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**IONIZING RADIATION-INDUCED DNA DAMAGE AND ITS REPAIR
IN HUMAN CELLS**

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by

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Abbreviations

GC/MS-SIM, gas chromatography/mass spectrometry with selected-ion monitoring; $\cdot\text{OH}$, hydroxyl radical; **5,6-diHThy**, 5,6-dihydrothymine; **5,6-diHUra**, 5,6-dihydrouracil; **5-OH-5-MeHyd**, 5-hydroxy-5-methylhydantoin; **5-OH-Hyd**, 5-hydroxyhydantoin; **5-OH-Ura**, 5-hydroxyuracil; **5-OH-Cyt**, 5-hydroxycytosine; **5-OH-6-HThy**, 5-hydroxy-6-hydrothymine; **5-OH-6-HUra**, 5-hydroxy-6-hydrouracil; **5-OHMeUra**, 5-(hydroxymethyl)uracil; **Thy glycol**, thymine glycol; **Ura glycol**, uracil glycol; **Cyt glycol**, cytosine glycol; **5,6-diOH-Ura**, 5,6-dihydroxyuracil; **FapyAde**, 4,6-diamino-5-formamidopyrimidine; **8-OH-Ade**, 8-hydroxyadenine; **2-OH-Ade**, 2-hydroxyadenine; **FapyGua**, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; **8-OH-Gua**, 8-hydroxyguanine; **Thy-Tyr crosslink**, 3-[(1,3-dihydro-2,4-dioxo-pyrimidin-5-yl)methyl]-L-tyrosine; **DMSO**, dimethyl sulfoxide; **J**, joule; **UNG**, uracil DNA *N*-glycosylase; **U**, unit.

1. REPORT

INTRODUCTION

The studies of DNA damage in living cells *in vitro* and *in vivo* were continued. A variety of systems including cultured mammalian cells, animals and human tissues were used to conduct these studies. In addition, enzymatic repair of DNA base damage was studied using several DNA glycosylases. To this end, substrate specificities of these enzymes were examined in terms of a large number of base lesions in DNA. In the first phase of the studies, we sought to introduce improvements to our methodologies for measurement of DNA damage using the technique of gas chromatography/mass spectrometry (GC/MS). In particular, the quantitative measurement of DNA base damage and DNA-protein crosslinks was improved by incorporation of isotope-dilution mass spectrometry into the methodologies. This is one of the most accurate techniques for quantification of organic compounds. Having improved the measurement technique, studies of DNA damage in living cells and DNA repair by repair enzymes were pursued.

The following report of accomplishments provides a summary of these studies with references to the original work. Most of this work was prepared for publication with some of it already published, or in press (see the attached list of papers).

ACCOMPLISHMENTS

A. QUANTITATIVE DETERMINATION OF DNA DAMAGE BY STABLE ISOTOPE-DILUTION MASS SPECTROMETRY

In recent years, we described the use of the technique of GC/MS for measurement of a variety of modified bases in DNA and chromatin [reviewed in Dizdaroglu (1991)]. Unlike the other techniques, GC/MS provides on-line structural evidence for the compounds analyzed and permits the measurement of a large number of pyrimidine- and purine-derived modified bases in the same sample of DNA or chromatin in a single analysis. Using GC/MS, the quantification of analytes is achieved by adding a suitable internal standard to DNA samples at an early stage of analysis such as prior to hydrolysis of DNA. In mass spectrometry, a stable isotope-labeled analog of an analyte can be used as an internal standard [reviewed in Watson (1990)]. This procedure is often called isotope-dilution mass spectrometry. The use of stable isotope-labeled analogues permits compensation of possible losses of the analyte during the sample preparation and GC/MS analysis.

because the analyte and its analog have essentially the same chemical and physical properties. With the use of the selected-ion monitoring (SIM) mode, an intense and characteristic ion of the analyte and that of its analog are simultaneously monitored for quantification (Watson 1990). Generally, stable isotope-labeled analogues of modified DNA bases are not available commercially. Moreover, they are very expensive to synthesize and purify. Nevertheless, we were recently able to obtain custom-synthesized, stable isotope-labeled analogues of modified bases which result from oxidative damage to pyrimidines and purines in DNA.

