Modification of Plasmid and Bacteriophage DNA by Aromatic Amines: Effects on Survival, Template Activity, and Mutagenicity

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The carcinogenic and mutagenic effects of the aromatic amines are believed to depend on their covalent modification of DNA, primarily through the formation of adducts at the C8 position of guanine. The actual biologic and biochemical responses to these adducts can be envisioned as the consequence of the abilities of the cell to repair the lesions, with or without fidelity, and the introduction of errors through bypass of the adducts by polymerases. A key question is whether changes in DNA sequence arise through the participation of common repair processes that cause mutations independent of adduct structure. Alternatively, do mutations arise through miscoding during polymerase bypass at the site of the adducts and are, therefore, more likely to produce sequence changes that are more characteristic of adduct structure? This question has been approached using single, site-specific, or randomly introduced aromatic amine DNA adducts in bacterial cells, and in vitro studies with DNA polymerases that employ site-specifically modified templates. The results of both approaches demonstrate that these adducts are distinguished readily by virtue of their structures, thus supporting the conclusion that mutagenic effects of the aromatic amines arise from their structures rather than from their triggering a common inaccurate repair response. — Environ Health Perspect 102(Suppl 8):217–220 (1994)

Key words: adducts, aromatic amine, mutagenicity, polymerase, SOS functions

Introduction

The carcinogenic and mutagenic effects of the aromatic amines are believed to depend on their covalent modification of DNA, primarily through the formation of adducts at the C8 position of guanine (1,2). While adduct formation is likely to be an obligatory event in these processes, the production of adverse biological responses is likely to be influenced by the repair of the lesion (3), the need for the cell to utilize the DNA as a template for semiconservative replication (4,5), and the bypass of unrepaired lesions by DNA polymerases (4,5). Each of these three factors will be affected by the structure of the bound moiety in both qualitative and quantitative ways. The objective of our studies is to attempt to establish the structural characteristics of aryamine adducts that determine their abilities to produce mutagenic effects.

Methodology

Adducts containing 2-aminofluorenyl or 4-aminobiphenyl moieties were synthesized by reaction of guanine-containing oligonucleotides or DNA with N-acetoxy-N-trifluorooracetyl derivatives—for preparation of the C8-substituted aryamine adducts—or with the N-acetoxy-N-acetylated aromatic amines to incorporate the ory lactamid group at this position (3). Oligonucleotides were reacted with 3-sulfagly-N-acetyl-4-aminobiphenyl to prepare C8-acylamino- biphenyl-substituted guanines (6). Adduct analyses of DNA (3) and oligonucleotides (4) were carried out as described. Modified DNA was used to transform bacterial cells by use of a calcium chloride technique (3,4). Mutations produced by randomly introduced adducts were detected by a phenotypic selection technique (7); mutations induced by single, site-specific adducts were identified by hybridization (4). Use of adduct-containing oligonucleotides as templates for DNA polymerases was accomplished by introducing a nonphosphorylated adducted 7-mer into a gapped heterohexaplex M13 DNA (8). The nicked DNA was linearized with Ava II, denatured, and then primed with an oligonucleotide that would yield a full-length 48-mer product or a 41-mer oligonucleotide with those molecules that had failed to ligate a 7-mer into the gapped heteroduplex DNA. Both DNA polymerases I (large fragment) and AMV reverse transcriptase were used for chain extension (9).

Survival and Mutagenicity of Randomly Adducted DNA

Previous studies have shown that, while 2-aminofluorene-substituted double-stranded bacteriophage DNA exhibits a decreased transfection in uvrC, but not uvrA or uvrB Uvr endonuclease deficient mutants, analogous N-acetylated aminofluorene adducts are essentially equally sensitive to these three mutants (10). Analogous studies with plasmid DNA (pBR322) comparing 4-aminobiphenyl and 2-aminofluorene adducts showed that the 2-aminofluorene C8 guanine adduct was the only one of the four to show minimal reduction in transformation efficiency in uvrA and uvrB cells (3). The D37 values of the two biphenyl adducts were essentially equivalent to that of the 2-aminofluorene in uvrC; the D37 of the acetylated aminofluorene adducts approached a value of one in all three mutants, suggesting that a single adduct was almost totally effective in preventing polymerase bypass. These results demonstrated that the Uvr endonuclease could distinguish between adducts that differed only by the single carbon atom that served to hold the fluorene ring in a more planar configuration than that preferred by the biphenyl moiety in which the two aromatic rings are not coplanar. Furthermore, the N-acetyl group of the biphenyl adduct was without apparent effect on polymerase bypass.

