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Base Flipping Free Energy Profiles for Damaged and Undamaged DNA

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Abstract

Lesion-induced thermodynamic destabilization is believed to facilitate β-hairpin intrusion by the human XPC/hHR23B nucleotide excision repair (NER) recognition factor, accompanied by partner-base flipping, as suggested by the crystal structure of the yeast orthologue (Min, J. H., and Pavletich, N. P. (2007) *Nature 449*, 570–575). To investigate this proposed mechanism, we employed umbrella sampling to compute partner base flipping free energies for the repair susceptible 14*R* (+)-*trans-anti*-DB[*a,l*]P-N^2-dG modified duplex 11-mer, derived from the fjord region polycyclic aromatic hydrocarbon dibenzo[*a,l*]pyrene, and for the undamaged duplex. Our flipping free energy profiles show that the adduct has a lower flipping barrier by ~7.7 kcal/mol, consistent with its thermally destabilizing impact on the damaged DNA duplex and its susceptibility to NER.

Nucleotide excision repair (NER) is a key cellular defense against bulky DNA lesions generated by polycyclic aromatic hydrocarbons (1). However the recognition mechanism of such lesions in successful NER is not well-understood. Here, we hypothesize that local thermodynamic destabilization is a hallmark of NER susceptibility, and that lesion partner-base flipping would be more facile in destabilizing repair-susceptible lesions than in unmodified DNA. This hypothesis is supported by the crystal structure of a truncated form of Rad4/Rad23, the *S. cerevisiae* homolog of XPC/hHR23B (the human global genomic NER recognition factor) (2), complexed with an oligonucleotide containing a cyclobutane pyrimidine dimer lesion. This structure revealed a β-hairpin of Rad4 to be inserted into the DNA helix; the lesion was separated from the unmodified strand and the two partner bases were flipped into the protein (2) (crystal structure shown in Figure S1, Supporting Information). It is notable that a β-hairpin insertion and base flipping recognition strategy is also utilized by the prokaryotic NER recognition factor UvrB (3,4).

To test this hypothesis, we have investigated a DNA lesion derived from dibenzo[*a,l*]pyrene (5), 14*R* (+)-*trans-anti*-DB[*a,l*]P-N^2-dG (14*R*-dG*) (Figure 1a). This adduct is highly repair-susceptible in the human HeLa cell extract assay ((6) and Geacintov et al. manuscript in preparation). Furthermore, the 14*R*-dG* duplex 11-mer is thermally destabilizing: ΔTm, the difference between the melting temperature of the unmodified and the damaged duplex, is ~ −9 °C (7). We determined the flipping free energy profile of the lesion partner base in the sequence shown in Figure 1b. For comparison, we also investigated the unmodified duplex.

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Supporting Information Available: Further experimental data, computational details, and additional data analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

The NMR solution structure of the 14*R*-dG* duplex 11-mer (7) adopts a classical intercalation structure with Watson-Crick hydrogen bonding maintained at the lesion site. Intercalation is from the minor groove on the 3' side of the modified strand in the 14*R*-dG* duplex 11-mer (Figure 1c).

Base-flipping free energy profiles have been investigated computationally (8–11) for unmodified DNA, but to our knowledge not for systems involving bulky DNA lesions. The goal of our work was to obtain a complete free energy profile for the flipping of the partner base C17 to the 14*R*-dG* (Figure 1c) and the C17 of the unmodified duplex (Figure 1d). We wish to elucidate the relationship between the barrier for flipping in the modified and unmodified duplexes, and relate the results to thermodynamic destabilization and NER.

