in Figure 2B, those shown in Figure 2A do not appear additive, an observation that is quantified by inspection of the data in Table 4B. We find that our ability to estimate in Ω -DNA's the magnitude of the thermodynamic impact of dual lesions from contributions exhibited by individual lesions is not as good as it is for the corresponding 22mers. Characteristically the ability to estimate the impact of double lesions from the sum of individual lesions declines the larger the energy impact of individual lesions; for example contributions from pairs of 8oxodG lesions can be reasonably well estimated based on the impact of the individual single lesion, while contributions from pairs of abasic sites can not. Excluded in this comparison are Ω -DNA's with abasic sites downstream of the loop domain due to the presence of two clearly separated melting domains. The most likely reason for the apparent "non-additivity" of lesion contributions is energy coupling between the lesions that is modulated by the bulge loop in Ω -DNA's. We favor an explanation based on allosteric coupling between distal lesions, since such an explanation would also be consistent with the differences in shape of the melting curves we observe (Figure 2A). Recall, we have shown above that energy coupling between lesions and the bulge loop impacts lesion thermodynamics. It is therefore reasonable to assume that the bulge loop also mediates energy coupling between the lesions themselves, although such impact should be reduced in magnitude by the distance between the lesion sites. Such coupling should be more pronounced the larger the energetic impact of the lesions. The critical observation here is that distal lesions that behave as isolated entities in duplex DNA become energetically linked as a consequence of insertion of a bulge loop in-between the lesions, an observation that will be of importance in the following section. Further evidence in support of this view will be presented in a subsequent paper on energetic coupling /allosteric interactions between lesion in the loop and the duplex domains bordering the bulge loop.

Positional Order matters: Disparate Lesion pairs are non-commutative in Ω -DNA's whereas they are commutative in duplex DNA: The case of 8oxodG upstream /F downstream vs. F upstream /8oxodG downstream

One of the most intriguing experimental observations for our dual lesion containing Ω -DNA constructs is the differences in melting behavior of Ω -DNA containing 8oxodG upstream/F downstream (Fig 3, blue trace) compared to the Ω -DNA containing F upstream/8oxodG downstream (Fig 3, red trace). These 8oxodG/F and F/8oxodG lesion pairs containing constructs can be considered models for partial lesion repair where a glycosylase has processed/excised one 8oxodG lesion, but not the other. As is evident from Figure 3, simply reversing the order in which these two lesions are arranged within the same DNA construct results in vastly different melting behavior. Whereas only a single transition is observed for the F upstream/8oxodG downstream Ω -DNA, the 8oxodG upstream/F downstream Ω -DNA transition breaks into two well-resolved melting domains. A similar dependence of melting

behavior on lesion order is not observed in the melting curves of the 22mer duplexes which lack the loop domain. The energetics of the 8oxodG/F lesion pair in the ordinary duplex is approximately *commutative*, the energetics of the same 8oxodG/F lesion pair becomes *non-commutative* due to the presence of the intervening bulge loop. As will be discussed in more detail below, such *non-commutative* lesion energetics likely will have a significant impact on how the DNA repair machinery will process lesions in the vicinity of bulge loop structures. It also highlights the importance of DNA conformation for lesion thermodynamics, recognition, and repair. The presence of the bulge loop introduces a new element into the lesion thermodynamics and, by inference into repair enzyme recognition, binding and processing that is not found in conventional duplex DNA.

To gain further insight into the origins of the differential melting behavior of 8oxodG/F vs. F/8oxodG Ω -DNA's we have dissected both melting curves into their relevant sub-transitions. To this end we make use of a previously described statistical mechanical deconvolution model, modified from the original developed by Wyman and Gill, to resolve the experimental melting curves into two partially overlapping two-state transitions^{20,63,64}. This formalism provides good fits for the calorimetric melting curves of all Ω -DNA constructs we have studied, including the F/8oxodG and 8oxodG/F Ω -DNA's. In the 8oxodG upstream/F downstream construct, the two fitted two state transitions are well separated, while in the F upstream/8oxodG downstream construct they are not. We note, however, that the fitting results are strongly influenced by the choice of baselines. In addition, our model assumes individual sub-transitions to be noninteracting, an assumption that likely is too simplistic to fully account for experimental realities. Also, such subtransitions resolved by fitting do not necessarily reflect independently populated discrete ensembles/conformational states. Accordingly, caution should be exercised when assigning subtransitions to specific conformational states, and/or when comparing fitting results of even closely related biopolymers that exhibit significant differences in thermal behavior. That said, we find in our fitting analysis that all lesions located in the same physical domain predominantly impact the same resolved two state peak²⁰. This observation provides a strong link between the mathematically resolved calorimetric peaks and specific physical domains, thereby justifying a comparison of the fitting results for 8oxodG/F Ω -DNA and F/8oxodG Ω -DNAs, as elaborated on below.

