A Novel, Sensitive, and Specific Assay for Abasic Sites, the Most Commonly Produced DNA Lesion†

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ABSTRACT: Free radicals produce a wide spectrum of damages; among these are DNA base damages and abasic (AP) sites. Although several methods have been used to detect and quantify AP sites, they either are relatively laborious or require the use of radioactivity. A novel reagent for detecting abasic sites in DNA was prepared by reacting *O*-(carboxymethyl)hydroxylamine with biotin hydrazide in the presence of carbodiimide. This reagent, called *A*ldehyde *R*eactive *P*robe (ARP), specificially tagged AP sites in DNA with biotin residues. The number of biotin-tagged AP sites was then determined colorimetrically by an ELISA-like assay using avidin/biotin complex conjugated to horseradish peroxidase as the indicator enzyme. With heat/acid-depurinated calf thymus or bacteriophage f1 DNA, ARP detected femtomoles of AP sites in DNA. Using this assay, DNA damages generated in calf thymus, ϕX174 RF, and f1 single-stranded DNA, X-irradiated in phosphate buffer, were easily detectable at 10 rad (0.1 Gy). Furthermore, ARP sites were detectable in DNA isolated from heat-inactivated X-irradiated (10 Gy) and methyl methanesulfonate (MMS)-treated (5 μM) *Escherichia coli* cells. The rate of production of ARP sites was proportional to the X-ray dose as well as to the concentration of MMS. Thus, the sensitivity and simplicity of the ARP assay should provide a potentially powerful method for the quantitation of AP sites or other DNA lesions containing an aldehyde group.

Abasic (AP)[1] sites in DNA can be generated spontaneously under physiological conditions by hydrolysis of the N-glycosylic bond. In humans, it has been estimated that about 10000 AP sites are produced in this manner per cell per day (Lindahl & Nyberg, 1972). AP sites can also be formed following chemical modification of DNA bases by DNA damaging agents such as bleomycin (Rabow et al., 1986) and alkylating agents (Loeb & Preston, 1986) as well as by ionizing radiation (von Sonntag, 1987). AP sites are also intermediates in the base excision repair pathway initiated by the action of DNA *N*-glycosylases. Thus, the cellular steady-state level of AP sites would be elevated as a consequence of base modifications and their subsequent repair (Friedberg, 1985; Weiss & Grossman, 1987; Wallace, 1988). If left unrepaired, AP sites can lead to cell death and/or mutation induction (Kunkel et al., 1983; Loeb & Preston, 1986). AP sites are strong blocks to DNA synthesis in vitro (Sagher & Strauss, 1983) and are cytotoxic lesions when present in phage-transfecting DNA (Schaaper & Loeb, 1981; Moran & Wallace, 1985). Although AP sites are bypassed inefficiently in bacterial cells (Schaaper & Loeb, 1981; Laspia & Wallace, 1988), when bypass occurs, mutation can result since AP sites are noninstructive lesions. It is therefore highly desirable to have a simple assay that can rapidly and accurately measure the level of AP sites in DNA.

There are several methods currently available to quantitate AP sites in DNA. These procedures usually involve the estimation of single-strand breaks formed as a result of alkali treatment of AP site-containing DNA (Brent et al., 1978; Kohn et al., 1981). Recently, Weinfeld et al. (1990), using a ^{32}P-postlabeling assay, were able to quantitate AP sites in DNA at the femtomole level. However, the procedure involves gel electrophoresis and HPLC separation of the end-labeled dinucleotides. AP sites can also be measured by the use of ^{14}C-labeled methoxyamine (Talpaert-Borle & Liuzzi, 1983; Liuzzi & Talpaert-Borle, 1988). Because [^{14}C]methoxyamine is only available at low specific activity, this method is relatively insensitive unless large quantities of DNA are available (Talpaert-Borle & Liuzzi, 1983; Liuzzi & Talpaert-Borle, 1988). We previously also demonstrated that *O*-(4-nitrobenzyl)hydroxylamine reacts with AP sites to produce *O*-(nitrobenzyl)hydroxylamine residues at the AP sites (Kow, 1989). The resulting *O*-(nitrobenzyl)hydroxylamine was then determined by a monoclonal antibody raised against 5′-phosphodeoxyribosyl *O*-(4-nitrobenzyl)hydroxylamine as a means of quantitating AP sites (Chen et al., 1991).

In order to simplify the procedure for the detection and quantitation of AP sites in DNA, as well as to increase the sensitivity for detection, we have prepared a biotin-tagged reagent specific for the aldehyde group, called *A*ldehyde *R*eactive *P*robe (ARP). After modification of the aldehyde group with ARP, the biotin-tagged AP site can then be easily quantitated by the use of avidin/biotin complex technology in an ELISA-like microtiter plate assay. This paper reports the properties of this reagent as a probe for AP sites and describes as well both its limitations and its sensitivity for quantitating AP sites in DNA.