The following stable isotope-labeled compounds were obtained: 5,6-dihydrothymine-1,3-¹⁵N₂-2-¹³C, 5,6-dihydrouracil-1,3-¹⁵N₂-2-¹³C, 5-hydroxy-5-methylhydantoin-1,3-¹⁵N₂-2-¹³C, alloxan-1,3-¹⁵N₂-2,4-¹³C₂, 5-hydroxyhydantoin-1,3-¹⁵N₂-2,4-¹³C₂, 5-hydroxyuracil-1,3-¹⁵N₂-2-¹³C, 5-hydroxycytosine-1,3-¹⁵N₂-2-¹³C, 5-(hydroxymethyl)uracil-2,4-¹³C₂- α,α -²H₂, cis-thymine glycol- α,α,α ,6-²H₄, 5,6-dihydroxyuracil-1,3-¹⁵N₂-2-¹³C (isodialuric acid-1,3-¹⁵N₂-2-¹³C), 4,6-diamino-5-formamidopyrimidine-1,3-¹⁵N₂-2-¹³C-(5-formamido-¹⁵N,²H), 8-hydroxyadenine-1,3,7-¹⁵N₃-2,8-¹³C₂, 2,6-diamino-4-hydroxy-5-formamidopyrimidine-1,3-¹⁵N₂-(5-amino-¹⁵N)-2-¹³C, 8-hydroxyguanine-1,3-¹⁵N₂-(2-amino-¹⁵N)-2-¹³C, xanthine-1,3-¹⁵N₂, and thymine-tyrosine crosslink-²H₂. Figure 1 illustrates the structures of these compounds.

The mass spectral fragmentation patterns of stable isotope-labeled analogues are similar to those of corresponding unlabeled compounds. Masses of most ions in the mass spectra of labeled analogues are shifted to higher masses according to their isotope contents. Typical examples of mass spectra are illustrated in Figure 2. Here are shown the mass spectra of the trimethylsilyl derivatives of 4,6-diamino-5-formamidopyrimidine (Figure 2A) and 4,6-diamino-5-formamidopyrimidine-¹⁵N₃,¹³C,²H (Figure 2B). In Figure 2A, characteristic ions appear at *m/z* 369, 368, 354 and 280. In Figure 2B, the masses of these ions are shifted by 5 Da to 374, 373, 359 and 285 Da, respectively. Ions at *m/z* 73 and 147 in Figures 2A and 2B are commonly observed with trimethylsilyl derivatives.

Unlabeled compounds co-elute with their ¹³C- and ¹⁵N-labeled analogues, indicating no isotope effect on elution behaviors of corresponding compounds (Dizdaroglu 1993). However, if a labeled analog contains several ²H atoms such as thymine glycol-²H₄, a slight resolution from the unlabeled material is observed, indicating a well known isotope effect on the elution behavior of ²H-containing compounds. For quantification, calibration plots are obtained prior to GC/MS analysis for the response of the mass spectrometer to known quantities of both the analyte and its stable isotope-labeled analog as the internal standard (Watson 1990). For this purpose, mixtures containing known quantities of the analyte and its analog are analyzed by GC/MS-SIM and a number of prominent characteristic ions are monitored. The ratios of ion currents at selected masses are plotted as a function of the ratios of the molar amounts of an analyte and its analog. A linear relationship of the ratio of ion currents to the ratio of quantities should be obtained. Subsequently, quantities of analytes in a given mixture

Figure 1. The structures of stable isotope-labeled compounds that are now available as internal standards for quantitative measurement of DNA base products by isotope-dilution mass spectrometry (labeled atoms are not indicated).