The initial structure for the 14*R*-dG* duplex 11-mer was the NMR solution structure (7), and the unmodified duplex was constructed with InsightII from Accelrys Inc. Both modified and unmodified 11-mers were subjected to 2ns of unrestrained MD simulations for the subsequent free energy profile calculations. These structures are shown in Figure 1c and Figure 1d respectively. Next, utilizing a restraint force constant of 10,000 kcal/(mol×radian^2), 0.5 ps simulations were performed serially for 72 windows at 5 degree intervals of the reaction coordinate (Figure 2) as the partner base cytosine is extruded, employing the last structure of the previous window to initialize the current window. This protocol provided the structures to begin the 3ns MD simulations for sampling each umbrella window. The Amber 10 software package (12) modified for base flipping along the pseudo-dihedral angle reaction coordinate (9,13) (definition shown in Figure S2, Supporting Information) was employed. Random snapshots from the MD simulations of the 14*R*-dG* duplex 11-mer along different reaction coordinate values (−180°, −90°, 0°, 90°) are depicted in Figure 2. The free energy profiles were computed with data collected between 1 and 3ns with 1.0 fs time step. Thus, a total of 2×10^6 structures were utilized to compute the free energy profile for each window, and for the 72 windows, the total number of structures was 1.44×10^8. The statistical errors were calculated based on the differences among different time periods (1 – 2ns versus 1 – 3ns, 2 – 3 ns versus 1 – 3ns, Figure S3, Supporting Information.) The force constants for the 72 windows were in the range of 50 to 100 kcal/(mol×radian^2) to ensure sufficient overlap between the windows. The free energy profiles along the reaction coordinate were obtained with the weighted histogram analysis method (WHAM) (14). The Cornell et al. force field (15), the parm99 parameter set (16), the parmbsc0 DNA parameters (17), and force field parameters developed for the 14*R*-dG* adduct were utilized, together with explicit solvent and counterions. Full details of the parameter development and the force field parameters for the lesion, as well as all other protocols are given in the Supporting Information.

The calculated free energy profiles for the 14*R*-dG* and unmodified duplex 11-mers are shown in Figure 3, which have converged reasonably well (details shown in Figure S3, Supporting Information). For the unmodified duplex 11-mer, the calculated free energy barrier is 18.1 ± 0.8 kcal/mol. This is in a similar range to the barriers computed in previous studies (~ 15 to 20 kcal/mol) (8,10,11). For the 14*R*-dG* duplex 11-mer, the calculated free energy barrier is 10.4 ± 0.6 kcal/mol.

Thus, the free energy barrier difference between the unmodified and the damaged duplex is ~ 7.7 kcal/mol, with the lower barrier for the repair susceptible 14*R*-dG* duplex 11-mer. This corresponds to a flipping rate that is ~400,000-fold higher for the C17 in the 14*R*-dG* than the unmodified case. We note that the profiles differ in shape depending on the flipping direction, but the barriers scarcely depend on the direction.

The significantly lower barrier for flipping the C17 base-partner to the 14*R*-dG* adduct than for the C17 in the unmodified duplex is consistent with the thermally destabilizing impact of the lesion on the duplex melting temperature; the destabilization stems from distortions due to the intercalation of the bulky aromatic ring system from the narrow minor groove side of the B-DNA duplex. The specific origins of the free energy profile differences between the adduct and the unmodified DNA may stem from differences in Watson-Crick hydrogen bonding, stacking interactions, and solvation effects, which are under investigation.

