Specificity of Mutagenesis by 4-Aminobiphenyl

A POSSIBLE ROLE FOR N-(DEOXYADENOSIN-8-YL)-4-AMINOBIPHENYL AS A PREMUTATIONAL LESION*

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Dana D. Lasko§§, Susan C. Harvey†, Susan B. Malaiakal‡, Fred F. Kadlubar*, and John M. Essigmann‡

From the †Department of Chemistry, and the Whitaker College of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 and the §Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, Arkansas 72079

Mutagenesis by N-acetoxy-N-trifluoroacetyl-4-aminobiphenyl, a reactive form of the human bladder carcinogen 4-aminobiphenyl (ABP), was studied in Escherichia coli virus M13mp10. N-acetoxy-N-trifluoroacetyl-4-ABP-treated DNA containing 140 lesions/duplex genome, when introduced into excision repair-competent cells induced for SOS mutagenic processing, resulted in a 40-fold increase in mutation frequency over background in the lacZa fragment. DNA sequence changes were determined for 20 independent mutants. G-C base pairs were the major targets for base pair substitution mutations, although significant mutagenic activity was also observed at certain A-T base pairs. Deletion and frameshift mutations also were found in this sample. The salient feature of this partial "mutational spectrum" was a hotspot that occurred at position 6357 (amino acid 30 of the β-galactosidase fragment encoded by M13mp10); this A-T to T-A transversion appeared in 6 of the 20 mutants. The property of ABP to mutate A-T base pairs was consistent with the result that N-hydroxy-ABP reverted Salmonella typhimurium strain TA104, which is presumed to revert primarily due to mutations at these sites. The ability of the major carcinogen-DNA adduct formed by ABP in vivo and in vitro, N-(deoxyguanosin-8-yI)-4-aminobiphenyl, to cause base pair substitution mutations was also investigated. This adduct was positioned specifically in the minus strand at position 6270 in duplex M13mp10 DNA. In the presence of the mutagenesis-enhancing plasmid pGW16 and UV induction of SOS mutagenic processing, it was shown that fewer than 0.02% of the adducts resulted in transversion mutations following transfection of DNA into excision-repair competent cells. Similar results were obtained in uvrA and uvrC backgrounds. Although the major adduct did not cause base substitution mutations under these experimental conditions, the contribution of this lesion to the entire spectrum of mutations in the lacZa fragment seems likely.

The DNA of cells exposed to a chemical carcinogen nearly always contains a set of structurally diverse carcinogen-nucleotide adducts (Basu and Essigmann, 1988). It is suspected that misreplication or mispair of a subset of these adducts gives rise to mutations, which in turn may be the genetic precursors of the cancer phenotype. Given the wide range of DNA adduct structures that usually forms, it is a challenging task to determine which lesion(s) pose the most significant mutagenic risks to the cell. One strategy for establishing such relationships is to use the mutational spectrum of the chemical carcinogen to formulate hypotheses about which lesions are premutagenic. These hypotheses are subsequently tested in a model system in which a single carcinogen-DNA adduct is situated at a unique site in a phage or plasmid genome and then replicated in vivo. The determination of the type and amount of mutation induced usually enables an assessment of the extent to which the adduct under examination could have contributed to the mutational spectrum induced by the collection of lesions formed by the DNA-damaging agent.

The goal of the study described here was to identify the premutagenic lesions of 4-aminobiphenyl (ABP), an aromatic amine used in industry until it was discovered to cause bladder cancer in exposed workers (Melick et al., 1971). Human exposure to ABP continues today owing to its presence in cigarette smoke (Patrianakos and Hoffmann, 1979; Bryant et al., 1987). ABP is mutagenic in Salmonella typhimurium (McCann et al., 1975; Kadlubar et al., 1982; Beland et al., 1980), in Escherichia coli (Pai et al., 1985), and in mouse lymphoma cells in culture (Oberly et al., 1984). When metabolically activated, ABP reverses S. typhimurium strains TA1538, TA98, and TA100 but not TA1535; in the presence of pKM101, ABP appears to cause either base pair or frameshift mutations. All of these strains are believed to detect mutagens that act primarily at G-C base pairs (Levin et al., 1982).

