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The Sequence Dependence of Human Nucleotide Excision Repair Efficiencies of Benzo[a]pyrene-Derived DNA Lesions: Insights into the Structural Factors that Favor Dual Incisions

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Summary

Nucleotide excision repair (NER) is a vital cellular defense system against carcinogen-DNA adducts, which, if not repaired, can initiate cancer development. The structural features of bulky DNA lesions that account for differences in NER efficiencies in mammalian cells are not well understood. *In vivo*, the predominant DNA adduct derived from metabolically activated benzo[a]pyrene (BP), a prominent environmental carcinogen, is the 10S (+)-*trans-anti*-[BP]-N²-dG adduct (G*), which resides in the B-DNA minor groove 5'-oriented along the modified strand. We have compared the structural distortions in double-stranded DNA, imposed by this adduct, in the different sequence contexts 5'-...CGG*C..., 5'-...CG*GC..., 5'-...CIG*C... ("I"= 2'-deoxyinosine), and 5'-...CG*C.... Based on electrophoretic mobilities, all duplexes manifest moderate bends except the 5'-...CGG*C...duplex, which exhibits an anomalous, slow mobility attributed to a pronounced flexible kink at the site of the lesion. This kink, resulting from steric hindrance between the 5'-flanking guanine amino group and the BP aromatic rings, both positioned in the minor groove, is abolished in the 5'-...CIG*C...duplex (the 2'-deoxyinosine group, I, lacks this amino group). In contrast, the sequence-isomeric 5'-...CG*GC...duplex exhibits only a moderate bend, but displays a remarkably increased opening rate at the 5'-flanking base pair of G*, indicating a significant destabilization of Watson-Crick hydrogen bonding. The NER dual incision product yields were compared for these different sequences embedded in otherwise identical 135-mer duplexes in cell-free human HeLa extracts. The yields of excision products varied by a factor of as much as ~4 in the order 5'-...CG*GC... > 5'-...CGG*C... ≥ 5'-...CIG*C... ≥ 5'-...CG*C.... Overall, destabilized Watson-Crick hydrogen bonding, manifested in the 5'-...CG*GC...duplex, elicits the most significant NER response, while the flexible kink displayed in the sequence-isomeric 5'-...CGG*C...duplex represents a less significant signal in this series of substrates. These results demonstrate that the identical lesion can be repaired with markedly variable efficiencies in different local sequence contexts that differentially alter the structural features of the DNA duplex around the lesion site.

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Keywords

Nucleotide excision repair susceptibility; benzo[a]pyrenyl-guanine lesion; structure-function relationship; flexible DNA bend; sequence-dependence; dual incision efficiencies

The global genome nucleotide excision repair (NER) mechanism is a repair pathway in eukaryotes that removes bulky DNA adducts *in vivo*.¹⁻³ The eukaryotic NER system consists of a set of ~30 distinct subunits⁴ that collaborate in the excision of lesion-containing oligonucleotides, 24-32 nucleotide-long, from the damaged strand. One of the key and vital features of the NER machinery is its ability to recognize and excise an astounding variety of bulky DNA lesions. The protein XPC, tightly associated with another protein, HR23B, *in vivo*⁵ and *in vitro*,⁶⁻⁹ plays an initial role in recognizing DNA lesions in cellular environments.^{7, 10-16} The XPC/HR23B complex initiates the recruitment of other NER factors that are essential for ultimately stimulating the dual incisions of the damaged strand.^{4, 12, 13, 17} The first among these other factors is the multi-protein transcription factor TFIIH.¹⁸⁻²⁰ The helicases XPB and XPD, components of TFIIH, cause the unwinding of a 20-25 nucleotide patch around the site of the lesion in an ATP-dependent manner.^{11, 14} The factor XPA then binds to this complex, presumably acting as a wedge to stabilize this “bubble”-like structure, and XPC/HR23B is released. Another factor, RPA, a single-strand binding protein, further stabilizes this nucleoprotein complex. Subsequently the structure-specific endonucleases XPG and XPF/ERCC1 bind to the complex and incise the damaged strand on the 3'- and 5'-sides of the lesion, respectively, thus releasing the 24-32 nucleotide-long fragments that are the hallmark of successful mammalian NER incision activity.^{11, 13, 18, 21, 22}

