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Conformational Properties of Equilenin-DNA Adducts: Stereoisomer and Base Effects

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Abstract

Equilin and equilenin, components of the hormone replacement therapy drug Premarin, can be metabolized to the catechol 4-hydroxyequilenin (4-OHEN). The quinoids produced by 4-OHEN oxidation react with dC, dA and dG to form unusual stable cyclic adducts, which have been found in human breast tumor tissue. Four stereoisomeric adducts have been identified for each base. These twelve Premarin-derived adducts provide a unique opportunity for analyzing effects of stereochemistry and base damage on DNA structure, and consequently its function. Our computational studies have shown that these adducts, with obstructed Watson-Crick hydrogen bond edges and near-perpendicular ring systems, have limited conformational flexibility, and near-mirror image conformations in stereoisomer pairs. The dC and dA adducts can adopt major and minor groove positions in the double helix, but the dG adducts are positioned only in the major groove. In all cases, opposite orientations of the equilenin rings with respect to the 5'→3' direction of the damaged strand are found in stereoisomer pairs derived from the same base, and no Watson-Crick pairing is possible. However, detailed structural properties in DNA duplexes are distinct for each stereoisomer of each damaged base. These differences may underlie observed differential stereoisomer and base-dependent mutagenicities and repair susceptibilities of these adducts.

The hormone replacement drug Premarin, whose use has been shown to increase breast cancer risk (1-6), contains the equine estrogens equilin and equilenin (Figure 1). This pair of substances can be metabolized to highly reactive 4-OHEN quinoids that can form unusual stable cyclic adducts with dC, dA and dG (7-9). There are four different stereoisomers for each base adduct (9-11), for a total of twelve distinct lesions (Figure 1). The structural properties of this set of twelve DNA adducts present a fascinating opportunity for elucidating how the stereochemical features and the nature of the damaged base differentially distort and destabilize the local structure of double-stranded DNA molecules around the lesion sites. Such differences

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Supporting Information Available: Details of the molecular dynamics protocol of 4-OHEN-G adducts in duplexes are provided. Table S1 gives glycosidic torsion χ values of the modified guanine, box sizes and numbers of waters in the MD simulation starting models. Table S2 gives added force field parameters for the modified guanine. Table S3 gives AMBER atom type, connection type, and partial charge assignments for the 4-OHEN-G adducts. Table S4 gives MM-PBSA free energy components of the lowest free energy conformation for each 4-OHEN-G stereoisomer modified duplexes. Table S5 gives solvent accessible surfaces of the equilenin rings of 4-OHEN adducts in 11 mer duplexes. Table S6 gives hydrogen bonds and occupancies at the lesion sites of 4-OHEN-G modified duplexes. Table S7 gives van der Waals interaction energies between the base pairs of the 4-OHEN-G modified duplexes. Table S8 gives trajectory average bending angles of the 4-OHEN-G modified DNA duplexes. Table S9 gives free energies for *syn* and *anti* conformations of each stereoisomeric adduct. Figure S1 shows starting structures for the MD simulations of the 4-OHEN-G stereoisomers in the DNA duplexes. Figure S2 shows RMSD vs time plots for each molecular dynamics simulation of the 4-OHEN-G adducts. Figure S3 shows torsion angle δ' (N1-C2'-C3'-N2) (degrees) vs time plots for each molecular dynamics simulation of 4-OHEN-G adducts. Figure S4 shows trajectory-average groove dimensions of 4-OHEN-G modified duplexes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

in distortion/destabilization could significantly affect the susceptibilities of the stereoisomeric 4-OHEN-DNA lesions to nucleotide excision repair (NER) in cellular environments. Furthermore, the absolute configurations and the nature of the base in these adducts can affect translesion bypass catalyzed by DNA polymerases and mutagenic specificity if bypass is successful, as well as transcription catalyzed by RNA polymerases. The biological impact of 4-OHEN-DNA adducts is of special interest because three stereoisomeric dG and two dA adducts have been found in the mammary fat pads of rats upon 4-OHEN injection (12), and dG, dA and dC adducts have been detected in human breast tissue of patients who were exposed to equilin and equilenin via hormone replacement therapy (13). Our detailed analysis of the structural features of the stereoisomeric 4-OHEN-DNA adducts reported here is important because differences in miscoding properties among the various adducts have been noted in *in vitro* systems (14-18) and, recently, differences in excision susceptibilities catalyzed by human and prokaryotic NER systems have been observed (17,19,20).