Experimental Procedures

Chemicals. *O*-(4-Nitrobenzyl)-, *O*-methyl-, and *O*-(carboxymethyl)hydroxylamines were purchased from Aldrich.

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[1] Abbreviations: ARP sites, aldehyde reactive probe detectable sites; AP sites, apurinic/apyrimidic sites.

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1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDAC), biotin hydrazide, and methyl methanesulfonate were obtained from Sigma.

Enzymes. Endonuclease III was prepared as described earlier (Katcher & Wallace, 1983; Kow & Wallace, 1987) from *Escherichia coli* strain $\lambda N99_{CI857}$ (Asahara et al., 1989), harboring plasmid pHIT1 which overproduces endonuclease III (obtained from R. P. Cunningham, SUNY, Albany), except that the DNA–agarose step was replaced with FPLC Mono S chromatography (Pharmacia) following Asahara et al. (1989). Uracil *N*-glycosylase was prepared according to the method of Lindahl et al. (1977) from *E. coli* strain BD438, carrying plasmid pBD15 which overproduces uracil–DNA *N*-glycosylase (Duncan & Chamber, 1984).

Nucleic Acids. Calf thymus DNA was obtained from Pharmacia Biochemicals. PM2 (Katcher & Wallace, 1983; Kow & Wallace, 1987), ϕX174 RF (Pagano & Hutchinson, 1971), and f1 (Messing, 1983) DNAs are prepared routinely in the laboratory. Calf thymus DNA containing AP sites was prepared by heat/acid depurination (Lindahl & Nyberg, 1972), and the number of AP sites was estimated by correlation with the rate of heat/acid depurination of PM2 DNA treated under the same conditions (Kow, 1989). The number of AP sites in PM2 DNA was assayed by alkali fluorometry (Futcher & Morgan, 1979; Kow, 1989). DNAs containing other lesions including thymine glycols (Katcher & Wallace, 1983; Kow & Wallace, 1987), pyrimidine dimers (Kow et al., 1990), uracil (Lindahl et al., 1977), urea residues (Kow, 1989), *O*-methylhydroxylamine residues (Kow, 1989), and reduced AP sites (Kow, 1989; Kow et al., 1990) were prepared as previously described. *E. coli* DNA was isolated after lysis of the cells with SDS and lysozyme (Ausubel et al., 1989). After the RNase and protease K treatment, the DNA was extracted with phenol twice and dialyzed extensively in 10 mM Tris-HCl, pH 7.5, plus 1 mM EDTA.

Aldehyde Reactive Probe. Biotin hydrazide was initially used as a reagent for detecting AP sites in DNA; however, due to the reactivity of hydrazide, the signal to noise ratio was poor for DNA-containing AP sites. In order to overcome this problem, biotin hydrazide was converted to an *O*-alkylhydroxylamine derivative by the reaction of *O*-(carboxymethyl)hydroxylamine with biotin hydrazide in the presence of carbodiimide (Rosenberg et al., 1986). *O*-(Carboxymethyl)hydroxylamine hydrochloride (1.365 g) was dissolved in 125 mL of distilled water. EDAC (2.395 g) was added, and the pH of the solution was adjusted with pyridine to between 4 and 5. Then 320 mg of biotin hydrazide was added, and the reaction was incubated overnight at room temperature. The pH was adjusted to 7 by NaOH, and the solution was extracted 3 times with an equal volume of $CHCl_3$. The chloroform phase was discarded, and the aqueous phase was evaporated to dryness under reduced pressure. The residue was extracted again with chloroform. The chloroform-extracted residue was then dissolved in 50 mL of distilled water, and the pH was adjusted to neutrality. Fifty grams of AG1-X8 (Cl^- form) was prepared by washing the resin twice with 200 mL of 2 M NaCl, and packed into a column (2.5 × 50 cm). The resin was washed with 4 bed volumes of distilled water. Crude ARP solution was loaded onto the column to remove unreacted *O*-(carboxymethyl)hydroxylamine. The first 50 mL of eluate was discarded, and subsequent fractions of 50 mL were collected. Each of the fractions was tested for its ability to produce a colorimetric reading with calf thymus DNA containing AP sites previously bound to a microtiter plate (for colorimetric quantitation, see following section). Active fractions were pooled (total about 300 mL) and used as a reagent for AP sites without further purification. The chemical structure of the ARP reagent has been determined by both NMR and mass spectroscopy (Hiroshi Ide, personal communication) and is shown in Figure 1.

