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Chemical and Biological Evidence for Base Propenals as the Major Source of the Endogenous M_1dG Adduct in Cellular DNA*

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The endogenous DNA adduct, M_1dG, has been shown to arise *in vitro* in reactions of dG with malondialdehyde (MDA), a product of both lipid peroxidation and 4′-oxidation of deoxyribose in DNA, and with base propenals also derived from deoxyribose 4′-oxidation. We now report the results of cellular studies consistent with base propenals, and not MDA, as the major source of M_1dG under biological conditions. As a foundation for cellular studies, M_1dG, base propenals, and MDA were quantified in purified DNA treated with oxidizing agents known to produce deoxyribose 4′-oxidation. The results revealed a consistent pattern; Fe^{2+}-EDTA and γ-radiation generated MDA but not base propenals or M_1dG, whereas bleomycin and peroxynitrite ($ONOO^-$) both produced M_1dG as well as base propenals with no detectable MDA. These observations were then assessed in *Escherichia coli* with controlled membrane levels of polyunsaturated fatty acids (PUFA). $ONOO^-$ treatment (2 mM) of cells containing no PUFA (defined medium with 18:0/stearic acid) produced 6.5 M_1dG/10^7 deoxynucleotides and no detectable lipid peroxidation products, including MDA, as compared with 3.8 M_1dG/10^7 deoxynucleotides and 0.07 μg/ml lipid peroxidation products with control cells grown in a mixture of fatty acids (0.5% PUFA) mimicking Luria-Bertani medium. In cells grown with linoleic acid (18:2), the level of PUFA rose to 54% and the level of MDA rose to 0.14 μg/ml, whereas M_1dG fell to 1.4/10^7 deoxynucleotides. Parallel studies with γ-radiation revealed levels of MDA similar to those produced by $ONOO^-$ but no detectable M_1dG. These results are consistent with base propenals as the major source of M_1dG in this model cell system.

There is now substantial evidence linking reactive oxygen and nitrogen species to aging and chronic diseases (1) as illustrated by the epidemiological evidence associating chronic inflammation with increased cancer risk (2–5). A variety of endogenous and exogenous oxidants react directly with bases in DNA to produce mutagenic lesions such as 8-oxo-dG and thymine glycol. The oxidants also react with lipids, carbohydrates, and proteins to generate electrophilic species capable of reacting with DNA bases to form adducts. This is illustrated by the reaction of a metabolite of hydroxynonenal, a product of polyunsaturated fatty acid (PUFA)[1] peroxidation, with dG, dA, and dC to form etheno adducts (6). A similar argument has been made for the PUFA peroxidation product, malondialdehyde (MDA), which reacts *in vitro* with dG to form M_1dG, the exocyclic pyrimido[1,2-α]purin-10-(3*H*)-one adduct (Fig. 1). M_1dG is present in normal human tissues at levels of 10–100 adducts/10^8 deoxynucleotides (nt) (7), and it is mutagenic in bacteria and mammalian cells (8, 9), causing base pair substitutions and frameshift mutations, as well as arresting transcription (10).

Recent studies suggest that deoxyribose oxidation may be an alternative to lipid peroxidation as a source of DNA-reactive electrophiles. Oxidation of deoxyribose in DNA produces a variety of oxidized abasic sites and strand breaks with different sugar residues, many of which are electrophilic and thus capable of reacting with local nucleophiles to form adducts. For example, the β-elimination product of the 5′-(2-phosphoryl-1,4-dioxobutane) residue arising from 5′-oxidation of deoxyribose (*trans*-1,4-dioxo-2-butene) reacts with dG, dA, and dC to form stable bicyclic adducts (11, 12). Similarly, we demonstrated that the base propenal products of deoxyribose 4′-oxidation, structural analogs of the enol tautomer of MDA (Fig. 1), also react with DNA to form M_1dG (13), although with significantly greater efficiency than MDA (13, 14). This may explain the 30–60-fold greater mutagenicity of base propenals than MDA (14).

We now report the results of studies aimed at defining the source of M_1dG under biologically relevant conditions. We first performed studies with purified DNA and oxidants known to cause 4′-oxidation of deoxyribose to define the relationship between generation of base propenals and M_1dG formation. These observations were then assessed in *Escherichia coli* cells in which the membrane content of PUFA was varied by growth in defined media. This model system provided an opportunity to compare MDA and M_1dG formation caused by exposure of the cells to different oxidants.