With these caveats noted above we compare below the low and high temperature deconvoluted two state transitions for 8oxodG/F Ω -DNA and F/8oxodG Ω -DNA with the corresponding transitions in the unmodified parent Ω -DNA, making the assumption that these different fitted transitions represent related entities. We find that for the F/8oxodG Ω -DNA (apparent single peak) the energetic impact that leads to the observed $\approx 13^\circ\text{C}$ decrease in (overall) T_m relative to the parent Ω -DNA can be almost entirely ascribed to the first (low temperature) two-state transitions ($\Delta\Delta H_{(1,\text{fit})} = -29 \text{ kcal mol}^{-1}$; $\Delta\Delta S_{(1,\text{fit})} = -80 \text{ cal mol}^{-1} \text{ K}^{-1}$), whereas the decrease in T_m for the higher temperature 2-state transitions is mostly entropic in origin ($\Delta\Delta H_{(2,\text{fit})} = 0.5 \text{ kcal mol}^{-1}$; $\Delta\Delta S_{(2,\text{fit})} = 11 \text{ cal mol}^{-1} \text{ K}^{-1}$). By contrast for 8oxodG/F Ω -DNA (resolved double peak) the enthalpy and entropy of both fitted two state transitions are impacted significantly by the lesions relative to the parent Ω -DNA. ($\Delta\Delta H_{(1,\text{fit})} = -38 \text{ kcal mol}^{-1}$; $\Delta\Delta S_{(1,\text{fit})} = -99 \text{ cal mol}^{-1} \text{ K}^{-1}$; and $\Delta\Delta H_{(2,\text{fit})} = -19.4 \text{ kcal mol}^{-1}$; $\Delta\Delta S_{(2,\text{fit})} = -56.3 \text{ cal mol}^{-1} \text{ K}^{-1}$). Despite the large thermodynamic impact, the second transition is shifted only by a modest 1.4°C in T_m , whereas the first transition shows a substantial T_m shift of 26°C . The upper transition in 8oxodG/F Ω -DNA melts almost 12°C above the upper transition in the F/8oxodG Ω -DNA but is significantly less stable in terms of enthalpy ($\Delta\Delta H_{(2,\text{fit})} = -19.8 \text{ kcal mol}^{-1}$) and entropy ($\Delta\Delta S_{(2,\text{fit})} = -67.5 \text{ cal mol}^{-1} \text{ K}^{-1}$). By contrast, the lower transition in 8oxodG/F Ω -DNA melts almost 13°C lower than that of F/8oxodG Ω -DNA, although the thermodynamic impact in terms of enthalpy and entropy ($\Delta\Delta H_{(1,\text{fit})} = -8.7 \text{ kcal mol}^{-1}$ and $\Delta\Delta S_{(1,\text{fit})} = -19.7 \text{ cal mol}^{-1} \text{ K}^{-1}$) is more modest. These

values are not corrected for any ΔC_p effects because of significant uncertainty in how ΔC_p values partition between individual fitted sub-transitions. The deconvolution model assumes that the ΔC_p values for the individual subtransitions scale with the fraction of total ΔH ascribed to the subtransition. It is of interest that the low temperature transition in 8oxodG/F appears essentially identical to the low temperature transition in the single lesion Ω -DNA containing a single abasic site upstream of the loop domain, whereas the upper transitions is shifted by only 1.4°C in T_m , with no detectable enthalpy/entropy change. Comparable behavior for F/8oxodG and the single lesion Ω -DNA containing either F upstream or 8oxodG downstream is not observed. (Fig 2/fig2 supplementary material)

