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Differences in the Access of Lesions to the Nucleotide Excision Repair Machinery in Nucleosomes

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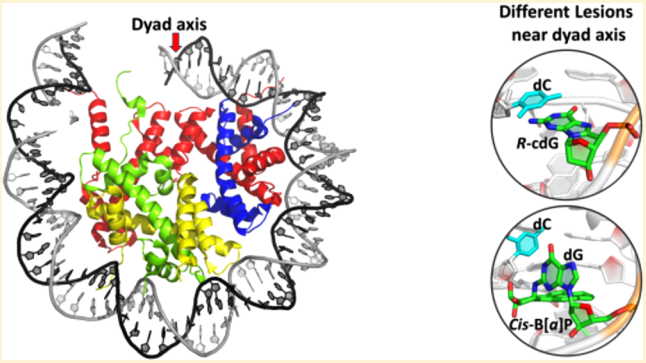
Supporting Information

ABSTRACT: In nucleosomes, the access of DNA lesions to nucleotide excision repair is hindered by histone proteins. However, evidence that the nature of the DNA lesions may play a role in facilitating access is emerging, but these phenomena are not well-understood. We have used molecular dynamics simulations to elucidate the structural, dynamic, and energetic properties of the *R* and *S* 5′-8-cyclo-2′-dG and the (+)-*cis*-*anti*-B[*a*]P-dG lesions in a nucleosome. Our results show that the (+)-*cis*-*anti*-B[*a*]P-dG adduct is more dynamic and more destabilizing than the smaller and more constrained 5′,8-cyclo-2′-dG lesions, suggesting more facile access to the more bulky (+)-*cis*-*anti*-B[*a*]P-dG lesion.



Nucleotide excision repair (NER) is a key mammalian defense mechanism against pro-mutagenic bulky DNA lesions. In NER, an oligonucleotide with 24−32 residues containing the lesion is excised.[1] Repair studies with nucleosome core particles have revealed that the nucleosome reduces NER efficiencies, compared with free DNA containing the same lesion, by factors of ∼2−10.[2−8] However, NER efficiencies are variable in the nucleosome, and the degree of lesion-induced distortion appears to be an important factor. For example, in the case of a pair of site-specific platinum-modified lesions embedded in nucleosomal DNA, the less distorting 1,2-d(GpG) cisplatin lesion is more resistant to NER than the more distorting 1,3-d(GpTpG) cisplatin case. The relative rates of removal of these lesions in free DNA duplexes are similar, and the rates of excision of both lesions are diminished similarly in the nucleosome environment.[9] Furthermore, Mann et al.[10] have shown that different lesions have a different impact on nucleosome stability: benzo[*a*]pyrene (B[*a*]P) diol-epoxide DNA adducts (predominantly the minor groove-aligned, *trans*-*anti*-N^2-dG adduct[11]) stabilize the nucleosome in the *Xenopus borealis* 5S rRNA gene by ∼0.3 kcal/mol per adduct, while lesions produced by UV light are destabilizing to an approximately similar extent.[5,10] In addition, it has been shown that nucleosomes containing destabilizing UV-induced DNA lesions manifest enhanced dynamics that promote nucleosome unwrapping,[12,13] while 1,2-d(GpG) and 1,3-d(GpTpG) cisplatin intrastrand cross-links change the DNA rotational setting.[14,15] However, when a *cis*-*syn*-cyclobutane pyrimidine dimer lesion was site-specifically incorporated into nucleosomal DNA near the dyad axis, the rotational setting of the DNA on the surface of the nucleosome did not change over at least one turn of the helix, as shown by hydroxyl radical footprinting studies.[5] In previous work,[16] we showed that a lesion that resists NER in free DNA diminishes the local nucleosome dynamics. The "access, repair, restore" paradigm pioneered by Smerdon[13,17−19] showed that DNA lesions in nucleosomes and chromatin must be accessible for NER to take place. However, the properties of lesions that trigger the initiation of the complex array of remodeling processes involved in permitting access to the lesion for NER are poorly understood. The finding that more distorting lesions are better substrates of NER in nucleosomes than the less distorting ones, even without remodeling,[9] suggests the hypothesis that more distorting or destabilizing lesions facilitate access and thus NER activity more than those that are less distorting. To pinpoint the intrinsic effect of the lesions on accessibility, we investigated the impact of two structurally very different types of DNA lesions on the local perturbations and dynamics in the nucleosome environment.