Figure 2. (A) Electron-ionization mass spectrum of the trimethylsilyl derivative of 4,6-diamino-5-formamidopyrimidine; (B) Electron-ionization mass spectrum of the trimethylsilyl derivative of 4,6-diamino-5-formamidopyrimidine-1,3-¹⁵N₂-2-¹³C-(5-formamido-¹⁵N, ²H).

Figure 3. Calibration plots for ratios of ion currents at indicated masses versus ratios of molar amounts of the trimethylsilyl derivative of 8-hydroxyguanine and its labeled analog (●, m/z 440/444), and the trimethylsilyl derivative of 4,6-diamino-5-formamidopyrimidine and its labeled analog (Δ, m/z 354/359).

are calculated using the areas of the ion-current profiles of the monitored ions and the corresponding calibration plots. Examples of calibration plots are illustrated in Figure 3. Although the data points were obtained by three independent measurements and contain standard deviations, the error bars are not discernable on these plots. This is because standard deviations were equal to, or less than 1%, indicating the precision of such measurements by GC/MS-SIM.

In conclusion, the results obtained in this study indicated that isotope-dilution mass spectrometry is an ideally suited technique for selective and sensitive quantification of modified bases in DNA. Isotope-dilution mass spectrometry using a large number of labeled compounds as internal standards is now routinely employed in my laboratory for quantitative measurement of DNA damage. Thus far, no other laboratory has reported the use of this variety of labeled compounds for quantitative measurement of DNA damage.

This work has been published in *FEBS Letters* (Dizdaroglu 1993). A brief description of this work was also given in a recent invited article published in *Methods in Enzymology* (Dizdaroglu 1994).

B. STUDIES OF DNA DAMAGE *IN VIVO* AND IN CULTURED CELLS

I. DNA BASE DAMAGE GENERATED *IN VIVO* IN HEPATIC CHROMATIN OF MICE UPON WHOLE BODY γ -IRRADIATION

There are limited data on ionizing radiation-induced DNA base damage in tissues *in vivo* (Kasai et al. 1986). In this work, we attempted to characterize and quantify *in vivo* DNA base modifications occurring in hepatic chromatin of mice upon the whole body irradiation with γ -rays. Animals were γ -irradiated in the dose range of 20 to 470 Gy. Chromatin was isolated from livers of irradiated and control animals. Chromatin samples were analyzed by GC/MS-SIM after hydrolysis and derivatization. Five pyrimidine-derived and five purine-derived modified DNA bases were identified and quantified in chromatin samples from irradiated animals as well as from control animals. These were 5-OH-5-MeHyd, 5-OH-Cyt, 5-OHMeUra, 5,6-diOH-Ura, Thy glycol, FapyAde, 8-OH-Ade, 2-OH-Ade, FapyGua and 8-OH-Gua. The control amounts and radiation yields of the modified bases are given in Table 1. The amounts of most products were increased significantly over the control levels at radiation doses of 200 and 470 Gy. As examples, dose-yields plots of 5-OH-Cyt and FapyGua are illustrated in Figure 4. At 100 Gy, the yields of 5-OH-5-MeHyd, 5-OHMeUra and 8-OH-Gua were significantly greater than control levels. Above 200 Gy, the product yields appeared to reach a plateau, although greater yields were observed at 470 Gy than at 200 Gy. At 470 Gy, percent increases over control amounts ranged from 65 to 110%, with the

Table 1. Yields^a of modified bases (molecules/10⁵ DNA bases) generated in hepatic chromatin of mice upon whole body γ -irradiation.