The major DNA adducts formed by ABP in vivo or by reaction of N-hydroxy-ABP in vitro have been identified (Fig. 1; Kadlubar et al., 1982; Beland et al., 1983; Beland and Kadlubar, 1985). The predominant lesion is N-(deoxyguanosin-8-yl)-4-aminobiphenyl (DG^ABP), which accounts for

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§ Present address: Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, U. K.

1 To whom correspondence should be addressed: Rm. 56-243, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139.

1 The abbreviations used are: ABP, 4-aminobiphenyl; bp, base pair; ss, single stranded; ds, double stranded; RF, replicative form; DG^ABP, N-(deoxyguanosin-8-yl)-4-aminobiphenyl; DG^ABP, N-(deoxyguanosin-N3-yl)-4-aminobiphenyl; DA^ABP, N-(deoxyadenosin-8-yl)-4-aminobiphenyl; N-OAC-TPAAPB, N-acetoxy-N-trifluoroacetyl-4-aminobiphenyl; M13mp10-ABP, duplex genome of M13mp10 in which the guanine at position 6270 in the minus strand has been replaced with N-(deoxyguanosin-8-yl)-4-aminobiphenyl; M13mp10-G, same, but where the genome contains guanine at this site (this genome has undergone the same recombinant DNA manipulations as M13mp10-ABP); M13mp10-ABP, randomly adducted duplex genome of M13mp10 containing an average of 140 ABP adducts; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; IPTG, isopropyl-β-D-thiogalactopyranoside; AP, apurinic/apyrimidinic; AF, 2-aminofluorene.

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Fig. 1. Structures of ABP-DNA adducts.

![N-(deoxyguanosin-8-yl)-4-aminobiphenyl](image)

![N-(deoxyadenosin-8-yl)-4-aminobiphenyl](image)

Fig. 2. Schematic outline of the experimental strategy allowing assessment of mutagenic potential of all ABP adducts, or of a single lesion. A, mutation assays using M13mp10 DNA randomly adducted with ABP lesions. DNA was treated with N-acetoxy-N-trifluorocetyl-ABP as described under “Experimental Procedures.” The adducted DNA was transfected into excision repair-proficient cells that were competent for SOS mutagenic processing due to the presence of the mutagenesis-enhancing plasmid, pGW16. Progeny phage were screened for the presence of colorless or pale blue mutant plaques, which indicated a defect in β-galactosidase activity. DNA sequencing of mutants allowed analysis of the mutational spectrum caused by ABP lesions. B, in parallel to mutation assays with randomly adducted DNA, the M13mp10 cloning vector was used to assess the potential of this adduct to cause base substitution mutations in the mutational spectrum caused by ABP lesions. DNA sequencing DNA of the fragment of M13mp10 resulting in a fragment of the lacZ gene. In the same vector, the ability of the major dGa-ABP adduct to induce base substitution mutations at the PstI site was assessed. An overview of the experimental strategy appears in Fig. 2.

Mutation Specificity of N-Acetoxy-N-trifluorocetyl-ABP, a Reactive Form of ABP—Reaction of double stranded DNA with N-acetoxy-N-trifluorocetyl-4-aminobiphenyl (Fig. 2A) produced 140 ABP residues/genome (M13mp10-ABP$_{\omega}$) or about 1 adduct/26 guanine residues. The accompanying number of abasic sites in the modified replicative form DNA was estimated to be 0.8 (±0.1)/genome. Under the same reaction conditions, single stranded DNA yielded 400 ABP adducts/genome. When calf thymus DNA modified in parallel to the above was transfected into host cells, either excision repair-proficient or -deficient, containing the mutagenesis-enhancing plasmid pGW16. Progeny phage were screened for base substitution mutations at the PstI site by iterative cleavage of replicative form DNA with PstI or by a plaque color assay that specifically detected G to T transversion mutations at position 6270.