We studied two categories of lesions that are NER-susceptible in free DNA, the bulky (+)-*cis*-*anti*-B[*a*]P-dG (*cis*-B[*a*]P-dG) and the non-bulky *R* and *S* diastereomeric 5′,8-

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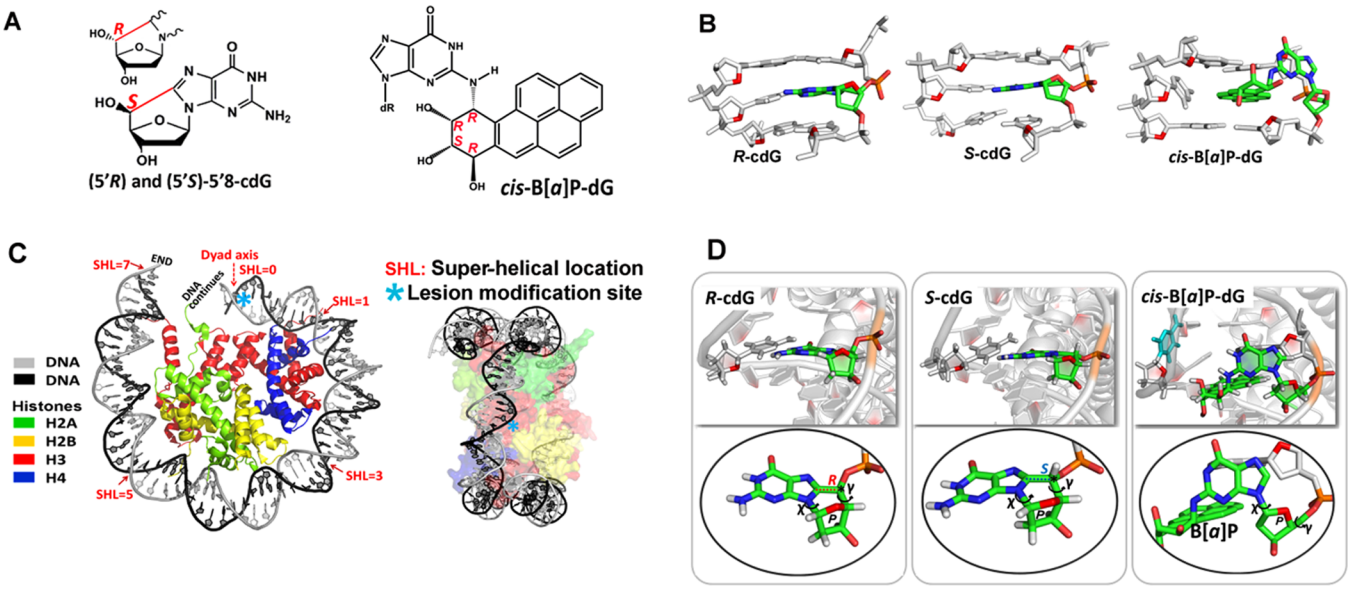

Figure 1. (A) Chemical structures of lesions investigated. (B) Nuclear magnetic resonance solution structure[21] of *cis*-B[*a*]P-dG and the best representative structures obtained from MD simulations of the *R*-cdG and *S*-cdG lesions,[28] modeled based on NMR solution structures.[38,39] The central CG*C duplex trimers (G* denotes modified base) are shown. (C) Structure of the nucleosome with PDB[34] entry 2NZD[35] used in this work. The location of the lesions at SHL ∼ 0 is designated by a cyan asterisk. Top (left) and side (right) views are shown. (D) Best representative structures from the nucleosome MD simulations of the *R*-cdG, *S*-cdG, and *cis*-B[*a*]P-dG adducts. The top panel shows the central trimer rendered in a half-transparency cartoon, with the damaged nucleotide shown as stick and colored by atoms. *R*-cdG and *S*-cdG are Watson−Crick-paired, and the partner cytosine is shown as gray sticks. The *cis*-B[*a*]P-dG is in a base-displaced/intercalated conformation, with the *cis*-B[*a*]P-dG colored by atom and the displaced partner cytosine cyan. The bottom panel shows the extracted lesions with their stereochemistry and key conformational features, glycosidic torsion χ angles (O4′−C1′−N9−C4), backbone torsion γ angles (C3′−C4′−C5′−O5′), and sugar pucker pseudorotation parameter P[40] designated. The dashed bond is the cross-link between C8 and C5′ in the cyclopurines. See also Movies S1−S6, corresponding to files bi5b00564_si_002.avi−bi5b00564_si_007.avi, respectively, of the Supporting Information.