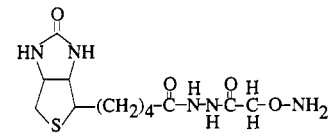

FIGURE 1: Structure for the ARP reagent.

Microtiter Plate Method for Assaying AP Sites. Immulon I microtiter plates were irradiated overnight with 40 W from an unfiltered germicidal lamp (254 nm) at a distance of 30 cm from the source (Zouali & Stollar, 1980). The irradiated plates could be used for up to 2 months with no loss in DNA binding efficiency. Two hundred microliters of calf thymus DNA containing AP sites (10 μg/mL) in PBS buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, and 1.4 mM KH_2PO_4) was added to each well, and incubated at 37 °C for 2–3 h. Alternatively, the microtiter plates were incubated at 4 °C overnight to facilitate DNA binding. The plates were then washed 4 times with PBS–Tween buffer (PBS buffer + 0.5% Tween 20). Then 100 μL of ARP reagent was added, and the plates were further incubated at 37 °C for 1 h. Using either procedure, approximately 70–100 ng of DNA was bound to each well. The plates were washed 4 times with PBS–Tween to remove any unreacted ARP reagent. Vectastain ABC (avidin-biotinylated horseradish peroxidase complex, prepared as described in the Vectastain ABC kit, Vector Laboratories), 50 μL, was added to each well, and the microtiter plates were incubated at 37 °C for another hour. Unbound Vectastain ABC reagent was removed by washing 4 times with PBS–Tween. Then 100 μL of horseradish peroxidase substrate (*O*-phenylenediamine) was added, and the color development was stopped with 50 μL of 5 N H_2SO_4 after an appropriate time interval, and the absorbance at 490 nm was taken. Standard curves were determined with either f1 or calf thymus DNA containing known amounts of AP sites.

For determining the number of intermediary AP sites produced by the action of endonuclease III (Katcher & Wallace, 1983; Kow & Wallace, 1987; Wallace, 1988), 200 μL of calf thymus DNA containing different amounts of thymine glycol (10 μg/mL) was added to each of the wells of a microtiter plate as described above. After incubation at 37 °C for 2 h, the plate was washed 4 times with PBS–Tween. One hundred microliters of appropriately diluted endonuclease III (endonuclease III was diluted into 10 mM Tris-HCl, 1 mM EDTA, and 0.1 M KCl at 37 °C) was added, and the plates were incubated at 37 °C for 10 min. The reaction was terminated by washing the microtiter plate 4 times with PBS–Tween after which 100 μL of ARP reagent was added and the number of AP sites were determined as described above.

For determining the number of AP sites produced after the action of uracil–DNA glycosylase (Lindahl et al., 1977; Duncan & Chamber, 1984), we used a procedure similar to that described for endonuclease III, except that uracil-containing f1 single-stranded DNA was used and uracil *N*-glycosylase was diluted appropriately into 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA.

X- and ^{60}Co-Irradiation. Calf thymus, ϕXRF, and f1 DNAs were X-irradiated at a concentration of 30 μg/mL in 10 mM potassium phosphate buffer (pH 7.5) using a Phillips X-ray generator with a beryllium window Machlett tube operated at 50 kVp and 2 mA (9.6 Gy/min). The dose rate is

Table I: Specificity of the ARP Reagent

DNA substrates	OD_{490nm}
undamaged calf thymus DNA	0.00[a]
uracil-containing f1 DNA[b]	0.00
UV (254 nm)-irradiated calf thymus DNA[c]	0.00
calf thymus DNA containing 1 AP site/molecule	0.52
calf thymus DNA containing reduced AP sites[d]	0.05
calf thymus DNA containing methoxyamine-modified AP sites[e]	0.00
calf thymus DNA containing 10 thymine glycols/molecule	0.05

[a] Control DNA. [b] DNA contained approximately 200 uracils per molecule. [c] DNA contained approximately 10 UV dimers per molecule. [d] DNA contained approximately 10 AP sites per molecule which were reduced with $NaBH_4$. [e] DNA contained approximately 10 AP sites per molecule which were modified with methoxyamine.

routinely determined in the laboratory by the Fricke ferrous sulfate dosimetry and phage T4 survival. ^{60}Co-irradiation was performed with a Theratron Junior ^{60}Co-irradiator (Atomic Energy of Canada Limited). The dose rate was adjusted to 0.2 Gy/min.

