Mutational and DNA Binding Specificity of the Carcinogen 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline*

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The mutagenic specificity of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), a food-borne mutagen and carcinogen, was studied. Plasmid pK19 was modified by photolysis with the 2-azido form of the carcinogen. High pressure liquid chromatography confirmed that the photoproduced azide formed primarily C8 and N2 guanyl adducts. Transformation of modified pK19 into excision repair competent Escherichia coli resulted in dose-dependent increases in genotoxicity and in mutagenesis within the lacZα target sequence. Upon induction of the SOS response, a 20-fold increase in mutation frequency over background was observed. A mutational spectrum for MeIQx, generated by sequencing 125 independent mutants, revealed base substitutions (41%), frameshifts (54%), and complex mutations (5.6%). >90% of the mutations occurred at G-C base pairs. Two hotspots were evident at runs of five or G-C base pairs; ~60% of the mutations occurred at the hotspot sites. The hotspot at position 2532 produced mainly base substitutions, while that at position 2576 gave exclusively frameshift mutations. A polymerase inhibition assay mapped the sites of MeIQx adducts. Arrest sites were primarily at or one base 3′ to a guanine residue, which correlated well with the distribution of mutations. No direct correlation was seen, however, between the intensity of modification and hotspots for mutation.

Heterocyclic aromatic amines form during the cooking of proteinaceous foods under normal household conditions (1). Two of these compounds (Fig. 1), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ, 1) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx, 2) are present in broiled fish (2) and fried beef (3), respectively, and are potent bacterial mutagens (2, 3). They are also multisite rodent carcinogens, inducing tumors in liver, hematopoietic tissue, lung, Zymbal gland, clitoral gland, forestomach, and small and large intestine (1, 4, 5). IQ is mutagenic to many mammalian cell lines (6), and MeIQx intensely induces hepatocellular carcinomas in nonhuman primates (6). Due to the presence of heterocyclic aromatic amines in many daily staples, and their carcinogenicity in animals, there is concern that these food mutagens may contribute to the development of human cancers.

The covalent binding of an aromatic amine or other carcinogens to DNA is widely regarded as the crucial event in cancer initiation (7). As with most chemical carcinogens, MeIQx and IQ require metabolic activation to exert their biological effects (7, 8). Heterocyclic aromatic amines so activated can covalently modify cellular macromolecules, including DNA. The metabolic steps to MeIQx activation are depicted in Fig. 1. Studies in rodents and human tissues reveal that the initial activation step involves oxidation of the exocyclic amino group by cytochrome P450 2A1 (9). Further studies to establish the mutagenicity of the activated form of MeIQx and IQ require metabolic activation to exert their effects (7, 8). Heterocyclic aromatic amines so activated can covalently modify cellular macromolecules, including DNA. The metabolic steps to MeIQx activation are depicted in Fig. 1. Studies in rodents and human tissues reveal that the initial activation step involves oxidation of the exocyclic amino group by cytochrome P450 2A1 (9). Further studies to establish the mutagenicity of the activated form of MeIQx and IQ require metabolic activation to exert their effects (7, 8).

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‡The abbreviations used are: IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; AAF, 2-(acetylamino)fluorene; ABP, 4-aminobiphenyl; bp, base pair; G2, 2-deoxyguanosin-8-yl; M1, MeIQx, N-(deoxyguanosin-8-yl)-2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; N2-MeIQx, 5-(deoxyguanosin-8-yl)-2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; HPLC, high pressure liquid chromatography; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline; Ras, oncoproteins; p53, tumor suppressor gene; ras, oncoprotein; lacI, gene (15). Frameshifts represent a minor fraction of the mutational spectrum. Similarly, G-C to T-A transversions dominate the spectrum of IQ-induced mutations in human fibroblasts (16). Base substitutions were also found to be the prevalent mutation in the activation of ras oncogenes and the inactivation of p53 gene.
confirmed the structure (10): molecular M⁺ ion at m/z 239.09307, calculated 239.09194; major fragment ions at m/z 211 [M – N₃]⁻ and the base peak observed at 184 [M – CH₃]⁻.

