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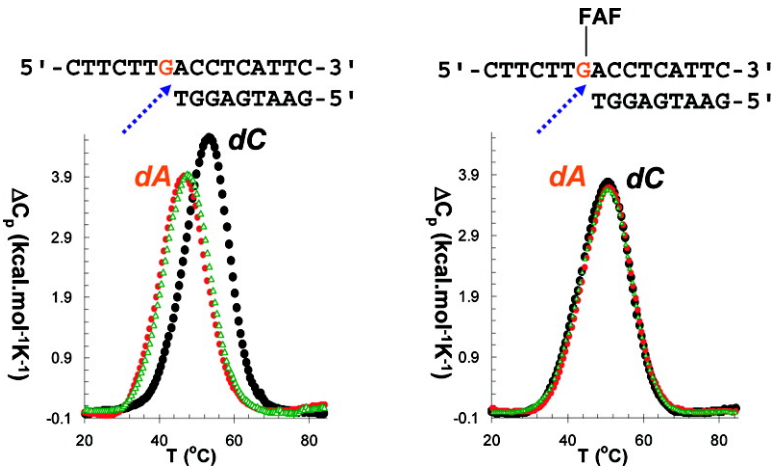

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Probing the Thermodynamics of Aminofluorene-Induced Translesion DNA Synthesis by Differential Scanning Calorimetry

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Accurate DNA replication is essential for maintaining genomic stability.[1] When a base mismatch or a mutagenic lesion is encountered at a replication fork, the polymerase stalls, which severely compromises the efficiency of nucleotide insertion,[2] and the effect lingers to several 5′-downstream nucleotides.[3] The resulting stalled replication forks are sensed and dealt with specialized bypass polymerases.[4] Although the mechanisms of mutagenesis have been studied extensively, the energetic aspects underlying the replication fidelity and the long-range effects of the mutation on translesion synthesis have not been resolved.[5] The enthalpy involved in an enzyme-directed insertion event has been shown to be similar to that involved in base pair addition, as predicted by nearest neighbor data derived from melting studies.[5c] Here, we used differential scanning calorimetry (DSC) to measure the thermodynamic changes associated with translesion synthesis across AF, [*N*-(2′-deoxyguanosin-8-yl)-2-aminofluorene], a major DNA adduct derived from the carcinogen 2-aminofluorene.[6] Two sets of successive template primers were designed to simulate AF translesion synthesis. The model probe FAF[7] was paired with matched dC and mismatched with dA (designated as the dC-match and dA-mismatch series, respectively) (Figure 1).

Figure 2a compares the DSC melting curves for the dC-match series. As expected, the melting point (T_m) and enthalpy (ΔH) values of the control series increased incrementally with primer extension. For example, insertion of a matched dC base at the primer terminus (n) resulted in significant gains in ΔH (7.6 kcal/mol), ΔG_{37} (1.6 kcal/mol), and T_m (5.9 °C), and the trend continued to $n + 6$, with all of the pairs from $n + 1$ to $n + 6$ comprising Watson−Crick base pairs (Table 1). The results were comparable to those values estimated by the nearest-neighbor parameters (Table S1, Supporting Information).[8] In contrast, incorporation of dC opposite the FAF lesion showed little change in the melting thermodynamics, and the impact persisted to $n + 2$. This long-range effect is in accord with previous steady-state kinetic results by Miller and Grollman[3] which showed significant reductions (10^2−10^6) in incorporation rates around the lesion and several 5′-downstream sites. The lesion at n may interfere with the ability of the modified dG to form Watson−Crick base pairs, thereby reducing overall fidelity of replication. While there was considerable entropy compensation (Table 1),[5a] the data showed the enthalpy terms are primarily responsible for the adduct-induced thermal and thermodynamic destabilization.

