Estimation of Apurinic/Apyrimidinic Sites and Phosphotriesters in Deoxyribonucleic Acid Treated with Electrophilic Carcinogens and Mutagens†

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ABSTRACT: The number of apurinic/apyrimidinic (AP) sites in supercoiled SV40 deoxyribonucleic acid (DNA) after treatment with several electrophilic mutagens was quantitated by electrophoretic analysis of the DNA after cleavage of the phosphodiester bonds adjacent to AP sites by a specific endonuclease. The compounds studied, in order of increasing yields of AP sites obtained on incubation with the DNA for 5 h at 37 °C, were dimethylcarbamoyl chloride, ethyl methanesulfonate, *N*-ethyl-*N*-nitrosourea, 2-(*N*-acetoxyacetylamino)fluorene, β-propiolactone, *N*-methyl-*N*-nitrosourea, methyl methanesulfonate, 1′-acetoxyestragole, 4-(*N*-acetoxyacetylamino)stilbene, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene, and 1-pyrenyloxirane. After a 5-h incubation at 37 °C and extraction of unreacted compound, further incubation at 70 °C generally increased the yield of AP sites; an exception was *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene-reacted DNA. Except for DNA treated with *N*-ethyl-*N*-nitrosourea and *N*-methyl-*N*-nitrosourea, which are known to bind to a significant extent to DNA phosphates, the number of alkali-labile lesions in the treated DNA was similar to the number of AP sites. For the compounds studied there was no direct correlation between the number of AP sites produced and missense mutagenic activity, as measured in *Salmonella typhimurium* strain TA100.

Substitution at certain sites on the purine or pyrimidine bases in DNA increases the rate of hydrolysis at neutrality of the N-glycosidic bond to yield AP[1] sites (Lawley & Brookes, 1963; Singer et al., 1978). At least for simple alkyl substituents, the order of hydrolysis rates is 7-adenyl > 3-adenyl > 7-guanyl > 3-guanyl > O^2-cytidyl > O^2-thymidyl (Lawley & Brookes, 1963; Lawley & Warren, 1976; Singer et al., 1978). The relatively rapid depurination of 3-alkyladenine and 7-alkylguanine adducts in DNA was first noted by Brookes and Lawley (Brookes & Lawley, 1961; Lawley & Brookes, 1963), who suggested that the resultant AP sites might be responsible for the mutagenic activities of simple alkylating compounds. However, subsequent studies indicated that quantitatively minor derivatives, such as O^6-alkylguanines, O^4-alkylthymines, and 3-alkylcytidines, which permit base-mispairing during DNA replication, played more important roles in mutagenesis (Abbott & Saffhill, 1979; Gerchman & Ludlum, 1973; Loveless, 1969; Singer, 1975, 1979). The primary biological effect of AP sites was considered to be DNA inactivation (Brakier & Verly, 1970; Lawley, 1976) owing to the production of strand breaks (Lindahl & Andersson, 1972) or interstrand cross-links (Burnotte & Verly, 1972).

The possible importance of AP sites in mutagenesis has been suggested again by Shearman & Loeb (1979), who demonstrated that the presence of AP sites can lead to base misincorporation during DNA synthesis. Moreover, despite the success of the base mispairing model in accounting for the mutagenic activities of methylating and ethylating agents, modification of DNA by some potent missense mutagens such as benzo[*a*]pyrenediol epoxide and 2-(*N*-acetoxyacetylamino)fluorene does not result in specific base mispairing (Grunberger & Weinstein, 1979). Since these compounds induce AP and/or alkali-labile sites in DNA (Deering et al., 1975; Osborne et al., 1978), we examined the possibility that AP sites might be significant sources of the mutagenic damage caused by compounds other than simple alkylating agents.

AP sites are characterized by the ease of hydrolysis of the adjacent phosphodiester bonds to yield single-strand breaks in DNA; the half-life for hydrolysis is ~200 h under physiological conditions (Lindahl & Andersson, 1972). The phosphodiester bonds adjacent to AP sites are rapidly hydrolyzed by alkali and are presumably responsible for at least some of the alkali-labile lesions observed in carcinogen-treated DNA by sedimentation (Kohn, 1975; Thielmann, 1977) and filter-retention (Kohn, 1975; Kohn & Grimek-Ewig, 1973; Kuhnlein et al., 1979) methods. These alkali-labile lesions may be distinguished from the alkali-labile alkyl phosphotriesters (Shooter, 1976) by the specific cleavage of phosphodiester bonds adjacent to AP sites by AP endonucleases (Lindahl, 1979).