Base	Radiation dose (Gy)					
	Control	20	50	100	200	470
5-OH-5-MeHyd	6.07 $\pm$ 0.13	6.30 $\pm$ 0.41	6.26 $\pm$ 0.25	7.12 $\pm$ 0.32†	8.27 $\pm$ 0.35†	11.4 $\pm$ 1.49†
5-OH-Cyt	0.83 $\pm$ 0.12	0.99 $\pm$ 0.16	0.80 $\pm$ 0.08	0.99 $\pm$ 0.25	1.43 $\pm$ 0.14†	1.37 $\pm$ 0.15†
5-OHMeUra	0.70 $\pm$ 0.22	0.89 $\pm$ 0.31	0.99 $\pm$ 0.17	1.14 $\pm$ 0.10†	1.53 $\pm$ 0.16†	1.34 $\pm$ 0.14†
5,6-diOH-Ura	1.34 $\pm$ 0.16	1.69 $\pm$ 0.38	1.40 $\pm$ 0.41	1.72 $\pm$ 0.60	1.34 $\pm$ 0.28	1.65 $\pm$ 0.76
Thy glycol	8.17 $\pm$ 0.48	9.32 $\pm$ 1.33	6.46 $\pm$ 0.10	7.92 $\pm$ 2.39	7.28 $\pm$ 0.14	9.44 $\pm$ 0.70
FapyAde	15.6 $\pm$ 1.75	13.6 $\pm$ 0.76	14.1 $\pm$ 2.38	13.6 $\pm$ 0.48	18.9 $\pm$ 1.08	27.6 $\pm$ 2.51†
8-OH-Ade	1.56 $\pm$ 0.14	1.37 $\pm$ 0.38	1.37 $\pm$ 0.16	1.88 $\pm$ 0.18	2.61 $\pm$ 0.16†	3.24 $\pm$ 0.07†
2-OH-Ade	5.69 $\pm$ 0.99	5.09 $\pm$ 0.86	5.37 $\pm$ 0.41	6.01 $\pm$ 0.57	7.44 $\pm$ 2.19	10.9 $\pm$ 0.70†
FapyGua	10.9 $\pm$ 0.38	9.22 $\pm$ 0.80	11.1 $\pm$ 2.32	9.38 $\pm$ 1.56	16.2 $\pm$ 1.49†	22.2 $\pm$ 1.72†
8-OH-Gua	3.69 $\pm$ 0.38	4.07 $\pm$ 0.64	3.59 $\pm$ 0.32	5.09 $\pm$ 0.54†	7.16 $\pm$ 0.29†	7.17 $\pm$ 0.73†

^aEach value represents the mean $\pm$ SD from three independent experiments using three animals.

†Significantly different from the control value ($p \leq 0.05$).

Figure 4. Radiation dose-yield plots of 5-OH-Cyt and FapyGua in hepatic chromatin of mice. Error bars represent standard deviations of the means from three independent experiments involving three animals.

highest increases in the amounts of 8-OH-Ade and FapyGua. The control amounts of Thy glycol and 5,6-diOH-Ura were not significantly affected upon irradiation (Table 1).

A similar pattern of base modifications has been identified previously in chromatin of γ -irradiated cultured mammalian cells (Nackerdien et al. 1992). Identified modified DNA bases are known to be typically produced by reactions of $\cdot\text{OH}$ with DNA bases [reviewed in Téoule (1987), Dizdaroglu (1982)]. This implies a role of $\cdot\text{OH}$ in modification of DNA bases *in vivo*. DNA base modifications produced *in vivo* may be due to $\cdot\text{OH}$ produced in the close proximity to DNA. The dose-yield responses of the modified bases were remarkably similar to those observed in chromatin of γ -irradiated cultured mammalian cells (Nackerdien et al. 1992). The yields approached a plateau at high doses. The reason for this deviation from linearity is not known. One possible explanation may be the generation of hypoxic conditions due to consumption of oxygen. Using isolated chromatin, we have recently shown that most of the DNA lesions measured in the present work are produced by γ -irradiation more abundantly in the presence of oxygen than in its absence (Fuciarelli et al. 1990, Gajewski et al. 1990).

These results show for the first time that all four DNA bases in genomic DNA undergo modifications *in vivo* upon exposure of animals to ionizing radiation. As a result, numerous DNA base lesions are generated at the same time. The guanine-derived lesions predominate the other base lesions. This may indicate the high reactivity of $\cdot\text{OH}$ toward guanine residues in chromatin of cells. The DNA base lesions produced *in vivo* may play a role in radiation-induced biological end-points such as mutagenesis and carcinogenesis. The formation of DNA lesions in a great variety may be a hampering factor in the assessment of their biological significance. The contribution of pyrimidine- and purine-derived DNA lesions to biological consequences of ionizing radiation is as yet unknown, and remains to be determined.