In conclusion, our free energy profiles reveal that the repair-susceptible 14*R*-dG* duplex 11-mer has a barrier for flipping of the partner-base C17 of ~ 10.4 kcal/mol while the barrier for the unmodified duplex is ~ 18.1 kcal/mol, which is ~ 7.7 kcal/mol higher. This lends support to the hypothesis that the thermodynamic destabilization of the 14*R*-dG* duplex 11-mer observed in thermal melting data is associated with enhanced flippability, which we hypothesize facilitates repair. We are currently investigating our further hypothesis that the repair-resistant 14*R* (+)-*trans-anti*-DB[*a,l*]P-N^6-dA (14*R*-dA*) adduct (6,18), which is thermally stabilizing by ~ 8 °C (19), manifests a higher flipping free energy barrier than the repair-susceptible 14*R*-dG* adduct. Repair resistant bulky lesions likely contribute significantly to cancer initiation via mutations by error-prone bypass polymerases during replication (20).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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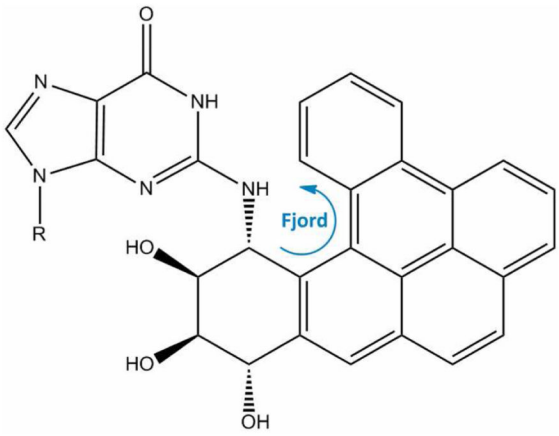
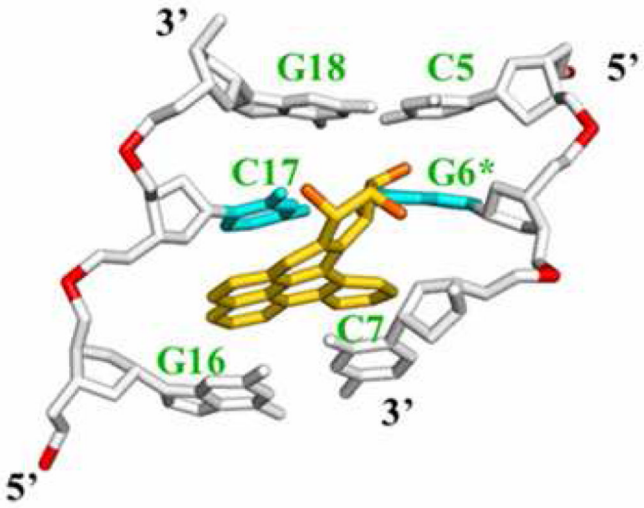
a.



b.

5’- C_1 C_2 A_3 T_4 C_5 G_6*C_7 T_8 A_9 C_{10} C_{11}-3’
3’-$G_{22}$$G_{21}$$T_{20}$$A_{19}$$G_{18}$ $C_{17}$$G_{16}$$A_{15}$$T_{14}$$G_{13}$ G_{12}-5’

c.



d.

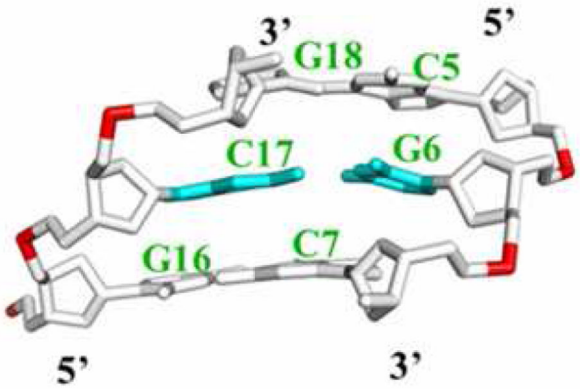


Figure 1.
(a) Chemical structure of the 14*R*-dG* adduct. (b) Sequence of the 14*R*-dG* duplex 11-mer. (c) Central trimer of the 14*R*-dG* duplex 11-mer classical intercalation structure (7). Intercalation is from the minor groove, and the view is into the minor groove. The DB[*a,l*]P ring system is shown in yellow. (d) Central trimer of the unmodified duplex 11-mer. The flipping base is C17.