Electrophoretic analysis of carcinogen-treated supercoiled DNA provides a facile means for quantitating the number of single-strand breaks in DNA. Introduction of a single-strand break in supercoiled form I DNA results in its conversion to nicked circular form II DNA, which has a lower electrophoretic mobility than form I DNA (Johnson & Grossman, 1977) (Figure 1). Alkali-denatured DNA can yield three bands on electrophoresis: single-stranded circular DNA, denatured form I, and single-stranded, unit-length, linear DNA (Johnson & Grossman, 1977). We have used this approach to determine the number of AP and total alkali-labile sites produced in supercoiled SV40 DNA by treatment with several electrophilic carcinogens and mutagens (Figure 2) which have very different structures and biological activities. The results were compared with the mutagenicities of the compounds in *Salmonella*

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[1] Abbreviations used: AP, apurinic/apyrimidinic; benzo[*a*]pyrenediol epoxide, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; EDTA, ethylenediaminetetraacetic acid; PEN, 10 mM sodium phosphate, 1 mM Na_2EDTA, and 0.1 M NaCl, pH 7.4; TAE, 50 mM Tris base, 20 mM sodium acetate, 2 mM Na_2EDTA, 18 mM NaCl, and 0.5 μg/mL ethidium bromide, adjusted to pH 8.05 with glacial acetic acid; *t*, Kendall's coefficient of rank correlation; *r*, correlation coefficient; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

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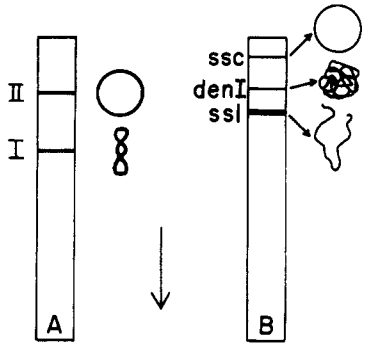

FIGURE 1: Schematic representation of the agarose gel electrophoresis of SV40 DNA. The arrow indicates the direction of electrophoresis. (A) 1% gel; I, supercoiled form I DNA; II, nicked, circular form II DNA. (B) 2% gel, alkaline-denatured DNA; ssc, single-stranded circular DNA; den I, denatured form I DNA; ssl, single-stranded, unit-length, linear DNA.

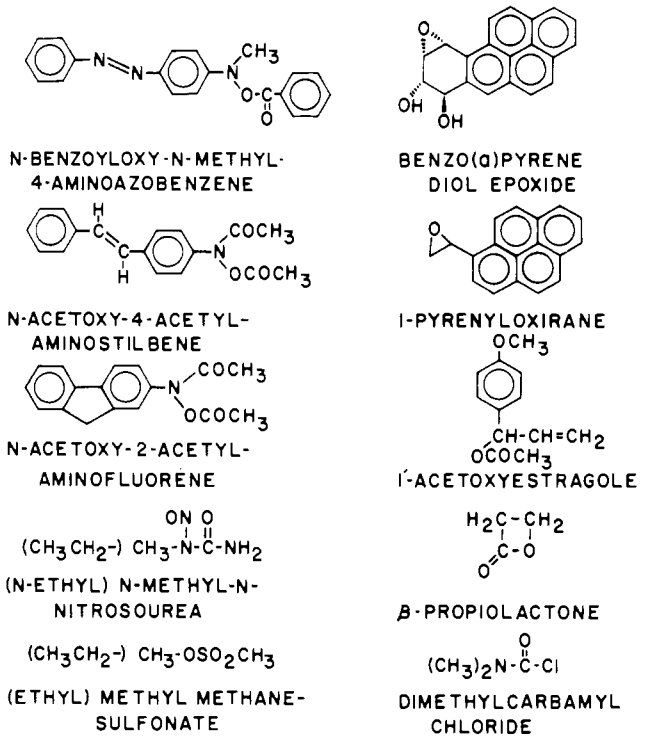

FIGURE 2: Structures of the reactive electrophiles used in this study.

typhimurium strain TA100, which detects missense mutagens.