EXPERIMENTAL PROCEDURES

Materials—Adenine propenal was purchased from Salford Ultrafine Chemicals (Manchester, UK). Thymine propenal and cytosine propenal were synthesized according to a published method (15). Bleomycin and calf thymus DNA were purchased from Sigma. Nitrocellulose membrane was obtained from Schleicher and Schuell. M_1dG monoclonal antibody and a lipid peroxidation kit were purchased from Oxford Biomedical Research (Oxford, MI). Peroxynitrite ($ONOO^-$) was synthesized by ozonolysis of sodium azide as described by Pryor *et al.* (47).

Instrumental Analyses—All HPLC analyses were performed on a Hewlett-Packard model 1100 HPLC system equipped with a Vydac C18 reversed phase column (250 × 4.6 mm) and a 1040A diode array

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[1] The abbreviations used are: PUFA, polyunsaturated fatty acid; GC, gas chromatography; HPLC, high-pressure liquid chromatography; MDA, malondialdehyde; M_1dG, pyrimido[1,2-α]purin-10-(3*H*)-one-2′-deoxyribose; MS, mass spectrometry; nt, deoxynucleotide; $ONOO^-$, peroxynitrite; TBA, thiobarbituric acid; Gy, gray; PBS, phosphate-buffered saline.

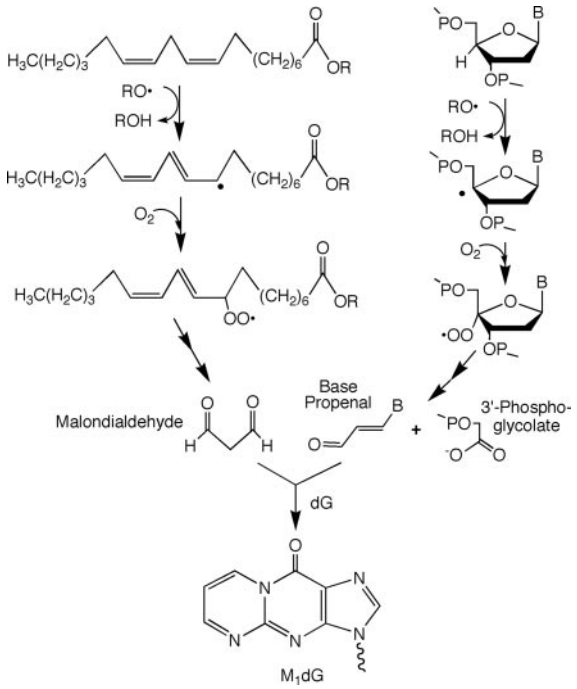

FIG. 1. **MDA from lipid peroxidation and base propenals from deoxyribose oxidation react with dG to form M_1dG.**

detector. Samples were resolved with the following gradient of acetonitrile in 10 mM sodium acetate buffer (pH 6.9): 0–18 min, 1–30%; 18–22 min, 30–50%; 22–30 min, 50–100%; 30–40 min, 100%. Gas chromatography (GC)/mass spectrometry (MS) analyses of fatty acid methyl esters were performed on a Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with a Hewlett-Packard 5872 mass selective detector. The operating parameters were as follows: 250 °C inlet (splitless mode); ionizing voltage, 70 eV; HP-5MS (cross-linked 5% phenylmethyl silicone) capillary column (0.25 mm × 30 m × 0.25 μm film thickness); oven temperature ramp of 10 °C/min from 100 to 310 °C.

Reaction of DNA with Oxidizing Agents—Calf thymus DNA was dissolved in Chelex-treated 50 mM potassium phosphate buffer (pH 7.4; see Ref. 16), and aliquots were stored at −80 °C. For γ-irradiation, DNA was exposed to 0–200 Gy in a ^{60}Co source at 2 Gy/min. For Fe^{2+}/EDTA treatment, a freshly prepared $FeSO_4$/EDTA solution (20 mM) was diluted with water, and 4-μl aliquots were added to the DNA solution (196 μl) to yield a final Fe^{2+}-EDTA concentration of 0–300 μM. For bleomycin treatment, 2-μl aliquots of an aqueous bleomycin solution (0–5 mM) were added to the DNA solution (196 μl) followed by addition of 2-μl aliquots of freshly prepared $Fe(NH_4)_2(SO_4)_2$ solution (0–5 mM) to initiate the DNA damage reaction. $ONOO^-$ damage reactions (0–300 μM) were carried out by adding 4-μl aliquots of the oxidant in 0.1 M NaOH to DNA dissolved in 100 mM potassium phosphate buffer (pH 6; higher buffer concentration and lower pH to compensate for the addition of 0.1 N NaOH). All DNA damage reactions were conducted at ambient temperature for 0.5 h followed by ultrafiltration using Microcon YM-10 centrifugal filters to separate MDA and base propenals from the DNA fragments.

