Base-displaced intercalation of the 2-amino-3-methylimidazo[4,5-f]quinolone N²-dG adduct in the NarI DNA recognition sequence

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ABSTRACT

2-Amino-3-methylimidazo[4,5-f]quinolone (IQ), a heterocyclic amine found in cooked meats, undergoes bioactivation to a nitrenium ion, which alkylates guanines at both the C8-dG and N²-dG positions. The conformation of a site-specific N²-dG-IQ adduct in an oligodeoxynucleotide duplex containing the iterated CG repeat restriction site of the NarI endonuclease has been determined. The IQ moiety intercalates, with the IQ H4a and CH₃ protons facing the minor groove, and the IQ H7a, H8a and H9a protons facing the major groove. The adducted dG maintains the anti-conformation about the glycosyl bond. The complementary dC is extruded into the major groove. The duplex maintains its thermal stability, which is attributed to stacking between the IQ moiety and the 5'- and 3'-neighboring base pairs. This conformation is compared to that of the C8-dG-IQ adduct in the same sequence, which also formed a ‘base-displaced intercalated’ conformation. However, the C8-dG-IQ adopted the syn conformation placing the Watson–Crick edge of the modified dG into the major groove. In addition, the C8-dG-IQ adduct was oriented with the IQ CH₃ group and H4a and H5a facing the major groove. These differences may lead to differential processing during DNA repair and replication.

INTRODUCTION

Although browning meats during cooking imparts flavor, it also leads to the formation of heterocyclic amines (HCAs) such as 2-amino-3-methylimidazo[4,5-f]quinolone (IQ) (1–5). IQ has been produced in cooked meats at ppb levels (6,7) and has also been detected in tobacco smoke (8). HCAs and their metabolites have been isolated from human urine (9). Human exposures to HCAs, estimated to be ~60 ng/day (10), are modest, but are likely to be involved in cancer etiology (11,12).

IQ is a potent mutagen (13). It is less prevalent than 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (14), but it is 200-fold more mutagenic in Salmonella reversion (Ames) assays (3) and it is an order of magnitude more mutagenic than aflatoxin B₁. In these assays (15–18), HCAs such as IQ are active in point and frameshift tester strains (19). In bacteria, mutations occur primarily at G:C base pairs (20,21). IQ is a potent inducer of two-base frameshifts in CG repeats. Similar levels of mutations are seen in mammalian hprt (22) and ef-2 (23) gene assays. Base-pair substitutions are the predominant mutations observed in mammalian cells (24–26). Sister chromatid exchange has been observed in rodent cells (27–29). IQ induces tumors in the organs of rodents and in the livers of monkeys (30–33). Liver, forestomach and lung tumors have been observed in IQ treated mice (34), whereas liver, intestine, zymbal gland, clitoral gland, skin (35), mammary glands, liver and ear ducts tumors have been observed in exposed rats (36). The TD₂₀ values for IQ are 0.7 and 14.7 mg/kg/day in rats and mice, respectively (37). Human exposures to HCAs have been associated with pancreatic (38), colon (39), prostate (40) and breast cancers (41,42).

The genotoxicity of IQ derives primarily from its oxidation by CYP P450 1A2 to an N-hydroxylamine (43–47) although extra-hepatic CYP P450s oxidize HCAs with lower efficiencies (Scheme 1) (48). The N-hydroxylamine is acetylated by N-acetyl transferases, particularly NAT2 (49–51). In humans, the NAT2 fast acetylator polymorphism correlates with increased genotoxicity and cancer (52–54). The nitrenium ion resulting from solvolysis of N-acetoxy-IQ is the ultimate electrophile (26,48). It reacts predominately at the C8 atom of guanine, while a minor alkylation product is formed at the N² atom of guanine (55–57). In addition, IQ can be converted to a...
reactive and genotoxic N-nitrosamine, which shows similar regioselectivity for DNA alkylation (58,59).

Levels of C8 and N2-dG-IQ adducts have been measured in rat and primate tissues using mass spectrometry (60,61) and 32P post-labeling methodology. Turesky et al. (62) have monitored C8-dG-IQ adduct formation in human hepatocytes using tandem liquid chromatography-electrospray ionization mass spectrometry. Levels range from 7 to 26 adducts per 107 bases. While less abundant, the N2-dG-IQ adduct is more persistent than is the C8-dG-IQ adduct. The N2-dG-IQ adduct may therefore play a significant role in the genotoxicity of IQ.