Materials and Methods

Chemicals. *N*-Ethyl-*N*-nitrosourea (Lawley & Shah, 1972), *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene (Poirier et al., 1967; Wislocki et al., 1975), 2-(*N*-acetoxyacetylamino)fluorene (Lotlikar et al., 1966), 4-(*N*-acetoxyacetylamino)stilbene (Andersen et al., 1964), and 1'-acetoxyestragole (Drinkwater et al., 1976) were synthesized by previously published methods. Benzo[*a*]pyrenediol epoxide and 1-pyrenyloxirane were kindly provided by Dr. N. C. Yang (University of Chicago). The remaining chemicals were obtained from commercial sources. All of the reactive compounds were stored desiccated at −20 °C, and each was judged to have a purity of >95% by its melting point, UV spectrum, and/or mobility on thin-layer or high-performance liquid chromatography.

Mutagenicity Assays. The compounds were assayed for mutagenicity toward *S. typhimurium* strain TA100, a histidine-requiring missense mutant, by methods adapted from those of McCann et al. (1975b). Although the mutagenic activities of most of the compounds for this bacterial strain have been reported (Drinkwater et al., 1976, 1978; McCann et al., 1975a), there were some differences in methodology. Accordingly, we redetermined the mutagenicities to facilitate comparisons. Briefly, 0.1 mL of bacterial culture (10^9 bacteria/mL) and the compounds, dissolved in 0.01 mL of dimethyl sulfoxide, were added to 2 mL of molten top agar, which was then poured on a minimal agar plate. After incubation for 2 days at 37 °C, the revertant bacterial colonies were counted. Each experiment consisted of duplicate or triplicate samples for each of 3–4 doses of the compound. The number of revertants induced per nanomole of compound over the linear portion of the dose–response curve was estimated by linear regression analysis of data from two or more experiments.

Quantitation of AP Sites. The compounds were each reacted with 0.075 μg of form I SV40 DNA (kindly provided by Dr. Janet Mertz, McArdle Laboratory, University of Wisconsin—Madison) for 5 h at 37 °C in 0.04 mL of PEN buffer containing 10% dimethyl sulfoxide. In separate experiments, the number of AP sites in the DNA at the end of the 5-h incubation and the total number of AP sites that could be obtained on hydrolysis were determined. For the former assay, the treated DNA sample was incubated at 37 °C for 15 min with 0.5 unit of exonuclease III (from *Escherichia coli*; New England Biolabs, Cambridge, MA), 0.3 μmol of $MgCl_2$, 0.05 μmol of dithiothreitol, and 5 μg of bovine serum albumin. Further nuclease action was then inhibited by the addition of 0.015 mL of 0.15 M EDTA. In addition to its 3'–5' exonucleolytic activity, exonuclease III is also an endonuclease specific for phosphodiesters at the 5' side of AP sites (Lindahl, 1979). The use of 0.5 unit of exonuclease III was based on preliminary experiments that showed that incubation with <0.25 unit did not hydrolyze all of the AP sites in the treated DNA and that treatment with >5 units resulted in extensive exonucleolytic digestion of the DNA adjacent to the sites of cleavage. The samples were then analyzed for the conversion of form I to form II DNA as described below.

To determine the total number of DNA–carcinogen adducts that could be hydrolyzed to yield AP sites, we removed unreacted compound by extraction of the sample 4 times with an equal volume of chloroform at the end of the 5-h incubation. The DNA was then incubated at 70 °C to effect the hydrolysis of the N-glycosidic bonds of the appropriately modified nucleotides; the length of the incubation for each compound was based on data from kinetic experiments to ensure the maximum yield of AP sites. The samples were then treated with exonuclease III as above.

For both assays, the relative amounts of form I and form II DNA were determined by electrophoresis of the treated samples on 8-cm 1% agarose gels with TAE buffer and a constant potential of 4 V/cm (Drinkwater et al., 1978; Johnson & Grossman, 1977). The gels, which contained 0.5 μg/mL ethidium bromide, were then photographed under 254-nm UV light with Tri-X film (Eastman Kodak, Rochester, NY) and a red filter (No. 23A; Tiffen Corp., Long Island, NY). The relative intensities of the form I and form II DNA bands in each gel were determined by microdensitometry (Model MkIIIC; Joyce, Loebl, and Co., Gateshead, England). For the exposure times used, the intensities of the photographed DNA bands were linearly related to the quantity of DNA present. On the assumption that the number of single-strand scissions per DNA molecule is Poisson-distributed, the mean number of strand breaks, m, per double-stranded SV40 molecule of $\sim 10^4$ nucleotides was estimated as

