Specificity of Base Substitutions Induced by the Acridine Mutagen ICR-191: Mispairing by Guanine N7 Adducts as a Mutagenic Mechanism

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Manuscript received February 21, 1991
Accepted for publication August 8, 1991

ABSTRACT

As the most nucleophilic site in DNA, the guanine N7 atom is a major site of adduction by a large number of alkylating mutagens and carcinogens. Aflatoxin B1, a powerful mutagen, is believed to act through its reaction with this DNA site. On the basis of the specificity of base substitutions induced by various adduct forms of aflatoxin, we have proposed that bulky guanine N7 adducts elicit base substitutions by two mechanisms. The first mechanism is similar to that observed for a number of bulky noninstructive lesions, whereas the second mechanism invokes mispairing between N7-adducted guanine and thymine. A prediction of the mispairing hypothesis is that diverse bulky guanine N7 adducts (regardless of structural similarities with the aflatoxins) should induce predominantly G-to-A transitions. Accordingly, we have recently observed that base substitutions induced by the acridine half-mustard ICR-191 in the M13 double-stranded DNA transfection system are predominantly G:C-to-A:T transitions. Here, by transfecting ICR-191-treated M13 AB28 single-stranded DNA into Escherichia coli, we show that base substitutions are predominantly targeted to guanines. Since the N7-adduct-guanine:thymine mispairing is proposed to require N1 deprotonation promoted by the primary N7 lesion, guanine imidazole ring-opening should abolish this mispairing property, and thereby alter the specificity of mutagenesis. Here, we show that the incubation of ICR-191-treated RF DNA at pH 10.5 results in a significant reversal of the specificity of G:C-targeted substitutions such that G-to-T transversions predominated over G-to-A transitions. These data suggest that the ring-opened forms may be processed as classical noninstructional lesions as previously deduced for ring-opened aflatoxin-guanine lesions. These findings raise the intriguing possibility that mispairing by guanine N7 adducts may be a source of induced as well as background mutagenesis.

P HYSICAL and chemical mutagens enhance mutagenesis by inflicting DNA damage (DRAKE and BALTZ 1976; SETLOW 1978). DNA damage is efficiently repaired by multiple (frequently overlapping) repair pathways (FRIEDBERG 1984; SANCAR and SANCAR 1988). Induced mutagenesis is generally attributed to residual DNA damage. Two general mechanisms by which DNA damage can lead to mutation are recognized (WALKER 1984). The miscoding or mispairing DNA lesions cause mutations because of an alteration in the specificity of basepairing (e.g., deamination of DNA cytosine to uracil). It is believed that these lesions do not stop replication, and that the "normal" replication machinery is sufficient for induction of mutations opposite such lesions.

On the other hand, the so-called noninstructional DNA lesions cause mutations because the basepairing potential is either lost (e.g., abasic sites; LOEB and PRESTON 1986) or is presumed to be inaccessible (e.g., UV photodimers and bulky chemical adducts; WALKER 1984). Noninstructional lesions are believed to block replication, leading to the induction of the SOS regulon in Escherichia coli (RADMAN 1974; WALKER 1984, 1985). DNA elongation past these lesions ("lesion bypass") requires factors (such as the products of SOS genes UmuD and UmuC) in addition to the replication machinery. An intriguing generalization ("the A rule") suggests that adenine is preferentially misinserted opposite noninstructional lesions regardless of the base affected or of the chemical modification involved: for example, adenine is misinserted opposite pyrimidine dimers, abasic sites as well as a number of bulky guanine adducts. The adenine preference is not absolute: the order of misinsertion frequency opposite noninstructional lesions is believed to be A>T>G>C. This bias in misinsertion opposite noninstructional lesions is proposed to be an intrinsic property of DNA polymerases (e.g., RABKIN and STRAUSS 1984).