We have synthesized phosphoramidite reagents of the C8- and N2-dG-IQ adducts in which the Buchwald-Hartwig palladium-catalyzed N-arylation was the key C-N bonding-forming step (64–67). These adducts have been site-specifically incorporated into the NarI restriction sequence, 5′-d(CG5G3C8CGX7C)-3′; 5′-d(G14T5G16G17C18G20C22G23)-3′; X = N2-dG-IQ adduct (Chart 1). This duplex contains the recognition sequence of the NarI restriction endonuclease, in which the G3 nucleotide (X3 in the NarI sequence and X7 in this study) represents a hot spot for two-base deletions. The IQ moiety intercalates, with the IQ H4a and CH3 protons facing the minor groove, and the IQ H7a, H8a and H9a protons facing the major groove. The added nucleotide maintains the anti-conformation about the glycosyl bond. The complementary dC is extruded into the major groove. Nevertheless, the duplex maintains its thermal stability. This is attributed, in part, to stacking between the IQ moiety and the 5′- and 3′-neighboring base pairs. The base-displaced intercalated conformation of the N2-dG-IQ adduct differs from that of the C8-dG-IQ adduct, and may also be compared with that of the N2-dG-AAF adduct (76), providing insight as to the persistence of the N2-dG-IQ adduct (63) and its processing during replication and repair events.

**MATERIALS AND METHODS**

**Sample preparation**

The N2-dG-IQ-adducted oligodeoxynucleotide 5′-d(CTC GGCCCACT)-3′ was synthesized as described (67). The complement strand 5′-d(GATGCGCCGAG)-3′ was synthesized by Midland Certified Reagents Co. (Midland, TX, USA) and purified by anion exchange chromatography. HPLC chromatographic utilized a Supelcosil LS-18-DB analytical base-deactivated C-18 column (Sigma-Aldrich Inc., St. Louis, MO, USA), using a gradient from 5% to 12% acetonitrile in ammonium formate buffer (pH 7), over 35 min, or a gradient from 5% to 30% acetonitrile in NaH2PO4 buffer (pH 5), over 25 min. The oligodeoxynucleotides were characterized by negative mode MALDI-TOF spectrometry in a hydroxypicolinic acid matrix. The oligodeoxynucleotides were annealed at 1:1 ratio at room temperature in 180 μl buffer containing 10 mM NaH2PO4, 100 mM NaCl and 5 μM Na2EDTA (pH 7).

**Thermal melting experiments**

UV melting temperatures were collected on Cary 100 Bio UV spectrometer using 0.5 OD of duplex in 1 ml of solution containing 0.1 M NaCl, 10 mM NaH2PO4 and 0.05 mM Na2EDTA (pH 7.0). The temperature was increased from 25 to 75°C at a rate of 1°C per min.

**NMR spectroscopy**

The N2-dG-IQ modified and the unmodified duplexes were prepared at concentrations of 570 and 810 μM, respectively, and placed into 3 mm diameter micro NMR sample tubes (77). The samples were prepared in 0.1 M NaCl, 50 μM Na2EDTA and 10 mM NaH2PO4 (pH 7.0). To observe non-exchangeable protons, the samples were
exchanged with D$_2$O. $^1$H NMR spectra were recorded at 600 or 800 MHz. The spectra were collected at 15°C; NOESY experiments were conducted at mixing times of 150, 200 and 250 ms with a relaxation delay of 1.8 s. Additional experiments were conducted with a longer relaxation delay to evaluate NOE distances arising from the adenine H2 protons, which typically exhibit longer $T_1$ relaxation values. The data were collected with 512 points in the $t_1$ dimension and 2048 points in the $t_2$ dimension. Chemical shifts were referenced to water. For the observation of exchangeable protons, the samples were dissolved in 9:1 H$_2$O:D$_2$O. $^1$H NMR spectra were recorded at 600 or 800 MHz at 15°C. The data were collected with 512 points in the $t_1$ dimension and 2048 points in the $t_2$ dimension. A mixing time of 250 ms was used. Water suppression was performed using the WATERGATE pulse sequence (78). The spectra were processed using the TOPSPIN software (Bruker Biospin Inc., Billerica, MA, USA).