$$m = -\ln f_I$$

Table I: Comparative Mutagenic Activities and Yields of AP Sites, Adducts That Hydrolyze to AP Sites at Neutrality, and Excess Alkali-Labile Lesions in Mutagen-Treated DNA[a]

compd	mutagenicity[b] (rev/nmol)	AP sites/(10^4 nucleotides·mM compd) (5 h, 37 °C)	total sites/(10^4 nucleotides·mM compd)	
			AP	excess alkali labile
ethyl methanesulfonate	0.010 ± 0.002	0.038 ± 0.004	0.56 ± 0.03	0.06 ± 0.06
dimethylcarbamoyl chloride	0.018 ± 0.001	0.002 ± 0.003	0.003 ± 0.003	0.005 ± 0.003
N-ethyl-*N*-nitrosourea	0.082 ± 0.007	0.103 ± 0.001	0.40 ± 0.05	0.51 ± 0.08
methyl methanesulfonate	0.19 ± 0.01	1.00 ± 0.02	13.2 ± 0.05	0 ± 0.6
N-methyl-*N*-nitrosourea	0.62 ± 0.07	0.99 ± 0.02	8.8 ± 0.8	2.8 ± 1.3
β-propiolactone	0.91 ± 0.06	0.94 ± 0.07	33.4 ± 1.5	0 ± 2.1
1′-acetoxyestragole	2.5 ± 0.1	0.85 ± 0.05	3.8 ± 0.1	0 ± 0.2
2-(*N*-acetoxyacetylamino)fluorene	3.0 ± 0.1	0.37 ± 0.02	4.1 ± 0.2	0.3 ± 0.3
N-(benzoyloxy)-*N*-methyl-4-aminoazobenzene	5.1 ± 0.4	19 ± 1[c]	19 ± 1[c]	0 ± 1.5
4-(*N*-acetoxyacetylamino)stilbene	22 ± 1	3.5 ± 0.1	6.4 ± 0.7	0.2 ± 0.8
1-pyrenyloxirane	650 ± 60	25 ± 1	102 ± 8	0.6 ± 13
benzo[*a*]pyrenediol epoxide	2300 ± 240	8.9 ± 0.5	11.9 ± 0.5	0.4 ± 1.0

[a] The mutagenicities of the compounds were determined in *S. typhimurium* strain TA100. Each chemical was reacted with supercoiled DNA at pH 7.4 at 37 °C. The number of AP sites was determined by cleavage with exonuclease III and electrophoresis, either at the end of the 5-h, 37 °C incubation or after a further incubation at 70 °C (total number of AP sites). The number of excess alkali-labile sites was calculated as the difference between the number of alkali-labile sites and the total number of AP sites. See text for further details. All values are the slope of the regression line ± the standard deviation. [b] The maximum dose (in nanomoles) analyzed for each compound was as follows: ethyl methanesulfonate, 40 000; dimethylcarbamoyl chloride, 25 000; *N*-ethyl-*N*-nitrosourea, 10 000; methyl methanesulfonate, 10 000; *N*-methyl-*N*-nitrosourea, 2500; β-propiolactone, 500; 1′-acetoxyestragole, 200; 2-(*N*-acetoxyacetylamino)fluorene, 160; *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene, 40; 4-(*N*-acetoxyacetylamino)stilbene, 20; 1-pyrenyloxirane, 0.5; benzo[*a*]pyrenediol epoxide, 0.2. [c] Corrected for the presence of 5.2 ± 0.55 (SD) direct strand breaks/(10^4 nucleotides·mM compound).

where f_I is the fraction of DNA in the sample migrating as form I.

Each experiment consisted of duplicate samples for each of four concentrations of the test compound and corresponding control samples treated in exactly the same manner except that the endonuclease treatment was omitted. The maximum concentration studied was chosen to yield a mean of <2 strand breaks/SV40 molecule. The number of AP sites per concentration of compound was estimated by linear regression analysis of data from two or more experiments.