Estrogens are metabolized by cytochrome P450 to 2- and 4-hydroxylated catechols (21,22). The 4-hydroxylated estrogens are likely to be more carcinogenic than the 2-hydroxylated ones. In the hamster kidney system 4-hydroxyestradiol was carcinogenic, while 2-hydroxyestradiol did not induce tumors (23). 4-hydroxyestradiol also showed much higher carcinogenic activity than 2-hydroxyestradiol in CD-1 mice (24). Furthermore, the levels of 4-hydroxyestrone/estradiol and derived conjugates were significantly higher in breast tissue from women with breast cancer than in controls (25). Recent studies have suggested that high levels of estrogen 4-hydroxylase expression are linked to an increased risk of developing breast cancer (26-28). Potential mechanisms of estrogen quinone carcinogenesis, focusing on DNA and protein damaging pathways, have been recently reviewed (8). Increased B ring unsaturation in equilin and equilenin (Figure 1a) significantly increases the deleterious 4-hydroxylation metabolic pathway. In human breast cancer cells (MCF-7), 4-hydroxyequilenin (4-OHEN) is the major phase I metabolite of both equilin and equilenin (22,29). 4-OHEN autooxidizes to the highly reactive and potent cytotoxic quinoids (22,30,31) that can cause a variety of DNA lesions *in vitro* and *in vivo* (12,32-36), including the stable dC, dG and dA adducts (9,10). The quinoid of 4-hydroxyequilin (4-OHEQ) derived from the metabolic activation of equilin oxidizes to the 4-OHEN o-quinone and forms the same DNA adducts as 4-OHEN (30). 4-OHEQ has been shown to be mutagenic in a supF shuttle vector plasmid system propagated in human cells, presumably due to adduct formation (37).

The chemical structures of the four stereoisomeric 4-OHEN adducts to dG, dA and dC have been determined (Figure 1) (9-11). The analogous dT adducts derived from 4-OHEN were not investigated here because none have been found experimentally at this writing (10). The Watson Crick hydrogen bonding edge of the base is obstructed by the formation of the cyclic adduct in all cases. While there are actually three chiral centers within the region connecting the nucleobase-4-OHEN ring system, the two covalent bonds formed between 4-OHEN and the base adopt only a *cis* configuration since the connection ring would be highly strained in a *trans* configuration (11). Thus, only four different stereoisomeric adducts are observed for each of the bases C, A, and G (9,10). The chiralities at C1' and C3' determine the stereochemistry of the 4-OHEN-G adducts, while the absolute configurations of substituents at C2' and C3' determine the stereochemical features of the 4-OHEN-C and A adducts.

We have previously investigated the twelve stereoisomeric adducts on the base level by QM calculations (38), and the dC and dA adducts in DNA duplexes with MD simulations (39,40). Here, we present new results for the 4-OHEN dG adducts in duplex DNA, and provide an integrated view with comparisons of our conformational analyses for the twelve unique 4-OHEN-G, -C, and -A adducts at the base level and in double-stranded DNA. We highlight both the common features as well as the structural distinctions of all twelve stereoisomeric adducts in the duplexes. As new biological data emerges, the specific structural hallmarks elucidated

in our studies should provide a structural perspective for the functional biological differences that are beginning to emerge (14-20).