In vitro and in E. coli, the major dGa-ABP adduct, which distorts DNA structure only to a minor extent, thus possibly avoiding recognition by DNA repair enzymes (Broyde et al., 1985; Shapiro et al., 1986), at the replication fork, low energy syn conformers were suggested as possibly being responsible for mutagenesis.

The ability of ABP to induce mutations at G-C targets led us to investigate the mutagenic activity of the dGa-ABP adduct as the initial step toward defining the mutagenic consequences of all ABP-DNA adducts. Although two guanine lesions have been identified, we chose to focus our investigation on the potential of this adduct to cause base pair substitution mutations at position 6270 in M13mp10 DNA containing dG8-ABP at position 6270 in the major groove and distort DNA structure only to a minor extent, thus possibly avoiding recognition by DNA repair enzymes (Broyde et al., 1985; Shapiro et al., 1986). At the replication fork, low energy syn conformers were suggested as possibly being responsible for mutagenesis.

Mutational Specificity

EXPERIMENTAL PROCEDURES

RESULTS

Previous studies have shown that ABP and its metabolite N-hydroxy-ABP are mutagenic, but there are few data available on the specificity of the mutational process. The mutational spectrum of ABP in E. coli was defined by introducing ABP adducts randomly into an M13 phage genome containing a fragment of the lacZ gene. In the same vector, the ability of the major dGa-ABP adduct to induce base substitution mutations at the PstI site was assessed. An overview of the experimental strategy appears in Fig. 2.

The “Experimental Procedures” are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
mutant plaques among 8268 examined, placing an upper 95% Poisson confidence limit for mutation frequency under these conditions of 4.3 x 10⁻⁶. In the absence of pGW16, mutagenesis of UV-treated M13mp10 single stranded DNA is detectable without prior irradiation of E. coli used for transfection assays. This property makes pGW16 a convenient tool for the analysis of mutagenesis by lesions that require SOS mutagenic processing (Walker, 1985). The mutation frequency among progeny phage increased when M13mp10-ABP₁₆ DNA was introduced into excision-repair competent cells containing plasmid pGW16 (Table I). The damaged DNA yielded a 25–50-fold increase in mutation frequency compared to control, untreated replicative form DNA. A separate mutation assay on DNA subjected to identical buffer and extraction conditions yielded no mutant plaques among 8268 examined, placing an upper 95% Poisson confidence limit for mutation frequency induced by buffer conditions of 4.3 x 10⁻⁶. In cells lacking pGW16, no mutant plaques were detected in 3421 examined; the upper 95% confidence limit for mutation frequency under these conditions was 1.1 x 10⁻⁶. Thus, SOS mutagenic processing was necessary for mutagenesis to be observed in M13mp10-ABP₁₆ DNA. Survival of phage immediately after heat shock, measured as infective centers, was 0.2% in DL7 (wur) cells, 0.3% in DL7/pGW16 cells, but less than 0.005% (no plaques detected) for both DL6 (wurA) and DL4 (wurC) cells. Survival in these experiments was the same as that of all other cells formed by added DNA compared to the number resulting from the same amount of unmodified DNA. In a separate experiment, buffer and solvent control DNA showed no reduction in survival compared to untreated DNA. These data show that, at 140 adducts/phage genome, excision repair proficiency markedly increased the survival of N-acetoxy-N-trifluoroacetyl-ABP-treated DNA. Mutagenesis was not investigated further in excision repair-deficient bacteria because of the low viability of the adducted DNA in these strains.

**Partial Mutational Spectrum of ABP Lesions**—Twenty independent lacZ mutants were collected from excision repair-proficient cells containing pGW16. Subsequently, their DNA was sequenced to provide a partial mutational spectrum for ABP lesions (Table II and Fig. 3). A background spectrum was not determined because of the low frequency of spontaneous mutations; however, because the mutation frequency in adducted DNA was on average 40-fold higher than control DNA, it was reasonable to assume that the mutants were mainly due to the ABP lesion.