For irradiation of *E. coli* cells, 20 mL of strain AB1157 cells in PBS (1×10^9/mL) was inactivated by heating at 90 °C for 2 min followed by quick-cooling to 4 °C. The dead cells were then diluted to a cell density of 2×10^8/mL and X-irradiated with the indicated doses at 10 °C. Due to the time required for irradiation of the cells, heat-inactivated *E. coli* cells were employed so as to minimize any possible postirradiation repair of the X-ray-induced lesions. Irradiated cells were then collected by centrifugation at 5000 rpm for 10 min, and the DNA was isolated as previously described (Ausubel et al., 1989).

Methyl Methanesulfonate Treatment. Ten milliliters (5×10^8 cells) of actively growing *E. coli* (AB1157) cells in PBS buffer was treated with different concentrations of MMS and incubated at 37 °C for 30 min. MMS was then removed by centrifugation (2000 rpm, SS34) at 4 °C, and the cell pellet was washed once with 10 mL of PBS. The cell pellet was collected at 2000 rpm (SS34) and resuspended in 10 mL of PBS. For each treatment, 5 mL of the washed cells was further incubated at 37 °C for 1 h, the other half was centrifuged at 2000 rpm, and the cell pellet was frozen at −20 °C. After 1 h, the post-MMS-treated cells were collected at 2000 rpm, and the cell paste was frozen at −20 °C. The frozen cell paste was later thawed and the DNA purified according to published procedures (Ausubel et al., 1989).

Results

Specificity and Sensitivity of the ARP Assay. ARP is a biotin-tagged derivative of *O*-(carboxymethyl)hydroxylamine, and the chemical specificity of ARP is expected to be similar to that of *O*-alkylhydroxylamine. To determine whether this was the case, DNA containing different unique base modifications was prepared. These included thymine glycols, urea residues, uracil, pyrimidine dimers, *O*-methylhydroxylamine-modified AP sites, and $NaBH_4$-reduced AP sites as well as the simple AP sites produced by heat/acid depurination. Microtiter plates coated with calf thymus DNA containing each of these lesions were incubated with the ARP reagent, and the amount of biotin bound in each of the wells was measured by enzyme assay using the avidin/biotin complex (ABC) conjugated to horseradish peroxidase. The signal produced was taken as a measure of the reactivity of the lesion with the ARP reagent. Table I shows that ARP reacted only with the simple AP site. No reactivity of the ARP reagent was observed with other lesions even after prolonged incubation with the reagent (data not shown). Modification of the AP sites by sodium borohydride reduction or methoxyamine completely eliminated the signal.

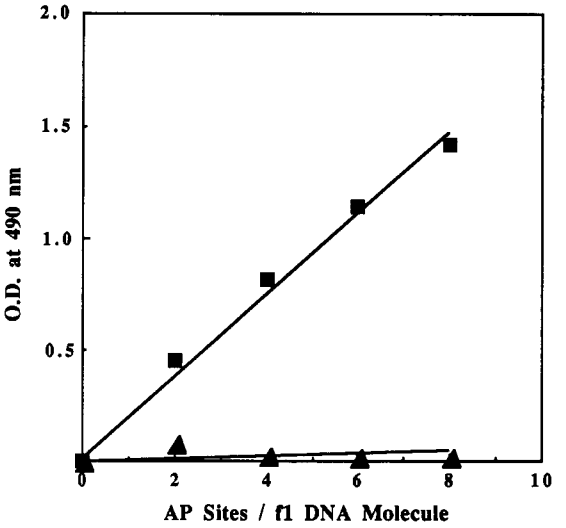

FIGURE 2: Detection of AP sites by the ARP reagent. f1 DNA containing AP sites was prepared by heat/acid depurination. Reduced AP sites were prepared by sodium borohydride reduction. f1 DNA containing different numbers of AP sites (■) or reduced AP sites (▲) was adsorbed to microtiter places as described under Experimental Procedures. The DNA on the plate was then allowed to react with the ARP reagent, and the amount of biotin on the plate was measured with avidin/biotin complexed to horseradish peroxidase as described under Experimental Procedures.