METHODS

Molecular modeling and MD simulations were carried out for the 4-OHEN-G adducts in DNA, as in previous studies of the 4-OHEN-C and A DNA adducts (39,40). Briefly, we started with an energy minimized B-DNA structure whose sequence is given in Figure 1 and replaced the guanine with the 4-OHEN-G. All the conformations of each 4-OHENG stereoisomer on the base level (38) were modeled into the DNA duplexes. A search was then made with glycosidic torsion χ rotated continuously over its 360 degree range to locate structures with minimal close contacts. Structures with both *syn* and *anti* glycosidic torsions between base and sugar were built as initial models for the MD simulations (Table S1 and Figure S1 in the Supporting information). Figure S1 shows *syn* and *anti* glycosidic torsion conformations. The MD protocol (41-52) is given in the Supporting Information. Force field parameters added for the 4-OHEN-G adducts in DNA are given in Tables S2 and S3. The PTRAJ and CARNAL modules of the AMBER 7 package (41) were employed for structural analyses. Stacking interactions were estimated by computing the van der Waals interaction energies between adjacent base pairs, including the damaged base and partner pair, with the program ANAL of AMBER. DNA duplex groove dimensions and bend angles were computed with the MD Toolchest (53,54) and CURVES (53) programs, respectively. The solvent accessible surface area was computed using the Connolly algorithm (55) implemented in Insight II (Accelrys Inc.) with a probe radius of 1.4 Å. The molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) method (56-59) in AMBER was employed for thermodynamic analyses. Full details of the structural and thermodynamic analysis methods are provided in (39,40).

RESULTS and DISCUSSION

Conformations of 4-OHEN base adducts

The 4-OHEN adducts have unusual cyclic linkage sites, which limit their flexibilities even on the base level. The conformations of the 4-OHEN base adducts were computed using the quantum mechanical Density Functional Theory (DFT) method (B3LYP/6-31G*) (60-62), as described fully in (38).

Connection ring conformational families—The 4-OHEN-C and 4-OHEN-A adducts have the same unsaturated bicyclo[3.3.1]nonane type linkage site, which makes the conformation highly rigid. This stems from the combined effects of the inflexible C1'-C2'-C3' bridge, the adenine or cytosine conjugated ring system and the unsaturated cyclohexene A ring. Therefore, each stereoisomer is restricted to a single conformational family (Figure 2), in which the dihedral angle θ' (C1'-N1-C6-N⁶) for adenine or (C1'-N1-C4-N⁴) for cytosine (Figure 1) is around 0°.

On the other hand, the reaction of 4-OHEN with guanine forms a relatively more flexible five membered connection ring. 4-OHEN-G adducts each have three base-level conformational families (except for 4-OHEN-G4 which has only two), defined by the conformations of the linking 5-membered E ring: negative envelope, positive envelope and planar conformational families. The dihedral angles δ' (N1-C2'-C3'-N²) and ϵ' (C1'-C2'-C3'-C4') (Figure 1) describe the conformations of the linking 5-membered E ring and the cyclohexene A ring, respectively. See Figure 2 of reference 38 for description of E and A ring conformations at the linkage site. In the negative envelope conformation, the E ring adopts an envelope conformation with negative δ' and ϵ' values. In the positive envelope, the E ring exhibits an envelope conformation with positive δ' and ϵ' values. In addition to the two envelope conformations, this 5-membered ring can also adopt a planar form with the cyclohexene A ring adopting the boat conformation.

For each 4-OHEN-G stereoisomer, the energy differences between the different connection conformational families are not great (< 4 kcal/mol), suggesting each conformation might be feasible in duplex DNA. Full conformational details for the 4-OHEN-G base adduct stereoisomers are given in reference 38. Figure 3 shows the lowest energy conformations. Others are given in Figures 3-6 of reference 38.

Structural features: stereochemistry and linkage—The chemical structures of the stereoisomeric adducts show that they are paired, with inverse *R* and *S* configurations at the linkage site. The four stereoisomers of each base adduct form two pairs. The chirality of the C3' atom determines the handedness of the equilenin ring systems. In each stereoisomeric pair, the equilenin rings adopt opposite orientations with respect to the attached base. Therefore, each pair adopts near mirror image conformations (Figures 2 and 3), with the symmetry broken only by the D ring with its C18' methyl group on the equilenin moiety. This is consistent with the near-mirror image CD spectra of each stereoisomeric pair that has been observed experimentally (63). The two stereoisomeric pairs of each base adduct differ in the orientations of one hydroxyl group at the linkage site. For the 4-OHEN-C and A adducts, the hydroxyl group at C2' is situated close to the base in the 4-OHEN-C1/C2 and A1/A2 stereoisomer pairs, while in the C3/C4 and A3/A4 pairs, it is directed away from the base and toward the equilenin moiety. In the 4-OHEN-G adducts, the OH group at C1' points outward from the ring system in the 4-OHEN-G1/G2 pair and is directed between the base and equilenin rings in the G3/G4 pair.