Introduction of 2-aminofluorene- and 4-aminobiphenyl-substituted single- or
double-stranded bacteriophage DNA (M13mp9) into the Uvr endonuclease-deficient cells provides insight into the extent of bypass without the possibility that differential excision repair contributed to D_{37} values. The data in Table 1 show that, as expected (11), transformation efficiency of adducted single-stranded DNA in uvr mutants is no lower than that observed in wildtype cells. In contrast to transfections of double-stranded DNA, the transfection efficiency of acetylaminobiphenyl-substituted single-stranded DNA is far lower than the comparable aminobiphenyl-modified DNA and approaches that of acetylaminofluorene-adducted DNA. The mutagenicities of the adducted aminobiphenyl DNA preparations in wildtype JM103 cells are shown in Table 2. These data, in agreement with earlier studies with the analogous fluorene adducts (7, 12), clearly demonstrate that both adducts are mutagenic, the mutagenicity is primarily an SOS-dependent phenomenon, and adducts in single-stranded DNA are much more effective in producing mutations. As compared with the earlier study

| Adduct | Genotype | DNA Adducts/DNA molecule |
|--------|----------|-------------------------|
| ABP    | JM 103   | Wild-type 5.5 34         |
| AB1886 | uvrA     | 5.6 8.5                |
| AB1884 | uvrC     | 5.3 7.0                |
| AABP   | JM 103   | Wild-type 2.0 > 34     |
| AB1886 | uvrA     | 2.0 12.0               |
| AB1884 | uvrA     | 2.1 6.5                |
| AF     | JM 103   | Wild-type 5.0 ND       |
| AB1886 | uvrA     | 5.2 ND                 |
| AAF    | JM 103   | Wild-type 0.8 ND       |
| AB1886 | uvrA     | 0.8 ND                 |
| AB1884 | uvrC     | 1.0 ND                 |

Abbreviations: ABP, 4-aminobiphenyl; AABP, N-acetyl-4-aminobiphenyl; AF, 2-aminofluorene; AAF, N-acetyl-2-aminofluorene; ND, not determined.

The Mutagenicity of 4-aminobiphenyl and N-acetyl-4-aminobiphenyl M13mp9 adducts in JM103 cells.

| Adduct | Adenine Adducts/DNA | % Mutation frequency |
|--------|---------------------|---------------------|
|        | -                   | 0.05 0.054          |
|        | AABP                | 15 0.014 0.027      |
|        | ABP                 | 29 0.053 0.082      |
|        | AABP                | 31 0.025 0.076      |
|        | ABP                 | 56 0.061 0.19       |

Abbreviations: ABP, 4-aminobiphenyl; AABP, N-acetyl-4-aminobiphenyl; ND, not determined.

These and modified versions. The data in Table 1 show that, as expected (11), transformation efficiency of adducted single-stranded DNA in uvr mutants is no lower than that observed in wildtype cells. In contrast to transfections of double-stranded DNA, the transfection efficiency of acetylaminobiphenyl-substituted single-stranded DNA is far lower than the comparable aminobiphenyl-modified DNA and approaches that of acetylaminofluorene-adducted DNA. The mutagenicities of the adducted aminobiphenyl DNA preparations in wildtype JM103 cells are shown in Table 2. These data, in agreement with earlier studies with the analogous fluorene adducts (7, 12), clearly demonstrate that both adducts are mutagenic, the mutagenicity is primarily an SOS-dependent phenomenon, and adducts in single-stranded DNA are much more effective in producing mutations. As compared with the earlier study

Table 1. Survival of aminylamine-substituted M13mp9 DNA.

| Adduct       | Genotype | DNA Adducts/DNA molecule |
|--------------|----------|-------------------------|
| ABP          | JM 103   | Wild-type 5.5 34         |
| AB1886       | uvrA     | 5.6 8.5                |
| AB1884       | uvrC     | 5.3 7.0                |
| AABP         | JM 103   | Wild-type 2.0 > 34     |
| AB1886       | uvrA     | 2.0 12.0               |
| AB1884       | uvrA     | 2.1 6.5                |
| AF           | JM 103   | Wild-type 5.0 ND       |
| AB1886       | uvrA     | 5.2 ND                 |
| AAF          | JM 103   | Wild-type 0.8 ND       |
| AB1886       | uvrA     | 0.8 ND                 |
| AB1884       | uvrC     | 1.0 ND                 |

Abbreviations: ABP, 4-aminobiphenyl; AABP, N-acetyl-4-aminobiphenyl; AF, 2-aminofluorene; AAF, N-acetyl-2-aminofluorene; ND, not determined.

The Mutagenicity of 4-aminobiphenyl and N-acetyl-4-aminobiphenyl M13mp9 adducts in JM103 cells.

| Adduct       | Adenine Adducts/DNA | % Mutation frequency |
|--------------|---------------------|---------------------|
| No adduct    | -                   | 0.05 0.054          |
| AABP         | 4.3 ND              | 0.14 0.37          |
| ABP          | 20 ND               | 0.45 0.56         |

Abbreviations: ABP, 4-aminobiphenyl; AABP, N-acetyl-4-aminobiphenyl; ND, not determined.