Preparation of MelQx-modified DNA—The plasmid pK19 (88 µg, 50 pmol) was dissolved in 1 ml of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.6) and N₂-MelQx (α⁴C-labeled) added in 20 µl of dimethylformamide and the solution briefly mixed (final concentrations were 17–430 µM for the toxicity study and 43 µM for mutational specificity and DNA binding studies). The solution was then irradiated (230 V, 50 Hz, 0.17 amps) at a wavelength of 366 nm for 40 min with a Spectroline ENF-260 UV lamp held 3 cm above the solution. The MelQx-modified DNA was separated from the unbound reaction products by solvent extraction three times with two volumes of chloroform. The DNA was then precipitated with ethanol (23) and resuspended in TE buffer. Unmodified control DNA was also produced by subjecting the plasmid to the modification conditions in the absence of N₂-MelQx. The concentration of recovered DNA was determined by UV absorption at 260 nm, and the extent of covalent binding was determined by liquid scintillation counting on a LKB 1219 Rackbeta scintillation counter.

DNA Digestion Conditions and Analysis by HPLC—MelQx-modified DNA (50 µg) was dissolved in 1 ml of reaction buffer (5 mM Tris-HCl, 10 mM MgCl₂, pH 7.5), and deoxyribonuclease I (0.04 U) was then added, followed by 1 unit of alkaline phosphatase, and incubation carried out for another 18 h at 37°C. The reaction mixtures were then added to 3 ml of ethanol and the proteins removed by precipitation on ice, followed by centrifugation. The supernatant, which contained greater than 95% of the radioactivity, was lyophilized to dryness.

HPLC analysis of the DNA digest was performed on a Hewlett Packard 1090 HPLC system with an online Berthold LB-1 radioactivity monitor. A TosoHaas TSKgel ODS-80TM HPLC column (4.6 mm × 25 cm, 5 µm particle size, Stuttgart, Germany) was used for all separations. A linear gradient that started at 90% solvent A (25 mM KH₂PO₄, pH 2.5, 5% CH₃CN) and 10% solvent B (100% CH₃CN) and proceeded to 40% solvent B after 40 min was employed to separate adducts. The flow rate was 1 ml/min. Fractions were taken every 30 s and measured for radioactivity by liquid scintillation counting.

SOS Induction and Transformation of E. coli—E. coli DL7/pGW16 cells were grown in 100-ml batches in Luria-Bertani (LB) broth (23) to a cell density of approximately 1 × 10⁸ cells/ml. A portion of the culture was then irradiated in 10 ml of MgSO₄₄, with UV fluxes of 30–45 mJ/cm² to induce the SOS response (20, 24). After a recovery period of 40 min at 37°C, the cells were harvested by centrifugation, washed with water, and resuspended in water as described by Yaron (24). Unmodified and MelQx-modified DNA samples were introduced into E. coli DL7/pGW16 cells by electroporation. The prepared cells (10⁶ viable cells in 90 µl) and 10–100 ng of unmodified or MelQx-adducted DNA were mixed and aliquoted into prestilled Bio-Rad GenePulser cuvettes (0.2-cm electrode gap). The cells were electroporated in a BTX Electrop Cell Manipulator 600 at 500 microfarads, 129 ohms, at 12.5 kV/cm for both SOS non-induced and induced cells. Immediately after electroporation, the cells were diluted with 1 ml of SOC medium (23) and allowed to recover for 1 h at 37°C. An aliquot of the transformed bacteria was then plated on LB plates containing kanamycin (50 µg/ml) to determine the number of transformants. Transformation efficiencies were 1–4 × 10⁸µg and 1–3 × 10⁹µg for unmodified DNA and MelQx-modified DNA, respectively. A portion of the remaining cells (0.5 ml) was diluted in LB (9.5 ml) containing kanamycin (50 µg/ml) and grown for 4 h at 37°C. The cells were then pelleted by centrifugation and plasmid DNA isolated from the preparations using Wizard mini prep kits (Promega). The isolated plasmid DNA was then retransformed into B2234 cells (made competent by CaCl₂; Ref. 23), and after heat shock allowed to incubate at 37°C for 1 h. The transformation mixture was then plated on minimal medium containing 5-bromo-4-chloro-3-indoyl-p-β-thiogalactopyranoside and isopropyl-p-β-thiogalactopyranoside. Mutant colonies in the selections using Wizard mini prep kits (Promega). The isolation and Sequencing of Mutants—Light blue or white colonies were picked and purified by three rounds of single colony purification. Double-stranded DNA isolated from mutant colonies (Wizard mini prep kits, Promega) was denatured and then sequenced according to the method of Sanger (25).