The noteworthy features of the ABP-induced spectrum were a hotspot (6 occurrences/20 independent mutants sequenced) for an A-T to T-A transversion at position 6357, other transversion mutations (mostly occurring at G-C base pairs), transition mutations, two (−1) and one (+1) frameshift mutations, and deletion mutations (Table II). Because adenine and guanine bases are known targets for modification by ABP, we assume that the transversions and −1 deletion frameshifts due to N-acetoxy-N-trifluoroacetyl-ABP treatment originated at these sites. We have not as yet determined whether or not the A-T to T-A hotspot exists in the background mutational spectrum. Confirmation of the 4-base deletion mutations by ABP is required since we cannot conclusively rule out the possibility of contamination of DNA or phage from site-specific mutagenesis experiments (see below).

The finding of a hotspot for mutagenesis by ABP lesions at an A-T base pair prompted an evaluation of mutagenesis by N-hydroxy-ABP in S. typhimurium strain TA104, a strain designed to detect mutation at A-T targets (Levin et al., 1982, 1984). Treatment of TA104 cells with N-hydroxy-ABP in a liquid suspension assay (Kado et al., 1983) at a dose of 50 μM resulted in a doubling of the number of revertant colonies at >60% survival; the dose-response curve was nearly identical to that of TA100 assayed in parallel. Although the interpretation of TA104 mutagenesis in terms of exact DNA sequence alterations is complicated by the occurrence of extragenic ochre suppressor revertants, these data generally support the conclusion that in an SOS-processing environment, ABP lesions cause base pair substitution mutations at both G-C and A-T targets.

**Site-specific Mutagenesis of dG⁻¹⁶t in M13mp10**—Seven of 14 base substitution mutations in the mutational spectrum of ABP occurred at G-C base pairs, suggesting that one or both of the two known guanine adducts (Fig. 1) may play a role in mutagenesis in an SOS-processing genetic background. As a first step toward dissecting the relative contributions of the 4-Aminobiphenyl

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**Table I**

| Expt. | Plaque yield mutant/ mutation | 10⁶ × mutation frequency | Plaque yield mutant/ mutation | 10⁶ × mutation frequency |
|-------|-------------------------------|--------------------------|-------------------------------|--------------------------|
| 1     | 41/7,595                      | 53 (38, 73)              | 2/8,104                       | 2.4 (0.3, 8.8)           |
| 2     | 54/5,132                      | 105 (72, 131)            | 2/11,271                      | 1.7 (0.2, 6.3)           |
| 3     | 58/7,899                      | 76 (53, 99)              | ND                            | ND                       |

**Table II**

| Mutant number | Occurrences | Mutation | Site | Consequence in protein |
|---------------|-------------|----------|------|------------------------|
| 1             | 1           | (CG)     | 5' upstream region |
| 2             | 1           | GC to TA | GAG (Glu) to TAG (amber stop) |
| 3             | 4           | (TGCA)   | 4-A reading frame |
| 4             | 1           | CG to TA | CAA (Gin) to TAA (ochre stop) |
| 5             | 1           | AT to CG | GCC (Asp) to GCC (Ala) |
| 6             | 1           | (+AT)    | 1 reading frame |
| 7             | 1           | GC to TA | TGG (Trp) to TTG (Leu) |
| 8             | 1           | GC to TA | TGG (Trp) to TGT (Cys) |
| 9             | 1           | (GC)     | 1 reading frame |
| 10            | 6           | TA to AT | CAT (His) to CAA (Glu) |
| 11            | 1           | GC to TA | GCC (Ala) to TCC (Ser) |
| 12            | 1           | GC to GC | TGG (Trp) to TAG (amber stop) |