The rate of repair of chemically and conformationally different lesions by the mammalian NER apparatus varies over several orders of magnitude.^{2, 21, 23} The structures of the DNA lesions that elicit efficient or inefficient NER repair have been a subject of considerable interest over the years.^{1-3, 21, 24} It has been suggested that the NER factors do not recognize the lesion itself, but the local distortions in the DNA that are associated with the lesions.^{7, 13, 18, 23, 25} Further insights into these issues is provided by the X-ray crystallographic structure of a truncated form of Rad4/Rad23 (the *S. cerevisiae* homologues of XPC and HR23B, respectively) in a complex with an oligonucleotide containing a cyclobutane pyrimidine dimer (CPD) lesion.²⁶ One of the three β -hairpin domains of Rad4 is inserted into the DNA helix, thus separating the CPD lesion from the unmodified strand. The CPD lesion is positioned in a disordered region of the crystal where it has no visible contacts with the protein.²⁶ In contrast, the two thymines in the complementary strand that were positioned opposite the CPD in the duplex, interact with Rad4 amino acid residues. As discussed by Schärer,^{24, 27} this structure indicates that the interaction of the unmodified complementary strand with the protein plays an important role in lesion recognition, which had been suggested by the experiments of Buterin *et al.*²⁸ The structure points to the necessity of local strand separation that should be facilitated by lesions causing significant local thermodynamic destabilization of duplex DNA. In turn, this local destabilization is affected by multiple factors such as the interactions of the lesions themselves with the local DNA residues that can cause the weakening of local hydrogen bonding and base stacking interactions. Thus, multiple structural deviations, or a multipartite mechanism, may be involved in the recognition of DNA lesions.^{29, 30} Other structural perturbations that lead to the recognition of DNA lesions have been considered. These include the recognition of flipped out bases from the complementary strand,²⁸ DNA flexibility around the lesion site,³¹ and oscillatory motions of the unmodified complementary strand.^{3, 32, 33} All of these phenomena involve lesion-induced deviations from the normal B-DNA duplex structural parameters.³⁴ Many of these parameters are coupled,^{35, 36} and the various deviations are therefore not mutually exclusive. Our interests have been directed towards an understanding of the multipartite changes in the DNA structural parameters and how they affect the recognition and

subsequent dual incisions that characterize both prokaryotic^{29, 30, 37-39} and eukaryotic NER.²⁹

In order to elucidate the relationships between NER efficiencies and the conformations of bulky DNA adducts and the DNA distortions they cause, we have been studying lesions derived from the binding of metabolites of polycyclic aromatic hydrocarbons (PAH) to DNA.⁴⁰ Adducts derived from the binding of the diol epoxide reactive intermediate 7*r*,8*t*-dihydroxy-*t*9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*anti*-BPDE), a metabolite of the cancer-causing compound benzo[*a*]pyrene,^{41, 42} are excellent substrates for probing the features of DNA lesions that are recognized by the NER apparatus. The most abundant stable adduct derived from the reaction of BPDE with DNA in mammalian cells^{43, 44} is the 10*S* (+)-*trans-anti*-[BP]-*N*²-dG adduct (Figure 1a). However, the reactions of BPDE with DNA also yield other, stereoisomeric adducts to dG and dA, with varying conformations and differences in structural perturbations of double-stranded DNA.⁴⁵ The susceptibilities of the different PAH-DNA adducts to NER depend on adduct stereochemistry^{46, 47} and PAH topology.⁴⁸ The [BP]-DNA adducts, unless removed by DNA repair mechanisms,⁴⁹ are highly mutagenic.^{50, 51}