Polymerase Arrest Assay—These assays were carried out as de-
scribed in Ross et al. (26) except that 33P-labeled primers were used. Primers were end-labeled with T4 polynucleotide kinase (New England Biolabs) and [γ-33P]ATP as described (23). DNA modified with MeQx (2 μg) was denatured with alkali and precipitated. The pellet was then resuspended in 2 μl of Sequenase reaction buffer (200 mM Tris-HCl, 100 mM MgCl2, 250 mM NaCl, pH 7.5) and 6.5 μl of water, prior to the addition of an equimolar amount of 33P-end-labeled primer. Annealing of the primer to the template was accomplished by heating the mixture to 65°C in a water bath for 2 min and then allowing the mixture to cool for 30 min. To the annealed template mixture was added 1 μl of 0.1 M dithiothreitol and 2 μl of Sequenase (T7 DNA polymerase) version 2.0, diluted 1:8 in enzyme dilution buffer (10 mM Tris-HCl, 5 mM dithiothreitol, 0.5 mM BSA, pH 7.5). A 3-μl aliquot of this mixture was added to a prewarmed tube containing 2.5 μl of 250 mM deoxynucleotide triphosphates and incubated for 5 min at 37°C. The reactions were terminated with 4 μl of stop solution containing 95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol FF. The samples were heated to 75°C for 2 min prior to loading 3 μl on a 6% polyacrylamide gel. The gel was dried before exposing it to a Molecular Dynamics PhosphorImager screen for analysis.

PhosphorImager Analysis—Polymerase arrest data were quantitated by using ImageQuant software on a Molecular Dynamics PhosphorImager 400S. Densitometry scans were recorded for each lane on the gel. Peaks were integrated and areas compared to unmodified pk19 run under identical conditions. The intensity of the separation of the termination sites diminished with increasing distance from the primer in an exponential manner. The scans were normalized by generating a best fit exponential curve determined by Cricket Graph (Computer Associates) to the density plot and then subtracting these values from each of the densitometry points. The relative intensities of the peaks (or stop sites) were then classified using five levels of intensity, where 1 was a weak arrest site and 5 was a strong arrest site.

RESULTS

The mutagenic consequences of MeQx damage were evaluated in a forward mutational assay based on α-complementation between the lacZα fragment on the kanamycin-resistant plasmid pk19 and the host, E. coli strain B2324, M15 protein. The lacZα gene fragment, which encodes a portion of β-galactosidase, was the genetic target used to measure MeQx-induced mutations. The azide of MeQx (N3-MeQx) was used to modify pk19 in vitro by photolyzing the compound under long wavelength UV light in the presence of the plasmid. Arylazides have been used recently for mutagenesis studies, because they generate short-lived arylnitrenium ions nonenzymatically. They have been shown to form the same DNA adducts as those of the putative in vivo activated species, N-acetoxy-MeQx (27). The mutagenic potency of N3-MeQx also correlates with N-acetoxy-MeQx in reversion assays (27), and N3-MeQx has been shown to form a similar ratio of N2 and C8 guanine adducts as the N-acetoxy compound.2

The modified plasmids were enzymatically digested and the resulting nucleosides analyzed by HPLC, as shown in Fig. 2B. The major peak observed, which represented 52% of the radioactivity incorporated into DNA, was identified as the dG-C8-MeQx adduct. The dG-N2-MeIQx adduct was also present at 8.6%. The major peak observed, which represented 52% of the radioactivity incorporated into DNA, was identified as the dG-C8-MeQx adduct. The dG-N2-MeIQx adduct was also present at 8.6%

used previously to determine the mutations from bulky adducts in excision repair competent cells where they increase SOS-associated mutagenesis (14, 15, 20). After a growth period, the cells were plated on LB or allowed to grow for subsequent DNA isolation. The toxicity of MeQx was assessed at this point by counting the number of colonies produced. The isolated DNA was retransformed into the strain B2324, which contains the complementary M15 protein (DL7/pGW16 does not provide α-complementation). Mutant colonies resulting from loss of α-complementation appeared as either light blue or white. A doxide-dependent increase in toxicity and mutagenicity was seen over the range 17-430 μM N3-MeQx. At a concentration of 17 μM, an 89% survival was observed (7.3 MeQx adducts/plasmid), which decreased to 2% at a dose of 43 μM (33 MeQx adducts/plasmid). At 430 μM MeQx, there was no viability. MeQx concentrations of 17 μM and 43 μM were chosen for subsequent electroporation to deduce mutational frequency. The mutational frequency (MF) was calculated by dividing the number of light blue plus white colonies by the total number of transformants. When MeQx-modified pk19 was transformed into uninduced DL7/pGW16 cells, a MF of 5.3 × 10−4 was found.8 In comparison, the background MF assessed using pk19 subjected to the same buffer and photolyzing conditions as the modified plasmid was 1.5 × 10−4. Upon transformation of the MeQx-adducted plasmid into SOS-inactivated cells, the MF increased to 7.5 × 10−4 for the plasmid containing 3.3 MeQx adducts and to 2.9 × 10−3 for the plasmid containing 33 MeQx adducts. The background MF did not increase significantly with the induction of SOS functions. Since the mutation frequency in the modified DNA was on average 20-fold higher than the control DNA, it was concluded that 95% of the mutations were caused by the MeQx modification.