Based on the results discussed here it is clear that the abasic site dominates the thermodynamics of the bulge loop constructs in either orientation, with the 8oxodG lesion contributing only a small amount to the overall dual lesion energetics. However, it is also clear that no simple model can readily explain the differential impact of the order (upstream vs. downstream) of the lesion pair on bulge loop thermodynamics. Even absent a detailed understanding of the underlying causes for this differential behavior, it is apparent that the observed effects are likely to have significant biological consequences. In particular, our results suggest that the order by which BER repair enzymes initially recognize, bind, and process clustered 8oxodG lesions is critical if lesions are found upstream/downstream of a repeat loop domain. This unexpected and intriguing feature is discussed below in more detail.

Implications for the repair of double lesions—The repair of closely spaced clustered oxidative lesions provides special challenges to the BER pathway due to possible cross interactions between lesions and between repair intermediates and repair complexes^{26,30-33,78,79}. We have shown here that 8oxodG and abasic sites that are one helical turn, 10 base pairs, apart in duplex DNA behave thermodynamically as isolated entities in the absence of proteins. Our results suggest that, absent steric clashes between proteins, the BER machinery ought to be able to recognize, bind and perhaps process pairs of 8oxodG/abasic sites 1 helical turn apart in duplex DNA as separate entities. By contrast, when these same lesions are separated by DNA bulge loops they no longer are thermodynamically isolated. Our results show that the presence of a triplet repeat bulge loop between lesion sites allows for energetic coupling between the lesion and the loop site such that lesions upstream are no longer energetically independent from lesions downstream. Such energy coupling between distal lesions modulated by bulge loops may impact BER protein recognition, binding and processing even absent steric clashes between proteins and binding induced distortions in the DNA. Based on X-ray crystallographic structural data of lesion containing DNAs bound to BER enzymes, it is known that structural (and likely energetic) perturbations of the DNA in the vicinity of the lesions site are part of the recognition and repair process⁸⁰⁻⁸³. It therefore is likely that energetic coupling between lesions and bulge loops will impact the ability of the various BER enzymes to recognize, bind and process single or dual lesions in the vicinity of repeat bulge loop structures⁸⁴. Such bulge loop induced impact on BER pathways may represent one possible avenue by which repair of oxidative lesions can lead to DNA expansion in triplet expansion diseases^{17,19}. The structural or energetic impact of BER proteins binding to single and dual lesions upstream and/or downstream of the bulge loop and/or steric clashes between BER proteins as well as the consequences of steric clashes between BER proteins for repair of clustered lesion will be the subject of future investigations using purified repair proteins.

Intriguingly, our results also suggest that the order by which the BER base excision process initiates lesion repair in bulge loop DNA structures containing multiple lesions (upstream first or downstream first) will modulate lesion repair at the second lesion site. The presence of the bulge loop introduces a new element into lesion thermodynamics and repair that is

absent in conventional duplex DNA. Specifically, we have shown here that excision of 8oxodG at lesion sites downstream of the loop domain has a very different consequence for the energetics of the DNA substrate than excision of 8oxodG upstream of the bulge loop. This differential impact of the first repair step upstream or downstream on the energetics of the DNA substrate likely has consequences for subsequent repair steps, and may determine the fate of repair at either or both lesion sites. How the BER enzymes deal with the conundrum posed by dual lesions separated by bulge loops will be the subject of future studies.

Finally, we note that formation of slipped DNA structures can cause lesions that are far apart in sequence space to be brought into close physical proximity in structure space. The formation of slipped structures in repeat sequences, perhaps in response to mechanical stress due to actions of the replication or repair machinery itself⁸⁵⁻⁸⁷, may create conditions that may prove challenging for the BER machinery to process correctly. Equally important, formation of the bulge loop may lead to conditions that favor staggered double stranded DNA breaks as a consequence of the action of the BER repair machinery due to the reduced thermodynamic stability of the DNA domain separating the lesion sites. For our system, glycosylase action at both upstream and downstream lesion positions would result in a staggered double stranded break held together by at most 10 base pairs (5 bp upstream/ 5 bp downstream) and the loop domain. Experimental results with Ω -DNA of identical sequence containing upstream and downstream duplex domains of only those 5 base pairs as a model for such staggered DSB breaks reveal these constructs to be thermodynamically unstable, and to be partially melted at physiological temperatures. By contrast, the corresponding 10-mer duplex lacking the bulge loop is thermodynamically quite stable (unpublished observations). As a consequence, simultaneous BER repair of lesions separated by slipped DNA bulge loops structures may lead to enhanced levels of double stranded DNA breaks, even absent bulge loop specific miscues in the BER process.