The essential structural feature of these 4-OHEN base adducts is that the equilenin rings are near-perpendicular to the covalently linked base, creating very bulky and conformationally restricted structures (Figures 2 and 3). Another important and characteristic feature of these base adducts is the orientations of the stereoisomeric adducts: stereoisomer pairs, with reverse chiralities at C1', C2' and C3' atoms, have opposite orientations of the equilenin rings with respect to the attached base.

4-OHEN adducts in DNA duplexes

In order to investigate the structural effects of the 4-OHEN adducts in DNA duplexes, molecular modeling and MD simulations were carried out. For each stereoisomeric adduct, we modeled each base conformer into the 11mer B-DNA duplex in the sequence of Figure 1, which had been under experimental investigation (63). Both *syn* and *anti* glycosidic bond conformations were studied (see Figure S1). In all cases an unmodified control was also investigated.

Connection ring conformations—On the base level, as mentioned above, there is only one conformational family of the connection ring for each of the stereoisomeric 4-OHEN-C and A base adducts. During the MD simulations, these adducts retain the same conformational family in the duplexes, due to their rigidity.

For each guanine stereoisomer, there are two or three different conformational families for the connection ring, as mentioned above and detailed in reference 38. These all served as initial conformations for the MD simulations of the duplexes (see Methods). In these duplex simulations, the initial conformations of the connection ring converged to one connection ring conformational domain in the case of each stereoisomer. This is shown in the time-dependence of the linkage site torsion angles (Figure S3). The domain is the same for both *syn* and *anti* glycosidic torsions and is in one of the base level domains, with just slightly altered pucker of the E and A rings at the linkage site. However, the connection ring conformations in the duplexes vary with stereochemistry, so that the guanine-equilenin ring systems are more or less open in the different isomers (Figure 4). We computed free energies for each case using

the MM-PBSA method (56-59) employed previously for the C and A adducts in DNA. The lowest free energy conformation in the *syn* and *anti* domain for each stereoisomer was selected for detailed analysis (Table S4).

Stereochemistry governed structural features—Stereochemistry plays a key role in determining the specific structural properties of each base adduct. The 4-OHEN-C and A adducts have the same connection ring, which is close to the major groove side of the C and A bases. Therefore these adducts have similar stereochemistry-determined conformations. However, the 4-OHEN-G adducts' connection ring is different: the linkage site of the modified guanine is close to the minor groove side of the guanine (Figure 1), resulting in distinct stereochemistry-governed structural properties for this base adduct.

The stereochemistry of the equilenin C3' and the glycosidic bond conformation determine the orientation of the bulky equilenin ring system. In the *syn* conformation, the equilenin rings are 3' directed in 4-OHEN-C1/A1 and C3/A3 with *R* stereochemistry at the C3' position, and 5' directed in 4-OHEN-C2/A2 and C4/A4 with C3' *S* (Figure 5). However, the equilenin rings are oriented toward the 5'-side of the modified strand in 4-OHEN-G1 and G3 with C3' *R*, and 3'-side in 4-OHEN-G2 and G4 with C3' *S*. The orientations of the equilenin rings in the *anti* glycosidic bond conformation adducts (Figure 6) are opposite to those of the *syn* structures. The stereochemistry also governs the orientations of the methyl and OH groups at the linkage site. These stereochemical characteristics of the 4-OHEN adducts are summarized in Table 1.