Although chemical mutagens are known to react with every oxygen, nitrogen and several carbon atoms in DNA, guanine N7, as the most nucleophilic atom in DNA, is especially prone to addition by a variety of chemicals (FREESE 1971; LAWLEY 1989). For example, alkylating agents, which encompass a number of endogenous mutagens as well as a large number of
exogenous mutagens (LAWLEY 1989) and chemotherapeutic agents, react predominantly at this position, as do a number of bulky mutagens such as the aflatoxins that contaminate food (BUSBY and WOGAN 1985). However, the contribution of guanine N7 addition to chemical mutagenesis has been difficult to assess because of several paradoxes. First, N7-methylation, but not N3-methylation of guanines has been correlated with mutagenesis, implying that N7-methylation has little mutagenic potential (LAWLEY 1974). The mutagenic potential of methylating agents (such as the many common laboratory mutagens) is widely believed to be mediated by methylation of oxygens (e.g., O3-methylguanine; BASU and ESSIGMANN 1990). Nevertheless, aflatoxin B1 (AFB1), among the most potent mutagenic carcinogens, is believed to exert its mutagenic activity through guanine N7 adduction (BUSBY and WOGAN 1984). A common property of all guanine N7 adducts, regardless of whether they are small methyl groups or bulky aromatic moieties, is the weakening of the N-glycosyl bond such as to potentiate deguaninylation (LAWLEY 1957, 1961). Bulky mutagens such as AFB1 have been proposed to cause mutagenesis through abasic sites resulting from the loss of adducted guanines (FOSTER, EISENSTADT and MILLER 1983; LOEB and PRESTON 1986). Elegant rationalizations of how a bulky adduct (as contrasted to a smaller adduct) can promote mutagenesis through an abasic site have been discussed (LOEB 1985).

AFB1 reacts avidly with double-stranded DNA (dsDNA) to almost exclusively yield bulky guanine N7 adducts. As expected for bulky noninstructional lesions, AFB1 induces the E. coli SOS functions, utilizes SOS functions for mutagenesis, and is repaired by UvrABC excision pathway (for brief review, see REFOLO, BENNETT and HUMAYUN 1987). Nevertheless, SAMBAMURTI et al. (1988) observed that the specificity of base substitutions induced by AFB1 lesions deviated from that predicted for a noninstructional lesion. Specifically, there was evidence that thymine was preferentially incorporated opposite primary guanine-AFB1 adducts. Upon conversion of the primary lesions to the guanine imidazole ring-opened formamidopyrimidine-AFB1 (FAPY-AFB1) lesions, the specificity reverted to that expected for noninstructional lesions. These and other observations suggest that bulky guanine N7 adducts may induce mutations by a combination of mispairing and “noninstructional” mechanisms. The noninstructional mechanisms are likely to be those considered above. The hypothesized mispairing mechanism proposes that primary (but not secondary) guanine N7 lesions can suffer deprotonation at the guanine N1 position (see Figure 1 for structures) and thereby mispair with thymine.

Since the proposed mispairing mechanism can in principle apply to any bulky guanine N7 adduct capable of transiently arresting DNA replication or locally denaturing the DNA helix, one simple prediction of the hypothesis would be that the base substitution specificity of primary and secondary lesions induced by bulky chemical mutagens structurally unrelated to AFB1 will be nevertheless similar to that of AFB1. As a test of this prediction, we have investigated the specificity of base substitutions induced by the laboratory mutagen ICR-191. ICR-191 consists of an acridine moiety attached to a long alkylation side-chain believed to react covalently with guanine N7 atom (see DISCUSSION). SASHASRABUDHE, LUO and HUMAYUN (1990) have recently demonstrated that in vitro addition of M13 double-stranded replicative form (RF) DNA with ICR-191 leads to both frameshift, and base substitution mutations, and that G:C to A:T events predominate among the substitutions. Here, we describe experiments showing that when ICR-191-added M13 RF DNA is incubated at pH 10.5, the specificity of substitutions is significantly altered. We also show, using M13 single-stranded DNA (ssDNA) added with ICR-191 in vitro, that base substitutions are targeted specifically to guanines.