Conclusions

In conclusion we have shown that clustered oxidative lesions and abasic sites (the universal BER repair intermediate) significantly impact the thermodynamics of DNA duplexes and triplet repeat bulge loops. Such impact on DNA thermodynamics is likely to have significant consequences on DNA repair. Specifically we have shown that triplet repeat bulge loops introduce unique features into DNA lesions thermodynamics that are not found in clustered lesions present in conventional duplex DNA. Energetic coupling between the repeat loop domain and lesions upstream and downstream lead to significant changes in lesion thermodynamics and may impact lesion recognition and processing by relevant repair enzymes. The bulge loop causes the same lesion to have a different thermodynamic impact depending on whether it is located upstream or downstream of the loop domain. More importantly, the triplet repeat bulge loop causes the energetics of a lesion to be influenced by a second unrelated lesion that is far away in sequence space, but not in structure space. The presence of the bulge loop and its impact on lesion thermodynamics imposes an asymmetry / directionality on lesion repair that is absent in conventional duplex DNA. Such asymmetry is likely to pose additional challenges for the DNA repair machinery optimized for processing lesions in conventional duplex DNA. The findings reported here provide insights as to why the repair of oxidative lesions at/near triplet repeat domains may lead to faulty repair and ultimately DNA expansion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Experimentally measured excess heat capacity curves for Ω -DNAs (A) and 22mers (B) with pairs of 8oxodG and/or abasic sites located upstream and downstream of the loop domain. The homo lesion pairs are shown in magenta (8oxodG/8oxodG) and green (F/F), whereas the hetero lesion pairs 8oxodG/F and F/8oxodG are shown in red and blue, respectively. Note the significant differences in shape of the excess heat capacity curves for the 8oxodG/F Ω -DNA compared to the F/8oxodG Ω -DNA. The excess heat capacity curve for the lesion free parent is shown in black for comparison.

Figure 2.

Comparison of the experimentally measured excess heat capacity curves for Ω -DNA's (A) and 22mers (B) with single lesions with the excess heat capacity curve for the corresponding double lesion construct. Figure 2 compares the measured excess heat capacity curves for a single abasic site upstream (red), a single 8oxodG lesion downstream (green) with the excess heat capacity curve for the hetero lesion construct with abasic site upstream/8oxodG lesion downstream (blue). The excess heat capacity curve measured for the lesion free parent construct is shown in black for comparison. The corresponding comparisons for 8oxodG/8oxodG, 8oxodG/abasic site and abasic site/abasic site pairs are shown in the supplementary material.

Figure 3.

Comparison of the experimental excess heat capacity curves measured for the 8oxodG/F (blue) and F/8oxodG (red) hetero lesion pairs in Ω -DNA (A) and 22mer constructs (B). Note that the lesion pairs in the 22mer behave essentially *commutative*, whereas in the Ω -DNA constructs the lesion pairs are clearly *non-commutative*. Such difference in lesion properties due to the absence or presence of an unrelated DNA structural element (the bulge loop) is likely to have significant implications for the biological fate of clustered lesions and their repair.

Scheme 1.

Schematic representation of the Ω -DNA (B) and 22mer (A) constructs we have studied. The position of the lesion, which can be either 8oxodG (O) or an abasic site (F), is indicated by the letter X. A lesion 5' of and in the same strand as the CAG repeat bulge loop is referred to as an upstream lesion, a lesions 3' of the CAG repeat bulge loop and in the opposing strand is referred to as downstream lesion. Lesions are listed in order upstream/downstream. Two 8oxodG or abasic sites upstream and downstream represent a homo-lesion pair, whereas a mixture of one 8oxodG and one abasic site represent a hetero-lesion pair. For simplicity we maintain the same upstream/downstream nomenclature for the 22mer duplexes even absent the repeat loop domain by reference to the strand/sequence which contains the lesion. Although our schematic represents the repeat loop domain as unstructured, experimental evidence suggests that the loop bases adopt a highly structured /base paired conformation. The nature of the pairing interactions in the loop and at the loop duplex junction is unknown.