cyclo-2′-deoxyguanosine (*R*-cdG and *S*-cdG, respectively) lesions (Figure 1A). The *cis*-B[*a*]P-dG adduct, derived from the well-known procarcinogen benzo[*a*]pyrene (B[*a*]P) via the widely studied diol epoxide pathway,[20] adopts a base-displaced/intercalated conformation (Figure 1B)[21] and is a good substrate of NER in free DNA in human HeLa cell extract assays.[22,23] The cyclopurines contain a cross-link between the C8 position of purines and 5′-deoxyribose groups of the same nucleotide in DNA. These lesions are derived from the reactions of hydroxyl radicals with guanine or adenine in DNA and are known to be produced in tissues during the inflammatory response.[24,25] The C5′−C8 cross-links cause an overtwisting of the DNA helix and an abnormal and rigid sugar pucker, but Watson−Crick base pairing is not ruptured (Figure 1B). These oxidatively generated lesions are good substrates of NER when present in free DNA, with the *R* stereoisomer being removed more efficiently than the *S* isomer by NER mechanisms in human HeLa cell extracts[26−28] and in CHO cell extracts;[29] they are also repaired by NER in wild-type mice but nevertheless accumulate with aging.[30] The cyclopurine lesions are suspected to play a causative role in neurological diseases that are manifested in xeroderma pigmentosum patients with defects in NER proteins.[31] The chemical and biological characteristics of these lesions have been reviewed by Chatgilialoglu et al.[32]

We have employed molecular dynamics (MD) simulations with binding energy calculations, using AMBER 11,[33] to investigate the structural, dynamic, and energetic properties of these two types of lesions in nucleosomes. We wished to determine whether these structurally very contrasting lesions evoke different signals that trigger access to them in this fundamental DNA packaging unit. We investigated the three lesions and an unmodified control in the nucleosome with Protein Data Bank (PDB)[34] entry 2NZD[35] (Figure 1C). The local 7-mer sequence context surrounding the lesion is given in Figure S1 of the Supporting Information. Full details concerning molecular modeling, MD simulations, force field parameters, structural analyses, the MM-PBSA binding energy calculations, and calculations of electrostatic surfaces are given in the Methods of the Supporting Information. Simulation root-mean-square deviations are given in Figure S2 of the Supporting Information. PyMOL (The PyMOL Molecular Graphics System, version 1.3.x, Schrödinger, LLC) was employed to make molecular images, electrostatic surfaces, and movies.

The results of our molecular dynamics simulations show that the base-displaced/intercalated *cis*-B[*a*]P-dG adduct in the nucleosome is more dynamic, more distorting, and more destabilizing than the Watson−Crick paired cdG lesions (Figure 1B). A hallmark of all 5′,8-cyclopurine lesions is the C5′−C8 cross-link (Figure 1A, D). This cross-link has an impact on a number of structural and dynamic characteristics of free double-stranded DNA in solution[28] that are carried over to the nucleosome environment. These effects are shown in Figure 2. (1) The cross-link enforces overtwisting at the lesion site. (2) The DNA sugar pucker (P) is locked in an unusual O4′-exo conformation (∼280°). (3) The glycosidic bond torsion angle χ is still in the normal *anti* domain but occupies a more narrow and shifted range as compared to the unmodified DNA, although Watson−Crick base pairing is maintained. (4) Backbone C4′−C5′ torsion angle γ is restrained by the cross-link and is locked in the *trans* domain in the case of the *R* stereoisomer (∼193°) and the *gauche*$^-$ domain in the case of