**MATERIALS AND METHODS**

**Strains, media and chemicals:** *E. coli* strain KH2AM (uvrA/mucAB*) and the phage M13 derivative AB28 have been described elsewhere (SAMBAMURTI et al. 1988). Media and reagents for transfection experiments were as described (SAMBAMURTI et al. 1988). 6-Chloro-9-(3-[N-(2-chloroethylamino)propylamino]-2-methoxyacridine dihydrochloride (CAS No. 17070-45-0), more commonly known as acridine mutagen ICR-191, was from Terochem (Alberta, Canada) or Sigma. For the purpose of spectrophotometric quantitation of ICR-191, an ε25 value of 45,600 was used.

**M13AB28 ssDNA preparation and ICR-191 treatment:** AB28 ssDNA was prepared as follows (K. SAMBAMURTI, unpublished data). Phage stocks were prepared by inoculating 1 liter of Luria broth (in a 4-liter flask) with 5 ml of a fresh overnight culture of *E. coli* NR3883 (from B. GLICKMANN) and 5 ml of TNE buffer (20 mM Tris-HCl, 10 mM NaCl, 1 mM EDTA, pH 8) containing a total of 1.5 × 107 plaque-forming units (pfu) of phage. The cultures were incubated at 37° with vigorous shaking (200 rpm) for 5 hr. Cells and debris were removed by centrifugation at 4000 × g for 30 min at 4°. The supernatant was mixed with 0.25 volume of a stock solution containing 2.5 M NaCl and 20% polyethylene glycol-8000 (PEG), and allowed to incubate at 4° overnight. The phage-PEG complex was “pelleted” by centrifugation at 4000 × g for 30 min at 4°, and the crude phage was resuspended in 20 ml of TNE buffer. Debris and residual bacteria were removed from the crude phage solution by centrifugation for 30 min at 4° in a Sorvall SS34 rotor (10,000 rpm), and the supernatant was subjected to a second round of “PEG precipitation” as described above. The final pellet was resuspended in 20 ml of TNE and was passed through a 5 ml (bed volume) column of DEAE-cellulose (Whatman DE-52; prewashed with 1 M Tris-HC1, pH 8, and equilibrated with TNE). The flow-through (which contained phage) was collected and the viral DNA was extracted by the following sequential steps: two phenol extractions, one chloroform extraction, three ether extrac-
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FIGURE 1.—Proposed mispairing mechanism of mutagenesis by bulky DNA adducts at the guanine N7 position. Structures of aflatoxin B1 (a) and ICR-191 (b) are shown at top. The so-called primary N7 adduct (d), which has acquired a positive charge in the imidazole ring as a result of the adduction, can suffer deprotonation at the N1 atom to form the zwitterion (e). The zwitterion form can no longer form a normal Watson-Crick G:C base pair, but can form a wobblelike pair with cytosine (g), and a mispair with thymine (h; LAWLEY and BROOKES 1961). When the primary adduct (d) is converted to the imidazole ring-opened secondary form (c), the positive charge of the imidazole ring as well as the potential for N1 deprotonation are lost. Base-substitution mutagenesis at the primary adduct is proposed to arise from a combination of mispairing and “A rule” mechanisms, whereas substitutions at the secondary adduct are proposed to arise exclusively by the “A rule” mechanism. The X in c, d, and e represents an alkyl group such as a methyl group, or a bulky adduct such as AFB.

Viral ssDNA prepared by the above procedure had greatly reduced levels of contaminating DNA (chromosomal DNA fragments and RF DNA) observed in ssDNA prepared by standard PEG precipitation procedures.