Mutational and DNA Binding Spectra of MeQx

FIG. 2. Structures of dG-C8-MeQx and dG-N2-MeQx (A) and HPLC analysis of an enzymatic digest of plasmid pk19 modified with 43 μM N3-MeQx (B).

2 R. Turesky, unpublished results.
3 It is possible that several of the other peaks observed in the chromatogram are oligonucleotides containing C8 or N2 dG adducts. At high levels of DNA modification, enzymatic hydrolysis may have been incomplete.
were purified by three rounds of single colony purification, and their DNA was isolated for sequencing. A 170-bp portion of the lacZ gene including the polylinker region and 93-bp host M15 deletion was analyzed for mutations. A total of 203 individual mutants were sequenced, and 125 (61%) contained mutations, which was consistent with the fact that only a portion of the β-galactosidase insert was sequenced. The mutational spectrum for MeIQx in SOS-induced DL7/pGW16 is shown in Fig. 3. The mutations induced by MeIQx are clearly not random; they are divided between frameshifts (54%) and single base substitutions (41%) with a small contribution from complex mutations (5.6%). With the exception of three A-T to T-A transversions, all of the mutations occurred at G-C base pairs. Ninety-three spontaneous mutants were also sequenced; however, only five spontaneous mutations were found in the region of interest. The spontaneous mutations were found at base pair 2576, which is situated in one of the hotspots for mutation and represents one of the 40 mutants found in this region.

The types of mutations observed in the mutational spectrum are summarized in Table I. Among the frameshift mutations, both single base additions and deletions were observed. Loss of a G-C base pair was the major frameshift mutation seen, accounting for 31% of the total mutations. The most frequent type of single base substitution was a transversion (25%), with G-C to T-A mutations dominating the spectrum. The few A-T to T-A transversions that were seen may have been caused by an MeIQx-adduct on the adjacent guanine interfering with proper replication at this adenine or may be due to modification of the adenine itself. The transitions seen were exclusively G-C to A-T and were present in 12% of the total mutations. Complex mutations consisted of double mutants, addition of AC, a TG to A mutant, and a GGTA to TT mutant; collectively these represented 5.6% of the mutants. Two hotspots were seen in the MeIQx-induced mutational spectrum. The first was at the 5’-GGG sequence starting at position 2532 and consisted mainly
of single base substitutions. At the second hotspot, position 2576, 5'-CCCCC, only 1 base frameshifts were seen.

Sites of Polymerase Arrest—Most bulky DNA adducts inhibit replication in vivo (24, 28, 29). To determine whether MelQx was also a replication blocking lesion and to examine the sequence specificity of the modified sites, a polymerase arrest assay was performed. Plasmid pk19 modified to 33 adducts/plasmid (the same level used for mutant isolation; approximately 2 MelQx adducts/170-bp plasmid (the same level used for mutant isolation; approximately 2 MelQx adducts/170-bp plasmid fragment) was used for this analysis. A 5'-end-labeled primer was annealed to denatured MelQx-modified plasmid template and extended with T7 DNA polymerase (Sequenase version 2.0, which contains no 3'-exonuclease activity). Exact polymerase stop sites resulting from the presence of MelQx adducts were visualized by electrophoresing the extension products and comparing their positions to dideoxy sequencing products of unmodified pk19 plasmid. In this manner the position of the stop sites was determined to the nearest base. As a control, the replication mapping assay was also conducted with unmodified pk19 and determined to the nearest base. As a control, the replication mapping assay was also conducted with unmodified pk19 and determined to the nearest base.

Electrophoresing the extension products and comparing their positions to dideoxy sequencing products of unmodified pk19 plasmid. As a control, the replication mapping assay was also conducted with unmodified pk19 and determined to the nearest base.