This work has been published in *International Journal of Radiation Biology* (Mori et al. 1993).

II. DNA BASE DAMAGE AND EVIDENCE FOR ITS REPAIR IN NUCLEATED BLOOD CELLS OF CANCER PATIENTS UNDERGOING RADIATION THERAPY

Ionizing radiation is one of the therapeutic agents of cancer. In general, approximately half of cancer patients receive radiation therapy for their disease management (Castro and Curtis 1982). The goal of radiation therapy is to kill malignant cells while maintaining the integrity of the normal cells. It is generally accepted that ionizing radiation kills mammalian cells by inducing damage to the nuclear DNA, although the ultimate cause of cell death in terms of DNA damage is a controversial subject (Painter 1980, Radford et al. 1988). Since ionizing radiation is mutagenic and carcinogenic by virtue of its ability to damage DNA in cells, radiation therapy is also associated with an increased risk of

incidence of secondary malignancies in cancer patients (Prasad 1984, Boice, Jr. et al. 1988). Determination of DNA damage in humans is of great interest because of its potential value in risk assessment. It may contribute to monitoring of radiation therapy patients and to optimization of irradiation conditions. Furthermore, determination of DNA damage *in vivo* may contribute to the understanding of mechanisms of radiation action on cells.

In this work, we investigated DNA damage in terms of a variety of modified bases in nucleated blood cells (lymphocytes) of numerous radiation therapy patients. The objective of the work was to see whether typical radiation-induced products of DNA bases are produced in genomic DNA of nucleated blood cells of cancer patients upon exposure to radiation. Furthermore, we wished to see whether removal (repair) of DNA base products occur in those cells during the course of the radiation therapy.

Blood samples from several radiation therapy patients with various types of cancers were collected. The types of cancers included breast cancer, bladder cancer, cervix cancer and lung cancer. Each patient had received daily fractions of therapeutic doses of ionizing radiation. Chromatin samples were isolated from lymphocytes in blood samples, which were drawn before and after exposure of patients to radiation. The samples were analyzed by GC/MS-SIM. A number of modified DNA bases were identified and quantified. As an example, Figure 5 illustrates the amounts of various modified bases in blood samples of a breast cancer patient. Significant increases in the amounts of the modified bases over control levels were observed. In some cases after irradiation, there were discernible decreases in the levels of modified bases, especially after the last step of exposure which took seven consecutive days of irradiation. Similar results were obtained with blood samples drawn from the other patients.

There are limited data on DNA base modifications in nucleated blood cells of radiation therapy patients. Wilson et al. (1993) reported increased levels of 8-OH-Gua in nucleated blood cells of several cancer patients following exposure to ionizing radiation. They compared the level of this modified base in treated patients with that of another group of nonirradiated individuals. Our results demonstrated, for the first time, increases in the control levels of a variety of typical $\cdot$ OH-induced DNA base products in lymphocytes of radiation therapy patients. Blood samples were taken from the same individuals prior to and following irradiation. Higher radiation doses were required to observe discernable DNA base damage in livers of mice (see above and Mori et al. 1993). This may be due to differences in radiation sensitivities between rodent liver cells and human lymphocytes as was previously reported in the case of chromosomal aberrations (Brooks et al. 1971, Brooks 1975). There were differences between responses of the patients. Observed decreases in the levels of modified bases depending on the time after radiation exposure may reflect their removal from DNA by cellular repair. Differences between the modified bases in terms of removal may be due to different kinetics of repair. Previously, it has been reported that the excretion in urine of typical $\cdot$ OH-