Quantification of MDA and Base Propenals—The ultrafiltrate prepared from the *in vitro* reactions above or from cell suspensions exposed to $ONOO^-$ (see below) were resolved by HPLC and MDA, and base propenals were isolated by collection of fractions bracketing their retention times: MDA, 6.4 min; C-propenal, 9.3 min; T-propenal, 13.0 min; A-propenal, 20.0 min. Following combination of the base propenal fractions, 1/25 volume of a thiobarbituric acid (TBA) suspension (10 mg/ml in 0.2 M HCl) was added to each MDA and base propenal solution followed by heating to 90 °C for 30 min. The quantities of the identical MDA and base propenal derivatives of TBA were then determined by absorbance at 532 nm ($\epsilon = 1.53 \times 10^5\ M^{-1}\ cm^{-1}$); if necessary, the TBA reaction solutions were concentrated under vacuum to increase the final concentration of the 532-nm absorbing species.

As an alternative to the HPLC/TBA method for the cell studies (see below), MDA (free and bound to proteins or other biological molecules) and other analogous lipid peroxidation products were quantified using a lipid peroxidation kit from Oxford Biomedical Research. Cells were washed twice (2050 × *g*, 15 min, 4 °C) with phosphate-buffered saline (PBS; 10 mM potassium phosphate, 137 mM NaCl, pH 7.4) and resuspended in 200 μl of water followed by sequential addition of 50 μl of butylated hydroxytoluene (40 mM in ethanol), 600 μl of diluted R1 reagent (3:1 *N*-methyl-2-phenylindole in acetonitrile:ferrous iron in methanol), and 150 μl of 12 N HCl. The mixture was mixed by vortexing and was incubated at 60 °C for 1 h followed by centrifugation (16,100 × *g*, 20 min) and removal of the supernatant for measurement of absorbance at 586 nm. A standard curve was prepared with solutions of 1,1,3,3-tetramethoxypropane (MDA precursor) in 200 μl of water.

The presence of MDA and base propenals was also established qualitatively by thin-layer chromatography (17). An aliquot of the ultrafiltrate prepared from the *in vitro* reactions above (5 μl) was loaded onto a silica gel IB-F TLC plate, resolved with ethyl acetate:isopropyl alcohol:water (74:17:9) and developed by spraying with 0.6% TBA and heating (20 min, 100 °C). R_F values were established using synthetic standards.

Quantification of M_1dG—The M_1dG content of all DNA samples was measured by an immunoblot assay (14). To construct standard curves, MDA-modified DNA (calibrated against a standard provided by Prof. Lawrence Marnett, Vanderbilt University) was diluted with calf thymus DNA to give 6.6 μg of DNA in 150 μl of PBS followed by sonication for 15 min at 4 °C, heating at 100 °C for 15 min, and cooling in ice water for 10 min. The solution was then immediately diluted by addition of 150 μl of 2 M cold ammonium acetate, and 90-μl aliquots (2 μg of DNA) were blotted in triplicate onto a nitrocellulose membrane using a Bio-Rad BioDot microfiltration system. The wells were washed twice with ammonium acetate (200 μl, 1 M), and the membrane was baked (80 °C, 90 min) and then blocked at ambient temperature for 1.5 h with PBS containing 0.1% Tween 20 and 5% nonfat dry milk. The membrane was washed twice for 5 min in blocking solution and then incubated with a 1:30,000 dilution of anti-M_1dG monoclonal antibody (0.3 mg/ml stock) in blocking solution containing 0.5% milk. Following agitation for 30 min at ambient temperature and overnight at 4 °C, the membrane was washed with blocking solution (four times, 5 min) and incubated at ambient temperature for 2 h with a 1:3,000 dilution of goat anti-mouse IgG horseradish peroxidase conjugate in blocking solution containing 0.5% nonfat dry milk. The membrane was again washed (four times, 5 min) with blocking solution followed by incubation in 10 ml of Super Signal West Dura substrate (Pierce) for 5 min. The chemiluminescence signal on the membrane was quantified using an Alpha Innotech Fluorochem charge-coupled device camera system (San Leandro, CA). The membrane was then washed in PBS overnight and stained with 5 μg/ml propidium iodide in PBS (3 h, ambient temperature, dark) followed by washing in PBS and quantification of the fluorescence using the Alpha Innotech Fluorochem charge-coupled device camera system. Standard curves were prepared by plotting the adduct level against the enhanced chemiluminescence signal following normalization of the latter to the propidium iodide staining intensity. The M_1dG levels of unknown samples were based on standard curves analyzed in parallel on the same blot.