We have shown recently by NMR methods that the identical 10*S* (+)-*trans-anti*-[BP]-*N*²-dG adduct (**G***, Figure 1a) positioned either at G6 or at G7 in the G6G7 duplex (Figure 1b) exhibits remarkably different, base sequence-dependent structural characteristics.⁵² The G6G7 dinucleotide sequence was initially selected for study because it constitutes a mutational hotspot in *E. coli*^{53, 54} and in mammalian cells.⁵⁰ The bulky aromatic residue in **G*** adopts a minor groove adduct conformation in both the G6**G7*** and the **G6***G7 12-mer duplexes. However, the characteristics of these two sequence-isomeric duplexes differ in significant aspects. Specifically, in the **G6***G7 case, the duplex exhibits a moderate bend, and the flanking C5:G20 base pair is destabilized at ambient temperatures, while all other base pairs flanking the **G6***:C19 base pairs remain intact.⁵² In contrast, the G6**G7*** 12-mer duplex manifests a pronounced flexible bend or kink at the site of the lesion, but all base pairs flanking the **G7***:C18 pair are intact.⁵² Since these duplexes are identical except for the positions of the modified guanine residues, we wished to determine whether the NER dual incision efficiencies are affected by these physical differences.

The relative NER efficiencies were determined by incubating the **G6***G7 and the G6**G7*** 12-mer duplexes (Figure 1b) embedded in otherwise identical 135-mer duplex sequences, in cell-free extracts from human HeLa cells, as described previously.^{46, 47} The yields of dual incision products were also investigated using the G6**G7*** duplex analog I6**G7***. In the latter, G6 in the G6**G7*** duplex was replaced by inosine (I6)[‡] and is of interest because the I6**G7*** duplex exhibits a moderate bend, rather than the pronounced kink/bend exhibited by the G6**G7*** duplex. Finally, the new NER results reported here are compared to the dual incision yields obtained with the CPD and 6-4<TT> photodimers,^{7, 13, 22, 55-57} and the same minor groove 10*S* (+)-*trans-anti*-[BP]-*N*²-dG adduct⁵⁸ in the CG*C sequence context (Figure 1b) reported earlier.^{46, 47}

An unusual flexible kink in the G6G7* duplex

The impact of the 10*S* (+)-*trans-anti*-[BP]-*N*²-dG adduct on the three-dimensional characteristics of the modified oligonucleotide duplexes can be assessed by comparing the electrophoretic mobilities of the BP-modified and unmodified duplexes in native (non-denaturing) polyacrylamide gels.⁵⁹⁻⁶² The **G6***G7, G6**G7*** and I6**G7*** duplexes (Figure 1b) exhibit extraordinary differences in electrophoretic mobilities (Figure 2). The mobility of the

[‡]Formally, inosine is the nucleoside, while hypoxanthine is the correct name for the corresponding base. For simplicity we utilize the term inosine only

12-mer **G6*G7** duplex is about 10% lower than that of the unmodified G6G7 duplex (Figure 2, lanes 3 and 4, respectively). These results are comparable to the mobility of the **CG*C** duplex (~ 5% lower mobility) and with **G*** in other sequence contexts.⁵⁹⁻⁶² Remarkably, the mobility of the **G6G7*** duplex is lowered by 42% (Figure 2, lanes 2 and 4, respectively). This result indicates that the lesion at **G7*** induces an usually strong bend⁵⁹ or kink⁵² in the **G6G7*** sequence context. Interestingly, the anomalous slow electrophoretic mobility of the **G6G7*** duplex is abolished by substituting the guanine base G6 by inosine. The mobility of the **I6G7*** duplex is identical to the mobility of the **G6*G7** duplex, suggesting that the overall three-dimensional shapes of the **G6*G7** and **I6G7*** duplexes are similar. Since inosine is identical to guanine except that it lacks the latter's exocyclic amino group, these results indicate that the exocyclic amino group of guanine at G6 plays a dominant role in causing the prominent kink in the **G6G7*** duplex.^{52, 63}

The difference between rigid, directed bends and flexible, hinge-like joints can be established by examining the relationships between the electrophoretic mobility patterns of self-ligation products and the phasing of the bends with respect to the helical repeat⁶⁴ (10.3-10.6 base pairs⁶⁵ per helical turn), as discussed by Hagerman.⁶⁴ These techniques were previously employed to demonstrate that the prominent kink induced by the 10S (+)-*trans-anti*-[BP]-N²-dG adduct in the **G6G7*** duplex is highly flexible.⁶⁶ In contrast, for the same 10S (+)-*trans-anti*-[BP]-N²-dG adduct in the **G6*G7** and **I6G7*** duplexes, the bending is moderate (Figure 2) and the bends are rigid rather than flexible, as shown earlier in our laboratory.⁶⁶ Furthermore, when **G*** is flanked by cytosine on both sides as in the **CG*C** sequence context, the bend is also rigid, as shown by phasing methods^{60, 62} In the case of the stereoisomerically different 10R (+)-*cis-anti*-[BP]-N²-dG adduct in the **CG*C** duplex, which adopts a base-displaced/intercalated conformation (Figure S1, Supporting Information),⁶⁷ the extent of bending is smaller than in the case of the (+)-*trans*-adduct in the same sequence context.⁶⁸

