**NMR SOLUTION STRUCTURES OF ADDUCTS FROM THE
BINDING OF POLYCYCLIC AROMATIC DIOL EPOXIDES TO DNA**

by

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Abstract. Site-specifically modified oligonucleotides were derived from the reactions of stereoisomeric polycyclic aromatic diol epoxide metabolite model compounds with oligonucleotides of defined base composition and sequence. The NMR solution structures of ten different adducts studied so far are briefly described, and it is shown that stereochemical factors and the nature of the oligonucleotide context of the complementary strands, exert a powerful influence on the conformational features of these adducts.

We have synthesized a variety of site-specifically modified oligonucleotides derived from the covalent binding reactions of different stereoisomeric polycyclic aromatic hydrocarbon diol epoxides (PAHDE) to short DNA fragments of defined base composition and sequence.¹ Using one- and two-dimensional NMR methods combined with molecular mechanics calculations, we have studied the solution structures of these oligonucleotide adducts and discovered a striking diversity of stereochemistry-dependent structural motifs.²⁻¹⁰ A comprehensive picture of the types of conformations of adducts derived from the binding of stereoisomeric PAHDE molecules to duplex DNA is beginning to emerge.²⁻¹³ In this article, we provide a short account of our own findings²⁻¹⁰ in this area of research.

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STRUCTURES OF DNA ADDUCTS: BPDE-N²-dG LESIONS.

The (+)- and (-)-enantiomers of *anti*-BPDE (7*R*,8*S*-dihydroxy-9*S*,10*R*-epoxy- and 7*S*,8*R*-dihydroxy-9*R*,10*S*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, respectively) bind covalently to the exocyclic amino group of guanine residues in DNA¹⁴ (BPDE-N²-dG lesions). There are four stereoisomerically distinct *anti*-BPDE-N²-dG lesions since both (+)- and (-)-*anti*-BPDE can add *trans* or *cis* to the C10 position of *anti*-BPDE¹⁴. We have studied the solution NMR conformations of these adducts in the duplex:

in which the non-modified strand is fully complementary to the *anti*-BPDE-modified strand; the *anti*-[BP]-N²-dG lesion is denoted by [BP^G]. The adduct conformations of the stereoisomeric, but chemically identical (+)-*trans*- (10*S* adduct stereochemistry at C-10 of the BP residue²), (-)-*trans*-(10*S*³), (+)-*cis*-(10*R*⁴) and (-)-*cis*-*anti*-[BP]-N²-dG lesions (10*S*) (Cosman et al., in preparation) in duplex I are remarkably different from one another.

The two *trans*-*anti*-[BP]dG adducts. In the (+)-*trans*- and (-)-*trans*-*anti*-[BP]dG adducts in duplex I, the pyrenyl residues are situated in the minor groove with one face exposed to the aqueous environment. In the (+)-*trans*-adduct the pyrenyl residue points to the 5'-end of the modified strand, while in the case of the (-)-*trans*-adduct, the pyrenyl ring system points toward the 3'-end.^{2,3} This structural motif, i.e. opposite orientations of adducts relative to the modified base reoccurs in other pairs of stereoisomeric adducts derived from chiral enantiomeric diol epoxides.^{9,10}

The two *cis*-*anti*-[BP]dG Adducts. In the (+)- and (-)-*cis*-*anti*[BP]-N²-dG adducts in I the pyrenyl residues are inserted into the duplex but the modified guanine residues are displaced into the minor groove ((+)-*cis*-adduct⁴) and into the major groove ((-)-*cis*-adduct). Furthermore, in the (+)-*cis*-adduct, the benzylic ring is in the minor groove with the polycyclic aromatic portion pointing towards the major groove.⁴ In the (-)-*cis*-adduct (M. Cosman, et al., in preparation) the BP assumes the opposite orientation with the benzylic ring situated in the major groove. Thus, the chirality of the two *anti*-BPDE enantiomers manifests itself in a plane

perpendicular to the helix axis, rather than along the minor grooves as in the case of the (+)- and (-)-*trans-anti*-[BP]dG adducts^{2,3} in sequence I.

abstracts, equations

Conformations of [BP]-N²-dG Lesions in Deletion Duplexes

11-mer/10-mer anti-[BP]dG deletion duplexes. The stalling of polymerases at the sites of the lesions can give rise to slipped, misaligned intermediates that can lead to deletions and point mutations.¹⁵ As a model system for such extended bulged intermediates, we investigated the high resolution NMR characteristics of sequence II, with the C opposite the lesion missing in the partner strand^{5,6} (11-mer/10-mer duplex):

The most striking finding is that the (+)-*trans-anti*-[BP]-N²-dG is no longer in the minor groove as in the full duplex² I, but is inserted into the helix with the modified guanine displaced into the major groove.⁵ With the stereoisomeric *cis*-adduct, the base-displaced intercalation conformation prevails in both the normal duplex⁴ I, as well as in the deletion duplex⁶ II; in both cases, the modified guanine residues are displaced into the minor groove. These results suggest that stable (-1) deletion structures can be formed upon extension of slipped frameshift intermediates. Consistent with this conclusion, Ya et al.¹⁶ showed that the deletion duplexes II are thermodynamically stabilized by the presence of the BP residues.