Modulation of the PUFA Content of E. coli—E. coli strain DH5α was grown at 37 °C in medium E (18) supplemented with 0.5% glucose, 0.5% casamino acids, 0.0015% thiamine and fatty acids to a final, total concentration of 0.05 g/liter. Fatty acids were added from stock solutions (100 ml, ethanol) with the following compositions: solution A, 0.1 g each of lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1); solution B, 0.5 g of stearic acid (18:0); solution C, 0.5 g of linoleic acid (18:2). Cells cultured in media with the three different fatty acid compositions grew at the same rate (data not shown; A_{600} doubling time ~1.8 h), and oxidant treatments were performed at a cell density of ~5×10^8 cells/ml (A_{600} = 0.6).

Determination of E. coli Fatty Acid Composition—The fatty acid composition of *E. coli* membranes was quantified by a modification of the method of Harley *et al.* (19). Following three washes in PBS (5000 × *g*, 15 min, 4 °C), the cells were resuspended in 1 N KOH in 90% ethanol and heated for 1 h at 75 °C. The solution was filtered, and 6 N HCl was added to the filtrate followed by two extractions with chloroform. The organic layers were combined and dried under a stream of N_2. To the white residue was added methanol and 12 N HCl (2:1, v/v), and the solution was refluxed at 75 °C for 1 h. The fatty acid methyl esters were extracted with petroleum ether, dried over anhydrous Na_2SO_4, and concentrated under vacuum, and the fatty acid methyl ester composition was analyzed by GC/MS following injection of 1-μl samples. Fatty acids were identified by retention time and mass using standards and by matching data against a standard electron impact ionization spectral library. GC retention times were: 14:0, 11.3 min; 16:1, 13.5 min; 16:0, 13.8 min; Cy17:0, 14.3 min; 18:2, 15.0 min; 18:1, 15.1 min; 18:0, 15.3 min.

γ-Radiation and $ONOO^-$ Treatment of E. coli—Cells grown to a

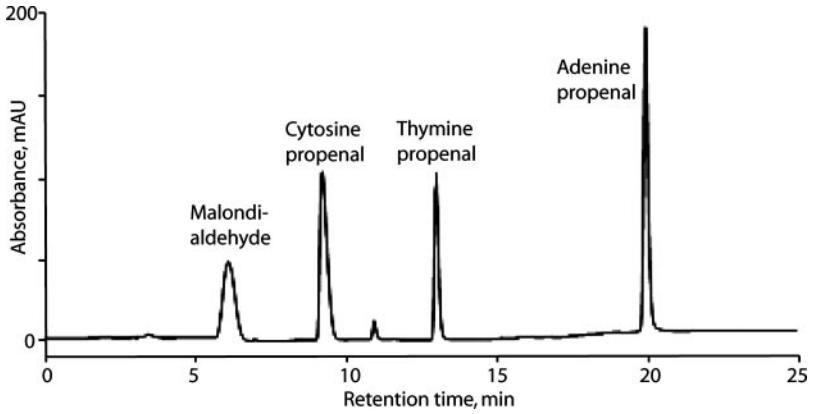

FIG. 2. **HPLC separation of MDA and base propenals for post-column detection.** This chromatograph was prepared by injection of chemical standards. See "Experimental Procedures" for details. *mAU*, milliabsorbance units.

density of ~5 × 10^8/ml were washed three times in PBS and then resuspended (A_{600} = 3) in 100 mM potassium phosphate buffer (pH 7). For γ-radiation, the cell suspension (5 ml) was irradiated (0–300 Gy) in a ^{60}Co source at 2 Gy/min at ambient temperature. For $ONOO^-$, 4.5 ml of cell suspension was mixed with 170 μl of 0.2 M HCl (to neutralize the 0.1 M NaOH added with the $ONOO^-$). This was followed quickly by addition of 0.33 ml of $ONOO^-$ solution (0–30 mM in 0.1 M NaOH; final $ONOO^-$ 0–2.2 mM) that was carefully transferred to the side wall of a 15-ml conical tube wall and rapidly mixed with cell suspension by vortexing. The mixture was incubated at ambient temperature for 1 h. The treated *E. coli* suspensions were then washed once with PBS (2050 × *g*, 15 min, 4 °C), and DNA was purified using a Qiagen cell culture DNA Midi kit (Valencia, CA), precipitated with isopropanol, and washed with 70% cold ethanol. The DNA pellet was air-dried, resuspended in 200 μl of PBS, and stored at −80 °C until used for the M_1dG immunoblot assay. For several samples of $ONOO^-$-treated *E. coli*, the exposed cells were pelleted by centrifugation and the supernatant subjected to YM-10 ultrafiltration, as described above, followed by quantification of MDA in the ultrafiltrate.