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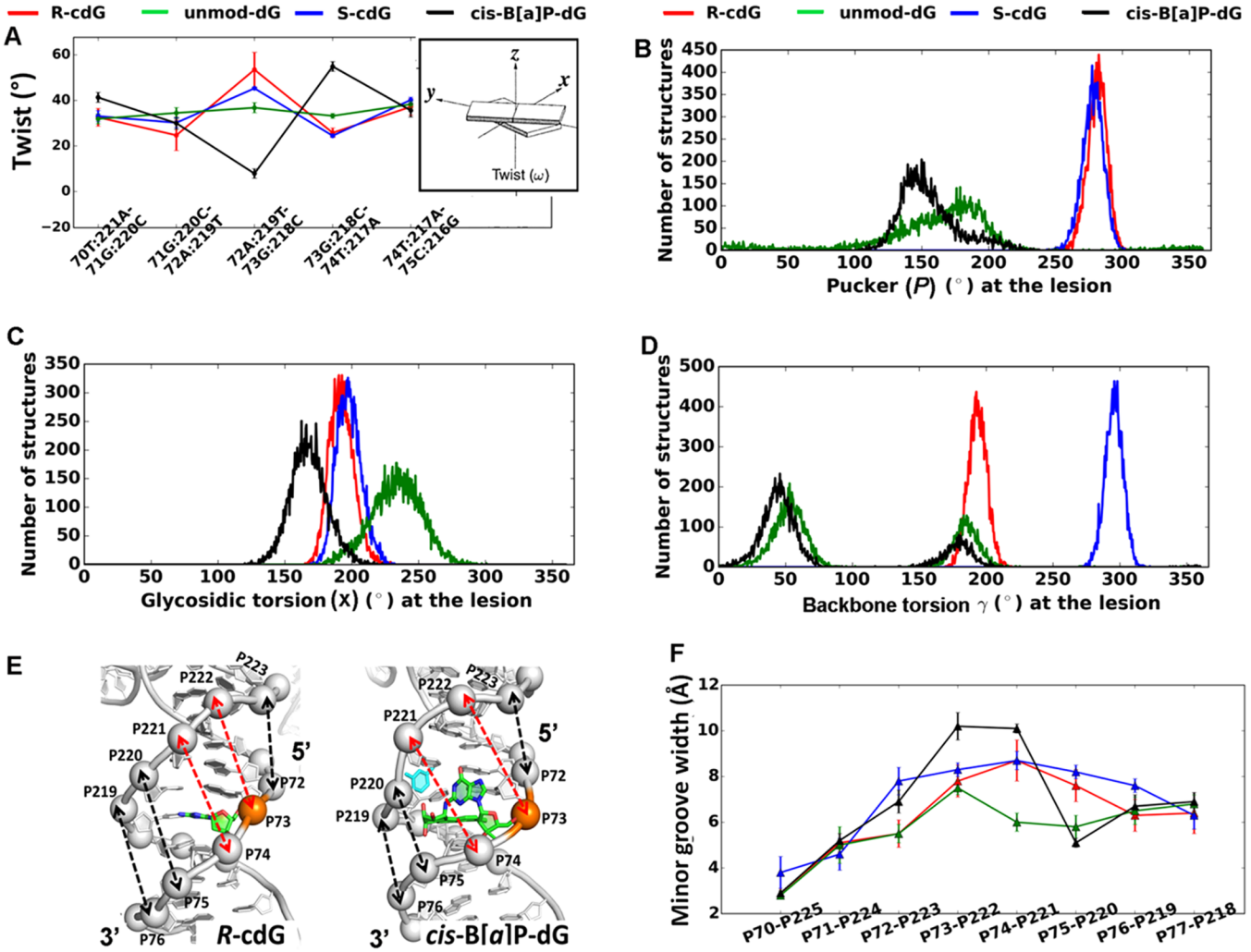

Figure 2. Distorted and dynamic *cis*-B[*a*]P-dG and Watson−Crick-paired and cross-link-locked 5′-8-cdG cyclopurines. (A) MD ensemble average values with block average standard deviations for the Twist. The cartoon illustrating Twist was adapted with permission from ref 41. (B−D) Population distributions from the MD ensembles for sugar pucker, glycosidic torsion, and backbone torsion γ, respectively. (E) Minor groove widths of *R*-cdG and *cis*-B[*a*]P-dG illustrating their differences, using the best representative structures from the MD simulations. Minor groove widths are designated by arrows, and the greatest enlargement is colored red. Values are given in panel F. Minor groove widths are distances between backbone phosphates P70 and P225, P71 and P224, P72 and P223, etc., less 5.8 Å to account for the van der Waals radius of the P atoms.[42] (F) Ensemble average values with block average standard deviations for minor groove widths. See Figure S1 of the Supporting Information for correspondence between residue numbers here (panels A and F) and in the crystal structure with PDB entry 2NZD.[35]

the *S* isomer (∼296°). (5) The cross-link also opens the minor groove as it draws the cross-linked base closer to the deoxyribose residue.