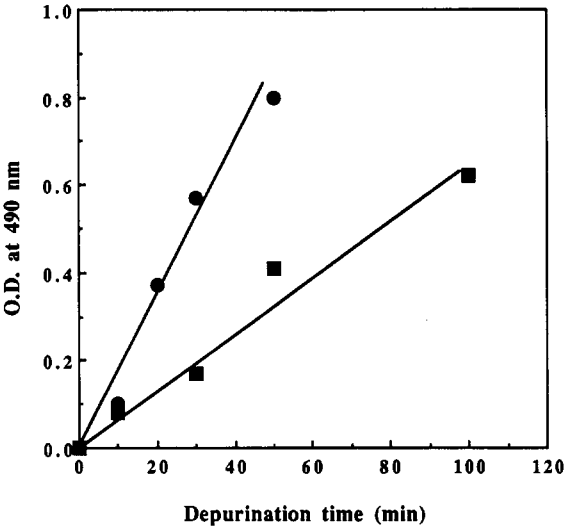

FIGURE 3: Rate of production of ARP-reactive sites in single- and double-stranded DNA. Two hundred microliters of partially depurinated f1 single-stranded (●) or calf thymus duplex DNA (■) was used to coat the wells of a microtiter plate at 4 °C overnight as described under Experimental Procedures. One hundred microliters of ARP reagent was added, and the amount of biotin on the plate was measured with avidin/biotin horseradish peroxidase as described under Experimental Procedures.

Figure 2 shows that the signal measured with ABC horseradish peroxidase was proportional to the number of AP sites in the DNA. Again, once the AP site was reduced with sodium borohydride, no reactivity with the ARP reagent was observed. We showed earlier that AP sites in DNA can be modified with *O*-benzylhydroxylamine and that the resulting *O*-benzylhydroxylamine residues on DNA can be measured with an antibody raised against *O*-benzylhydroxylamine residues (Chen et al., 1991). When DNA containing AP sites was modified with *O*-benzylhydroxylamine and used in antibody assay, a similar level of detection was observed (data not shown). The ARP reagent was able to detect AP sites in both single- and double-stranded DNA (Figure 3); however, the reaction was much faster with single-stranded DNA than with double-

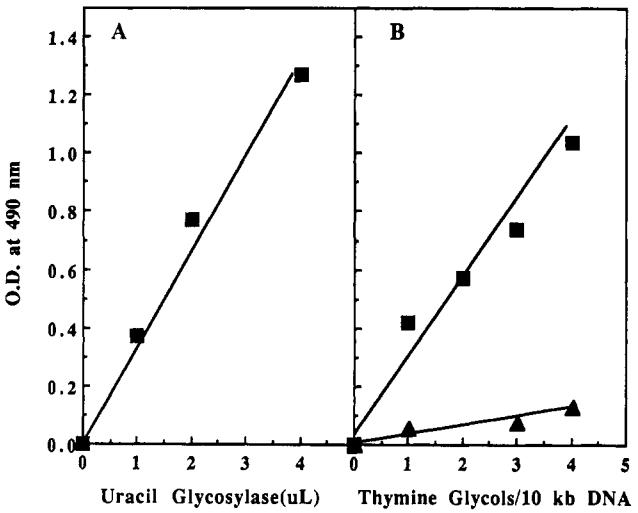

FIGURE 4: Detection of intermediary AP sites generated by DNA *N*-glycosylase by the ARP reagent. Microtiter plates were precoated with f1 DNA containing 2% uracil (panel A) or calf thymus DNA containing different amounts of thymine glycol (panel B). For panel A, uracil *N*-glycosylase was added and incubated at 37 °C for 10 min. The enzyme was removed by washing the plates, and the ARP-reactive sites were then detected by the ARP assay. For panel B, 100 μL of endonuclease III (■) was added in a buffer containing 0.1 M KCl/10 mM Tris–EDTA, pH 7.5, while to control plates was added 100 μL of the same buffer without endonuclease III (▲). The plates were then incubated at 37 °C for 0.5 h, the wells washed, and the AP sites determined by the ARP assay.

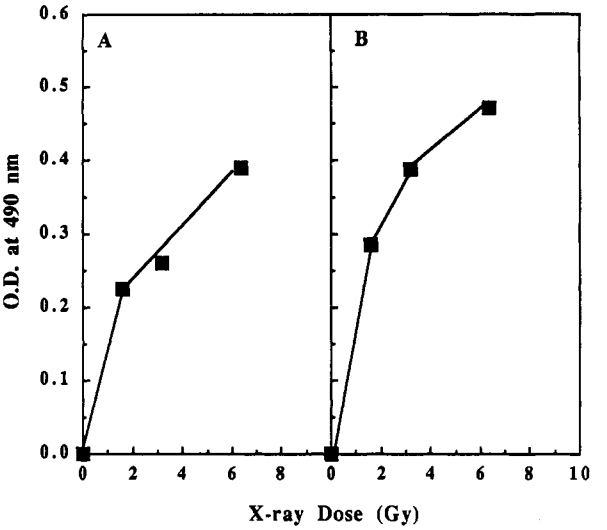

FIGURE 5: Production of ARP-reactive sites in X-irradiated DNA. Calf thymus duplex (panel A) or f1 single-stranded (panel B) DNA was X-irradiated in phosphate buffer at a concentration of 30 μg/mL at the indicated X-ray dose. The DNA was then adsorbed onto the microtiter wells, and the number of ARP-reactive sites produced was determined by the ARP assay as described under Experimental Procedures.

stranded DNA (data not shown). Further, the signal produced with depurinated f1 DNA was about twice that produced with depurinated calf thymus DNA at all depurinating time intervals. This is in agreement with the fact that the rate of depurination of single-stranded DNA is about 2-fold higher than that of double-stranded DNA (Lindahl & Nyberg, 1972).