Figure 5. The amounts of modified base in genomic DNA of nucleated cells in blood samples of a breast cancer patient undergoing radiation therapy. Radiation dose: 2 or 3 doses a day, each 200 cGy. 1: control; 2: second day of irradiation, total 800 cGy; 3: three consecutive days of irradiation, total 1200 cGy; 4: four consecutive days, total 1600 cGy; 5: four consecutive days, total 2400 cGy; 6: seven consecutive days, total 4200 cGy. 1 nmol of a modified base/mg of DNA corresponds to ≈ 32 modified bases per 10^5 DNA bases.

induced DNA base products such as 8-OH-Gua and Thy glycol was elevated in patients after exposure to therapeutic doses of radiation, but returned to normal after a period of time following exposure (Bergtold et al. 1990, Shinega et al. 1989). It is thought that this phenomenon was due to DNA repair. DNA base products that we identified in the present work may play a role in secondary malignancies often observed with radiation therapy patients (Prasad 1984, Boice, Jr. et al. 1988). Some of these compounds have been shown to possess premutagenic properties (Wallace 1987, Loeb et al. 1991, Grollman and Moriya 1993, Purmal et al. 1994).

This work is still in progress. It is being carried out in collaboration with Prof. R. Olinski of the Medical Academy in Bydgoszcz, Poland. Dr. Olinski is the deputy dean of this medical school and has access to cancer patients undergoing radiation therapy. All blood samples used in this work were obtained and all chromatin samples were isolated at the aforementioned medical school.

III. DNA BASE DAMAGE IN RADIATION-SENSITIVE MUTANT M10 CELLS AND IN PARENT MOUSE LYMPHOMA L5178Y CELLS

A number of human disorders with a high incidence of malignancy such as ataxia telangiectasia (AT) and Fanconi's anemia show an increased sensitivity to ionizing radiation (Taylor et al. 1975, Setlow 1978). There are also mutants of other mammalian cell lines that are more radiation-sensitive than their parent cell lines. The knowledge of the molecular basis of radiation sensitivity may contribute to the understanding of carcinogenesis, predisposition to cancer, responses to radiation therapy and mechanisms of radiation-induced DNA damage and its cellular repair (McMillan and Peacock 1994). M10 cells isolated from mouse lymphoma L5178Y cells show hypersensitivity to the lethal effects of ionizing radiation (Sato and Hieda 1979). In its sensitivity, the M10 cell line resembles the human AT cell line (Taylor et al. 1975). No reports exist on radiation-induced damage to DNA bases in such cell lines and on comparison of DNA base damages between hypersensitive cells and their parent cells.

We investigated radiation-induced DNA base damage in M10 cells and their parent L5178Y cells. The objective of this work was to characterize and quantify DNA base damage at the molecular level and, as a result, to see whether differences exist between these two cell lines in terms of base damage to their nuclear DNA. Cultured L5178Y cells and mutant M10 cells were exposed to γ -radiation. Chromatin was extracted from unirradiated and irradiated cells and analyzed by GC/MS-SIM after hydrolysis and derivatization. Ten DNA base products were identified and quantified. These products were FapyGua, 8-OH-Gua, FapyAde, 8-OH-Ade, 2-OH-Ade, 5-OH-Cyt, 5-OH-Ura, 5-OHMeUra, 5,6-diOH-Ura and 5-OH-5-MeHyd. Their yields are given in Table 2. As examples, the

Table 2. Yields^a (molecule/10⁵ DNA bases) of DNA base products in M10 and L5178Y cells.