BPDE-N²-dG Lesions at Single Strand/Double Strand Junctions.

13-mer/9-mer partial replication fork model sequence. At the single-strand/double strand junction in a polymerase replication complex, the complementary strand on the 5'-side of the modified template strand is missing. In order to investigate the conformations of [BP]-N²-dG lesions at such junctions, we investigated the solution structure of the following 13-mer/9-mer modified template/primer sequence⁷:

The primer strand extends up to the lesion site in III, and there is no complementary

base opposite the BP-modified guanine residue. A detailed analysis of the NMR solution conformations shows that there is more conformational flexibility in the case of the (-)-*trans*-adduct III than in the case of the (+)-*trans*-adduct III. In the latter case, the glycosidic torsion angle of the modified guanine residue assumes a *syn* rather than the usual *anti* conformation⁷; the modified guanine is thus displaced, and the pyrenyl ring system of the BP moiety is stacked over the 3'-terminal guanine on the primer strand. This structural motif shares features of base displacement and BP-base stacking with the deletion structures, but employs a *syn*-modified guanosyl residue. In the (-)-*trans*-adduct III, there is a striking loss of resolution of the NMR resonances of the protons in the immediate vicinity of the BP-modified guanine residue that extends over 2-3 bases flanking the lesion on either side. These data suggest a significant conformational heterogeneity in the case of the (-)-*trans*-adduct III and the exact conformation of the BP residue could not be established.⁷

5-Methylchrysene diol epoxide-N²-dG adducts: effects of a methyl group.

Methyl groups, depending on their positions in a polycyclic aromatic ring system, can exert powerful effects on the biological activities of these compounds.¹⁷ The effects of a methyl group on the solution structure of duplex I containing a (-)-*trans-anti*-[MC]-N²-dG lesion was investigated in detail⁸ ([MC]: residue obtained from the reaction of 1*S*,2*R*-dihydroxy-3*R*,4*S*-epoxy-1,2,3,4-tetrahydro-5-methylchrysene, a metabolite of 5-methylchrysene¹⁷, to N²-dG in DNA). The [MC] residue is situated in the minor groove and points into the 3'-direction of the modified strand,⁸ as in the structurally related (-)-*trans-anti*-[BP]-N²-dG adduct duplex³ I. The -CH₃ group, however, causes a buckling of the [MC]-modified dG-dC base pair, a local widening of the minor groove and a compression in the major groove, thus leading to a wedge-shaped local structure and a large bend at the binding site.⁸

Conformational Adduct Heterogeneity.

In the case of the (+)-*cis*-[BP]-N²-dG adduct duplexes I, there is evidence of a second, minor conformation (~ 15% at 10 °C), that bears the characteristics of a non-intercalated structure. NMR exchange crosspeaks suggest that the major base-displaced intercalative conformation and this minor adduct conformation are in

exchange with one another.⁴ Fountain and Krugh¹¹ studied the solution structure of duplexes with the (+)-*trans-anti*-[BP]-N²-dG lesion in the sequence context 5'-.....T[^{BP}G]C.....-3' which differed from the 5'-.....C[^{BP}G]C.....-3' sequence context in I. In the 5'-.....T[^{BP}G]C.....-3' sequence context, a minor groove structure with the pyrenyl residue pointing into the 5'-direction of the modified strand was the major observed conformation,¹¹ consistent with the structure observed in duplexes² of I. However, a minor structure in equilibrium with the major structure was also observed; this conformational heterogeneity was attributed to the dT base on the 5'-side of the lesion,¹¹ since only the minor groove structure is observed when dC bases flank the modified guanine residue on both sides.² Conformational heterogeneity that is sensitive to base sequence context may thus be an important structural feature of such adducts.

PAH DIOL EPOXIDE-N⁶-dA ADDUCTS.

We synthesized a pair of stereoisomeric adducts, derived from the binding of 4*r*,3*t*-dihydroxy-2,1*t*-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (*anti*-BPhDE) to the exocyclic amino group of adenine in the duplex¹⁸:

The NMR solution structures of the (+)- and (-)-*trans* adducts^{9,10} IV derived from the reaction of the 4*S*,3*R*,2*R*,1*S*- and 4*R*,3*S*,2*S*,1*R*-BPhDE enantiomers to the oligonucleotide d(CTCTCACTTCC), respectively, exhibit structural motifs quite different from those exhibited by the [BP]-N²-dG adducts.²⁻⁴ These structures^{9,10} are the closest to those of classical intercalation complexes (modified classical intercalation) normally associated with the insertion of flat polynuclear aromatic drug molecules between adjacent base pairs of duplex DNA. The aromatic BPh residues in both the (+)-*trans*- and (-)-*trans-anti*-[BPh]-N⁶-dA adducts IV, with T opposite the lesions in the complementary strands, are stacked essentially between the T and G residues on the complementary strands.^{9,10} In contrast to the base-displaced intercalative conformations observed in the case of the *cis-anti*-[BP]-N²-dG adducts,⁴ the modified base is *not* displaced, and all 11 base pairs in IV are intact.