AP Sites Formed in DNA by the Action of DNA N-Glycosylases Are Detectable by the ARP Assay. Base excision repair enzymes, such as endonuclease III and uracil *N*-glycosylase, excise the damaged base from DNA, leaving behind an AP site. Figure 4 shows that the ARP reagent exhibited no reaction with DNA containing uracil (A) or thymine glycol (B); however, upon digestion of these substrates with endonuclease III and uracil *N*-glycosylase, respectively, the intermediary AP sites formed were readily detectable with the ARP reagent. Using uracil-containing f1 single-stranded DNA, the number of AP sites detected was proportional to the amount of uracil–DNA *N*-glycosylase added (Figure 4A). Similarly, with calf thymus DNA containing thymine glycols, the ARP signal observed after endonuclease III digestion was linearly proportional to the original number of thymine glycols in the DNA (Figure 4B).

ARP Sites Detected in X-Irradiated DNA. Ionizing radiation produces a wide spectrum of DNA damages including DNA strand breaks and base damages as well as AP sites. Figure 5A shows that when calf thymus DNA was exposed to ionizing radiation, the number of ARP sites detected was proportional to the X-ray dose. Similarly, production of ARP sites in single-stranded f1 DNA (Figure 5B) was proportional to the dose of X-rays delivered. However, in both cases, the production of ARP sites appeared to be biphasic, with a higher rate of production in the lower dose range (below 2 Gy). This may be due to the fact that the ARP assay detects more than one type of lesion such as single-strand breaks with an aldehyde group and abasic sites. However, (formamido)pyrimidine, which contains an aldehyde group, is not reactive with the ARP reagent (Hiroshi Ide, personal communication). It is also possible that during the irradiation period, some damages may be converted to lesions that are not detectable by the ARP reagent, thus giving apparent lower yield at higher doses. For example oxidation of aldehydes to carboxylic acids could convert an ARP-reactive site to a non-ARP-reactive site (von Sonntag & Schulte-Frohlinde, 1978).

In order to examine the rate of production of ARP sites at low doses (below 2 Gy), calf thymus DNA was irradiated with γ rays delivered by a ^{60}Co source. Figure 6A shows that the ARP sites generated by γ-irradiation were linearly proportional to the dose. The ARP assay was able to detect ARP sites produced by γ rays at a dose as low as 0.1 Gy (Figure 6A).

Since thymine glycol is also a major product of ionizing radiation, it was of interest to compare the rate of production of thymine glycols to that of ARP sites in X-irradiated calf thymus DNA. In Figure 6B, it can be seen that the rate of production of thymine glycols by ionizing radiation, as detected by an anti-thymine glycol antibody (Hayes et al., 1988), was significantly less than the rate of production of ARP sites. At 10 Gy, about seven ARP sites were produced per f1 DNA molecule as compared to one thymine glycol. Again, an apparent biphasic production of ARP sites was observed. In contrast, thymine glycol production was linearly proportional to the X-ray dose (Figure 6B).

ARP Sites in DNA Extracted from X-Irradiated E. coli Cells. When actively growing wild-type *E. coli* cells (starved at 37 °C for 30 min in PBS buffer) were X-irradiated, little or no detectable ARP signal was observed, suggesting that the lesions may have been repaired while the irradiated cells were processed to obtain the DNA sample. In order to circumvent this problem, *E. coli* cells were preheated at 90 °C for 2 min to inactivate the repair enzymes and then irradiated with the appropriate dose of X-rays. Figure 6C shows that the rate of production of ARP sites and thymine glycols in heat-inactivated X-irradiated *E. coli* cells was proportional to the X-ray dose. Under these conditions, ARP sites were detectable at about 10 Gy (1 krad), while thymine glycol was barely detectable even at 100-Gy (10-krad) X-rays. It is interesting to note that the D_{37} for *E. coli* is about 100 Gy (10 krad).