RESULTS

Correlation of M_1dG Formation with Oxidant-induced Generation of MDA or Base Propenals in DNA—We first undertook experiments to define the deoxyribose 4′-oxidation chemistry for several oxidizing agents in purified DNA and to correlate these products with the formation of M_1dG. These studies are based on disparate reports that: 1) γ-radiation produces MDA rather than base propenals as the product of 4′-oxidation of deoxyribose that accompanies the 3′-phosphoglycolate residue (20); 2) γ-radiation does not cause formation of M_1dG in DNA (13); 3) Fe^{2+}-EDTA causes lower levels of M_1dG to form in DNA than does Fe^{2+}/H_2O_2 (21); and 4) $ONOO^-$ causes formation of base propenals (17). The goal was to define the relationship between M_1dG formation and the generation of MDA or base propenals by deoxyribose 4′-oxidants.

As shown in Fig. 2, HPLC resolution provided a means to separate MDA and the base propenals for subsequent quantification by TBA derivatization. This method was used to quantify these species in the various DNA oxidation reactions as shown in Fig. 3. Although G-propenal was not quantified in these studies because of the lack of a synthetic standard, quantification of the other three base propenals represents a rigorous metric because γ-radiation, Fe^{2+}-EDTA, and $ONOO^-$ are sequence non-selective deoxyribose oxidants (22, 23), and bleomycin causes formation mainly of C- and T-propenals and some A-propenal (20). The graphs in Fig. 3 reveal that the various oxidizing agents produced either MDA or base propenals but did not simultaneously produce detectable levels of both products. It is also apparent that agents producing base propenals also caused M_1dG formation and those agents that produced MDA did not.

For unknown reasons, we were unable to detect base propenals in the $ONOO^-$ reactions with DNA using the HPLC/TBA method. However, the presence of adenine and thymine propenals was established (and the absence of MDA confirmed) by TLC (data not shown). Although the detection of the base propenals by TLC is qualitative, the results confirm published

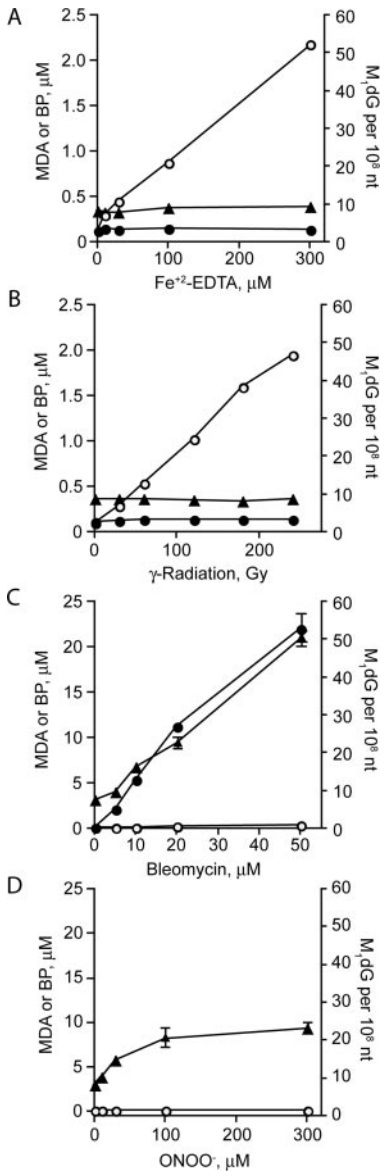

FIG. 3. **Correlation between M_1dG (▲) and the generation of either MDA (○) or base propenals (*BP*) (●) in purified DNA treated with different oxidants.** *A*, Fe^{2+}-EDTA; *B*, γ-radiation; *C*, Fe^{2+}-bleomycin; and *D*, $ONOO^-$. Reactions were performed as described under "Experimental Procedures." Data represent mean ± S.D. for three experiments.

TABLE I
Fatty acid composition (mol %) of E. coli cells grown in defined media

The abbreviations used are: n.d., not detected; Cy17:0, *cis*-9,10-methylene hexadecanoic acid; UFA, unsaturated fatty acid.

Component	Fatty acid supplement[a]		
	Mixture	18:0	18:2
14:0	4.1	3.0	1.5
16:1	13	9.1	3.3
16:0	52	28	24
Cy17:0	3.9	3.3	5.7
18:2	0.5	n.d.	54
18:1	20	3.4	9.5
18:0	6.2	53	1.6
Total % UFA	33	12	67
% PUFA	0.5	n.d.	54

[a] Sole fatty acid source in defined culture media (see "Experimental Procedures").

studies of thymine propenal formation in the reaction of 2′-deoxythymidine with $ONOO^-$ (17). One possible explanation for the lack of detection of the base propenals by HPLC with TBA reaction is their oxidation by excess $ONOO^-$, which is consistent with the non-linear dose-response curve in Fig. 3*D*.