Syn and anti conformations: structures and energetics—In the *syn* conformation, in all cases the equilenin rings are situated externally in the DNA major groove without much duplex distortion (Table 2): Watson-Crick hydrogen bonding of the adjacent base pairs is retained and less disturbed; base pair stacking is mainly retained; and groove dimensions and bending are comparable to the unmodified control. However, the equilenin rings are mostly exposed to solvent (Figure 7 and Table S5). On the other hand, less solvent exposure and larger duplex distortions are observed in the *anti* case. Table 2 and Figure 7 show these differences between the *syn* and *anti* conformations. Experimental thermal melting data in the same sequence contexts we investigated here indicate that these adducts are quite destabilizing as compared to unmodified DNA (63), which is consistent with our structural analyses. The structural perturbations summarized in Table 2 can account for the destabilizations of the 4-OHEN-modified duplexes.

The 4-OHEN-C and A adducts in DNA occupy similar *syn*/major groove and *anti*/minor groove positions (Figures 5 and 6). However, there are subtle differences that are governed by the nature of the damaged base. Compared to the C adducts, the equilenin rings of the A adducts are positioned further away from the DNA in the major groove due to the larger purine ring. (40) Thus, the equilenin rings are more solvent exposed and distort the major grooves less. Generally the larger 4-OHEN-A adducts disturb the stacking more and the DNA duplexes bend more toward the major groove. In the *anti* conformation, the equilenin rings insert well into the smaller minor groove, providing favorable hydrophobic interactions for both A and C adducts. However, the 4-OHEN-A adducts often cause the nearby bases to tilt in order to accommodate their larger bulk (Figure 6), causing weaker stacking interactions and larger groove distortions. In the *anti* 4-OHEN-A1 and -A3 cases, a dangling end is observed because the 5'-directed adducts are positioned only three base pairs away from the terminus in the current sequence context (Figure 6).

The structural features of the 4-OHEN-G adducts in DNA are distinct from those of the C and A adducts, since the connection ring differs and the linkage site is closer to the minor groove side of the base (Figure 1). The specific orientations of the 4-OHEN-G adducts are opposite to the 4-OHEN-A and C adducts with the same stereochemistry at C3', (Table 1). Also, the

equilenin rings are in the major groove in both *syn* and *anti* conformations; this arises because the equilenin distal rings are oriented toward the guanine major groove edge while being oriented toward the minor groove edge in C and A adducts (Figure 1). The *syn* guanine adducts mainly disturb the 5' side base pair of the modified strand, and the disturbance is greater than for the cytosine and adenine adducts, because the purine ring of the 5' side adenine stacks with the *syn* guanine. The details of the hydrogen bonding, van der Waals interactions and bending angles in 4-OHEN-G modified duplexes are shown in Tables S6-8. In the *anti* 4-OHEN-G adducts, the equilenin rings protrude into and widen the major groove and there are no favorable and compensating hydrophobic interactions (Figure 6). The two hydroxyl groups and the carbonyl group at the linkage site of the guanine adducts all participate in hydrogen bonding interactions with the partner C and nearby bases (Table S6).

Thermodynamic analyses of the simulated structures, using the MM-PBSA method (56-59), were carried out. Relative free energies of *syn* and *anti* conformations were calculated to compare their conformational stabilities (Table 3). The MM-PBSA free energy components for 4-OHEN-G modified duplexes are given in Table S4. The *syn* conformers are mainly favored by less distortion; the *anti* structures are favored by diminished solvent exposure. Moreover, the *anti*/minor groove conformations of 4-OHEN-C and A adducts have enhanced favorable hydrophobic contacts. However, each stereoisomer of each base adduct perturbs the structure of the DNA duplex differently. Each adduct selects a different balance between the following competing features: distortion, adduct solvent exposure, and favorable hydrophobic contacts. Generally, the 4-OHEN-A adducts mainly favor the *anti*/minor groove conformation due to less solvent exposure with attendant favorable hydrophobic interactions. The 4-OHEN-G adducts prefer the *syn*/major groove conformation due to lesser extents of distortion of the duplexes. This effect arises because the more distorting *anti* conformation, in contrast to 4-OHEN-C and A adducts, places the equilenin residue in the major groove where its ring systems cannot participate in the attendant favorable hydrophobic interactions. For the 4-OHEN-C case, in C2 and C4 the favorable hydrophobic contacts in *anti* dominate; in C1 and C3 the lack of distortion with favorable van der Waals interactions between the inward-facing equilenin methyl group and adjacent base in *syn* dominate (Figure 5).