Quantitation of Alkali-Sensitive Sites. Tritiated form I SV40 DNA (0.075 μg, ~10^4 dpm; kindly provided by Dr. Bill Sugden, McArdle Laboratory for Cancer Research, University of Wisconsin—Madison) was reacted with each test compound under the conditions described above. Following incubation of the sample for 5 h at pH 7.4 at 37 °C, the unreacted compound was extracted 4 times with an equal volume of chloroform, and the treated DNA was incubated at 70 °C to obtain the maximum yield of AP sites. After the addition of 0.004 mL of 1 N NaOH, the sample was incubated at 70 °C for an additional 45 min. These conditions were adequate to ensure complete hydrolysis of the alkali-sensitive sites in the treated DNA. The sample was then neutralized with HCl and electrophoresed on an 8-cm 2% agarose gel with TAE buffer and a constant 4 V/cm. The DNA bands were located by photography with 254-nm light and a red filter. The quantity of ^3H in each 2-mm section of the top 3 cm of the gel was determined after solution of the agarose in 1 mL of water at 100 °C and the addition of 10 mL of RIA Solve II (Research Products International, Elk Grove, IL) as the scintillation cocktail.

Instead of analyzing the entire gel, the quantity of DNA in each of the three bands (single-stranded circular, denatured form I, and unit-length, single-stranded, linear DNA) was determined. The presence of two or more scissions on the same DNA strand resulted in the production of single-stranded, linear DNA chains of less than unit length. These shorter DNA strands were distributed between the unit-length, single-stranded DNA band and the bottom of the gel. The number of strand breaks per double-stranded SV40 molecule is assumed to follow a Poisson distribution, and the scissions are assumed to be binomially distributed between the two strands. Thus, for an average of m strand breaks per SV40 molecule, the fraction of DNA migrating as denatured form I relative to the total amount of DNA in the three observed bands is

$$f_I' = e^{-m} / \sum_{s=0}^{\infty} \frac{e^{-m} m^s}{s!} \sum_{n=0}^{s} \binom{s}{n} 0.5^s \psi_n$$

where s is the number of strand breaks per DNA molecule, n is the number of scissions per DNA strand, and ψ_n is the probability that a DNA strand with n breaks will be of near unit length. The following approximation, obtained by curve-fitting using values of $m < 2.0$, was used to estimate the mean number of strand breaks per molecule:

$$m = -\ln(-0.0365 + 0.869 f_I' + 0.426 f_I'^2 - 0.263 f_I'^3)$$

This approximation is accurate to within 2% for values of m less than 2.

Each experiment consisted of triplicate samples for each of four concentrations of compound; the maximum concentration was one which yielded <2.0 strand breaks/molecule. The number of alkali-labile lesions induced per concentration of compound was determined by linear regression analysis of data from two or more experiments.

Results

Mutagenicity. The missense mutagenic activities of the compounds varied over a range of ~10^5-fold (Table I). The most mutagenic compounds were benzo[*a*]pyrenediol epoxide and 1-pyrenyloxirane, which induced 2300 and 650 strain TA100 revertants/nmol, respectively. Treatment of the bacteria with 1′-acetoxyestragole, 2-(*N*-acetoxyacetylamino)fluorene, *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene, and 4-(*N*-acetoxyacetylamino)stilbene resulted in 2.5 to 22 revertants/nmol. Less than 1 revertant/nmol was obtained for each of the remaining compounds.

In some cases the mutagenic activities of the compounds observed in these studies differed somewhat from those previously reported for the same compounds (Drinkwater et al., 1978; McCann et al., 1975a). However, the relative activities

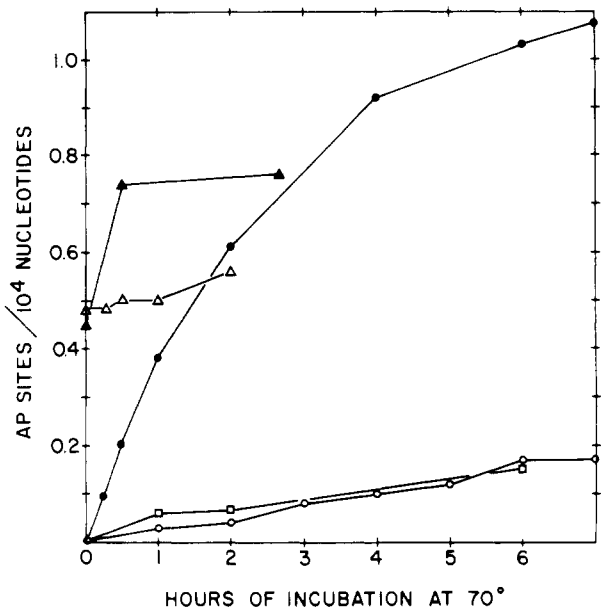

FIGURE 3: Time course of the production of AP sites. Supercoiled SV40 DNA was incubated with the compound for 5 h at 37 °C at pH 7.4. After unreacted compound was extracted, the treated DNA was incubated for various times at 70 °C at pH 7.4 and the number of AP sites was determined as described under Materials and Methods. (□) 1.56 mM dimethylcarbamoyl chloride; (●) 0.028 mM β-propiolactone; (△) 0.025 mM *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene; (▲) 0.053 mM benzo[*a*]pyrenediol epoxide; (○) solvent control.

of the compounds, where data for several may be obtained from the same source, are similar to those observed in this study.