By contrast, in the case of the *cis*-B[*a*]P-dG adduct, the sugar pucker is quite flexible near the C2′-endo domain. The glycosidic torsion of the displaced guanine is also quite flexible in the high-*anti* domain ($\chi \sim 168°$). The C4′−C5′ backbone torsion γ is flexible, being mainly in the *gauche*$^+$ domain (∼44°), with a small population in the *trans* region (∼181°). The base-displaced/intercalated *cis*-B[*a*]P-dG adduct causes significant abnormalities in the twist angle with severe overtwisting at the lesion site and undertwisting of the base pair on its 5′-side. These twist abnormalities stem from the presence of the displaced guanine and the three OH groups in the minor groove (Figure 2E). The hydroxyl groups also cause severe minor groove opening. Hence, compared to the rigid cdG lesions, the *cis*-B[*a*]P-dG adduct greatly distorts the DNA and enhances its local flexibility. The time dependence of these structural parameters of the lesions, together with the means and standard deviation of block averages, is shown in Figures S3−S5 as well as Tables S2 and S3 of the Supporting Information.

To gain further insights into the structural and dynamic differences between the 5′-8-cdG and the *cis*-B[*a*]P-dG lesions embedded in the nucleosome, we evaluated DNA−histone interactions and local electrostatic potentials. We found that the normal DNA with the unmodified guanine base has a hydrogen bond between Lys115 of histone H3 and a backbone phosphate oxygen atom of the nucleotide on its 5′-side. In the *cis*-B[*a*]P-dG adduct, however, this hydrogen bond is disrupted, while it remains intact in the *R*- and *S*-cdG lesions. Furthermore, the electrostatic surface facing the histones in the *cis*-B[*a*]P-dG lesion is less negative than in the *R*- and *S*-cdG and unmodified guanine cases, because of the presence of the bulky and essentially uncharged B[*a*]P aromatic ring system, which likely causes loss of the hydrogen bond. In addition, we computed the binding energy ΔG of the lesion-containing 5-mer duplex with the nearby histones H2A and H3 (Figure 3A), as well as the

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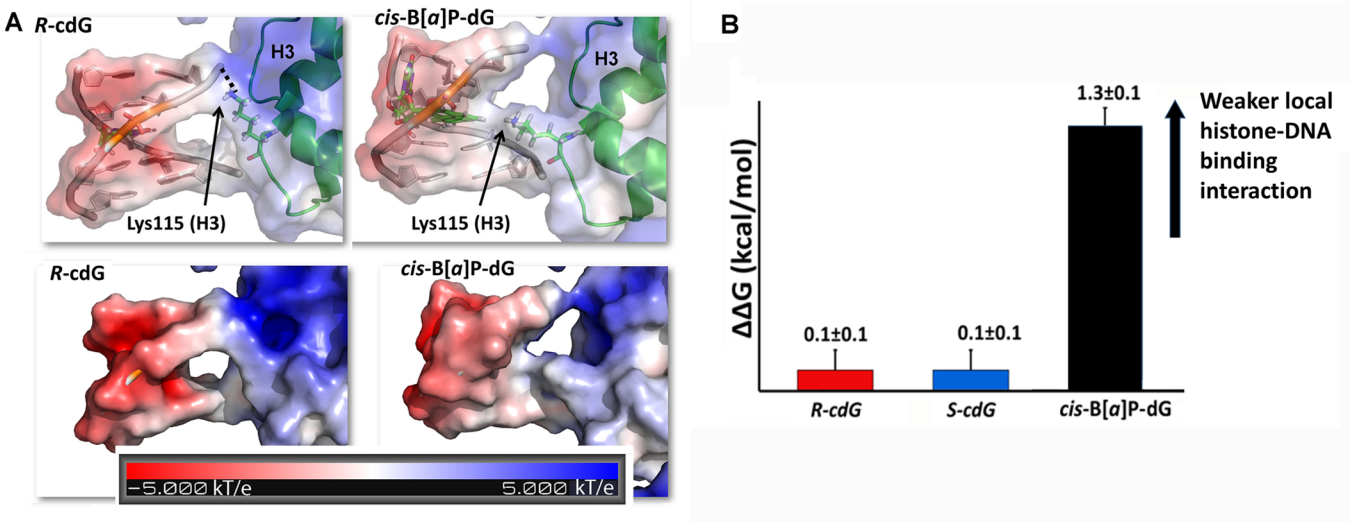