Mutagenicity of Single, Site-specific Adducted DNA

Studies with oligonucleotides and DNA into which a single adduct had been introduced at a specific site provide the opportunity to experimentally determine the behavior of these adducts in biochemical systems in vitro as well as their mutagenic effects in vivo (4, 5, 13). Use of DNA constructs that carry replication-inhibiting lesions in the strand opposite the adduct serves to maximize the use of the adducted strand as a template in vivo (4, 5). Identification of mutants by virtue of the inability of clones and plaques to hybridize to oligonucleotide probes under stringent conditions serves to eliminate selection bias in these forward mutation systems (4, 8). Introduction of fluorene-guanine adducts into a plasmid system incorporating these features demonstrated that the aminofluorene adduct exhibited SOS-dependent mutagenicity, yielding primarily frameshift mutations with loss of the adducted guanine (4). The analogous acetylaminofluorene adducts produced no mutations; i.e., they were less than 15% as mutagenic as the deacetylated adduct. Introduction of these adducts into a plasmid that contained uracil in the opposite strand increased the mutagenicity of the aminofluorene by approximately 10-fold, to a mutation frequency of 2.9%. Interestingly, the mutations produced by aminofluorene in the uracil-containing DNA were exclusively base substitutions at the adduct site, primarily G:C to T:A transversions. In contrast to the experiments with the nonuracil-containing DNA, the acetylaminofluorene adducts produced a 0.75% mutation frequency, which included both base substitutions and frameshifts, primarily at sites near to but not at the adduct site.

Virtually all of these mutations were observed in host cells that had been exposed to UV irradiation prior to transformation so as to induce SOS functions. Thus, the mutations are believed to be produced by an active process rather than by passive bypass by a DNA polymerase complex or by the introduction of structural artifacts in constructing the DNA vectors. The differential response to the two adducts clearly illustrates that the mutations are structure-specific. Why mutagenic specificity of the aminofluorene adduct changes when presented to the cell in uracil-containing DNA is not known, but it is interesting to note that this adduct produced only base substitutions upon introduction into human cells (14). Attempts to further enhance mutagenesis by transforming Uvr endonuclease-deficient uvrA cells (AB1886) produced the unexpected result of a decreased mutagenic response equivalent to that observed in wild-type cells (NM522). Whether this decreased response comes from the absence of a component required for the cells' mutagenic response will require further experimentation. This strain of uvrA cells, however, had produced mutagenic responses equivalent to wild-type AB1157 cells in previous studies with acetylaminofluorene-adducted plasmids (15).

Effects of Adducts on Polymerases

Experiments designed to explore distinctions between adduct structures, which might be evident by virtue of alterations in the abilities of enzymes to use them as substrates, were patterned on previous studies by Strauss (16) and Fuchs (17). At the oligonucleotide level, both acetylaminofluorene (4, 8) and aminobiphenyl-substituted molecules were readily digested by the exonuclease activity of T4 polymerase (17); acetylaminofluorene (4, 8) and acetylaminobiphenyl derivatives were resistant to digestion. Thus, these susceptibilities did not distinguish between the aryl moieties, as was evident in the differences in D_{37}, in vivo as described previously (3) and above.

Use of single, site-specific adducts as templates for DNA polymerases (13, 16) revealed that both types of fluorene and
biphenyl adducts could result in hesitation points, as detected on autoradiograms, as well as full-length extension products (Figure 1). The data obtained with AMV reverse transcriptase are shown in Figure 1, but similar data also were obtained with DNA polymerase I (large fragment). The hesitation points corresponded to the base prior to the adduct, the adduct site, and the site 5' to the adduct. The hesitation points prior to the adduct site were most prominent with the acetylaminofluorene adduct.

The aminofluorene adduct produced less hesitation than the other three adducts. The acetylaminofluorene adduct produced the most prominent hesitation points 5' to the adduct. These data demonstrate that bypass of each of these adducts can occur and that the presence of the adduct on the template can affect polymerization at positions 5' to the adduct. Although previous studies with site-specific adducts have demonstrated the polymerase bypass of the aminofluorene adduct, fidelity (13), evidence for the bypass of acetylaminofluorene adducts has been less compelling (9,18). Further studies with site-specific modified templates will permit a better understanding of the potential for bypass that appears required for expression of the mutagenicity of these compounds.

The in vitro nature of the polymerase systems described here are not comparable to those responsible for the production of mutations in the cell. However, they do demonstrate that these macromolecules can, under certain circumstances, distinguish between adducts. It is possible that similar approaches might provide tools to evaluate and identify those structures responsible for the induction of specific mutations in cellular systems.

**Conclusions**

The studies described here support the idea that aromatic amine adducts at the C8 position of guanine in DNA exert their mutagenic effects because of the specific structures of the adduct, not as a result of faulty repair of the lesion through a general surveillance system. The mutations are produced in bacteria by an active process involving elements of the SOS system. Whether similar systems exist in mammalian cells has not yet been adequately explored. In addition to the specificities of the adduct structures, the mutagenicities of these adducts also are dependent on the sequences into which the adducts are placed and the poorly defined characteristics of these cellular systems involved in the processing of the damaged DNA. Comprehension of these potentials will only come as these systems are identified and characterized.

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