Control of the Fatty Acid Composition of E. coli Membranes—As a model system to define the role of PUFA-derived MDA in M_1dG formation, *E. coli* DH5α cells were cultured in defined media containing different fatty acids. As shown in Table I, GC/MS analysis of *E. coli* extracts revealed a PUFA content consistent with the growth conditions. Although cells grown in the 18:0-enriched medium did not contain detectable PUFA, the level of PUFA increased to 53.9% of fatty acid content for cells grown in the 18:2-medium (Table I). This level of linoleic acid incorporation is similar to that reported by Harley *et al.* (45%) in experiments performed with an unsaturated fatty acid auxotroph of *E. coli* (19). As expected, an intermediate level of PUFA was present in the medium containing a mixture of fatty acids.

Attempts to grow *E. coli* DH5α in minimal media containing linolenate (18:3) or arachidonate (20:4) were unsuccessful in that the membrane levels of each fell below the detection limit of the GC/MS assay at non-toxic concentrations of the fatty acids. One possible explanation for this result is that, unlike the unsaturated fatty acid auxotrophs used in the studies of Harley *et al.* (19), *E. coli* DH5α is able to metabolize PUFA and thus possibly maintain low levels of 18:3 and 20:4.

Correlation of M_1dG Formation with Oxidant-induced Lipid Peroxidation in E. coli—To assess the role of MDA in the formation of M_1dG in living cells, *E. coli* grown in the three defined media were treated with γ-radiation or $ONOO^-$, and the level of M_1dG was quantified by an immunoblot assay. As shown in Fig. 4, $ONOO^-$ treatment of *E. coli* grown in the absence of PUFA led to a dose-dependent increase in M_1dG, which is consistent with the results obtained with purified DNA (Fig. 3*D*). It is also apparent that there is an inverse correlation between PUFA content and M_1dG formation (Fig. 4*D*), whereas there is a direct correlation between PUFA content and MDA formation as expected (Fig. 4*C*).

Exposure to γ-radiation did not induce detectable levels of M_1dG in *E. coli* grown with any of the fatty acids (Fig. 4*B*), which is again consistent with the *in vitro* results (Fig. 3). However, γ-radiation did produce MDA as a result of lipid peroxidation in amounts directly proportional to the PUFA content of the cells (Fig. 4*A*). Similar results were obtained by treating the three cell types with hydrogen peroxide with or without added Fe^{2+} (data not shown).

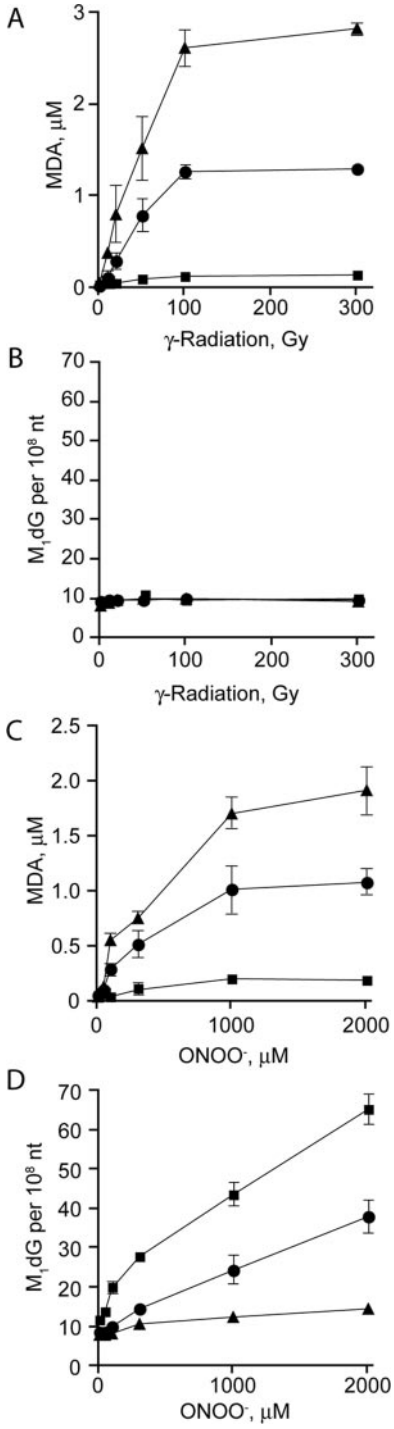

FIG. 4. **Correlation between M_1dG (*B* and *D*) and lipid peroxidation (*A* and *C*) in *E. coli* with controlled levels of PUFA, treated with either γ-radiation (*A* and *B*) or $ONOO^-$ (*C* and *D*).** *E. coli* grown in a defined medium containing a mixture of fatty acids (●), stearic acid (■; 18:0), or linoleic acid (▲; 18:2) were treated with oxidants. M_1dG, MDA, and lipid peroxidation products were quantified as described under "Experimental Procedures." Data represent mean ± S.D. for three experiments.