Figure 3. (A) Electrostatic surfaces of most representative structures in the nucleosome from the MD simulations. Top: *cis*-B[*a*]P-dG shows a ruptured hydrogen bond between Lys115 of histone H3 and the DNA backbone that is present in *R*-cdG (shown here), as well as *S*-cdG and the corresponding unmodified sequence (shown in Figure S6 of the Supporting Information). Bottom: electrostatic surfaces reveal that the *cis*-B[*a*]P-dG local region is less negative than the *R*-cdG case (shown here), as well as *S*-cdG and the corresponding unmodified sequence (shown in Figure S6 of the Supporting Information). The top panel is rendered as a half-transparency surface to reveal the molecular interactions, while the bottom panel without transparency emphasizes the electrostatic surface difference. See also Movies S7−S10, corresponding to bi5b00564_si_008.avi−-bi5b00564_si_011.avi, respectively, of the Supporting Information. (B) Impact of the lesions on the local histone−DNA binding interaction energies. The *cis*-B[*a*]P-dG lesion destabilizes the histone−DNA interactions by 1.3 kcal/mol, while the cycloguanines are only very modestly destabilizing by 0.1 kcal/mol. $\Delta\Delta G = \Delta G$ (adduct) $-\Delta G$ (unmodified control).

ΔG for the corresponding unmodified 5-mer duplex. The impact of each lesion on the binding energy is $\Delta\Delta G = \Delta G$ (lesion) $- \Delta G$ (unmodified). The higher the $\Delta\Delta G$ for a lesion, the weaker the local DNA−histone interactions. We obtained $\Delta\Delta G$ values of 0.1, 0.1, and 1.3 kcal/mol for *R*-cdG, *S*-cdG, and *cis*-B[*a*]P-dG, respectively, showing that the binding between local DNA and protein is hardly destabilized in the cycloguanines, while it is weakened by 1.3 kcal/mol in the *cis*-B[*a*]P-dG case (Figure 3B).

It has been shown that the 5′-8-cyclopurine lesions are removed by NER in wild-type mice and that the *R* stereoisomers are better repaired than the *S* isomers,[30] although these non-bulky lesions do persist in aging mice.[30] The relative NER efficiencies observed with the same lesions embedded in free DNA in HeLa cell extracts are consistent with these *in vivo* observations:[28] in the HeLa cell extracts, the *R* lesions are better repaired than the *S* stereoisomers by a factor of ∼2.[28]

Our results indicate that *R* and *S* stereoisomers are equally weakly destabilizing in the nucleosome before remodeling for access; however, once access is gained, the *R* stereoisomer is better repaired in the mice.[30] Our observations suggest the hypothesis that the partial persistence of the 5′-8-cyclopurine lesions *in vivo*[30] might be due to their greater resistance to NER when embedded in nucleosomes, even after remodeling of the chromatin environment occurs that renders the nucleosomes accessible to the DNA repair system.[36,37] This hypothesis is supported by our results that suggest that 5′-8-cyclopurine lesions cause modest local structural distortions and little local destabilization of the nucleosome, and the local dynamic structural fluctuations of the damaged DNA are constrained. By contrast, the *cis*-B[*a*]P-dG lesion destabilizes the nucleosome more, by causing severe local structural distortions and greater dynamics than the 5′-8-cyclopurines. Hence, we hypothesize that access to the lesion is achieved more readily in the case of this bulky adduct. This is consistent with the observations that the *cis*-B[*a*]P-dG adduct in DNA embedded in natural nucleosomes (extracted from HeLa cells and thus assembled with post-translationally modified histones) exhibits an NER efficiency in HeLa cell extracts that is smaller than that observed in free DNA by a factor of only ∼2[36,37] (manuscript in preparation). Thus, in contrast to the 5′-8-cyclopurine lesions, it is likely that the bulky *cis*-B[*a*]P-dG adduct will be cleared more rapidly *in vivo* because its destabilizing effect in nucleosomes would promote rapid access to the NER machinery.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary methods, tables, figures, and movies. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.5b00564.

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Notes

The authors declare no competing financial interest.

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