NMR experimental restraints

The spectral data were evaluated using the program SPARKY (79). The intensities of NOE cross peaks were measured by volume integrations. The bounds for overlapped peaks were optimized manually. Noise was assigned half the intensity of the weakest peak, and motion was assumed to be isotropic. Experimental intensities were combined with intensities obtained from complete relaxation matrix analysis (CORMA) of starting model to generate a hybrid intensity matrix (80,81). The intensities were converted to distances with the program MARDIGRAS (82), which refined the hybrid intensity matrix. Calculations were performed using 150, 200 and 250 ms mixing time data and 2, 3 and 4 ns isotropic correlation times. Evaluation of the resulting distance data allowed creation of upper and lower bound distance restraints that were used in restrained molecular dynamics (rMD) calculations.

Restrained molecular dynamics calculations

An unmodified B-DNA model (83) was used as a starting structure. The guanine at position G7 was replaced by the N2-dG-IQ adduct using the program INSIGHT II (Accelrys Inc., San Diego, CA, USA). Partial charges for the N2-dG-IQ adduct were calculated with the B3LYP/6-31 G* basis set in GAUSSIAN (84). The starting structure was energy minimized for 1000 cycles. Simulated annealing protocols (85) used for the rMD calculations were conducted with the parm99 force field (86), using the program AMBER (87). Force constants of 32 kcal/mol/Å$^2$ were applied for distance and torsion angle restraints. The generalized Born model (88) was used for solvation. The salt concentration was 0.1 M. The molecule was coupled to the bath to control the temperature during simulated annealing (89). First, calculations were performed for 20 ps (20,000 steps) and recording data every ps by the following protocol: during steps 0–1000, the system was heated from 0 to
600 K with a coupling of 0.5 ps. During steps 1001–2000, the system was kept at 600 K. The system was then cooled from 600 to 100 K during steps 2001–18 000 with a coupling of 4 ps. Further cooling from 100 to 0 K occurred during steps 18 001–20 000 with a coupling of 1 ps. After initial cycles of refinement a longer 100 ps (100 000 steps) calculation was performed by the following protocol: During steps 0–5000, the system was heated from 0 to 600 K with a coupling of 0.5 ps. During steps 5001–10 000, the system was kept at 600 K. The system was cooled from 600 to 100 K during steps 10 001–90 000 with a coupling of 4 ps. Additional cooling from 100 to 0 K occurred during steps 90 001–100 000 with a coupling of 1 ps. Structure coordinates were saved after each cycle and were subjected to potential energy minimization. CORMA (80,81) was used to compare intensities calculated from these emergent structures with the distance restraints. Helicoidal analysis was performed using the CURVES+ web server (90,91).

RESULTS

Oligodeoxynucleotide containing the N2-dG-IQ adduct

The N2-dG-IQ adduct was incorporated into 5′-d(CTCGG CXCCATC)-3′ using automated solid-phase synthesis (67). The position of the N2-dG-IQ adduct was located at position X7, corresponding to position G3 in the NarI sequence. The modified oligodeoxynucleotide was purified by C18 reverse phase HPLC and characterized by MALDI-TOF mass spectrometry in negative ion mode (56). After initial cycles of refinement a longer 100 ps (100 000 steps) calculation was performed by the following protocol: During steps 0–5000, the system was heated from 0 to 600 K with a coupling of 0.5 ps. During steps 5001–10 000, the system was kept at 600 K. The system was cooled from 600 to 100 K during steps 10 001–90 000 with a coupling of 4 ps. Additional cooling from 100 to 0 K occurred during steps 90 001–100 000 with a coupling of 1 ps. Structure coordinates were saved after each cycle and were subjected to potential energy minimization. CORMA (80,81) was used to compare intensities calculated from these emergent structures with the distance restraints. Helicoidal analysis was performed using the CURVES+ web server (90,91).

NMR

The modified duplex yielded well-resolved NMR spectra with narrow line shapes for the non-exchangeable protons at 15°C. The best spectral quality for the exchangeable protons was obtained at 5°C.

Non-exchangeable DNA protons

The base aromatic and deoxyribose anemic protons were assigned using established procedures (Figure 1) (92,93). The intensity of the X7 H8 to X7 H1′ NOE was not changed in the presence of the adduct, indicating minimal change in the conformation of the glycosyl torsion angle. In the complementary strand, the intensity of the NOE between C18 H1′ and G19 H8 was weakened. The N2-dG-IQ adduct did not induce breaks in the sequential pattern of NOEs between the aromatic base protons and the anemonic protons. With the exception of the adduct site, the internucleotide NOEs were characteristic of a B-type duplex. The adenine H2 protons were assigned based upon NOEs to the thymine imino protons of the respective A:T base pairs. With the deoxyribose H1′ assignments in hand, the remainder of the deoxyribose protons was assigned from a combination of NOESY and COSY data. The assignments of the non-exchangeable DNA protons are summarized in Supplementary Table S1.