DISCUSSION

There is strong evidence for the existence of a host of endogenous DNA adducts (reviewed in Ref. 25), yet the mechanisms of their formation have not been rigorously defined. The goal of the present studies was to address this problem for M_1dG. The chemical species known to form M_1dG *in vitro*, namely MDA and base propenals, arise from different sources. Lipids represent a major target for free radicals (26), and peroxidation of PUFA generates a host of reactive electrophiles, many of which have been implicated in the formation of DNA adducts, such as the etheno adducts of dG, dA, and dC (27). In a similar manner,

MDA is ubiquitously present in cells and tissues and has been demonstrated to react with dG to form M_1dG *in vitro* (28–30); hence the proposal that it is involved in M_1dG formation *in vivo* (7, 31, 32). We previously demonstrated that base propenals derived from deoxyribose 4′-oxidation in DNA served as an alternative source of M_1dG (13, 33). The higher reactivity of base propenals than MDA toward dG (>100-fold; Refs. 13 and 33) and their proximity to guanine bases in DNA suggested that base propenals might be a significant source of M_1dG formation *in vivo*. The results of the present studies support this conclusion.

As a foundation for interpretation of results obtained *in vivo*, a systematic comparison of the DNA 4′-oxidation chemistry caused by bleomycin, γ-radiation, Fe^{2+}-EDTA, and $ONOO^-$ confirmed fragmentary observations in the literature and revealed a trend that amounts to a third pathway for 4′-oxidation chemistry in DNA. Treatment of DNA with bleomycin and $ONOO^-$ gives rise to base propenals (Fig. 3; Ref. 34) in addition to 3′-phosphoglycolate residues (23, 35) but did not yield detectable amounts of MDA. On the other hand, γ-radiation and Fe^{2+}-EDTA produce damage consisting of 3′-phosphoglycolate residues (reviewed in Ref. 36) and MDA but no detectable base propenals (Fig. 3). The results with γ-radiation confirm the observations of von Sonntag and co-workers (20), with the Fe^{2+}-EDTA results extending this novel partitioning of 4′-oxidation of deoxyribose to another commonly used DNA oxidant. At least with this small set of oxidizing agents, the partitioning between base propenals and MDA appears to be complete, with no detectable amount of the other species being formed.

The chemical basis for the two phosphoglycolate-generating pathways is not known and is not apparent from the currently proposed mechanisms for 4′-oxidation chemistry (35, 37). However, there is one mechanistic distinction between the two sets of oxidants used in the present studies. Current evidence suggests that DNA-bound bleomycin participates in the subsequent chemistry of the deoxyribose oxidation it initiates (38, 39), whereas degradation of $ONOO^-$ simultaneously produces both hydroxyl radical and nitrogen dioxide radical (reviewed in Ref. 40). In both cases, secondary reactions with the oxidants or degradation products of the oxidants could alter the chemistry subsequent to formation of the 4′-carbon radical. On the other hand, the negative charge of Fe^{2+}-EDTA likely precludes any binding interaction with DNA and thus any chemical cycling of oxidant or its derivatives. Furthermore, neither the sparsely ionizing γ-radiation nor Fe^{2+}-EDTA simultaneously produces significant amounts of other DNA-proximate radical species that could participate in the deoxyribose degradation. This model can be tested by comparing other soluble and DNA-binding oxidants capable of 4′-oxidation of deoxyribose.