Relative NER Efficiencies

The relative efficiencies of dual incisions of the modified strand of **G6*G7**, **G6G7***, **I6G7*** and **CG*C** 135-mer duplexes containing identical single 10S (+)-*trans-anti*-[BP]-N²-dG adducts were compared in cell-free HeLa cell extracts (details in Supporting Information). Briefly, the NER activities were assessed using denaturing gel electrophoresis to resolve the characteristic 24-32 oligonucleotide dual incision products. Phosphorimager methods were then employed to evaluate the yields of these products (radioactivity in the 24-32 band region divided by the total radioactivity in the same lane). These yields varied in different cell extracts (2-6%), and this variability was accounted for by including experiments with a standard stereoisomeric 10R (+)-*cis-anti*-[BP]-N²-dG adduct in the **CG*C** sequence context, in each set of experiments. Thus, in experiments conducted with different cell extract preparations, the dual incision efficiencies for the 10S (+)-*trans* adduct in different sequence contexts were normalized with respect to the 10R (+)-*cis* positive control standard. The 10R (+)-*cis*-adduct, with its base-displaced/intercalated conformation⁶⁷, is removed ~5 times more efficiently than the minor groove 10S (+)-*trans*-adduct⁵⁸ in the same **CG*C** sequence context in HeLa cell extracts, and by a factor of ~2.5 by a set of reconstituted and purified NER factors.⁴⁷ We compared the time dependence of NER dual incision efficiencies of the 10S (+)-*trans-anti*-[BP]-N²-dG adduct in the **CG*C** duplex with those characterizing the extensively studied *cis-syn* cyclobutane pyrimidine dimer (CPD) and the 6-4 UV photodimer under identical conditions.^{13, 22, 55, 57, 69} Typical densitometry traces are shown (Figure 3a) and were used for the quantitative evaluation of relative NER efficiencies. The time dependence is linear up to 40 minute incubation time (Figure 3b). The relative rates decreased in the order (6-4 <TT>) ~ 10R (+)-*cis-anti*-[BP]-N²-dG > 10S (+)-*trans-anti*-[BP]-N²-dG > CPD <TT>. The [6-4 <TT>]/[CPD <TT>] ratio of dual incision rates is ~ 19. This compares with ratios of ≥ 10 also

found in human cell extracts *in vitro*⁵⁷ and *in vivo* in UVB/UVA-irradiated human skin,⁵⁶ and a ratio of ~ 8 observed in Cho cell extracts.²²

Sequence-context dependent differential excision

The excision efficiencies of the **CG***C, **G6***G7, and **G6G7*** sequences embedded in otherwise identical 135-mer duplexes are compared in Figure 4a. The same 10S (+)-*trans-anti*-[BP]-N²-dG adduct embedded in different sequence contexts is excised with different efficiencies in the order **G6***G7 > **G6G7*** > **CG***C (Figure 4). Both XPC, a critical component of the global genome NER apparatus, and XPA, are essential to the NER activities shown. In XPC-deficient, or XPA-deficient cell lines, the NER dual incision efficiencies are reduced by 90% or more, but are restored in mixtures of cell extracts from XPA and XPC cells, as expected if an NER mechanism is operative (Figure S2, Supporting Information).

In order to compare the initial rates of excision of these sequences, and to take into account possible variations obtained with different cell extracts, we examined the time course of incisions in the **CG***C, **G6***G7, **G6G7*** and **I6G7*** 135-mer duplexes. A representative example of a gel is shown in Figure 5a. Results from multiple experiments with different cell extracts and substrate samples are summarized in Figure 5b, together with the standard deviations of each set of time-dependent data points. The key feature of the findings (Table S1, Supporting Information) is that the NER efficiencies of the identical 10S (+)-*trans-anti*-[BP]-N²-dG lesions are markedly dependent on the bases flanking the lesion, spanning a factor of as much as ~4 (**G6***G7 vs. **CG***C duplexes). Particularly remarkable are the differences between the sequence isomers **G6***G7 and **G6G7***, the **G6***G7/**G6G7*** ratios of dual incision efficiencies being 2.4 ± 0.3 .