Table 1. Thermal melting temperatures (Tm values) of NarI duplexes containing the N2-dG-IQ adducts

<table>
<thead>
<tr>
<th>NarI</th>
<th>N2-dG-IQ modified duplex</th>
<th>Tm (°C)</th>
<th>ΔTm* (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5′-CTCXGCGCCCATC-3′</td>
<td>62</td>
<td>-1</td>
<td></td>
</tr>
<tr>
<td>5′-GAGCCGCGTAG-5′</td>
<td>64</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>5′-GAGCXCXCCATC-3′</td>
<td>63</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5′-GAGCCGCGTAG-5′</td>
<td>63</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Exchangeable DNA protons

The imino and amino proton regions of the NOESY spectrum are shown in Figure 2. The assignments were made using established methods (94). The N2-dG-IQ adduct perturbed Watson–Crick hydrogen bonding. At the X7:C18 base pair, the X7 imino proton resonance was broadened, probably due to an enhanced rate of exchange with water. The amino protons for C18 were not detected. No NOE was observed between the X7 and G19 imino protons, perhaps due to the broadening of the X7 imino proton. The chemical shifts of the X7 and G19 imino protons were almost isochronous. It was not possible to determine if a NOE between these two protons existed. All other base pairs were assigned, with the exception of the two terminal base pairs C5′:G3′ and C12′:G3′. The imino protons from the terminal base pairs were exchange broadened. Overall, the data suggested that the duplex maintained Watson–Crick hydrogen bonding, with the exception of the modified base pair (Figure 2B). The assignments of the exchangeable protons are summarized in Supplementary Table S2.

IQ protons

The IQ protons, consisting of the CH3 group, the H4a proton, and the H7a, H8a and H9a spin system, were assigned from a combination of COSY and NOESY data (Figure 3). The CH3 resonance was observed at 3.57 ppm. It displayed an intense NOE to the H4a proton, whose resonance was observed at 8.55 ppm. A 3J coupling between the H8a proton (δ 6.55 ppm) and the H9a proton (δ 7.65 ppm) was observed in the COSY spectrum. The H8a proton also exhibited a NOE to the H7a proton (δ 7.6 ppm). The 3J coupling between H8 and H7a exhibited weak intensity in the COSY spectrum. This was attributed to presence of the nitrogen atom in the ring, which broadened the H7a resonance. This effect


was also observed for the C8-dG-IQ adduct, for which the COSY cross-peak between H7a and H8a was only observed between 25 and 35°C (95). The IQ amine proton was not assigned.

Chemical shift perturbations
The N2-dG-IQ adduct resulted in localized chemical shift perturbations, involving the modified base pair X7:C18 and the neighboring C6:G19 and C8:G17 base pairs (Figure 4). At the modified X7:C18 base pair, the X7 H8 resonance shifted 0.4 ppm downfield relative to the G7 H8 resonance in the unmodified duplex. In contrast, the C18 H6 and C18 H1' resonances shifted 1 and 0.8 ppm downfield, respectively. At the 5'-neighbor C6:G19 base pair, the C6 H6 resonance shifted upfield by 0.2 ppm, whereas the C6 H1' resonance shifted downfield by 0.4 ppm. The G19 H8 and H1' resonances each shifted upfield by 0.4 ppm. At the 3'-neighbor C8:G17 base pair,
the C8 H6 resonance shifted downfield by 0.3 ppm, whereas the C8 H1 resonance shifted upfield by 0.2 ppm.

The G17 H8 resonance shifted downfield by 0.1 ppm. The G17 H10 resonance showed negligible chemical shift perturbation. The resonances for the remaining base pairs in the duplex also showed negligible chemical shift perturbations.

In the imino proton region of the spectrum, the X7 and G17 N1H imino resonances, at 11.57 and 11.59 ppm, respectively, exhibited upfield chemical shifts of >1 ppm from those of the unmodified duplex, at 13.24 and 13.16 ppm, respectively. The X7 N2H amine resonance was observed at 9.5 ppm.