Fifty micrograms of ssDNA were incubated with 13.85 μM ICR-191 in the dark in 400 μl of 20 mM Hepes (pH 6.8) for 1 hr at room temperature. The DNA was freed from small molecules by three successive ethanol precipitation steps and resuspension in 200 μl of 20 mM Hepes, pH 6.8. Mock-treated DNA was subjected to identical procedures except for omitting ICR-191. The amount of DNA recovered was ascertained by UV spectrophotometry.

RF DNA preparation and ICR-191 treatment: M13AB28 (RF) DNA was prepared as described (SAMBAMURTI et al. 1988) using the E. coli strain KH2A as the host cells, and was purified by cesium chloride-ethidium bromide gradients according to standard procedures (HEILIG 1989). RF DNA (50 μg) was incubated with 6.95–27.7 μM ICR-191 (delivered from a freshly prepared aqueous stock solution) at room temperature in 400 μl of 20 mM Hepes, pH 6.8, in foil-wrapped 1.5-ml Eppendorf test tubes. The DNA was recovered by three cycles of precipitation with ethanol, and the pellet was resuspended in 0.11 M glycine-sodium hydroxide buffer (pH 10.5), and incubated for 30 min at 37°. DNA was recovered by ethanol precipitation and resuspended in 10 mM Hepes, pH 6.8. This procedure is known to effectively convert primary guanine N7 adducts to their guanine imidazole ring-open (FAPY) form (e.g., CHETSANGA, POLODORI and MAINWARING 1982; HENDLER, FURER and SRINIVASAN 1970; HERZOG, LINDAY-SMITH and GARNER 1982; IRWIN and WOGAN 1984; LIN, MILLER and MILLER 1977). We have confirmed, by adduct analysis, that the above incubation conditions quantitatively convert primary AFB adducts to the corresponding FAPY adducts (SAMBAMURTI 1989). It should be noted that even though the reaction of ICR-191 with guanine N7 atoms is deduced from a number of observations (see DISCUSSION), the DNA adducts induced by ICR-191 have not been isolated.

Transfection, mutant identification and DNA sequencing: Procedures for transfection of mock-treated and ICR-191 treated RF DNA into E. coli KH2AM cells, method for identification of LacZ(a-) mutant phages, and for DNA sequencing have been described (SAMBAMURTI et al. 1988). UV-irradiation (10 J/m²) conditions for SOS induction were determined as described (BENNETT et al. 1988).

RESULTS

Phage M13AB28 is a derivative of phage M13mp2 except for the presence of a polylinker region derived from M13mp8 (SAMBAMURTI et al. 1988). M13AB28 can grow in suppressor-less (Sup') E. coli cells, and can provide for LacZ a-complementation. When plated on an appropriate LacZ a-complementing host strain, M13AB28 yields dark blue plaques in the presence of a Lac inducer and a chromogenic substrate for β-galactosidase. The experimental system used here consists of in vitro mutagenic treatment of M13 ssDNA or RF DNA followed by transfection in to E. coli cells. Survival is determined as the transfection efficiency
of treated DNA as compared to mock-treated DNA. Forward mutations in the LacZ(α) gene are detected from the colorless/light blue phenotype of phage plaques on Xgal indicator plates, and are confirmed by a second round of plating in the presence of wild-type phage as described. Mutation frequency is determined as the ratio of plaques with LacZ(α-) phenotype and wild-type (dark blue) plaques. The specificity of base changes associated with the mutations is determined by DNA sequence analysis.