Production and Repair of AP Sites in MMS-Treated E. coli Cells. MMS alkylates both adenine and guanine at

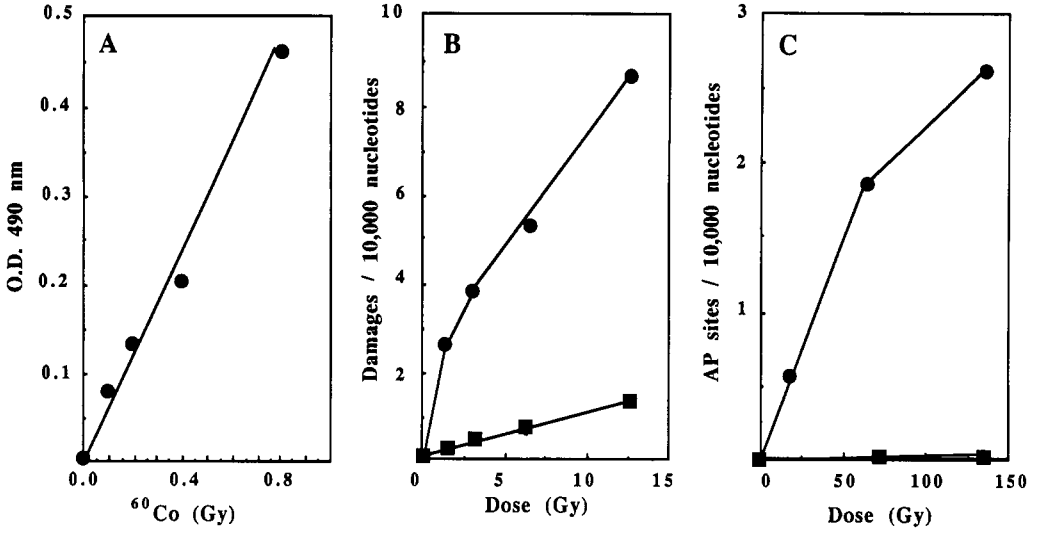

FIGURE 6: (A) Production of ARP-reactive sites by ^{60}Co γ-irradiation. Calf thymus duplex DNA was γ-irradiated in phosphate buffer at a concentration of 30 μg/mL at the indicated doses. The DNA was then adsorbed onto the microtiter wells, and the number of ARP sites produced was determined by the ARP assay as described under Experimental Procedures. (B) Relative production of thymine glycols and ARP sites in X-irradiated DNA. Calf thymus DNA (30 μg/mL) was X-irradiated in phosphate buffer at the indicated dose. The DNA was adsorbed onto the microtiter wells as described under Experimental Procedures. The number of ARP-reactive sites formed was determined by the ARP assay (●), and the number of thymine glycols formed (■) was measured using an anti-thymine glycol antibody following procedures described earlier (Hubbard et al., 1989). (C) Production of ARP sites and thymine glycols in X-irradiated *E. coli* cells. Wild-type *E. coli* (AB1157, 2 × 10^9/mL in PBS buffer) was heat-inactivated at 90 °C for 2 min and then X-irradiated at 4 °C at the indicated doses. DNA was then isolated, and the number of ARP-reactive sites formed (●) was determined by the ARP assay and the number of thymine glycols formed (■) by an anti-thymine glycol antibody in an ELISA assay (Hubbard et al., 1989).

multiple positions. Upon alkylation of the purines, the N-glycosylic bond becomes more labile, leading to an increased production of AP sites (Loeb & Preston, 1986). Furthermore, the repair of these alkylated DNA bases by *N*-glycosylases, such as 3-methyladenine–DNA *N*-glycosylase (Friedberg, 1985), should lead to a further increase in the production of AP sites. To determine the number of AP sites formed in DNA after MMS treatment of *E. coli* cells, DNA was isolated from cells treated with different concentrations of MMS. Figure 7B shows that the steady-state production and subsequent repair of AP sites in *E. coli* cells were clearly detectable by the ARP assay. At 25 μM MMS, where the survival of *E. coli* was at about 80%, a strong signal was observed (Figure 7A).