**NOEs between IQ and DNA**

The CH3, H4a, H7a and H8a protons of IQ exhibited NOEs to the C8, G17, C18 and G19 bases (Table 2). The pattern of NOEs involving H9a was difficult to establish due to resonance overlap with G17. The CH3 group showed medium strength NOEs to X7 H1′ and C8 H6 and weak NOEs to C8 H1′ and C8 H5. It also showed an NOE to the G19 N1H imino proton. The H4a proton showed a strong NOE to X7 H1′, and medium NOEs to C8 H5, the X7 N2H amine proton and the G19 N1H imino proton and weak NOEs to X7 H2′ and X7 H2′′. The H7a proton exhibited weak NOEs to G17 H1′, and the X7 N2H amine proton. Three NOEs from X7 to C18 were observed; these were of medium strength NOE between H9a and C18 H1′, and H8a and medium strength between C18 H2′ and H2′′. The H8a proton showed a medium strength NOE to G19 H3′ and weak NOEs to G17 H1′ and G19 H8. Some 30% of the NOEs from the IQ ring were to protons in the complementary strand, whereas another 46% were to other IQ protons and protons of the modified base. The remaining 24% of the NOEs were to neighbor bases in the modified strand.

**Conformational Refinement**

After the unmodified duplex was constructed using B-DNA coordinates (83), the guanine at position G7 was replaced by the N2-dG-IQ adduct. The partial charges for the N2-dG-IQ adduct are provided in Supplementary Figure S2. Potential energy minimization provided an energy minimized starting duplex. A total of 329 distance restraints consisting of 127 inter- and 202 intra-nucleotide distances (Table 3) were obtained using the program MARDIGRAS (81,82), from 15°C NOESY data. Similar distance restraints were obtained if the data were collected at 150, 200 or 250 ms mixing times. These restraints included 16 DNA-IQ distances. A total of 49 Watson–Crick hydrogen-bonding restraints were applied for all of the base pairs except for the modified X7:C18 base pair. An additional 100 phosphodiester backbone and 20 deoxyribose pseudorotation restraints for base pairs not proximal to the site of modification were obtained from canonical values derived from B-DNA (83), consistent with the spectroscopic data indicating that the duplex maintained a B-DNA like structure. A series of rMD calculations were performed using a simulated annealing protocol in which the generalized Born solvation model (88) was used, with a salt concentration of 0.1 M. The emergent structures were subjected to potential energy minimization before further analysis, which involved a 100 ps rMD calculation using the protocol described above, again followed by potential energy minimization.

The pairwise rmsd analysis of structures emergent from the rMD calculations was used to measure the precision of the structural refinement. Ten structures were chosen...
based on the lowest deviations from the experimental distance and dihedral restraints (Figure 5). These exhibited an rmsd of 0.012 Å in distances and 2.5° in torsion angles (Table 3). There were 56 distance violations with a maximum penalty of 0.187 kcal/mol and a total distance penalty of 2.3 kcal/mol. There were 50 torsion angle violations with a maximum penalty of 0.177 kcal/mol and a total torsion angle penalty of 2.8 kcal/mol. The maximum pairwise rmsd distances were 1.12 Å. These structures were averaged and subjected to potential energy minimization.

The accuracy of the refined structures was assessed by complete relaxation matrix analyses (80,81), which compared intensities calculated from the refined structures with the distance restraints (Figure 6). The sixth root residual $R_{1x}$ value of the average structure was 8.4%, and the individual values for intra-nucleotide restraints (8.5%) and inter-nucleotide restraints (8.3%) were of similar magnitudes. This indicated agreement with the NOE data. Nucleotide G19 exhibited a greater $R_{1x}$ value of 17.1%, suggesting that it was not as well-refined. This was attributed to several NOEs involving G19 being overlapped with other resonances. The structural statistics are summarized in Table 4.

Conformation of the $N^2$-dG-IQ Adduct

The modified nucleotide (X7) remained in the anti-conformation about the glycosyl bond. It was displaced toward the major groove. The IQ ring was intercalated and oriented such that the H4a proton and the CH$_3$ group faced into the minor groove, whereas the H7a, H8a and H9a protons faced into the major groove (Figure 7). The IQ ring was angled by ~15° with respect to the modified guanine, but otherwise remained largely in plane with the damaged base. The helix was unwound between C6 and X7, with a reduced helicoidal twist of 30°. This was partially compensated by an increased twist of 9° between X7 and C8. At base pair X7:C18, the roll of the X7 purine decreased by 24°. This was compensated at base pair C8:G17, where the roll decreased by 12°. Consequently, the $N^2$-dG-IQ adduct induced a bend of 10° to the duplex. The IQ ring exhibited stacking with the flanking base pairs (Figure 8). IQ was stacked between G17 and C18 of the complementary strand. The complementary nucleotide, C18, extruded into the major groove and did not exhibit stacking with the neighboring base pairs. The base opening between X7 and C18 increased