Production of AP Sites. Except for dimethylcarbamoyl chloride, incubation of form I SV40 DNA with each of the reactive compounds for 5 h at 37 °C resulted in a dose-dependent production of AP sites (Table I). Although the more mutagenic compounds tended to give higher yields of AP sites, the ordering of the compounds with respect to the two activities was not identical. 1-Pyrenyloxirane, *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene, and benzo[*a*]pyrenediol epoxide caused the greatest production of AP sites; these compounds yielded 25, 19, and 8.9 AP sites/(10^4 nucleotides·mM compound), respectively. Treatment of the DNA with β-propiolactone, *N*-methyl-*N*-nitrosourea, methyl methanesulfonate, 1′-acetoxyestragole, and 4-(*N*-acetoxyacetylamino)stilbene resulted in 0.94–3.5 AP sites/(10^4 nucleotides·mM compound). The remaining compounds gave yields of AP sites that were <0.4/(10^4 nucleotides·mM).

The rates at which the N-glycosidic bonds of the DNA–carcinogen adducts hydrolyzed to yield AP sites differed, as may be seen from representative time courses for the production of AP sites for four of the compounds (Figure 3). In these experiments, each compound was reacted with form I SV40 DNA for 5 h at 37 °C. The number of AP sites in the treated DNA was then measured at various times during a subsequent incubation at 70 °C. The production of AP sites was most rapid in DNA treated with *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene: the maximal yield of AP sites was achieved by the end of the 5-h incubation at 37 °C. No further sites were obtained after incubation at 70 °C. Depurination of benzo[*a*]pyrenediol epoxide adducts was also rapid; it was complete within 1 h of incubation at 70 °C. With the exception of dimethylcarbamoyl chloride, which did not result in a significantly increased production of AP sites even after incubation for 6 h at 70 °C, time courses for the production of AP sites in the DNA treated with any of the remaining compounds were similar in shape to that shown for β-propiolactone. Maximal yields of AP sites were obtained after an incubation at 70 °C for 3 h in the case of 1-pyrenyloxirane, 4 h for the ethylating and methylating agents studied, 5 h for 1′-acetoxyestragole and 4-(*N*-acetoxyacetylamino)-stilbene, 6 h for β-propiolactone, and 7 h for 2-(*N*-acetoxyacetylamino)fluorene.

The total yields of AP sites after incubation at 70 °C were not directly related to the mutagenicities of the compounds or to the number of AP sites obtained after incubation at 37 °C for 5 h (Table I). Thus, although 1-pyrenyloxirane resulted in the greatest yield of AP sites both after incubation for 5 h at 37 °C and after incubation at 70 °C, β-propiolactone, which was of only moderate activity in the former assay, was the second most active compound with respect to the yield of AP sites after incubation at 70 °C. Under conditions which yielded maximum numbers of AP sites, these two compounds resulted in 102 and 33 AP sites/(10^4 nucleotides·mM), respectively. The remaining active compounds yielded 0.4–19 AP sites/(10^4 nucleotides·mM) after incubation at 70 °C.

The strand breaks observed in the above assays were, in all cases but one, apparently due solely to the hydrolysis of the phosphodiester backbone adjacent to AP sites. The exception was *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene, which produced 5.2 ± 0.55 (SD) direct (i.e., in the absence of subsequent endonuclease treatment) strand breaks/(10^4 nucleotides·mM) after incubation with the DNA for 5 h at 37 °C. Further incubation of the treated DNA at 70 °C did not result in any increase in the number of direct strand breaks induced by this compound.