Differences in conformations dictated by base sequence context

The NMR solution structures of the 10S (+)-*trans-anti*-[BP]-N²-dG adduct embedded in the **G6***G7,⁵² **G6G7***,⁵² **I6G7***,⁷⁰ and **CG***C⁵⁸ duplexes have been investigated and the results show that there are subtle sequence-dependent conformational differences among double-stranded DNA duplexes. In all cases the bulky BP aromatic ring system is positioned in the minor groove, pointing in the 5'-direction relative to the modified guanine base. However, only the **G6***G7 duplex manifests a remarkable destabilization of the base pair flanking the lesion on its 5'-side in a temperature range much lower than the global melting temperature of the modified duplex. Specifically, the overall thermal stabilities of the **G6***G7 and **G6G7*** 12-mer duplexes ($T_m = 55 \pm 1$ °C and 57 ± 1 °C, respectively) are not very different from one another.⁵² But the temperature-dependence of the line widths and chemical shifts of the G20 imino proton NMR resonances in the **G6***G7 duplex suggest that the C5:G20 base pair is destabilized within the temperature interval of 18-30 °C. Furthermore, analysis of the Molecular Dynamics (MD) – refined structure based on the NMR data shows that the **G6G7*** duplex shows a unique local untwisting accompanied by an enlarged Roll⁵² (Roll is the angle between two neighboring base pair planes and produces bending into the major or minor groove.⁷¹ See also Figure S5, Supporting information). Enlarged Roll coupled with untwisting is known to be largely responsible for DNA bending or kink formation^{35, 36, 72} There are also changes in local base-base stacking interactions, but without a strong impact on Watson-Crick pairing except for a modest perturbation at the lesion site itself.⁶³ The properties of the **I6G7*** duplex were also studied by NMR methods.⁷⁰ Multiple conformations were found, although two major conformers with the BP residues positioned in the minor groove and oriented towards the 5'-direction of the modified strand could be identified.⁷⁰ The **CG***C and **G6G7*** duplexes exhibit only a single conformer and all neighboring Watson-Crick base pairs remain intact.^{52, 58}

Extensive structural analyses,⁶³ utilizing MD simulation methods, of the 10S (+)-*trans-anti*-[BP]-*N*²-dG adduct in the duplexes investigated here have provided further molecular insights into the origins of the experimentally observed sequence-dependent structural properties.⁶³ The critical elements that produce differences in the structural perturbations are the variable steric hindrance effects from the guanine amino groups that are positioned in the vicinity of the bulky aromatic BP ring system. Both compete for accommodation in the duplex minor groove. For the **G6*G7** duplex, steric hindrance from the guanine amino group of G7 causes the episodic rupturing of the Watson-Crick hydrogen bonds of the C5:G20 base pair 5'-flanking the lesion. The G7 guanine amino group crowds the BP benzylic ring on the 3'-side of the lesion, causing the BP moiety to rotate about the linkage site; as a result, the BP distal aromatic ring system episodically intrudes into the hydrogen-bonded region of the C5:G20 base pair, flanking the lesion on its 5'-side (Figure S6, Supporting Information). Furthermore, in the **G6G7*** duplex, the anomalously slow electrophoretic mobility associated with the flexible kink, manifested in our MD simulation,⁶³ as a dynamic untwisting and enlarged Roll at the C5:G20/G6:C19 base pair step, is caused by steric hindrance between the G6 guanine amino group and the BP aromatic ring system (Figure S6, Supporting Information). This role of the G6 guanine amino group was also substantiated by MD studies of the dynamics of the **I6G7*** duplex⁶³ which does not exhibit untwisting or enlarged Roll. In contrast, in the **CG*C** sequence, the amino groups of the guanines G18 and G16 in the C5:G18 and C7:G16 base pairs, respectively, flanking the lesion, tend to place the BP moiety in a more confined region of the minor groove, thus causing the bending to be rigid.