Table 2. Summary of NOEs observed between $N^2$-dG-IQ(X7) adduct protons and oligodeoxynucleotide protons and their intensities

<table>
<thead>
<tr>
<th>IQ proton</th>
<th>NOEs to oligodeoxynucleotide protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>X7 H1’: medium; C8 H6: medium; C6 H1’: weak; C8 H5: weak</td>
</tr>
<tr>
<td>H4a</td>
<td>X7 H1’: strong; C8 H5: medium; X7 H2’: weak; X7 N2H: medium; G19 N1H: medium</td>
</tr>
<tr>
<td>H7a</td>
<td>G17 H1’: weak; X7 H2: weak</td>
</tr>
<tr>
<td>H8a</td>
<td>G19 H3: medium; C18 H2’: medium; C18 H2’: medium; G17 H1: weak; G19 H8: weak; G17 H2’: weak</td>
</tr>
<tr>
<td>H9a</td>
<td>C18 H1’: medium</td>
</tr>
</tbody>
</table>

Table 3. NMR restraints used for the $N^2$-dG-IQ structure calculations and refinement statistics

<table>
<thead>
<tr>
<th>NOE restraints</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Internucleotide</td>
<td>127</td>
</tr>
<tr>
<td>Intranucleotide</td>
<td>202</td>
</tr>
<tr>
<td>Total</td>
<td>329</td>
</tr>
<tr>
<td>Backbone torsion angle restraints</td>
<td>100</td>
</tr>
<tr>
<td>H-bonding restraints</td>
<td>49</td>
</tr>
<tr>
<td>Deoxyribose restraints</td>
<td>20</td>
</tr>
<tr>
<td>Total number of restraints</td>
<td>498</td>
</tr>
</tbody>
</table>

| Refinement statistics           |          |
| Number of distance restraint violations | 56       |
| Number of torsion restraint violations | 50       |
| Total distance penalty/maximum penalty (kcal/mol) | 2.3/0.187 |
| Total torsion penalty/maximum penalty (kcal/mol) | 2.8/0.177 |
| r.m.s. distances (Å)            | 0.012    |
| r.m.s. angles (°)               | 2.5      |
| Distance restraint force field (kcal/mol/Å$^2$) | 32       |
| Torsion restraint force field (kcal/mol/deg$^2$) | 32       |
by 76°. This disrupted Watson–Crick hydrogen bonding. The other base pairs maintained Watson–Crick hydrogen bonding.

**DISCUSSION**

The \( \Lambda^2 \)-dG-IQ DNA adduct has been of interest following reports that it is more persistent than the C8-dG-IQ adduct in rodents and primates that were fed IQ in their diet (63). The synthesis of this adduct into oligodeoxynucleotides (67) has allowed the conformation of the \( \Lambda^2 \)-dG-IQ adduct at the G3 position of this sequence to be determined. This is a hot spot for two-base frameshift deletions in bacterial mutagenesis assays (68–71,73,74). In addition, human DNA polymerase (hpol) \( \text{Z} \) produces two-base deletions when replicating past the \( \Lambda^2 \)-dG-IQ adduct at the reiterated G3 position of the NarI sequence, *in vitro* (72).

**Conformation of the \( \Lambda^2 \)-dG-IQ adduct**

The IQ ring intercalates when the \( \Lambda^2 \)-dG-IQ adduct is positioned at the frameshift-prone G3 position of the NarI sequence (Figure 7). The strong NOE intensities of the IQ H4a and CH3 protons to the X7 and C8 H1′

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**Table 4. Structural statistics for the \( \Lambda^2 \)-dG-IQ modified duplex**

<table>
<thead>
<tr>
<th></th>
<th>Intranucleotide</th>
<th>Internucleotide</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMS pairwise difference</td>
<td></td>
<td></td>
<td>1.12</td>
</tr>
<tr>
<td>RMS difference from</td>
<td></td>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td>average structure</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CORMA analysis for average structure**

\[ R_1^b = \frac{\sum \left( X_1 - Y_1 \right)^6 - \left( \sum X_1 \right)^6}{\sum \left( \sum X_1 \right)^6} \]

**Average error**

\[ \frac{\sum (I_e - I_o)}{n} \]

where the \( I_e \) are NOE intensities calculated from the refined structure, the \( I_o \) are experimental NOE intensities.