Production of Alkali-Labile Sites. AP sites, which were assayed above by the formation of single-strand breaks on endonucleolytic cleavage, are also rapidly hydrolyzed by alkali (Lindahl & Andersson, 1972). Thus, the minimum number of alkali-labile lesions is equal to the number of endonuclease-sensitive sites. However, two of the compounds studied, *N*-methyl-*N*-nitrosourea and *N*-ethyl-*N*-nitrosourea, yielded 2.8 and 0.5, respectively, alkali-labile sites/(10^4 nucleotides·mM compound) in excess of the number of AP sites observed. Dimethylcarbamoyl chloride also induced a small, though significant, number of excess alkali-labile sites. The number of alkali-labile lesions was not significantly different from the number of AP sites in the DNA treated with each of the other compounds.

Discussion

Our data indicate that electrophilic carcinogens and mutagens of widely different structural types, including simple alkylating agents, reactive arylamine derivatives, and hydrocarbon epoxides, induce significant quantities of AP sites within a few hours after reaction with DNA under physiological conditions. Within the same time frame, mutagenic damage resulting from these chemicals should be fixed in the test organism, *S. typhimurium*, used in the mutagenicity assays since the small amount of histidine present in the medium enables the bacteria to undergo a limited number of replications (McCann et al., 1975b). Reversion of the *hisG46* missense mutation in strain TA100 could result from base misincorporation at AP sites (Shearman & Loeb, 1979) or from error-prone *recA*-dependent repair of single-strand breaks obtained by hydrolysis of AP sites (McCann et al., 1975b).

If AP sites were a major source of the missense mutagenic damage caused by the compounds in strain TA100, a high degree of correlation would be expected between mutagenic activity and the yield of AP sites after incubation for 5 h at

37 °C. A strong positive correlation does exist for the ordering (Bradley, 1968) of the compounds with respect to these two activities ($t = 0.62, p < 0.003$). However, quantitative analysis reveals large deviations from the correlation line ($r = 0.35$, $p > 0.3$). For example, 2-(*N*-acetoxyacetylamino)fluorene and benzo[*a*]pyrenediol epoxide are 3- and 100-fold more mutagenic than would be expected on the basis of the number of AP sites they induce. Since AP sites should be equivalent in their induction of mutations regardless of their source, the poor quantitative correlation argues against a causal relationship between AP sites and missense mutagenesis for the compounds studied. The positive rank correlation between mutagenic activity and the yield of AP sites presumably reflects the relationship of the electrophilic reactivities of the compounds to both of these activities. On the other hand, the lack of a quantitative relationship between the formation of AP sites and mutagenicity cannot be used as evidence that AP sites have no mutagenic effect. The data of Shearman & Loeb (1979) that the presence of AP sites in DNA resulted in base misincorporation during DNA replication indicate that these lesions could be a major source of the mutagenic damage caused by some compounds.

The AP sites induced in DNA by treatment with chemical carcinogens are most commonly a consequence of reaction of these compounds at the N-3 position of adenine or N-7 position of guanine bases (Brookes & Lawley, 1961; Lawley & Brookes, 1963). Among the simple alkylating agents studied, these two adducts account for between 17%, for *N*-ethyl-*N*-nitrosourea, and 97%, for methyl methanesulfonate, of the total binding of the compounds to DNA (Singer, 1975). Osborne and co-workers (King et al., 1979; Osborne et al., 1978) have tentatively identified N-7 substitution on guanine as a result of the reaction of benzo[*a*]pyrenediol epoxide with DNA; this adduct accounts for ~20% of the total binding at neutrality. The latter result, together with the data presented above, provides strong evidence that the strand breaks observed in benzo[*a*]pyrenediol epoxide treated DNA by Gamper et al. (1977) and Kakefuda & Yamamoto (1978) resulted from hydrolysis of AP sites and were not, as suggested by these authors, due to phosphotriester hydrolysis.

The source of the AP sites in 1′-acetoxyestragole-treated DNA is not known, but it appears to result from a minor adduct. Data of Phillips et al. (1980) indicate that as much as 85% of the binding of this compound to DNA may occur at N^2 of guanine. Both *N*-(benzoyloxy)-*N*-methyl-4-aminoazobenzene and 2-(*N*-acetoxyacetylamino)fluorene bind predominantly at the N^2 and C-8 positions on guanine in DNA (Beland et al., 1980; Kriek, 1974; Lin et al., 1975; Tarpley et al., 1980), but recent work in our laboratory (W. G. Tarpley, E. C. Miller, and J. A. Miller, unpublished experiments) has provided evidence for the release of purine derivatives from DNA treated with these compounds. On the basis of estimates of the total binding of 2-(*N*-acetoxyacetylamino)fluorene to SV40 DNA from the degree of unwinding (Drinkwater et al., 1978), ~3% of the adducts may be hydrolyzed to AP sites.