Product	Cell line	Radiation dose (Gy)				
		Control	48	100	200	400
FapyGua	M10	3.64 ± 0.13	5.78 ± 0.69 ^b	9.64 ± 0.35 ^{b,c}	12.18 ± 0.72 ^{b,c}	14.76 ± 0.85 ^{b,c}
	L5178Y	2.89 ± 0.25	5.97 ± 0.50 ^b	6.88 ± 0.06 ^b	7.07 ± 0.09 ^b	7.47 ± 0.47 ^b
8-OH-Gua	M10	2.25 ± 0.80	2.95 ± 0.35	3.64 ± 0.38 ^b	4.58 ± 0.41 ^{b,c}	5.15 ± 0.09 ^{b,c}
	L5178Y	2.39 ± 0.22	2.83 ± 0.16	3.49 ± 0.28 ^b	3.67 ± 0.19 ^b	3.83 ± 0.13 ^b
FapyAde	M10	6.34 ± 0.16	7.44 ± 0.11 ^b	7.79 ± 0.97	9.33 ± 0.31 ^{b,c}	12.53 ± 0.31 ^{b,c}
	L5178Y	6.50 ± 0.72	6.69 ± 0.19	6.37 ± 0.25	7.32 ± 0.35 ^b	8.13 ± 0.31 ^b
8-OH-Ade	M10	0.57 ± 0.06	0.91 ± 0.06 ^b	1.32 ± 0.03 ^b	1.48 ± 0.03 ^{b,c}	1.88 ± 0.22 ^{b,c}
	L5178Y	0.47 ± 0.05	0.88 ± 0.06 ^b	0.94 ± 0.10 ^b	0.91 ± 0.02 ^b	0.95 ± 0.06 ^b
2-OH-Ade	M10	2.36 ± 0.41	2.67 ± 0.13	3.99 ± 0.06 ^b	5.06 ± 0.28 ^{b,c}	6.28 ± 0.94 ^{b,c}
	L5178Y	2.29 ± 0.35	2.61 ± 0.04	2.98 ± 0.41	3.52 ± 0.22 ^b	4.18 ± 0.19 ^b
5-OH-Cyt	M10	2.07 ± 0.16	3.17 ± 0.03 ^{b,c}	3.05 ± 0.22 ^{b,c}	3.30 ± 0.04 ^{b,c}	4.90 ± 0.04 ^{b,c}
	L5178Y	2.01 ± 0.04	2.36 ± 0.19 ^b	2.26 ± 0.06 ^b	2.72 ± 0.17 ^b	3.33 ± 0.10 ^b
5-OH-Ura	M10	3.61 ± 0.13	3.49 ± 0.09	3.71 ± 0.75	6.15 ± 0.57 ^{b,c}	8.35 ± 1.07 ^{b,c}
	L5178Y	3.60 ± 0.28	3.50 ± 0.11	3.89 ± 0.16	4.36 ± 0.28	5.65 ± 0.57 ^b
5-OHMe-Ura	M10	1.41 ± 0.05	1.57 ± 0.35	2.45 ± 0.25 ^b	2.54 ± 0.16 ^{b,c}	3.52 ± 0.31 ^{b,c}
	L5178Y	1.44 ± 0.19	1.48 ± 0.19	1.41 ± 0.35	1.73 ± 0.05 ^b	1.82 ± 0.04 ^b
5,6-diOH-Ura	M10	0.97 ± 0.04	1.60 ± 0.01 ^b	1.61 ± 0.47	1.70 ± 0.11 ^b	2.04 ± 0.16 ^b
	L5178Y	0.98 ± 0.09	0.94 ± 0.04	1.10 ± 0.03	1.41 ± 0.12 ^b	1.70 ± 0.22 ^b
5-OH-5-MeHyd	M10	2.43 ± 0.35	3.17 ± 0.17	2.98 ± 0.31	3.11 ± 0.25	4.71 ± 0.50 ^b
	L5178Y	2.64 ± 0.28	4.36 ± 2.14	3.42 ± 0.38	3.39 ± 0.79	3.93 ± 0.01 ^b

^aEach value represents the mean ± standard error from three independent experiments.

^bSignificantly different from the control value ($P \leq 0.05$ by the Student's *t* test).

^cSignificantly different from the value in L5178Y cells ($P \leq 0.05$ by the Student's *t* test).