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known ABP adducts to mutagenesis, the ability of dG8-ABP to induce base pair substitution mutations at the PstI site in M13mp10-ABP, was investigated (Fig. 4). This was found to be the case even when a sufficient number of infective centers was examined so that the limit of detection with 95% confidence was below a mutation frequency of 0.02%. By contrast, single stranded DNA treated with 72 J/m² UV light as a positive control produced a mutation frequency of 9 x 10⁻⁴, demonstrating that the cells were indeed capable of the SOS processing required for UV- and ABP-induced mutagenesis. In excision repair-deficient backgrounds (uvrA and uvrC), induced base pair substitution mutagenesis was below 0.2%. In the two cases where mutation appeared to rise above background levels (DL4 cells + UV and DL6 cells - UV), DNA sequencing of six PstI-resistant phage from control tubes were found to be A-T to T-A mutants at the PstI site. The frequency of G to T transversion at the PstI site. The frequency of G to T transversion at position 6271. It is conceivable that the in-frame deletion mutations were induced by the adduct but, as explained below, complete analysis of frameshift and deletion mutagenesis was beyond the scope of this study.

The G-C to T-A transversions at position 6271 were relatively frequent (0.3-4.0%) and they yielded colorless plaques, it was impossible to screen easily for frameshift or deletion mutations induced by the adduct. In the minus UV, DNA repair phenotype was not elevated above the background (less than 0.03%). A complication of this screen resulted from the presence among the progeny phage of deletion mutants missing the central four bases (5'-TGCA-3') of the PstI site; these mutants were shown earlier to result from the genetic engineering procedures used to prepare the singly adducted genome (Loechler et al., 1984). Because these small deletions were relatively frequent (0.3-4.0%) and they yielded colorless plaques, it was impossible to screen easily for frameshift or deletion mutations induced by the adduct.

**DISCUSSION**

**ABP as a Versatile Mutagen** — The most striking feature of the mutational spectrum is that ABP is a very versatile mutagen. An evaluation of a sample of 20 changes induced by type that had evaded PstI selection. Two PstI-resistant phage from control tubes were found to be A-T to T-A mutants at the PstI site. The frequency of G to T transversion at position 6271. It is conceivable that the in-frame deletion mutations were induced by the adduct but, as explained below, complete analysis of frameshift and deletion mutagenesis was beyond the scope of this study.

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**DISCUSSION**

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ABP lesions in a genetic background that included the mutagenesis-enhancing plasmid pGW16 showed that base substitution mutations occurred at both G-C and A-T base pair targets (Table II). This was consistent with earlier studies and with our observations on mutagenesis by ABP and its metabolites in S. typhimurium his tester strains. In addition, the mutational spectrum revealed frameshift mutations, again consistent with results from S. typhimurium reversion assays.

Comparative Mutagenesis of ABP Lesions throughout the Genome and at a Specific Site—The ability of the major DNA lesion caused by ABP in vivo or by reaction of activated forms of the chemical with DNA in vitro, dG⁴-ABP, to induce base substitution mutations was evaluated at a specific site in the M13mp10 genome. The singly adducted genome yielded no increase in base pair substitution mutagenesis over background (less than 2 × 10⁻⁴ mutants/progeny phage in excision repair-proficient cells, and less than 2 × 10⁻³ mutants/progeny phage in excision repair-deficient cells). This negative result raises the question as to what frequency of mutation was expected for the “average” ABP lesion, based on our results with M13mp10-ABP₁₄₀. When a series of assumptions is made, including random modification, equal probability of repair at any target site, and equal probability of mutation at any given target, it is possible to calculate the “average” mutagenic efficiency of an ABP lesion (Schaeper and Loeb, 1981; Koffel-Schwartz et al., 1984). Given the mean induction of mutagenesis above background of 80 × 10⁻⁴ and the mean number of adducts per target of 6, the average adduct results in mutation 1.4 times out of every 1000; i.e., the mutation frequency in vivo is ≈0.14%. Thus, the results of the site-specific mutagenesis study indicate that the mutagenic efficiency of dG⁴-ABP built into the PstI site was at least 7-fold lower than this value under the conditions of the experiment, and below the limit of detection of our mutation assay. This result could indicate that the dG⁴-ABP adduct is not highly mutagenic. However, the partial mutational spectrum in the lacZa fragment, in combination with the results of many other workers on other mutagens (notably Benzer, 1961; Miller, 1983; and, with other aromatic amine lesions, Koffel-Schwartz et al., 1984) indicate that the effect of neighboring DNA sequence is important. A crucial future experiment will be to evaluate mutagenicity by an ABP adduct at a known “hotspot” for mutagenesis, as deduced from Table II.