Table I

| Types of mutations | Number of mutants (%) |
|--------------------|-----------------------|
| Single base substitutions |                       |
| Transversions       |                       |
| G-C → T-A           | 31 (24.8)             |
| G-C → C-G           | 2 (1.6)               |
| A-T → T-A           | 3 (2.4)               |
| Transitions         |                       |
| G-C → A-T           | 15 (12.0)             |
| Frameshifts         |                       |
| Single base deletion| 64 (51.2)             |
| Single base insertion| 3 (2.4)        |
| Complex*            | 7 (5.6)               |
| Total               | 125 (100)             |

*Complex mutations include double mutations.

and the fact that point mutations at the third positions of codons generally do not score. We also observed that the intensities of the sites of modification did not necessarily correlate with the mutational intensities. For example, comparison of the mutational hotspots at positions 2532 and 2576, reveals that the hotspot for (−1) frameshift mutations at position 2576 was only moderately blocking to the polymerase, with the highest intensity being two for this sequence. In contrast, the mutational hotspot for base substitutions at position 2532 was also a hotspot for polymerase arrest with intensities of 4 and 5, the strongest sites in this assay.

**DISCUSSION**

The heterocyclic aromatic amines have attracted much recent interest because of suspicion that they may be responsible for the induction of genetic diseases, especially cancer, in humans. A representative of this class of carcinogens, MelQx, is one of the most abundant dietary heterocyclic aromatic amines, with dietary intakes estimated at 0.2–2.6 μg/person/day (12).

As a first step toward understanding the mode of action of MelQx, we have investigated its effects on DNA polymerases in vitro and in vivo.

Modification of a plasmid with the azide of MelQx generated a genome that contained both C8 and N2 guanine adducts. Subsequent transformation of this modified DNA into E. coli produced a dose-dependent increase in toxicity. Increased mutagenicity was also observed and, as with many bulky adducts including 4-amino-2-methylimidazo[4,5-f]quinoline (4-AM1Q) and 2-acetylaminofluorene (AAF), MelQx required SOS induction for its mutagenesis (20, 30). The SOS dependence of the MelQx-induced mutations (MF of 2.9 × 10−3 at 33 adducts/plasmid) was detected as a 5-fold increase in MF over that of uninduced cells and a 20-fold increase in MF over that of unmodified DNA. These results are comparable to those of King and co-workers who found a mutation frequency of 1.3 × 10−8 for AAF, a 6.2-fold increase over uninduced cells, in the lacZ gene of M13mp9 with 63 adducts/molecule under similar host conditions (31). Lasko et al. (20) found a MF of 8.0 × 10−4 for ABP corresponding to a 25-fold increase in MF over uninduced cells. If the MFs are taken on a per adduct basis within the lacZ mutational target, a single MelQx adduct had a MF of 0.10%. By comparison, AAF and ABP had mutation frequencies of 0.019% and 0.14%/adduct/lacZ mutational target, respectively, indicating that the mutation frequency of MelQx is comparable to other carcinogenic aromatic amines.

While the mutation frequencies are similar, the lacZ mutational spectrum of MelQx is unique when compared with other bulky adducts. MelQx was able to induce frameshifts and base substitutions at an almost equal frequency, with 91% of the mutations occurring at G-C base pairs. The most abundant mutations seen were single base deletions at G-C, G-C to T-A transversions, and G-C to A-T transitions. Of the mutations that did not occur at G-C base pairs, all occurred at a base pair adjacent to a G-C base pair. The mutational spectrum generated here differs from previous studies in bacteria on the structurally related compound, MelQ, where base substitution mutations are the major mutagenic event. In addition, in mammalian cells, IQ induces primarily base substitution mutations with very little contribution from frameshift mutations (15, 16). It would appear that MelQx produces a very distinct mutational spectrum, although neither of these previous studies employed the lacZ gene as a reporter of mutations so a direct comparison cannot be made.

A substantial portion of the MelQx-induced mutations (60%) was clustered at positions 2576–2581 and 2532–2534. Both of these sites are in G-C clusters adjacent to runs of adenines, which may influence the reactivity to the activated MelQx at
these sites, since runs of adenines are known to bend DNA and thus structurally influence adjoining sequences (32). Interestingly, different types of mutations occurred at these two sites. The sequence at position 2532 produced mainly base substitutions (although some frameshifts were also detected), whereas the sequence at position 2576 produced exclusively single base frameshift mutations. Of the five spontaneous mutations found, only one, a frameshift at 2576, occurred at a site in the MeIQx-induced spectrum.