An identical set of measurements was obtained for the dA-mismatch series (Figure 2b). Unlike the dC-match cases above, addition of dA at n resulted in reduction of ΔH, ΔG_{37}, and T_m by 1.5 kcal/mol, 0.1 kcal/mol, and 0.9 °C, respectively. Although Watson−Crick base pairs followed the mismatch site, the ΔH values during the $n - 1$ to $n + 2$ extension were increased by only 5.6 kcal/mol, and the normal trend resumed at $n + 3$. This is clearly in contrast to the value (∼20 kcal/mol) observed with the

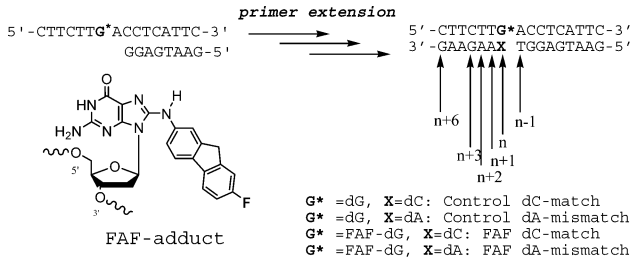

Figure 1. (a) Structure of FAF adduct, [*N*-(2′-deoxyguanosin-8-yl)-7-fluoro-2-aminofluorene]. (b) Template primer sequences; *n* is the site of a lesion or a mismatch. See Supporting Information Figures S1−5.

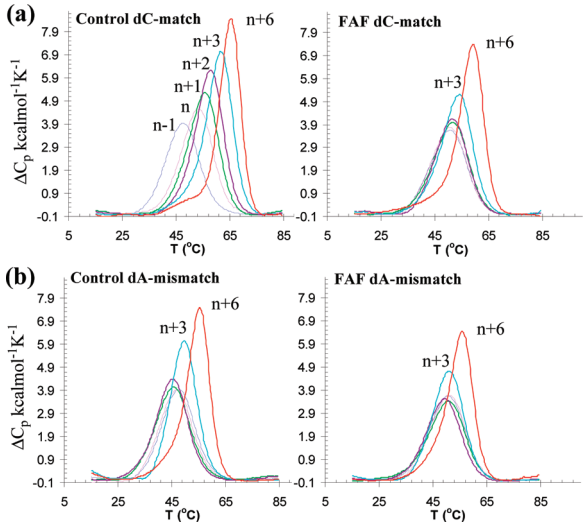

Figure 2. Differential scanning calorimetry (DSC) curves in 20 mM phosphate buffer containing 0.1 M NaCl at pH 7. The total heat of the helix-coil transition of (a) dC-match, (b) dA-mismatch template primer series was measured and the excess heat capacity ΔC_p^{ex} is plotted versus temperature.

aforementioned control dC-match series (Table 1). Gibbs free energy ΔG_{37} and T_m from $n - 1$ to $n + 2$ were also decreased by 0.2 kcal/mol and 2.2 °C, respectively. These results indicate the complexity of a G:A mismatch in terms of H-bonding, stacking, geometric fit, and cooperativity with neighboring base pairs.[9] Johnson and Beese[10] have termed this phenomenon a "short-term memory effect" of replication errors. A similar distortion effect has also been observed in RNA.[11] Interestingly, FAF adduction did not alter significantly the overall DSC profiles (Figure 2b) and resulted in consistent increases in thermal stabilities (2.1−5.0 °C) between $n - 1$ and $n + 2$ relative to the controls.

Stacking is an important contributor to the thermal and thermodynamic stabilities of a DNA duplex.[12] In the present case, the interaction between the bulky AF and neighboring bases could be modulated by both favorable stacking and unfavorable steric

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Table 1. Thermodynamic Parameters Derived from DSC Experiments for Simulated AF-Induced Translesion DNA Synthesis[a]

sequence	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (eu)	$-\Delta G_{37}°$ (kcal/mol)	T_m[b] (°C)
$n-1$	60.8 (61.6)	168.2 (172.5)	8.6 (8.0)	50.6 (47.5)
		dC-match		
n	61.2 (69.2)	169.4 (192.3)	8.7 (9.6)	50.7 (53.4)
$n+1$	64.8 (76.8)	180.0 (214.0)	9.0 (10.4)	51.5 (55.6)
$n+2$	65.6 (83.4)	182.6 (232.7)	9.0 (11.2)	51.3 (57.9)
$n+3$	77.4 (92.4)	216.9 (256.5)	10.1 (12.9)	54.1 (61.6)
$n+6$	93.2 (110.3)	260.8 (306.1)	12.4 (15.4)	59.2 (65.5)
		dA-mismatch		
n	59.7 (60.1)	164.5 (168.4)	8.7 (7.9)	51.1 (46.6)
$n+1$	58.4 (64.9)	160.8 (184.0)	8.5 (7.8)	50.7 (45.7)
$n+2$	58.0 (67.2)	160.3 (191.5)	8.3 (7.8)	49.4 (45.3)
$n+3$	68.8 (76.5)	192.7 (217.4)	9.0 (9.0)	50.9 (49.6)
$n+6$	81.1 (86.9)	227.0 (245.0)	10.7 (10.9)	55.7 (55.3)