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**Figure 6.** Sixth root residuals \( (R_1^b) \) calculated using complete relaxation matrix calculations from the average of 10 structures emergent from the rMD calculations of the \( \Lambda^2 \)-dG-IQ modified duplex. The black bars represent intra-nucleotide sixth root residuals, and the gray bars represent inter-nucleotide sixth root residuals. (A) Nucleotides C1–C12 in the modified strand. (B) Nucleotides G13–G24 in the complementary strand.
protons (Table 1) indicate that these protons face into the minor groove and establish the conformation about the bond between N2-dG and C5 of the IQ moiety. In contrast, NOEs involving the H8a proton of the IQ ring are primarily to bases G1, C18, and G19 of the complementary strand (Table 1). The chemical shifts of the IQ H7a, H8a and H9a protons are observed between 6.5 and 8.0 ppm, which is 1.3–2.0 ppm upfield as compared to the N2-IQ-dG nucleoside. This is consistent with the intercalated conformation and stacking of the IQ ring below the 5'-neighboring G19 of the complementary strand and above the 3'-neighboring C8:G17 base pair (Figure 8). Chemical shift perturbations corroborate the NOE data (Figure 4). The IQ H4a proton resonance, observed at 9.6 ppm, is 0.4 ppm upfield from the resonance observed for the modified N2-dG-IQ nucleoside (67), consistent with its location below G19 and above C8 (Figure 8). The IQ moiety displaces the complementary C18 base from the duplex, and flips it into the major groove. This is supported by smaller perturbations in chemical shifts for the H4a and CH3 protons as compared to the H7a, H8a and H9a aromatic protons of IQ. The displacement of the modified nucleotide X toward the major groove (Figure 8) is supported by the downfield chemical shift change of 0.4 ppm for the X7 H8 and H1' protons of the modified base. The C8 H6 proton resonance also experiences a downfield shift of 0.3 ppm. The stacking interactions of the IQ ring with the flanking bases C8, G17, and G19 are reflected in the thermodynamic analysis of the adduct, in which the thermal melting temperature of 63°C is unchanged from that of the unmodified duplex.

Comparison to the N2-acetylaminofluorene-dG adduct

The other N2-dG arylamine adduct that has been subjected to conformational analysis, although not in the NarI sequence of interest herein, is that arising from N-acetylaminofluorene (AAF; Chart 1) (76). The N2-dG-AAF adduct conformation has also been examined using computational approaches (96). Zaliznyak et al. (76) have shown that the AAF moiety resides in the minor groove with its long axis directed toward the 5'-end of the modified strand. This shields the hydrophobic AAF ring from water. Similar to the N2-dG-IQ adduct, the modified nucleotide maintains the anti-conformation about the glycosyl bond. Notably, the N2-dG-AAF adduct increases the stability of the DNA, which has been attributed to a favorable entropic effect (76). The present data reveal that the base-displaced intercalated conformation of the N2-dG-IQ adduct at position G3 of the NarI sequence differs from that of the N2-dG-AAF adduct, suggesting that the conformations of N2-dG arylamine adducts vary rather than following a common motif. At the molecular level, the factors governing whether planar aromatic molecules such as AAF or IQ favor DNA groove binding versus intercalation are not well established, but may be influenced both by their electronic structures and their respective geometries (97). Replication bypass studies have revealed that the N2-dG-AAF adduct largely blocked DNA synthesis, but with some bypass and misincorporation of dATP opposite the lesion (98).