**Mutations induced by ICR-191 in M13 ssDNA are predominately guanine-targeted:** In order to determine whether, as expected, ICR-191 induces mutations predominantly at guanines, M13AB28 ssDNA was treated in vitro with ICR-191 and transfected into E. coli KH2AM. Table 1 summarizes the effects of ICR-191 treatment on survival, mutation frequency and specificity. The DNA sequence changes in 16 mutants (Table 1; also see Figure 2) show that: (a) 9 of the 12 substitutions affect guanines; of the remaining three, one each affects cytosine, adenine and thymine; and (b) only about 20% of the mutations are frameshifts (4 of 16, Table 1). Figure 2 shows that three of these frameshifts occur at the stretch of 4 guanines found at bases 6239-42, whereas only one occurs at the major hotspot found for RF DNA (5 guanines in the complementary strand, bases 6343-6347). Since ICR-191 treatment enhances mutagenesis by threefold (Table 1A), up to a third of the 23 mutants analyzed in this study may represent background events. Furthermore, in dsDNA, primary lesions are known to predominantly induce G-to-A mutations (SAHASRABUDEH, LUO and HUMAYUN 1990). Here, there are five G-to-A mutations and 4 G-to-T events. Whether this is attributable to the very small numbers of sequenced mutants or to an unrecognized experimental difference between the ds and ssDNA experiments is not known. The above caveats aside, the data summarized in Table 1 and in Figure 2 are consistent with the possibility that ICR-191 mutations are predominantly targeted to guanines, and not to cytosines.

**Incubation of ICR-191-treated RF DNA at pH 10.5 significantly alters the specificity of base substitution mutations:** SAHASRABUDEH, LUO and HUMAYUN (1990) have previously reported the specificity of mutation induced by ICR-191 in the RF DNA system. The in vitro ICR-191 treatment was carried out at a pH of 6.8, a condition at which guanine N7 adducts are likely to be predominantly the so-called primary lesions (Figure 1d). A general property of primary guanine N7 lesions is that they suffer imidazole ring-opening at mildly alkaline conditions (Figure IC; LAWLEY and BROOKES 1963). For example, incubation of DNA bearing primary AFB lesions at pH 10.5 for 30 min can quantitatively convert the lesions to imidazole ring-open secondary adduct forms (e.g., IRWIN and WOGAN 1984; LIN, MILLER and MILLER 1977). Even though DNA adducts induced by ICR-191 have not been isolated, guanine N7 adduction is deduced from a number of experimental observations (see DISCUSSION). Table 2 summarizes the effects of ICR-191 treatment with or without subsequent incubation at pH 10.5 on survival and mutagenesis of AB28 RF DNA. Table 2A shows that when ICR-191-treated/pH 10.5-incubated DNA is transfected into unirradiated or UV-irradiated cells, there is a similar, dose-dependent drop in survival. The loss
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FIGURE 2.—A part of the DNA sequence (viral strand) of the LacZ(α) gene contained in phage M13AB28 showing the sequence changes associated with mutations induced by ICR-191 under various conditions. For comparative purposes, base substitutions induced by the primary ICR-191 adducts in unirradiated cells (data of SAHASRABUDHE, LUO and HUMAYUN 1990) are shown in lowercase letters immediately above the affected upper strand base. Frameshift mutations are shown by placing an uppercase letter preceded by a plus or minus symbol (e.g., +G) above the affected base run. Data from the present study are shown below the sequence as follows: mutations obtained by transfecting ICR-191-treated, pH 10.5 incubated RF DNA are shown by the conventions described above. Mutations obtained by transfection of adducted ssDNA are enclosed within square brackets (e.g., [t]), but otherwise follow the same conventions.
Effect of pH 10.5 incubation on survival and mutagenic effects of ICR-191 in M13 RF DNA