Previous studies have demonstrated that the sequence 5′-CCTCCCC, within the same context of the lacZ in M13, is prone to frameshift mutations when bulky adducts are present. Gupta et al. found a high frequency of single base deletions at this sequence in their work on AAF (31). Misincorporation of nucleotides at homopolymeric sequences, resulting in frameshift mutations, is thought to be a common occurrence in DNA synthesis, potentially arising from slippage of the two DNA strands during replication (33, 34). Fuchs and co-workers have studied the frameshift mutations induced by AAF in site-specifically modified DNA duplexes (34). They speculate that destabilization of the helix by AAF at the sequence 5′-CGGGGA is relieved by formation of a bulged intermediate; rotation of the AAF moieties inside the helix stabilizes the mutagenic intermediate (34). Interestingly, both the hotspots for MeIQx-induced mutations are at 5′-GGGA sequences and frameshift mutations were found at both positions. The C8 adduct of MeIQx is known to rotate into the syn orientation on a deoxyguanosine in an analogous manner to the AAF C8 adduct. It is plausible that MeIQx may also induce destabilization in a similar manner to AAF, producing a bulged intermediate that causes misinsertion of cytosine opposite the lesion.

Single base substitutions were seen at a frequency of 41% in the mutational spectrum of MeIQx with the most dominant being G-C to T-A transversions followed by G-C to A-T transitions. The high frequency of G-C to T-A transversions may be explained by the known propensity of DNA polymerase to insert adenine opposite a noninstructional lesion such as a bulky adduct or an apurinic site (35). If the premutagenic bulky adducts dG-C8-MeIQx or dG-N2-MeIQx evade repair, they may induce a G-C to T-A transversion by forcing a replication error during DNA synthesis leading to preferential insertion of adenine. Alternatively, formation of an apurinic site may have occurred. Apurinic sites are thought to be formed from alkylolation of the guanine N-7 position of heterocyclic aromatic amines and subsequent loss of the base (36). In fact, if one compares the distribution of base substitution mutations seen by Kunkel for AP site mutagenesis (59% A, 28% T, and 11% G incorporated opposite a template G) and those observed for MeIQx (64% A, 31% T, and 4% G incorporated opposite a template G), the distribution is quite comparable (37). The similarity supports the idea that the base substitutions observed for MeIQx may be due to AP site mutagenesis, an observation that has also been made for the induction of base mutations by MeIQx (15).

A polymerase arrest assay was used to determine the binding specificity of MeIQx and the ability of MeIQx-modified DNA to inhibit polymerization. MeIQx proved to be a replication blocking lesion with arrest sites at or 3′ to guanine residues. While there was overall convention between the positions of mutation and the sites of adduct formation, there was little correlation between intensity of modification and mutational hotspots. This is especially evident when observing the hotspot for mutations at base pairs 2576–2581. That site was only a weak arrest site in the polymerase arrest assay, yet 32% of the MeIQx-induced mutations occurred at this position. Similar observations have been made by others (29, 30).

One important feature of MeIQx is its mutagenic versatility, inducing both frameshift and base substitutions almost equally. The conformation of an MeIQx adduct is likely to influence its persistence and its mutagenic impact. NMR studies on the 2′-deoxyguanosine MeIQx-adduct at C8 have shown that the preferred glycosidic conformation is syn (11). Similarly, dG-C8-AAF, which induces mainly frameshift mutations, displays the syn conformation both at the nucleoside (38) and duplex DNA (39) levels. It is conceivable that the dG-C8-MeIQx adduct in the syn conformation is responsible for the observed frameshift mutations. It is reasonable to speculate that the base substitutions may be derived from the corresponding dG-N2-MeIQx adduct. In contrast to the C8 adduct, the conformational preference was shown to be anti for the N2 adduct of MeIQx on 2′-deoxyguanosine (11). Although, there have been no NMR investigations on the structural effects of bulky heterocyclic aromatic amine N2 adducts in DNA, modeling shows that the N2 adduct should be accommodated within the minor groove and be available for base pairing. Induction of base substitution mutations by N2 adducts would result when polymerases traverse the lesion but fail to replicate it faithfully. Deciphering the conformations of the adducts of MeIQx will require synthesis of site-specifically modified DNAs followed by NMR structural investigations. This information in conjunction with mutagenesis studies involving singly modified genomes will be an important next step toward understanding the details underlying the mutagenic activity of the adducts of MeIQx.

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