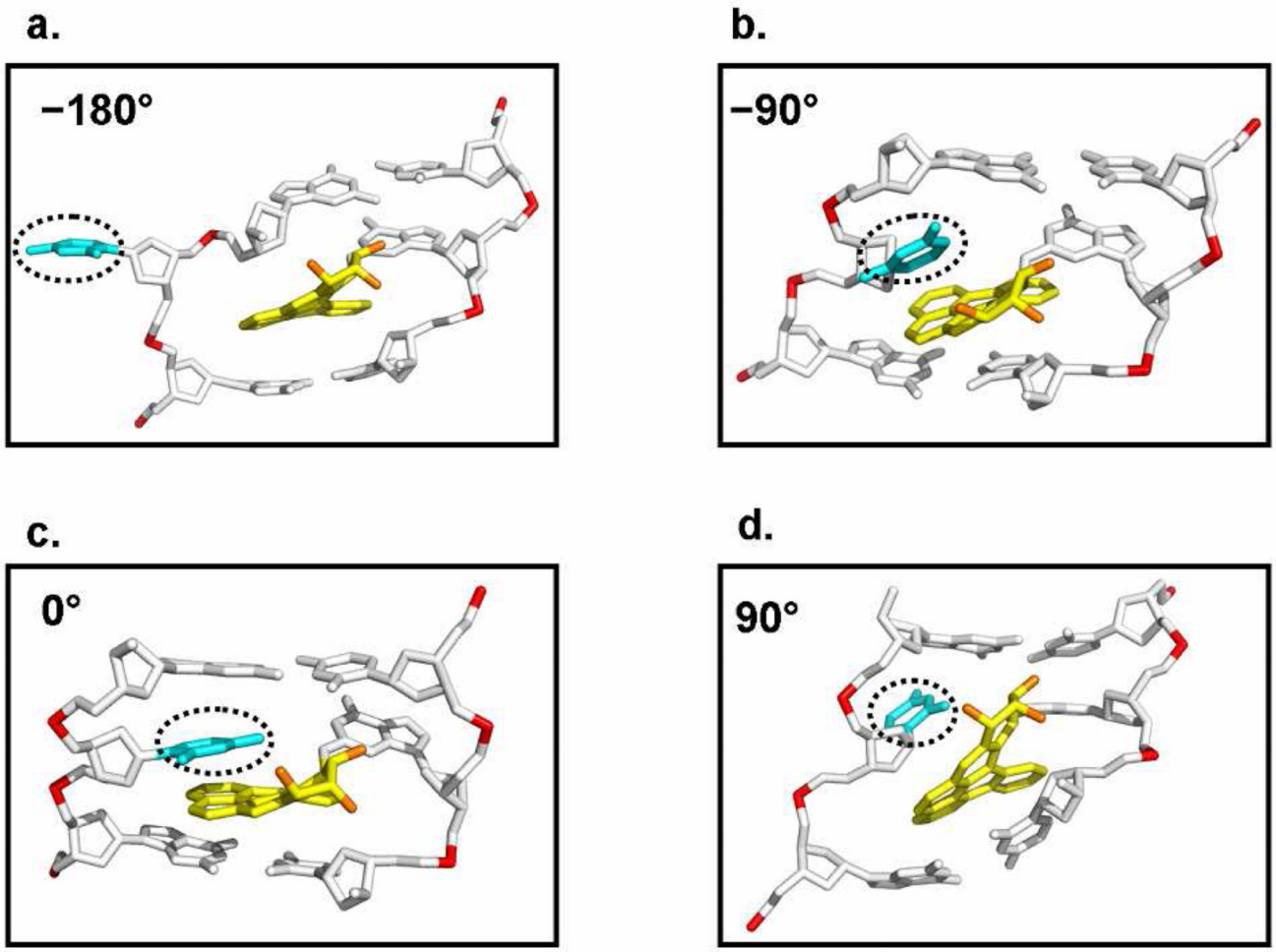


Figure 2.

Snapshots of the 14*R*-dG* duplex 11-mer along different reaction coordinate values. These are random snapshots from the MD simulations which illustrate the particular reaction coordinate values: (a) pseudo-dihedral = −180°. (b) pseudo-dihedral = −90°. (c) pseudo-dihedral = 0°. (d) pseudo-dihedral = 90°. See Figure S2 in Supporting Information for the definition of the pseudo-dihedral angle reaction coordinate. The flipping C17 is circled.