An additional explanation for the inability to detect induced mutagenesis by dG⁴-ABP at position 6270 concerns the kinetics of DNA repair. The large number of lesions per genome in M13mp10-ABP₁₄₀ may well have saturated the repair system(s) for this type of damage, while the comparatively low dose of one ABP adduct per genome in M13mp10-ABP¹ may have resulted in rapid repair of the single lesion and thus no observed mutagenesis. Experiments in wuA and wuC cells revealed no consistent increase in dG⁴-ABP-induced base pair substitution mutagenesis compared to excision repair-proficient cells (Fig. 4). This may have resulted from leakage of the repair-deficient phenotype or from the inability of another cellular repair system to act on the single ABP lesion. In the case of O⁴-methylguanine (Loechler et al., 1984) for which there is a well characterized, suicidal repair enzyme (Lindahl, 1982), mutagenesis in single stranded DNA modified specifically at the PstI site was elevated from 0.4 to ~20% when the repair system for this lesion was compromised. Further experimentation under conditions of saturated repair and SOS mutagenic processing may resolve this question.

Another plausible reason for lack of mutagenesis by the single dG⁴-ABP lesion in the duplex M13 genome is that there may have been a bias in favor of recovering progeny phage replicated from the unadducted plus strand of M13mp10-ABP¹. Since double stranded DNA was used for transfections in the site-specific mutagenesis assays, progeny could come either from the intact plus strand or the adducted minus strand. Penetration of the minus strand phenotype is at least ~30% (Hill-Perkins et al., 1986; Kunkel and Alexander, 1986) and possibly higher (Easigmann et al., 1986). Because no direct measurement of the penetration of the plus versus minus strands was made, a correction has not been applied to the raw mutation frequencies reported here. This correction would also alter the control mutation frequency estimates by the same factor. An additional and more severe bias in favor of progeny of the unadducted plus strand could have resulted from selective blockage of DNA polymerase III by the adduct in the minus strand. Consequently, few if any progeny phage may have been produced with the genotype of the adduct-containing strand. Such a model is strongly suggested by recent results of Koffel-Schwartz et al. (1987).

Biochemical Mechanism of ABP Mutagenesis: Comparison with Other Aromatic Amines—Fuchs and colleagues have investigated the mutational specificity of the related aromatic amines, N-acetoxy-N-acetyl-2-aminofluorene (N-acetoxy-N-acetyl-2-aminofluorene; Koffel-Schwartz et al., 1984) and N-hydroxy-2-aminofluorene (N-hydroxy-AF; Bichara and Fuchs, 1985), in pBR322. For N-acetoxy-N-acetyl-AF, these workers concluded that frameshifts comprise 90% of the mutations, in contrast to N-hydroxy-AF, which was found to cause 85% base pair substitutions (mostly G-C to T-A transversions). N-acetoxy-N-acetyl-AF-induced mutagenesis was highly se-
quence-dependent and had a component that was independent of an umuDC processing environment; N-hydroxy-AF-induced mutagenesis was less governed by sequence context but was found to be umuDC-dependent.