The adducts are characterized by strikingly different orientations in that the BPh residue is ~~intercalated on the 5'-side~~^{abstracts, equations} of the modified adenine residue in the (+)-*trans*-, and on the 3'-side in the case of the (-)-*trans*-adduct.^{9,10} These differences, just as in the case of the opposite orientations of the (+)-*trans*- and (-)-*trans-anti*-[BP]-N²-dG lesions,^{2,3} are a consequence of the chiral characteristics of the enantiomeric PAH diol epoxide adduct precursors. In order to accommodate the bulky BPh residues, the helix is stretched, and the base pairs at the site of the lesion are buckled in opposite directions.

Recently, the NMR solution structures of (-)-*trans-anti*- and (+)-*trans-anti*-[BP]-N⁶-dA lesions^{12,19} opposite G, and a (-)-*trans-syn*-[BP]-N⁶-dA lesion¹³ and a (-)-*trans-anti*-[BP]-N⁶-dA lesion^{20,21} opposite the normal complementary T, were determined. The conformations are intercalative in all four cases *without* base displacement, and the sites of insertion on the 5'- or 3'-side of the modified adenine residues,^{12,19-21} are consistent with those observed with the stereochemically analogous (+)- and (-)-*trans-anti*-[BPh]-N⁶-dA adducts^{9,10} in duplex IV.

CONCLUSIONS

The types of structural motifs discovered up till now are summarized in Table 1. The rapidly growing number of established PAH diol epoxide-DNA adduct structures^{2-13,19-21} will ultimately lead to a better understanding of the mechanisms of mutations associated with this class of PAH metabolites. The remarkable effects of the different configurations of substituents about the four chiral carbon centers in the benzylic ring on adduct conformation indicates that the impact of stereochemical factors on the biological activities of PAH diol epoxides will be eventually elucidated.

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Table 1. Observed Structural Motifs For Conformations of PAH-DNA Adducts^a

Structural Motif	PAH Orientation	Adduct	DNA Context	Reference
Minor groove	5'-orientation ^b	(+)- <i>trans</i> -[BP]-N ² -dG	normal duplex	Refs. 2, 11.
Minor groove	3'-orientation ^b	(-)- <i>trans</i> -[BP]-N ² -dG (-)- <i>trans</i> -[MC]-N ² -dG	normal duplex normal duplex	Ref. 3 Ref. 8
Base-Displaced Intercalation ^b	from minor to major groove ^c	(+)- <i>cis</i> -[BP]-N ² -dG (+)- <i>cis</i> -[BP]-N ² -dG	normal duplex (-1)deletion duplex	Ref. 4 Ref. 6
Base-Displaced Intercalation	from major to minor groove ^c	(-)- <i>cis</i> -[BP]-N ² -dG (+)- <i>trans</i> -[BP]-N ² -dG	normal duplex (-1)deletion duplex	Cosman et al (1996) Ref. 5
Modified Classical Intercalation	5'-insertion ^b	(+)- <i>trans</i> -[BPh]-N ⁶ -dA (-)- <i>trans</i> -[BP]-N ⁶ -dA (-)- <i>trans</i> -[BP]-N ⁶ -dA (-)- <i>trans-syn</i> -[BP]-N ⁶ -dA	normal duplex mismatch, duplex normal duplex normal duplex	Ref. 9 Ref. 12 Ref. 20, 21 Ref. 13
Modified Classical Intercalation	3'-insertion ^b	(-)- <i>trans</i> -[BPh]dA (+)- <i>trans</i> -[BP]dA	normal duplex mismatch, duplex	Ref. 10 Ref. 19
Stacking with terminal primer base at single strand - duplex junction; modified G is <i>syn</i>	from modified template strand towards primer strand	(+)- <i>trans</i> -[BP]-N ² -dG	13-mer/9-mer (modified template)-(primer) junction	Ref. 7
Conformational heterogeneity	multiple	(-)- <i>trans</i> -[BP]-N ² -dG	13-mer/9-mer	Ref. 7

^aAll adducts derived from *anti*-PAH diol epoxides (DE 2), unless noted "*syn*" (DE 1). [BP] = from benzo[a]pyrene diol epoxide; [BPh] = from benzo[c]phenanthrene diol epoxide. [MC] = from 5-methylchrysene diol epoxide. ^bOrientation of aromatic PAH residue relative to modified base and the 5'- or 3'-direction of the modified DNA strand. ^cOrientation is from benzylic ring to the pyrenyl moiety. Modified base displaced into major or minor groove.