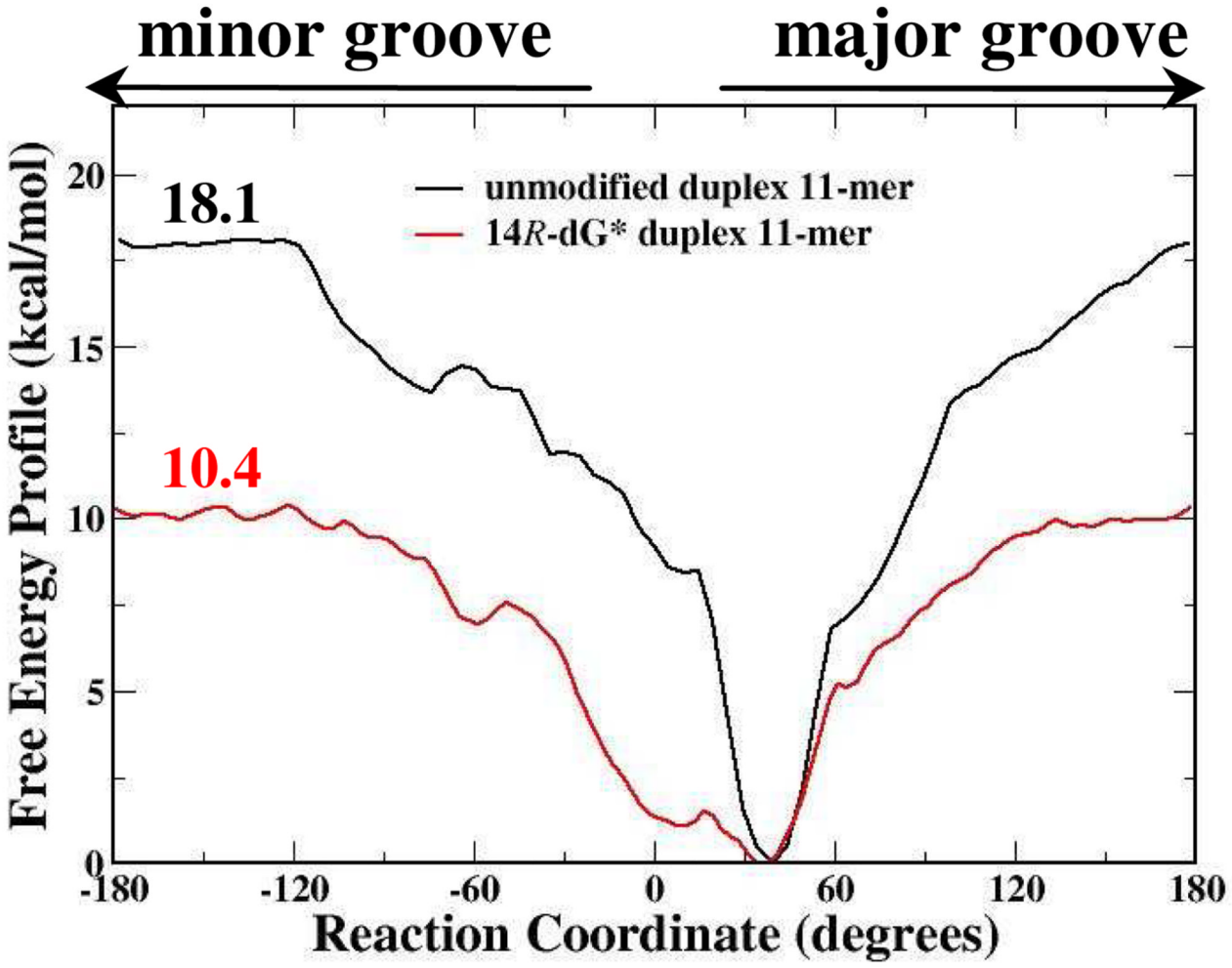


Figure 3.
Calculated free energy profiles for flipping of the C17 base in the 14*R*-dG* duplex 11-mer (red) and the unmodified duplex (black). The barrier difference is ~ 7.7 kcal/mol.