Whatever the basis for the different DNA oxidation chemistries, the results from purified DNA serve as a benchmark for interpreting the studies in *E. coli*. The fatty acid requirements of *E. coli* and *Saccharomyces cerevisiae* differ from those of mammalian cells in that they do not require PUFA for growth and their membranes normally contain mainly saturated and monounsaturated fatty acids (41, 42). (The observation by Fridovich and co-workers that *E. coli* synthesize linoleic acid during late stationary phase (43) has been shown to be an artifact of contamination (44)). However, these organisms readily incorporate PUFA into cell membranes when the fatty acids are supplied in the growth medium (19, 45). Upon challenge with oxidizing agents, the PUFA undergo peroxidation that results in the formation of thiobarbiturate-reactive species (*e.g.* MDA) (19, 45), products that are not generated when PUFA are not provided (43, 45). We were able to control the levels of PUFA as indicated by the GC/MS analysis presented in Table I, which confirmed an absence of PUFA in the stearate-containing medium, intermediate levels in the fatty acid mixture, and high levels in the linoleate-containing medium. The choice of linoleic acid is based on the fact that it is the major PUFA in mammalian cell membranes (*e.g.* Ref. 46). Although it has been claimed that linoleate peroxidation does not produce MDA (47, 48), recent studies using more rigorous analytical methods proved that MDA formation does occur (49, 50). In a similar manner, we used HPLC with post-column detection to quantify MDA in suspensions of linoleic acid-labeled *E. coli*.

This well characterized model cell system was used to assess the role of MDA in the formation of M_1dG with two important observations. The first involves an inverse relationship between the level of lipid peroxidation, as measured by both MDA formation and generation of thiobarbiturate-reactive species, and the level of M_1dG in DNA from $ONOO^-$-exposed cells (Fig. 4). If MDA were responsible for the bulk of M_1dG formation, then a direct relationship would be expected. A lack of correlation between M_1dG and lipid peroxidation, however, is consistent with deoxyribose oxidation and base propenals as the source of M_1dG in the *E. coli* cells. The results with γ-irradiation further strengthen this argument. There was a high level of MDA produced upon irradiation of the 18:2-labeled cells yet no increase in M_1dG in any of the three cell cultures. This is entirely consistent with the results obtained with purified DNA (Fig. 3; Refs. 13 and 20) in which γ-irradiation caused MDA formation but not base propenals or M_1dG. The results suggest that lipid peroxidation alone is insufficient to induce M_1dG formation if the oxidizing reagent cannot generate base propenals by direct oxidation of deoxyribose in DNA.

It is important to point out that our results do not rule out MDA as a source of M_1dG in human cells. Indeed, we have observed that exposure of *E. coli* to 10 mM MDA (37 °C, 24 h) caused a doubling of the M_1dG adduct level to 2 lesions/10^7 nt,[2] which is similar to the trebling of the M_1dG level (1.2–3.9/10^7 nt) observed by Marnett and co-workers (51) in studies of *Salmonella typhimurium* exposed to 10 mM MDA. However, given the small increases in M_1dG occurring with these highly non-physiological MDA concentrations, we argue that base propenals arising from oxidative DNA damage make the major contribution to the cellular burden of M_1dG. This model is supported by the lack of a correlation between M_1dG and lipid peroxidation-derived etheno adducts and by the positive correlation between M_1dG and 8-oxo-dG, a DNA oxidation product, in human pancreas (7). Similar results have been obtained in the SJL/RcsX mouse model of nitric oxide overproduction in which it was observed that etheno-dA adducts increase severalfold in inflamed spleens (52), whereas M_1dG levels were unchanged from values obtained in non-inflamed mice.[2]

The second notable observation was the apparent protective effect of PUFA with regard to M_1dG formation. There are several possible explanations for the observed inverse correlation between PUFA content and M_1dG formation. One involves differences in the rate of uptake of $ONOO^-$ into cells containing different levels of PUFA. Increases in membrane fluidity caused by incorporation of PUFA into *E. coli* membranes have been shown to increase the rate of diffusion of glycerol into the cells (*e.g.* Ref. 24). However, if $ONOO^-$ behaved in a similar manner, we would have expected an increase in M_1dG formation in the 18:2-labeled cells because of an increase in the quantity of intracellular $ONOO^-$; this assumes that M_1dG is derived from DNA oxidation. The most likely explanation for the results with $ONOO^-$ involves preferential reaction of

[2] X. Zhou and P. C. Dedon, unpublished observations.

$ONOO^-$ with the PUFA either as a result of a first encounter phenomenon as the $ONOO^-$ diffuses into the cells or as a result of a thermodynamic preference for reaction of $ONOO^-$ with PUFA compared with deoxyribose in DNA. The greater reactivity of PUFA can be rationalized by the stability conferred to the initial oxidant-induced radical by the conjugated system carbon-carbon double bonds that define PUFA (Fig. 1). Such electron delocalization is not possible with a radical centered at the 4′-position in deoxyribose.

In conclusion, the results from studies in purified DNA and an *E. coli* model suggest that base propenals, and not MDA, are the major source of M_1dG in biological systems. Furthermore, PUFA appear to protect from M_1dG formation, possibly by virtue of their location in cells relative to DNA or their higher reactivity with oxidants than deoxyribose in DNA.

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