A subtle interplay of adduct-induced distortion and adduct solvation and hydrophobic interactions determine the conformational preference of each stereoisomeric adduct. Hence, environmental conditions, such as salt concentration, base sequence context, duplex length and lesion position in the duplex (eg. central or close to an end), could readily influence the preferred glycosidic domain and attendant positioning of the equilenin moiety.

Stereoisomer and base effects in lesion processing

The twelve 4-OHEN adducts investigated share the obstructed Watson-Crick hydrogen bond edge and near-perpendicular ring systems. However, our modeling studies indicate structural properties in DNA duplexes that are distinct for each stereoisomeric adduct and its attached nucleobase. These structural differences can produce differences in biochemical function. In vitro primer extension studies conducted with several Y-family bypass polymerases indicate that various 4-OHEN-C and A DNA lesions are differentially bypassed, depending on stereochemistry and the base damaged (14-17,19). Bypass efficiency in pol η is highly dependent on adduct stereochemistry. Specifically, the bypass frequency in this enzyme differed by two orders of magnitude in the members of a pair of 4-OHEN-dC stereoisomers whose CD spectra were opposite in sign (14). With 4-OHEN-dA adducts, the bypass frequency past one stereoisomer was approximately 3 times higher than for another (15). Using pol κ and the pair of 4-OHEN-dC adducts with opposite sign CD spectra, mismatched dCMP and dAMP were inserted opposite both stereoisomers; however, chain extension with dCMP was much higher than with dAMP (14). Insertion of dGMP, the correct base, was highly inefficient. For

this 4-OHEN-dC pair with pol η , insertion of dAMP and subsequent extension were both higher than for the correct base dGMP. By contrast, for the pair of 4-OHEN-dA adducts with opposite sign CD spectra, both pols κ and η preferentially incorporated dTMP, the correct base, opposite these lesions; mismatched dAMP and dCMP were also incorporated by pol κ and η , respectively (15). Clearly the nature of the polymerase also governs the biological effects. Current studies also suggest damaged base-specific repair susceptibilities, with certain dA adducts less repaired than certain dC adducts in prokaryotic and eukaryotic nucleotide excision repair (NER) assays (17,19,20) (Kropachev, K., Chen, D., Kolbanovskiy, M. and Geacintov, N., to be published).

CONCLUSION

Conformations of the 4-OHEN base adducts have been determined using quantum mechanical methods, followed by structural and thermodynamic studies of these adducts in 11-mer DNA duplexes using molecular modeling and MD simulations. Adduct stereochemistry-governed unifying conformational features were delineated involving opposite orientations of stereoisomeric pairs of adducts; these characteristics are found in all modified bases. The absence of Watson-Crick base pairing due to the obstructed edge is also common to all adducts. However, each individual stereoisomer and base adduct has a unique impact on DNA structure which is manifested in an accumulating set of experimental biochemical data revealing different lesion-specific responses to DNA repair systems and polymerases. The emerging integrated structure-function knowledge base could potentially stimulate the design of improved hormone replacement therapy agents with lower reactivities of their metabolites with DNA and with lower mutagenic potentials, thus diminishing the genotoxic impact. Furthermore, an improved understanding of structure-function relationships may permit the biomonitoring of those Premarin-derived DNA lesions that are most genotoxic and mutagenic, and thus pose a greater risk for developing human cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

4-OHEN, 4-hydroxyequilenin
4-OHEN-G, 4-hydroxyequilenin-guanine
4-OHEN-A, 4-hydroxyequilenin-adenine
4-OHEN-C, 4-hydroxyequilenin-cytosine
dC, 2'-deoxycytosine
dA, 2'-deoxyadenosine
dG, 2'-deoxyguanosine
DFT, Density Functional Theory
MD, molecular dynamics
QM, quantum mechanics
RMSD, root-mean-square deviations
MM-PBSA, molecular mechanics Poisson-Boltzmann surface area

NER, nucleotide excision repair

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(a)

equilin **equilenin** **4-OHEN**

(b)

4-OHEN-G **4-OHEN-A** **4-OHEN-C** **4-OHEN-G1** **4-OHEN-G2** **4-OHEN-G3** **4-OHEN-G4** **4-OHEN-A1/C1** **4-OHEN-A2/C2** **4-OHEN-A3/C3** **4-OHEN-A4/C4**

(c)

5'-CCATCGC*TACC-3'

3'-GGTAGCG*A*TTGG-5'

Figure 1.