<table>
<thead>
<tr>
<th>Assay</th>
<th>No UV irradiation to cells (-SOS)</th>
<th>UV irradiation of cells (+SOS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary(^x) (ICR-191)</td>
<td>Secondary(^y) (ICR-191/pH 10.5)</td>
</tr>
<tr>
<td>A. pfu/μg DNA [x 10(^2)] (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mock-treated DNA</td>
<td>4.65 (100)</td>
<td>6.76 (100)</td>
</tr>
<tr>
<td>6.93 μM ICR-191</td>
<td>2.06 (44)</td>
<td>2.71 (44)</td>
</tr>
<tr>
<td>13.85 μM ICR-191</td>
<td>1.49 (32)</td>
<td>1.47 (22)</td>
</tr>
<tr>
<td>27.7 μM ICR-191</td>
<td>1.03 (22)</td>
<td>0.71 (11)</td>
</tr>
<tr>
<td>B. Mutation frequency [x 10(^{-4})]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mock-treated DNA</td>
<td>0.80 (1.0)</td>
<td>1.32 (1.0)</td>
</tr>
<tr>
<td>6.93 μM ICR-191</td>
<td>9.53 (9.5)</td>
<td>4.28 (3.2)</td>
</tr>
<tr>
<td>13.85 μM ICR-191</td>
<td>11.3 (11.3)</td>
<td>16.14 (12.2)</td>
</tr>
<tr>
<td>27.7 μM ICR-191</td>
<td>10.28 (10.3)</td>
<td>18.43 (14.0)</td>
</tr>
</tbody>
</table>

\(^x\) ICR-191-treated RF DNA not subjected to guanine imidazole ring-opening conditions (data of Sahasrabudhe, Luo and Humayun, 1990).

\(^y\) ICR-191-treated RF DNA subjected to guanine imidazole ring-opening treatment (incubation at pH 10.5 for 30 min at 37°C; this work). Values shown are means of two transfections.

\(^z\) Values in parentheses are fold enhancements in mutation frequency over that observed in the corresponding mock-treated DNA.

\(^z\) It is not clear why the mutation frequencies are low for the 6.93 μM ICR-191-treated, secondary lesion-bearing DNA. Since the primary lesion-bearing DNA and the secondary lesion-bearing DNA were obtained in separate experiments, it is possible that DNA adduction in the secondary lesion-bearing DNA was lower.

Table 3

Summary of DNA sequence changes induced by ICR-191 in M13 RF DNA in unirradiated (-SOS) cells

<table>
<thead>
<tr>
<th>Assay</th>
<th>Primary(^x) (ICR-191)</th>
<th>Secondary(^y) (ICR-191/pH 10.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. pfu/μg DNA [x 10(^2)] (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mock-treated DNA</td>
<td>4.65 (100)</td>
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</tr>
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<td></td>
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\(^x\) ICR-191-treated RF DNA not subjected to guanine imidazole ring-opening conditions (data of Sahasrabudhe, Luo and Humayun, 1990).

\(^y\) ICR-191-treated RF DNA subjected to guanine imidazole ring-opening treatment (incubation at pH 10.5 for 30 min at 37°C; this work). Values shown are means of two transfections.

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An interesting feature of the data shown in Figure 2 is that a majority of G-to-T mutations occur in the minus strand, whereas most G-to-T mutations occur...
TABLE 4
Comparison of the specificity of G:C targeted base substitutions induced by ICR-191 under various conditions

<table>
<thead>
<tr>
<th>ICR-191 pH 10.5 UV treatment</th>
<th>Row</th>
<th>Treatment</th>
<th>UV to cells</th>
<th>G:C &gt; A:T</th>
<th>G:C &gt; T:A</th>
<th>x² (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>12</td>
<td>7</td>
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<tr>
<td></td>
<td>C</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

Data shown for row A are from Table 3 of this work; data for rows B and C are from Table II of Sahasrabudhe, Luo and Humayun (1990).

* The x² value shown in row B is for the null hypothesis that the observed transition/transversion ratio in row A is the same as that expected from the data in row B. The x² value shown in row C similarly compares the data in rows A and C. The degrees of freedom are taken to equal 2 even though G-to-C transversions have not been observed.

in the plus strand. It is not clear whether this strand bias is significant.