Structural DNA distortions/destabilizations recognized by the human NER apparatus

The highest NER efficiency in this series of minor groove-aligned, stereochemically identical 10S (+)-*trans-anti*-[BP]-*N*²-dG adduct in the different sequence contexts considered is observed in the **G6*G7** duplex; in this case, the efficiency is ~2.4 times greater than in the sequence-isomeric **G6G7*** duplex and ~4.1 times greater than in the **CG*C** sequence context (Figure 6 and Table S1, Supporting Information). In terms of structural distortions, only the **G6G7*** duplex displays a significant destabilization and episodic denaturation of Watson-Crick hydrogen bonding at the 5'-side base pair flanking the lesion modification site; this is correlated with the highest NER efficiency in this series of BP-modified duplexes. In contrast, only the **G6G7*** duplex has the hallmarks of a flexible kink or bend,⁶⁶ manifested in the anomalous electrophoretic mobility observed in Figure 2 (lane 2); this is correlated with the second highest repair efficiency in this series of substrates. Therefore, in this set of minor-groove aligned sequence-isomeric BP-modified guanine lesions, the weakening of Watson-Crick base pairing seems to be a more effective factor for eliciting mammalian NER than a flexible, sharply bent or kinked duplex. The **I6G7*** duplex lacks this flexible kink, but its repair efficiency is similar to that of the sharply bent **G6G7*** duplex substrate with the flexible kink. However, replacing G6 by I6 changes other, local interactions that could affect NER, since the conformation of the **I6G7*** duplex is conformationally heterogeneous and displays at least two different minor groove conformations.⁷⁰ By contrast the **G6G7*** duplex has only one predominant conformation.⁵² The conformational heterogeneity of **I6G7*** is attributed to a weaker I6:C19 base pair, with only two hydrogen bonds flanking the lesion on the 5'-side. Furthermore, MD studies⁶³ show that this I6:C19 base pair has one of the two hydrogen bonds episodically ruptured, which could account for its moderate repair susceptibility.⁶³ The least structurally distorted **CG*C** duplex, without disrupted Watson-Crick hydrogen bonding or flexible kink, exhibits the lowest susceptibility to NER in this series. The repair in this case seems to be related to its enlarged minor groove,⁴⁷ and modestly perturbed Watson-Crick hydrogen bonding at the lesion site;⁶³ these two features are common to all of the substrates

we considered here. These structure/function correlations are summarized in Figure 6 and Table S1, Supporting Information.

The DNA duplex with the stereoisomerically different 10*R* (+)-*cis-anti*-[BP]-*N*²-dG adduct, with base-displaced/intercalated conformation is excised with similar efficiencies as the 6-4 <TT> dimer, and about ~5 times better than the stereoisomeric 10*S* (+)-*trans-anti*-[BP]-*N*²-dG adduct in the CG**C* sequence context.^{46, 47} The large distortions associated with this conformation, namely, the prominently flipped out base pair,²⁸ with completely ruptured Watson-Crick hydrogen bonding as well as the enlarged minor groove,⁴⁷ can provide the significant recognition signals in the family of base-displaced/intercalated [BP]-*N*²-dG adducts; this also fits well with the hierarchy of NER susceptibilities for the cases investigated here.

Conclusions

Analyses of the structural properties of the series of minor groove 10*S* (+)-*trans-anti*-[BP]-*N*²-dG adducts positioned in different sequence contexts of double-stranded DNA by NMR methods, electrophoretic mobilities and MD simulations,⁶³ have provided new insights into the structural distortions/destabilizations that elicit and modulate the efficiencies of the initial steps in NER (Figure 6). In the case of the [BP]-*N*²-dG adduct studied in this work, the episodic denaturation of Watson-Crick hydrogen bonding in the base pair flanking the lesion on the 5'-side provides a stronger recognition signal than a flexible kink/bend. The origins of these structural differences stem from steric hindrance between the exocyclic amino groups of unmodified guanines in the vicinity of the bulky [BP]-*N*²-dG adducts in the minor groove. The impact of these exocyclic amino groups on the physical properties of the duplexes depends on the positions of the unmodified guanines in the duplexes. Our results are consistent with the finding that in this family of bulky lesions, differences in the local structural distortions and destabilizations caused by the adducts are correlated with local helix opening patterns caused by the XPC/HR23B binding and their differential dual incision efficiencies.⁴⁷ Depending on the sequence context, the lesion-containing duplexes manifest different structural distortions/destabilizations.⁶³ In turn, strand separation, base flipping, and β -hairpin insertion by the Rad4²⁶ or XPC recognition factors may depend on the local base sequence context. Future detailed studies of the structural properties of different PAH-derived lesions with different conformations in selected base sequence contexts, should provide further clues to the mechanisms underlying the recognition and excision of bulky DNA lesions by the mammalian NER apparatus, and their mutagenic and tumorigenic properties.