(a) Chemical structures of equilin, equilenin and 4-OHEN. (b) Chemical structures and stereochemical characteristics of the 4-OHEN-G, -A and -C adducts. (c) Sequence of the 11 mer B-DNA duplex for the MD simulations. The asterisk denotes the damaged position.

Figure 2.

Optimized conformations of 4-OHEN-C and 4-OHEN-A base adducts. The color code is as follows: 4-OHEN-C, blue; 4-OHEN-A, cyan; hydrogens are white and the C2' OH group is red.

Figure 3. Lowest energy optimized conformations of 4-OHEN-G base adducts. The color code is as follows: 4-OHEN-G, yellow; hydrogens are white and the C1' OH group is red.

Figure 4. Conformations of the 4-OHEN-G adducts in the trajectory-average structures for 4-OHEN-G modified duplexes.

Figure 5.

Five base pair segments with modified bases at center in the trajectory-average structures for the 4-OHEN modified duplexes in *syn* conformation. 4-OHEN adducts are in CPK. The color code is as follows: 4-OHEN-C, blue; 4-OHEN-A, cyan; 4-OHEN-G, yellow; hydrogens are white, the methyl group is orange and the OH group (at C1' in 4-OHEN-G or C2' in 4-OHEN-C and A) is red.

Figure 6.

Five base pair segments with modified bases at center in the trajectory-average structures for the 4-OHEN modified duplexes in *anti* conformation. In 4-OHEN-A1 and — A3 6 pb segments at the 5'-end of the modified strand are shown. 4-OHEN adducts are in CPK. The color code is the same as in Figure 5.

Figure 7.
Trajectory-average solvent accessible surfaces of the equilenin rings in 4-OHEN Adducts modified 11mer duplexes.

Table 1
Stereochemistry-Determined Structural Properties of 4-OHEN Adducts

Stereochemistry	Syn Conformation			Anti Conformation		
	Equilenin rings orientation ^a	C18' methyl group ^b	OH group ^{b,c,d}	Equilenin rings orientation ^a	C18' methyl group ^b	OH group ^{b,c,d}
4-OHEN-A/C1	2S-3R					
4-OHEN-A/C2	2R-3S	M	A	5'	M	A
4-OHEN-A/C3	2R-3R	S	A	3'	P	A
4-OHEN-A/C4	2S-3S	S	E	3'	M	E
					P	E
4-OHEN-G1	1'R-3R					
4-OHEN-G2	1'S-3S	M	S	3'	S	P
4-OHEN-G3	1'S-3R	S	S	5'	S	P
4-OHEN-G4	1'R-3S	S	M	3'	S	M

^a with respect to the modified strand

^b S, toward solvent; M, toward modified strand; P, toward partner strand

^c OH at C2' in 4-OHEN-C and A; OH at C1' in 4-OHEN-G

^d A, near axial; E, near equatorial

Table 2
Comparison of Structural Features of 4-OHEN-C, A^a and G Adducts with the Unmodified Duplex