DISCUSSION

The possibility that bulky guanine N7 adducts might promote base substitution mutagenesis by both noninstructive and mispairing mechanisms was suggested by the observation of the specificity of substitutions induced by primary and FAPY-AFBl lesions (Sambamurti et al. 1988) and the known chemical properties of N7-adducted guanines (Lawley and Brookes 1961). Since the hypothesized mechanisms should apply to mutagens structurally dissimilar to AFBl, we have sought to test whether key predictions of the hypotheses are fulfilled by the commonly used laboratory mutagen ICR-191. We have previously shown that in vitro adduction of M13 RF DNA with ICR-191 leads to base substitutions predominantly at G:C pairs, and that these substitutions are predominantly G:C to A:T transitions (Sahasrabudhe, Luo and Humayun 1990). Here, we have shown that ICR-191-induced substitutions are predominantly guanine-targeted, and that, subjecting ICR-191-treated DNA to pH 10.5 incubation (guanine imidazole ring-opening condition) brings about a shift in the specificity of substitution mutagenesis. These observations suggest that the mechanisms for base substitution mutagenesis may be similar for AFBl and for ICR-191, and are consistent with the hypothetical mechanisms outlined in Figure 1.

The major caveat in our interpretation of the results presented here is the fact that DNA adducts induced by ICR-191 have not been isolated. That ICR-191 induces covalent adducts is deduced from the following lines of evidence. (a) ICR-191 is a far more potent mutagen than the 9-aminocaridine core (Ames, Lee and Durston 1973), a difference traditionally attributed to formation of covalent DNA adducts through the reaction of the alkylating side chain of ICR-191 (see Figure 1a) with nucleophilic centers in DNA. Furthermore, replacement of the terminal chlorine atom by an OH group to create ICR-191-OH appears to abolish mutagenic activity (Creech et al. 1972). (b) ICR-191, but not 9-aminocaridine, is an effective inducer of the E. coli SOS response (Podger and Hall 1985). (c) By analogy to other alkylating agents, guanine N7, the most nucleophilic center in DNA, is expected to be the major adduction target. This possibility is supported by the induction of alkali-labile sites in DNA by ICR-191 (Sahasrabudhe, Luo and Humayun 1990), and of mutations targeted to guanines (this work).

As briefly reviewed earlier, imidazole ring-opening by mild alkali is believed to be a general property of guanine N7 adducts. Base substitutions induced by ICR-191-treated DNA (neutral pH) are predominantly G:C to A:T transitions, whereas upon incubation of the DNA at pH 10.5, the predominant substitutions are G:C to T:A transversions. Even though other possibilities cannot be eliminated by the data presented here, available information is consistent with the possibility that both noninstructive and mispairing mechanisms (Figure 1) operating at guanine N7 adducts contribute to base substitution mutagenesis induced by ICR-191.

Finally, it is possible that the G:C to A:T mutations induced by primary guanine N7 adducts may arise by mechanisms other than the N1-deprotonation model depicted in Figure 1. The anti-tumor compound cis-diaminedichloroplatinum(I1) (cisplatin), is known to cross-link adjacent (or nearby) N7 atoms of purines in the 5'AG, GG and GNG sequences. Since cisplatin adduction involves chelation, N1 deprotonation as depicted in Figure 1 is unlikely. It appears that most cisplatin mutations occur at adenines in the AG sequence and at the 5' guanine in the GG sequence (Burnouf, Daune and Fuchs 1987), but they may also occur in GNG sequences (Brouwer et al. 1981; Brouwer, Adhin and van de Putte 1983). The direct sequencing data of Burnouf, Daune and Fuchs (1987) show that of the ten sequenced mutations at guanine, four are G-to-A mutations, five are G-to-T, and one is a G-to-C. The LacI genetic assay data of Brouwer et al. (1981) and Brouwer, Adhin and van de Putte (1983) suggest that both G-to-A and G-to-T events are induced by cisplatin. Although it is hard to determine from the above data whether G-to-A events occur at a level higher than what is expected for bulky (noninstructive) lesions, it is conceivable that a fraction of the transitions induced by cisplatin arise by an unknown mechanism which does not involve N1 deprotonation. At present there is no evidence to suggest that excess transitions induced by the two adduct classes represented by cisplatin and AFBl arise by a single mechanism. On the other hand, it is reasonable to assume a fraction of the mutations
induced by both adduct classes arises from the "A rule" (i.e., "A>T>G>C rule") mechanism.