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Abbreviations

BP

benzo[*a*]pyrene

(+)-*anti*-BPDE

(benzo[*a*]pyrene diol epoxide):(+)-(7*R*,8*S*,9*S*,10*R*)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene

dG

2'-deoxyguanosine

NER

nucleotide excision repair

CPD

cyclobutane pyrimidine dimer

5'-C1 A2 T3 G4 C5 G6 G7 C8 C9 T10 A11 C12	G6G7
3'-G24 T23 A22 C21 G20 C19 C18 G17 G16 A15 T14 G13	
5'-C1 A2 T3 G4 C5 G6* G7 C8 C9 T10 A11 C12	G6*G7
3'-G24 T23 A22 C21 G20 C19 C18 G17 G16 A15 T14 G13	
5'-C1 A2 T3 G4 C5 G6 G7* C8 C9 T10 A11 C12	G6G7*
3'-G24 T23 A22 C21 G20 C19 C18 G17 G16 A15 T14 G13	
5'-C1 A2 T3 G4 C5 I6 G7* C8 C9 T10 A11 C12	I6G7*
3'-G24 T23 A22 C21 G20 C19 C18 G17 G16 A15 T14 G13	
5'-C1 C2 A3 T4 C5 G6* C7 T8 A9 C10 C11	CG*C
3'-G22 G21 T20 A19 G18 C17 G16 A15 T14 G13 G12	
5'-G C A A G T T G G A G	<TT>
3'-C G T T C A A C C T C	

Figure 1.

Structure and stereochemical properties of the 10S (+)-trans-anti-[BP]-N²-dG adduct (top). The base sequence contexts of the different duplexes studied and their abbreviations are defined here where **G*** = 10S (+)-trans-anti-[BP]-N²-dG. The NMR properties^{52, 58, 70} and the gel electrophoresis results (Figure 2) were obtained with the duplexes shown. The NER studies were conducted with the same duplexes embedded in 135-mer duplexes that otherwise had the same base sequence (Supporting Information).

Figure 2.

Relative electrophoretic mobilities of unmodified and the 10S (+)-*trans-anti*-[BP]-*N*²-dG adduct-modified oligonucleotide duplexes defined in Figure 1 with their natural complementary strands in native 8% polyacrylamide gels at room temperature. The faint bands at the top of the gel represent the wells. Table: % decrease in electrophoretic mobility^a: (Mobility of the modified duplex – mobility of the unmodified duplex) × 100 / mobility of the unmodified duplex. ^bData from Xu *et al.*⁶⁶

Figure 3.

(a) Comparison of nucleotide excision repair results in HeLa cell extracts for 135-mer duplexes containing the UV photodimer CPD <TT> or 6-4 <TT> duplexes, the 10*S* (+)-*trans-anti*-[BP]-*N*²-dG (CG*⁺C), or the stereoisomeric (+)-*cis* adducts in the same CG*⁺C sequence context (*cis*-CG*⁺C). The (+)-*cis*-duplex was used as a reference standard in each set of experiments to adjust for variabilities in the NER activities of cell extracts prepared at different times (see text for details). The overall radioactivity levels in each lane were comparable, to allow for a visual inspection of the differences in dual incision efficiencies. Lanes 1, 2, 3, and 4 represent incubation times of 10, 20, 30, and 40 minutes respectively. The lane marked <TT> represents parallel incubations with unmodified <TT> duplexes, while lane M contains unmodified oligonucleotide markers 32, 30, 28, 26, and 24 nucleotides in length. In this experiment, the maximum extent of NER incisions (observed in the case of the 10*R* (+)-*cis-anti*-[BP]-*N*²-dG adduct in the *cis*-CG*⁺C duplex at 40 min), was 4.6%. (b) Time dependence of formation of dual incision products. The average values and standard deviations are based on five independent experiments in the case of the two UV photodimers, and eight experiments in the case of the 10*S* (+)-*trans-anti*-[BP]-*N*²-dG adduct in the CG*⁺C sequence. The straight lines are the least square fits to the data points, and the relative values of the slopes and the standard errors are summarized in Table S1, Supporting Information. In all these independent experiments, the data points for the the CPD <TT>, 6-4 <TT>, and CG*⁺C (10*S* (+)-*trans-anti*-[BP]-*N*²-dG adduct) were normalized to the 40 min value of the 10*R* (+)-*cis-anti*-[BP]-*N*²-dG adduct-containing CG*⁺C duplex (open circles) obtained in the same experiment. The inset in Figure 3b shows the densitometric tracings of the 40 min lanes shown in the gel (panel (a)).