Alkali-labile lesions in excess of those accounted for as AP sites may reasonably be interpreted as resulting from phosphotriesters in the treated DNA (Kuhnlein et al., 1979; Lindahl & Andersson, 1972; Shooter, 1976). The relative amounts of excess alkali-labile lesions and AP sites observed in DNA treated with the methylating and ethylating agents studied are consistent with this interpretation (Singer, 1977). However, there are two difficulties in the quantitation of the extent of reaction at DNA phosphates as excess alkali-sensitive sites. First, hydrolysis to yield a strand scission is possible for only two of the three ester bonds of phosphotriesters. Swenson & Lawley (1978) found that alkaline hydrolysis of ethyl and methyl phosphotriesters under similar conditions to those used here yielded strand breaks with a frequency of 0.8. This fraction is not known for the phosphate adducts of other compounds. For example, the acylating agent, dimethylcarbamoyl chloride, would be expected to yield a labile diester phosphoanhydride. To the extent that the anhydride bond was preferentially hydrolyzed, substitution on the phosphate would not be recognized by our assay. Second, since the AP sites present in the treated DNA generally constitute a large background, the assay for phosphotriesters is not very sensitive. Thus, no significant excess of alkali-labile sites was observed in methyl methanesulfonate treated DNA, although phosphotriesters account for ~1% of the total methylation by this compound (Singer, 1977). Only with *N*-ethyl-*N*-nitrosourea and *N*-methyl-*N*-nitrosourea, for which 59% and 16%, respectively, of the total binding is on the phosphate backbone (Swenson & Lawley, 1978), were appreciable fractions of the total number of alkali-sensitive lesions not accounted for as AP sites.

Since most chemical carcinogens require metabolic activation to electrophilic derivatives to exert their biological effects (Miller, 1978), attempts were made to extend these methods to the study of procarcinogens by the use of liver microsomes or semipurified microsomal mixed-function oxidases. These met with little success because of nonspecific endonuclease activity in the enzyme preparations (N. R. Drinkwater, E. C. Miller, and J. A. Miller, unpublished data). Two alternative approaches may be feasible. Treatment of *E. coli* containing the plasmid ColE1 with procarcinogens in the presence of tissue preparations might yield modified supercoiled DNA suitable for analysis by the methods described above. Mizusawa et al. (1977) have observed alkali-labile lesions in ColE1 DNA isolated from *E. coli* treated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Similarly, Wilkinson et al. (1975) found direct strand breaks in supercoiled mitochondrial DNA obtained from the livers of rats treated with dimethylnitrosamine.

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Isolation and Characterization of Complementary Deoxyribonucleic Acid Complementary to the Highly Abundant Class of Poly(adenylic acid)-Containing Ribonucleic Acid from Oocytes of *Drosophila melanogaster*†

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ABSTRACT: The complementary deoxyribonucleic acid (cDNA) complementary to the highly abundant class of poly(adenylic acid)-containing ribonucleic acid [poly(A+) RNA] from *Drosophila malanogaster* oocytes has been isolated and characterized. Analysis of the kinetics of hybridization of this cDNA ($cDNA_{HA}$) to total poly(A+) RNA of oocytes indicates this class contains ~86 different sequences. Hybridization kinetics of $cDNA_{HA}$ annealed to poly(A+) RNA from 19-h-old embryos is essentially the same as that of oocyte poly(A+) RNA. This suggests the highly abundant class of poly(A+) RNA persists in approximately the same frequency through early development. Analysis of the hybridization of $cDNA_{HA}$ to genomic DNA suggests that the highly abundant poly(A+) RNA from oocytes is not enriched for transcripts from repetitive sequences of the genome.

Our approach to the investigation of the early developmental processes in *Drosophila melanogaster* has centered on the programmed use of stored maternal messenger RNA. Early embryogenesis (pregastrular) is dependent upon the informational molecules placed in the oocyte during oogenesis (Lovett & Goldstein, 1977), since very little zygotic transcription is believed to occur during the first 2 h after egg deposition (Anderson & Lengyel, 1979; Lamb & Laird, 1976; Zalokar, 1976). Thus the oocyte provides an excellent opportunity to investigate the role of both maternal and zygotic

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