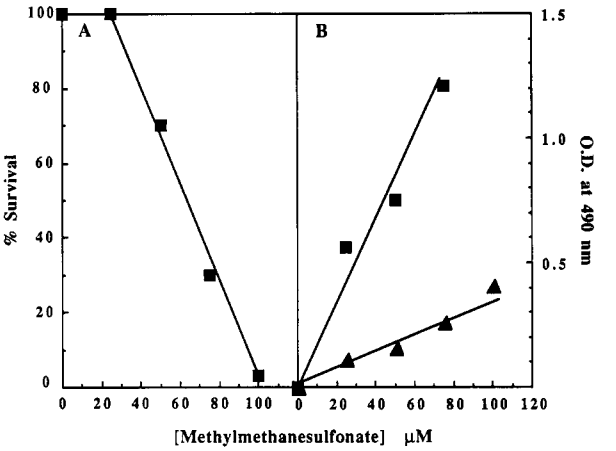

FIGURE 7: Production of ARP-reactive sites in methyl methanesulfonate-treated *E. coli* cells. (A) Wild-type *E. coli* (AB1157, 5 × 10^9 cells in 10 mL of PBS buffer) was treated with different concentrations of methyl methanesulfonate (MMS) for 30 min at 37 °C. MMS was then removed by centrifugation followed by washing with PBS buffer. Survival of *E. coli* cells was scored by plating then on LB plates. (B) Wild-type *E. coli* (AB1157, 5 × 10^9 cells in 10 mL of PBS buffer) was treated with different concentrations of methyl methanesulfonate (MMS) for 30 min at 37 °C. MMS was then removed by centrifugation followed by washing with PBS buffer. DNA was extracted from half of the cell pellet (■), while the other half was further incubated at 37 °C for an additional 1 h to allow for repair. Cells were then collected by centrifugation, the DNA was extracted (▲), and the number of ARP-reactive sites formed was determined by the ARP assay.

Discussion

Specificity of the ARP Assay. We have shown that the ARP reagent can detect AP sites generated in vitro by heat/acid depurination (Table I and Figure 2), by ionizing radiation (Figures 5 and 6A,B), and by *N*-glycosylase action on DNA base damages (Figure 4). Cellular DNA damage produced by methyl methanesulfonate modification of purines (Figure 7) or by ionizing radiation (Figure 6C) was also measured. The ARP reagent did not cross-react with base damage such as thymine glycols, pyrimidine dimers, or uracil (Table I and Figure 4). Modification of AP sites either by methoxyamine, which blocks the aldehyde group, or by sodium borohydride, which reduces it to an alcohol, resulted in a loss of the ARP signal (Table I and Figure 2). Thus, as predicted from the chemistry, the lesion of interest must contain an aldehyde group. However, ARP reagent was unable to detect (formamido)pyrimidine, an imidazole ring opened purine produced by ionizing radiation, which contains an aldehyde group. The inability of ARP reagent to detect (formamido)pyrimidine could be due to the low reactivity of the aldehyde, which is present in (formamido)pyrimidine as a formamido group. Therefore, the ARP sites detected in X-irradiated DNA probably included AP sites (alkali-labile sugar lesions) and strand breaks containing AP sites at the terminus. Certain classes of AP sites and strand breaks which do not contain an aldehyde group would not be detected (von Sonntag, 1987; Povirk & Steighner, 1989). Because more than one type of X-ray damage can be measured by this method, the ARP assay should be able to provide a simple and sensitive means for determining the presence of X-ray-induced DNA damage. This is reflected by the fact that the rate of formation of ARP sites in both X-irradiated DNA and *E. coli* cells is much higher

than thymine glycols (Figure 6B,C).

Comparison of the ARP Assay with Other Methods for Measuring AP Sites. The ARP assay reported here makes use of the same chemical specificity as *O*-alkylhydroxylamine; that is, it reacts with the aldehyde group present in AP sites. In addition, it combines the ease and sensitivity of the avidin/biotin assay in an ELISA-like microtiter plate assay. The ARP assay can detect 1 AP site per 10^4 nucleotides in the direct binding assay with approximately 70 ng of DNA bound to each well. This detection limit translates to about 15 fmol of AP sites. As currently used, the sensitivity of the ARP assay is comparable to other methods for quantitation of AP sites (Brent et al., 1978; Birnboim & Jevcak, 1981; Kohn et al., 1981; Weinfeld et al., 1990; Chen et al., 1991). However, procedures such as alkali elution (Brent et al., 1978) and DNA unwinding (Birnboim & Jevcak, 1981; Kohn et al., 1981) require radioactively labeled DNA, and they can be cumbersome to set up (Brent et al., 1978; Birnboim & Jevcak, 1981; Kohn et al., 1981). Although the ^{32}P-postlabeling assay for AP sites developed by Weinfeld et al. (1990) obviates the need for prelabeled DNA, it is not as simple to set up as an ELISA-type assay. The simplicity and sensitivity of the ARP assay are comparable to the ELISA assay using a monoclonal antibody to determine the *O*-(nitrobenzyl)hydroxylamine-tagged AP site (Chen et al., 1991). However, the ARP assay obviates the need of both primary and secondary antibody for detection, and the procedure reduces to a mere detection of the amount of biotin and DNA by using an avidin/biotin complex conjugated with an indicator enzyme. In summary, the ARP assay is rapid, simple, specific, and sensitive, and offers the possibility of processing a large number of DNA samples through automation.

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