[a] Values of unmodified control duplex are given in parentheses. Standard deviations for $\Delta H°$, $\Delta S°$, $\Delta G_{37}°$, and T_m are ±2 kcal/mol, ±6 eu, ±0.3 kcal/mol, and ±0.2 °C, respectively. [b] At 0.1 mM.

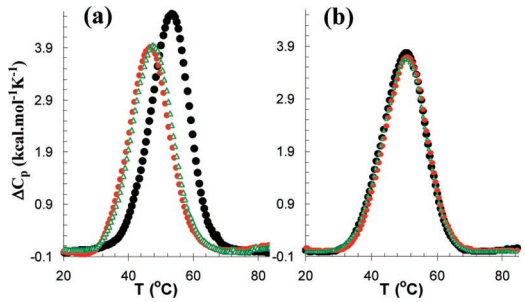

Figure 3. DSC curves of an $n-1$ (green) → n transition with insertion of dC (black), and dA (red) opposite (a) unmodified and (b) FAF-modified G at the primer terminus junction (n).

interactions.[9a] As such, AF induced only a slight change in melting enthalpy (Table 1) at the $n-1$ and n positions but substantially changed melting enthalpy (6−9 kcal/mol) from $n+1$ to $n+6$. This suggests that the entropy around the lesion plays a critical role in the increased adduct-induced thermodynamic stability.

The above DSC results, albeit with no polymerases involved, suggested the possibility of a significant thermodynamic contribution that distinguishes between dCTP and dATP insertion opposite the lesion. Figure 3a and b shows the calorimetric profiles measured for nucleotide insertion at n for the unmodified control and FAF-modified series, respectively. Without the lesion, the addition of a matched dC base was clearly favored over insertion of dA ($\Delta\Delta G° = 1.7$ kcal/mol) (Figure 3a, Table S2, Supporting Information). These values were larger than those reported previously (0.1−1.0 kcal/mol) in various sequence contexts.[13] The discrimination enthalpy difference $\Delta\Delta H°$ was 9.1 kcal/mol with considerable enthalpy−entropy compensation ($\Delta\Delta S° = 23.9$ eu) (Table S2, Supporting Information). It has been proposed that the effect of enthalpy−entropy compensation is suppressed in the enzyme catalytic pocket; if so, then insertion preference is primarily modulated by the enthalpy term.[13] Therefore, our findings are in accord with the generally low dA misinsertion mutational specificity (~0.5%) detected for unmodified controls.[14] In contrast, the FAF-modified template (Figure 3b) under the same experimental conditions resulted in comparable thermodynamic parameters ($\Delta\Delta G° \sim 0$ kcal/mol) regardless of whether dC or dA was inserted at n. Shibutani et al.[14] have shown that a single AF lesion in simian COS cells produces a wide range (2−59%) of sequence-dependent G → T mutational specificity, the most targeted base mutation by this lesion.

In summary, we have demonstrated the utility of DSC methods for evaluating the detailed thermodynamics of translesion synthesis across carcinogen-induced lesions. The thermodynamic paucity generated by either a lesion or mismatch might be responsible for the short-term memory effects observed with replicative polymerases.[3,7c] The equilibrium thermodynamic data also provide insight into the most prevalent dA mismatch induced by AF. However, kinetic effects undoubtedly play a key role in the processing of this bulky lesion by polymerases. The nature of the polymerase is likely to govern and to determine the balance between kinetic and thermodynamic effects. This is a significant future challenge, and the current findings provide the key data for such future studies.

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Supporting Information Available: Experimental procedures (Figures S1,2), mass characterization (Figures S3−5), plots (Figures S6−19) and tables (Tables S1,2) of thermodynamic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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