	Adjacent Base Pair ^b	Number of Adduct Hydrogen Bonds ^c	Stacking Energy Difference ^d (kcal/mol)	Bend Angle Difference ^e (degrees)	Major Groove Distortion ^f (Å)	Minor Groove Distortion ^f (Å)
<i>syn</i>						
4-OHEN-C1	intact	0	5.5	11.9	3.1	0.7
4-OHEN-A1	intact	0	5.9	14.3	1.8	-1.3
4-OHEN-G1	intact	2	3.2	4.2	-5.7	1.6
4-OHEN-C2	intact	1	4.6	11.0	3.1	2.0
4-OHEN-A2	intact	2	4.7	8.7	2.3	2.1
4-OHEN-G2	distorted	1	5.4	0.7	3.0	2.4
4-OHEN-C3	intact	0	5.3	-0.7	-2.2	-2.3
4-OHEN-A3	intact	1	6.6	15.6	1.0	1.8
4-OHEN-G3	distorted	3	5.1	-2.5	2.8	3.6
4-OHEN-C4	distorted	2	9.2	0.1	5.3	-6.0
4-OHEN-A4	intact	2	3.1	7.5	2.9	2.7
4-OHEN-G4	distorted	2	3.4	4.3	4.0	2.1
<i>anti</i>						
4-OHEN-C1	distorted	0	22.2	4.1	5.2	8.8
4-OHEN-A1	broken	2	- ^g	26.3	1.4	6.2
4-OHEN-G1	broken	3	12.8	13.5	10.7	2.2
4-OHEN-C2	broken	1	19.7	15.8	3.7	-2.6
4-OHEN-A2	broken	2	22.1	38.9	4.1	9.5
4-OHEN-G2	broken	4	11.3	7.0	4.7	3.5
4-OHEN-C3	broken	3	22.6	38.4	2.2	8.2
4-OHEN-A3	broken	1	- ^g	32.9	4.7	6.1
4-OHEN-G3	distorted	3	12.6	26.4	7.1	2.7
4-OHEN-C4	broken	2	15.9	40.1	2.8	1.6
4-OHEN-A4	broken	0	20.9	39.8	13.4	-1.9
4-OHEN-G4	broken	4	20.8	28.5	5.4	3.2

Color code: most distorted, red; somewhat distorted, orange; least distorted, cyan.

^aData for 4-OHEN-C and A from (39,40).

^bWatson-Crick hydrogen bonds: intact, occupancy of all > 90%; distorted, occupancy of any one 50%-90%; broken, occupancy of all 0%. Distorted or broken hydrogen bonds are at the base pair adjacent to the lesion in the direction of the equilenin ring orientation in 20 of 24 cases, excepting *syn* 4-OHEN-G2, G4 and C4, and *anti* C1; for these the disturbance is at the base pair adjacent to the lesion in the direction opposite to the equilenin ring orientation due to specific hydrogen bonding and stacking interactions (39). The unmodified duplex has all base pairs intact.

^cNumber of hydrogen bonds involving 4-OHEN base adduct with occupancy > 50% in stable time frame of trajectory (see Methods and Table S6).

^dDifference between stacking interaction energy of modified duplex and its unmodified counterpart (Table S7). Larger energies show more perturbed stacking.

^eDifference between the trajectory average bend angle of the adduct and the unmodified duplex (Table S8).

^fUsing the unmodified duplex as reference, we calculated the groove distortions of the modified duplexes as (d-d0), where d is a groove dimension of the modified duplex, and d0 is the corresponding value for the unmodified duplex (Figure S4). The groove dimension difference with the largest absolute value is shown. A negative sign indicates groove closing compared to the unmodified control.

^gStacking cannot be calculated due to the dangling end.

Table 3Relative Free Energies for *syn* and *anti* Conformations of Each Stereoisomeric Adduct.^{a,b}

	4-OHEN-C1	4-OHEN-C2	4-OHEN-C3	4-OHEN-C4
<i>syn</i>	0.0	3.9	0.0	5.0
<i>anti</i>	1.5	0.0	6.8	0.0
	4-OHEN-A1	4-OHEN-A2	4-OHEN-A3	4-OHEN-A4
<i>syn</i>	3.6	0	5.4	0.6
<i>anti</i>	0	6.3	0	0
	4-OHEN-G1	4-OHEN-G2	4-OHEN-G3	4-OHEN-G4
<i>syn</i>	0	0	0	0
<i>anti</i>	1.0	7.0	6.2	5.0

^aFor each stereoisomeric adduct, the conformation with the lower energy is assigned $\Delta G = 0$. Energies are in kcal/mol.^bThe calculated free energy values and errors in the mean for each entry are given in Table S9.