Comparison to the C8-dG-IQ adduct

When the C8-dG-IQ adduct was placed into the NarI sequence at the frameshift-prone G3 position, the IQ...
ring also intercalated into the duplex and the complementary C\textsuperscript{18} base was extruded into the major groove. The conformation of the C8-dG-IQ adduct also was characterized as base-displaced intercalated (75). Thus, at the G\textsuperscript{1} position within the NarI sequence, both the C8-dG-IQ and N\textsuperscript{2}-dG-IQ adducts share a motif in which the IQ ring intercalates and C\textsuperscript{18} is extruded into the major groove. However, the two conformations are distinctive. Apart from the difference in the regiochemistry of alkylation (C8 versus N\textsuperscript{2}; Scheme 1), a major difference between the C8-dG-IQ and N\textsuperscript{2}-dG-IQ adducts is that the C8-dG-IQ-modified guanine adopts a syn conformation about the glycosyl bond, whereas the N\textsuperscript{2}-dG-IQ-modified guanine maintains the anti-conformation about the glycosyl bond (Figures 8 and 9). In addition, for the C8-dG-IQ adduct, rotation of the glycosyl bond into the syn conformation places the Watson—Crick hydrogen bonding edge of the modified dG into the major groove. The X' imino and amino protons are exposed to solvent. For the C8-dG-IQ adduct the orientation of the IQ ring with respect to the base is opposite to that of the N\textsuperscript{2}-dG-IQ adduct, such that the IQ CH\textsubscript{3} group and H4a and H5a protons face the major groove rather than the minor groove (75). The orientation of the C8-dG-IQ adduct in the duplex rotates the bulk of the IQ aromatic ring away from the flanking bases, resulting in a loss of base-stacking interactions, as shown in Figure 8. In comparison, the N\textsuperscript{2}-dG-IQ adduct appears to have more favorable stacking interactions with G\textsuperscript{19}. These differences may lead to differential processing during both DNA repair and DNA replication.

Structure—activity relationships

The N\textsuperscript{2}-dG-IQ adduct is less efficiently removed from genomic DNA by nucleotide excision repair (63,99). The NER machinery is thought to recognize bulky DNA damage that is destabilizing and distortive to the duplex (100–103). It has been proposed that the thermal stabilization of the N\textsuperscript{2}-dG-AAF adduct hinders NER (76). We observe that the T\textsubscript{m} of the N\textsuperscript{2}-dG-IQ adduct at position G\textsuperscript{1} within the NarI sequence does not destabilize the duplex (Table 1), correcting our original report (67). The T\textsubscript{m} of the N\textsuperscript{2}-dG-IQ modified duplex is 63°C, and does not differ significantly from the unmodified duplex. This is remarkable given that the intercalated IQ moiety disrupts Watson—Crick hydrogen bonding and that the complementary C\textsuperscript{18} base is displaced into the major groove. The stability of the N\textsuperscript{2}-dG-IQ modified duplex likely arises from favorable stacking between the IQ moiety and the neighboring base pairs (Figure 8). It is also interesting to note that unlike the N\textsuperscript{2}-dG-IQ adduct, the C8-dG-IQ adduct, which does not stack with the neighboring bases as well at this position (Figure 8) thermally destabilizes the duplex, reducing the T\textsubscript{m} by 4°C. Yeo et al. (104) examined AAF and AF C8-dG adducts within the NarI sequence and observed a correlation between the degree of destabilization induced by the lesions, binding affinities to the damage recognition protein XPC-RAD23B and overall NER efficiencies. Likewise, Zaliznyak et al. (76) attributed the increased
G\(^3\). While further studies will be necessary to probe the basis for these observations, it is of interest to note that the stability of the \(N^2\)-dG-IQ adduct placed opposite a 2-bp deletion increases as compared to the fully complementary duplex, suggesting that the adduct may stabilize a 2-bp strand slippage intermediate (67). At the G\(^1\) position, the \(N^2\)-dG-IQ adduct is bypassed and extended by the \(E.\ coli\) polymerases and the Dpo4 polymerase, and error-free product is observed. Thus, it will also be of interest to examine the structure of the \(N^2\)-dG-IQ adduct when positioned at position G\(^1\) of the \(NarI\) sequence.

Summary

Analysis of the \(N^2\)-dG-IQ adduct placed at position G\(^3\) of the \(NarI\) sequence (68–71,73,74), where it has been observed to cause −2 bp deletions when bypassed by hpol \(\eta\) (72), reveals that it adopts a base-displaced intercalated conformation in which the H4a and CH3 protons of the IQ ring face the minor groove and the H7a, H8a and H9a protons face the major groove. The IQ ring is shielded from water and stacks with the 5'- and 3'-neighbor base pairs. Remarkably, despite this conformational perturbation, the \(N^2\)-dG-IQ adduct does not destabilize the duplex, which may correlate with the observation that it is refractory to repair by NER (63,99). In addition, the IQ moiety disrupts the potential for Watson–Crick hydrogen bonding with incoming dNTPs, which perhaps explains why this lesion blocks DNA synthesis by many polymerases.

ACCESION NUMBERS

The structural coordinates were deposited in the Protein Data Bank (www.rcsb.org): The PDB ID code for the \(N^2\)-dG-IQ duplex is 2MAV.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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