Relative contribution of mispairing and noninstructional mechanisms to mutagenesis induced by guanine N7 adducts: The mispairing mechanism discussed in this communication is available only to the primary lesions. The noninstructional lesion mechanism, on the other hand, is available to the primary lesions as well as to the secondary lesion forms considered below.

Guanine N7 adducts are relatively unstable, and can suffer a number of spontaneously occurring secondary reactions. For example, primary AFB1 lesions have an in vitro half-life of about 2 days; almost all of the adduct loss can be attributed to the following three processes: (a) chemical reversal, leaving behind an intact guanine on DNA; (b) depurination, leaving behind an abasic site; and (c) guanine imidazole ring-opening, converting the primary lesions to FAPY lesions (Groopman, Croy and Wogan 1981). FAPY lesion forms are believed to predominate in later stages in vivo (Groopman, Caine and Kensler 1988).

While the relative stability of the primary lesions is expected to vary depending on the actual guanine N7 adduct, formation of abasic sites and ring-open forms are believed to be general features of guanine N7 adducts. Any evaluation of the relative contributions of the above two mutagenic mechanisms is complicated by the proportions of various adduct forms at the time of bypass. Nevertheless, the results presented here and elsewhere (Sambamurti et al. 1988; Sahasrabudhe, Sambamurti and Humayun 1989; Sahasrabudhe, Luo and Humayun 1990) suggest that bulky guanine N7 adducts induce base substitutions by more than one major mechanism, and that mispairing might be one of those major mechanisms.

Significance of mispairing by guanine N7 adducts: What makes some guanine N7 adducts (e.g.; aflatoxin B1) but not others (e.g., N2-methylguanine) highly mutagenic? N1 deprotonation of N7 adducted guanines is favored in single-stranded regions (see Sambamurti et al. 1988 for brief review of evidence). Thus, among the expected properties of the more mutagenic lesions are an ability to act as strong blocks to replication (e.g., aflatoxin B1; Refolo et al. 1985; Jacobsen et al. 1987) or an ability to facilitate localized DNA denaturation. Both the above conditions should result in exposure of adducts (in single-stranded conformation) to water, and facilitate N1 deprotonation. In contrast, small adducts such as N7-methylguanine are tolerated because they neither stop replication (Larson et al. 1985; J. S. Jacobsen and M. Z. Humayun, unpublished results) nor significantly affect DNA duplex stability. Since DNA synthesis by E. coli DNA polymerase III is fast (over 500 nt/sec; Kornberg 1980), it is conceivable that N2-methylguanine is replicated before suffering deprotonation. It is alternatively possible that specific mechanisms exist in vivo to suppress N1 deprotonation in N2-methylguanine. It is assumed that these hypothetical mechanisms are inefficient in suppressing deprotonation at bulky guanine N7 adducts.

If one assumed that prolonged exposure in single-stranded conformation can lead to N1 deprotonation of N2-methylguanine, it follows that this normally innocuous adduct can act as a mispairing mutagenic lesion under certain conditions. In theory, such a possibility exists for N2-alkylguanine adducts occurring within heavily transcribed sequences, the loops of cruciform DNA structures, B-Z DNA junctions, and other unusual DNA structures. While the fraction of N2-alkylguanines in such special sequence environments may be small, the total amount of chemical modification of guanine N2 atom is likely to be high. The prevalence of endogenous and unavoidable exogenous alkylating agents raise the intriguing possibility that a significant fraction of background as well as induced mutagenesis may derive from miscoding by guanine N7 adducts.

The authors are grateful to John Callahan for assistance in early phases of this work, and to anonymous referees for useful suggestions. This work was supported by U.S. Public Health Service grant CA27735 and in part by a grant from American Cancer Society.

LITERATURE CITED


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Communicating editor: N. R. DRINKWATER