In view of the structural similarity between the major AF and ABP adducts, our results for ABP and those reported for AF provide an interesting comparison. The mutational efficiency that we found for ABP was similar to those reported for other aromatic amine lesions (Koffel-Schwartz et al., 1984; Bichara and Fuchs, 1985). Moreover, in our hands ABP-induced mutagenesis was similar to that induced by AF, in that it was dependent on SOS mutagenic processing. With ABP, however, we observed a lower proportion of the G to T-A transversion and the presence of a hotspot at an A-T base pair target. The appearance in the mutational spectrum of ABP (Table II) of a hotspot at an A-T base pair, coupled with the knowledge that a d\(\text{A}^\bullet\text{ABP}\) adduct is among the lesions produced by ABP, suggests that site-specific mutagenesis in this DNA sequence context would be informative.

One plausible cause of the transversion mutations is an apurinic site formed either before or after entry of the adducted DNA into the cell (Schaeper and Loeb, 1981; Kunkel, 1984). The former possibility is unlikely, however, in view of the low number of apurinic/apyrimidinic sites per molecule of M13mp10-ABP, prior to transfection (determined empirically to be 0.8) and the low mutation frequency in the solvent control. The average apurinic/apyrimidinic site formed prior to transfection would need to have a mutagenic efficiency of \(20\%\) in double stranded DNA in order to have been responsible for this mutation; this is much higher than has been reported thus far in any forward mutation assay. To the extent that apurinic/apyrimidinic sites may have acted as intermediates in the mutagenic activity of ABP, it is more likely that they formed inside the cell, probably via the action of repair enzymes or, less likely, by spontaneous depurination. In support of the involvement of apurinic/apyrimidinic sites in mutagenesis by ABP is the observation that ABP induced transversion mutations (Table II) that presumably originated from purines (ABP forms no known pyrimidine adducts). These data are in accord with the established mutagenic specificity of apurinic/apyrimidinic sites (Loeb and Preston, 1986).

A second possible mechanism of mutagenesis by ABP would require that the premutagenic adduct(s) evade repair and induce mutation by following a replication error during DNA synthesis. In this regard it is noteworthy that the hotspot for the A-T to T-A mutation is adjacent to a run of 5 G-C base pairs, and it is tempting to speculate that the presence of the G-C run may act as a "clamp" that offers this sequence decreased susceptibility to the action of DNA repair enzymes on d\(\text{A}^\bullet\text{ABP}\). It is also noteworthy that the prevalence of transversion mutations for ABP, as well as for AF and other aromatic amines, is consistent with conformational changes in DNA that can arise from carcinogen modification at C-8 of guanine and adenine (Swenson and Kadlubar, 1981). From theoretical as well as spectroscopic studies, it appears that d\(\text{G}^\bullet\text{ABP}\) can readily adopt a syn conformation about the guanine-deoxyribose linkage (Broyde et al., 1985; Shapiro et al., 1996). This has been suggested to occur preferentially with a destabilized or unwound DNA helix such as that obtained during replication. The syn conformation allows carcinoma stacking with a neighboring base and provides the potential for frameshift or missense mutations. However, such a conformational change (Fig. 5) also places the \(\text{G}\) and \(\text{N}\) atoms of the modified guanine in a position to mispair with N-1 and N-7 of a guanine or with an N-2 and N-1 of an adenine (iminotautomer) in the complementary strand (Drake and Baltz, 1976; Topal and Fresco, 1976), resulting in G-C to C-G or G-C to T-A transversions, respectively. Similarly, d\(\text{A}^\bullet\text{ABP}\) might be expected to be converted to its syn conformation in which a mispair could occur between N-8 and N-7 of the modified adenine and N-8 and N-1 of a complementary adenine, resulting in an A-T to T-A transversion. The existence of such base pairs is supported by \textit{ab initio} self-consistent field and dispersion energy calculations that predict the relative stability of hydrogen bonds at \(\text{O}'\) and N-7 of guanine and at N-8 and N-7 of adenine (Holba and Sandoz, 1987).

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