Table 1

Thermodynamic Properties of Omega DNA sequences

Sample	T _m [°C]	ΔH _{cal} [kcal mol ⁻¹]	ΔS _{cal} [cal mol ⁻¹ K ⁻¹]	ΔC _p [cal mol ⁻¹ K ⁻¹]	ΔG _{cal} [kcal mol ⁻¹]
Parent	62.3 ± 0.3	171.6 ± 8.5	511.5 ± 25.5	1550 ± 230	15.7 ± 0.9
O-Upstream	61.8 ± 0.3	174.1 ± 8.7	519.7 ± 26.0	1772 ± 265	15.4 ± 0.9
O-Downstream	59.3 ± 0.3	171.3 ± 8.5	515.3 ± 25.7	1736 ± 260	14.5 ± 0.9
F-Upstream	54.0 ± 0.3	141.7 ± 7.0	433.0 ± 21.6	1187 ± 178	11.0 ± 0.7
F-Downstream ¹	65.9 ± 0.3 36.6 ± 0.3	131.2 ± 6.6	386.8 ± 19.3	N/D	(14.3)
O-Upstream O-Downstream	59.2 ± 0.3	170.4 ± 8.5	512.5 ± 25.0	1680 ± 250	14.4 ± 0.9
O-Upstream ² F-Downstream	65.0 ± 0.3 36.6 ± 0.3	130.4 ± 6.5	385.6 ± 21.1	N/D	(13.6)
F-Upstream O-Downstream	51.7 ± 0.3	139.8 ± 7.0	430.2 ± 22.3	1643 ± 246	9.6 ± 0.6
F-Upstream F-Downstream	40.0 ± 0.3	107.9 ± 5.4	344.5 ± 17.2	1495 ± 220	4.6 ± 0.4

¹Two well resolved transitions. The ΔH, ΔS and ΔG values are derived for the combined transitions, the ΔG value is calculated without ΔC_p correction and listed in parenthesis

²Two well resolved transitions. The ΔH, ΔS and ΔG values are derived for the combined transitions, the ΔG value is calculated without ΔC_p correction and listed in parenthesis

Table 2

Thermodynamic Properties of 22mer control sequences

Sample	T_m [°C]	ΔH_{cal} [kcal mol ⁻¹]	ΔS_{cal} [cal mol ⁻¹ K ⁻¹]	ΔC_p [cal mol ⁻¹ K ⁻¹]	ΔG_{cal} [kcal mol ⁻¹]
22mer	79.1 ± 0.3	172.2 ± 2.6	488.8 ± 24.4	822 ± 120	22.8 ± 1.3
O-Upstream	78.4 ± 0.3	172.5 ± 1.5	490.7 ± 24.5	799 ± 120	22.7 ± 1.3
O-Downstream	77.6 ± 0.3	171.1 ± 3.1	487.8 ± 24.4	756 ± 113	22.5 ± 1.3
F-Upstream	69.6 ± 0.3	154.6 ± 1.6	451.0 ± 22.6	811 ± 120	17.6 ± 1.1
F-Downstream	72.2 ± 0.3	146.9 ± 0.6	425.4 ± 21.2	931 ± 139	16.9 ± 1.0
O-Upstream O-Downstream	76.8 ± 0.3	175.2 ± 3.5	500.6 ± 25.0	833 ± 124	22.5 ± 1.3
O-Upstream F-Downstream	70.9 ± 0.3	145.5 ± 2.9	422.9 ± 21.1	746 ± 111	17.0 ± 1.1
F-Upstream O-Downstream	67.3 ± 0.3	151.9 ± 3.0	446.2 ± 22.3	636 ± 95	17.1 ± 1.1
F-Upstream F-Downstream	56.8 ± 0.3	131.2 ± 2.6	397.6 ± 18.4	818 ± 122	11.3 ± 0.7