Figure 4.

Comparison of dual incisions elicited by the 10S (+)-*trans-anti*-[BP]-*N*²-dG adduct in CG*C, G6G7* (abbreviated as G7*), and G6*G7 (abbreviated as G7*) 135-mer duplexes in HeLa cell extracts after an incubation time of 40 min. Control samples: G6G7, unmodified duplex without or after treatment with cell extracts: CG*C, G6G7*, and G6*G7: untreated controls. The overall radioactivity levels in each lane were comparable, to allow for a visual inspection of the differences in dual incision efficiencies. Densitometry tracings of the lanes in panel comparing dual incision efficiencies of the same 10S (+)-*trans-anti*-[BP]-*N*²-dG adduct embedded in the different sequence contexts are shown in Figure S4, Supporting Information.

The relative NER efficiency of the (+)-*cis-anti*-[BP]- N^2 -dG adduct used as a standard (See text) is also shown.

Figure 5.

(a) Denaturing gel showing the appearance of dual excisions elicited by the 10*S* (+)-*trans-anti*-[BP]-*N*²-dG adducts in the I6G7*, G6*G7, G6G7* and CG*C duplexes as a function of incubation time in HeLa cell extracts. Lanes 1, 2, 3, and 4 represent incubation times of 10, 20, 30, and 40 min, respectively. Representative densitometry tracings, adjusted for the total radioactivity in each lane to compensate for loading factors and differences in the radioactivities of the samples (the concentration of the I6G7* was lower than in the case of the other samples). (b) Time course of dual incision product formation elicited by the 10*S* (+)-*trans-anti*-[BP]-*N*²-dG adduct in the G6*G7, G6G7*, I6G7* and CG*C duplexes. The experimental points are averages of seven independent experiments, and the error bars represent the standard deviations. The error bars for the I6G7* data points are omitted for clarity, but they do overlap with the error bars of the G6G7* and CG*C data points. The straight lines are the least square fits to the data points, and the relative values of the slopes and the standard error are summarized in Table S1, Supporting Information. In all these experiments, the data points were normalized to unity based on the 40 min value of the 10*R* (+)-*cis-anti*-[BP]-*N*²-dG in the CG*C duplex obtained in each individual experiment with a given cell extract (not shown). The lanes for the I6G7* duplex are uniformly lighter (panel a, left) than in the case of the other sequences; this was due to the lower quantities of I6G7* sample available. However, this difference is not reflected in panel b, since these data represent ratios of [dual excision products]/[total DNA substrates] (see text).

Figure 6.

Summary and comparisons of structural effects on relative NER efficiencies. Differential steric hindrance by nearby guanine amino groups governs sequence-dependent distortions and duplex destabilization^{63,70} that influence the DNA sequence-governed NER dual incision efficiencies. In the series of minor groove lesion-containing duplexes studied here, the strongest signal that elicits the NER dual incision response is a disrupted Watson-Crick base pair in the **G6*G7** duplex. The flexible kink in **G6G7*** appears to be less important since the bends are more rigid in **I6G7*** and the other two duplexes studied (see text). Minor groove enlargement^{47,63} together with modest perturbation of Watson-Crick base pairing at the lesion site⁶³ is common to all DNA sequences examined here.