Table 3

Differential thermodynamic parameters at 55°C

Lesion Type and Position		ΔT_m [°C]	$\Delta\Delta H_{cal}$ [kcal mol ⁻¹]	$\Delta\Delta S_{cal}$ [cal mol ⁻¹ K ⁻¹]	$\Delta\Delta G_{cal}$ [kcal mol ⁻¹]
O - Upstream	22mer	-0.7	+1.4	+5.0	-0.2
	Ω -DNA	-0.5	+1.7	+5.8	-0.2
O - Downstream	22mer	-1.5	+1.6	+6.8	-0.6
	Ω -DNA	-2.9	+3.5	+15.2	-1.4
F - Upstream	22mer	-9.5	-9.6	-14.8	-4.7
	Ω -DNA	-8.2	-17.5	-41.1	-4.0
F - Downstream	22mer	-6.9	-21.5	-52.8	-4.2
	Ω -DNA ³	+3.6/-25.7	-35.4	-109.5	0.5
O - Upstream· O - Downstream	22mer	-2.3	4.6	16.5	-0.7
	Ω -DNA	-3.1	2.9	13.7	-1.5
O - Upstream· F - Downstream	22mer	-8.2	-18.7	-42.9	-4.6
	Ω -DNA ⁴	+2.7/-25.2	-37.2	-114.0	-0.1
F - Upstream· O - Downstream	22mer	-11.8	-8.3	-7.8	-5.7
	Ω -DNA	-10.6	-15.1	-30.8	-5.0
F - Upstream· F - Downstream	22mer	-22.3	-22.6	-37	-10.3
	Ω -DNA	-22.3	-30.0	-63.2	-9.3

³Two well resolved transitions. $\Delta\Delta H$, $\Delta\Delta S$ and $\Delta\Delta G$ values are calculated for the overall transition, allowing for considerable uncertainty due to unknown ΔC_p effects

Table 4A

Differential Thermodynamic Parameters at 55°C

Lesion Type and Position		$\Delta\Delta H_{cal}$ [kcal mol ⁻¹]	$\Delta\Delta S_{cal}$ [cal mol ⁻¹ K ⁻¹]	$\Delta\Delta G_{cal}$ [kcal mol ⁻¹]
22mer				
O - Upstream		1.4	5.0	-0.24
O - Downstream		1.6	6.8	-0.62
F - Upstream		-9.6	-14.8	-4.75
F - Downstream		-21.5	-52.8	-4.17
O - Upstream	Measured	4.6	16.5	-0.75
O - Downstream	Summed	3.0	11.9	-0.86
	Difference	1.6	-4.5	0.12
O - Upstream	Measured	-18.7	-43.0	-4.64
F - Downstream	Summed	-20.0	-47.7	-4.41
	Difference	1.3	4.7	-0.22
F - Upstream	Measured	-8.3	-7.8	-5.74
O - Downstream	Summed	-8.0	-8.0	-5.38
	Difference	-0.3	0.2	-0.35
F - Upstream	Measured	-22.6	-37.4	-8.93
F - Downstream	Summed	-31.1	-67.6	-8.47
	Difference	8.4	30.2	-1.44

Table 4B

Differential Thermodynamic Parameters at 55°C

Lesion Type and Position		$\Delta\Delta H_{cal}$ [kcal mol ⁻¹]	$\Delta\Delta S_{cal}$ [cal mol ⁻¹ K ⁻¹]	$\Delta\Delta G_{cal}$ [kcal mol ⁻¹]
Ω -DNA				
O - Upstream		1.7	5.8	-0.19
O - Downstream		3.5	15.2	-1.44
F - Upstream		-17.5	-41.1	-4.01
F - Downstream		-35.5	-109.5	0.51
O - Upstream·	Measured	3.0	13.7	-1.50
O - Downstream	Summed	5.3	21.0	-1.63
	Difference	-2.3	-7.4	0.13
O - Upstream·	Measured	-37.3	-114.0	0.13
F - Downstream	Summed	-33.7	-103.7	0.31
	Difference	-3.5	-10.3	-0.18
F - Upstream·	Measured	-15.1	-30.8	-5.04
O - Downstream	Summed	-13.9	-25.9	-5.44
	Difference	-1.2	-4.9	-0.4
F - Upstream·	Measured	-30.0	-63.1	-9.29
F - Downstream	Summed	-52.9	-150.6	-3.50
	Difference	22.9	87.5	-5.79