Figure 6. Radiation dose-yield plots of FapyGua and 8-OH-Gua identified in chromatin of M10 and L5178Y cells. Error bars represent standard errors of the means from three independent experiments.

dose-yield plots of FapyGua and 8-OH-Gua are illustrated in Figure 6. In all cases, the amounts of the individual products in unirradiated M10 and L5178Y cells were similar (Table 2). FapyGua was the major product in both cell lines and at all radiation doses used. A significant formation of 8-OH-Ade and 5-OH-Cyt were also observed in both cell lines at all doses. At 48 Gy, no significant increases in the amounts of other products over control levels were observed in L5178Y cells. At 400 Gy, the amounts of all products in both cell lines were significantly larger than in control cells. Significantly higher yields of 5-OH-Cyt and FapyGua in M10 cells than in parent cells were observed starting at 48 Gy and 100 Gy, respectively. The yields of 8-OH-Gua, FapyAde, 8-OH-Ade, 2-OH-Ade, 5-OH-Ura and 5-OHMe-Ura were significantly higher in mutant cells than in parent cells at 200 Gy and 400 Gy.

The results of this work present evidence, for the first time, for more abundant formation of DNA base products in radiation-sensitive mutant M10 cells than in their parent L5178Y cells. At the highest dose used, the differences in yields between the two cell lines varied from 1.5- to 3-fold, with formamidopyrimidines (FapyGua and FapyAde) having the highest difference of 3-fold. FapyGua was the major product in both cell lines. The GSH content of M10 cells was found to be lower than that of L5178Y cells, in agreement with the results reported previously (Nakazawa et al. 1982). These data, however, provide no information on the intracellular localization of GSH. DNA base products detected in the present work typically result from reactions of $\cdot\text{OH}$ with DNA bases. Only those hydroxyl radicals generated in the immediate vicinity of DNA should be able to react with DNA, because of the short diffusion distance of $\cdot\text{OH}$ in cells (Ward et al. 1985). Thus GSH may not be capable of interfering with reactions of $\cdot\text{OH}$ generated in the immediate vicinity of DNA in a cellular environment. Taken together, the difference between the GSH contents of L5178Y and M10 cell lines is unlikely to account for the observed greater DNA base damage in mutant cells than in parent cells. The reason for induction of greater base damage in M10 cells and whether this phenomenon plays a role in their radiation sensitivity remain to be determined.

This work has been published in *Radiation Research* (Mori and Dizdaroglu 1994).

IV. DNA BASE DAMAGE IN HUMAN LYMPHOBLASTOID CELLS AT LOW RADIATION DOSES

During the previous period of this project, we identified and quantified a number pyrimidine- and purine-derived DNA lesions in cultured human K562 cells (Nackerdien et al. 1992). More recently, we measured DNA base lesions in L5178Y mouse lymphoma cells (see above, Section 1.B.III, and Mori and Dizdaroglu 1994). In these studies, radiation doses above ≈ 50 Gy were required to measure significant increases in the product yields above intrinsic levels

of lesions in DNA. In this period of the project, we attempted to measure DNA base damage in human lymphoblastoid cells at low radiation doses such as 10 Gy. Preliminary results indicated that these cells may be more sensitive to ionizing radiation than cancer cells. The following Table shows some preliminary results:

product	amount (molecule/ 10^5 DNA bases)		
	control	10 Gy	50 Gy
5,6-diOH-Ura	4.67 $\pm$ 0.88	8.70 $\pm$ 0.54	26.8 $\pm$ 0.69
FapyAde	2.98 $\pm$ 0.31	4.14 $\pm$ 0.16	25.4 $\pm$ 2.36
2-OH-Ade	1.66 $\pm$ 0.09	2.51 $\pm$ 0.20	8.76 $\pm$ 0.72

This work is still in progress and will be continued if the project is renewed.

C. STUDIES OF DNA DAMAGE *IN VITRO*

I. MONOMERIC PRODUCTS FROM ADENINE, GUANINE AND THYMINE INDUCED BY EXPOSURE OF DNA TO ULTRAVIOLET RADIATION

The main biological effects of ultraviolet (UV) radiation result from its interaction with DNA in cells. Exposure of DNA to UV-radiation generates a number of products. The major photoproducts are pyrimidine dimers and the so-called pyrimidine photohydrates, mainly cytosine hydrates [reviewed in Fisher and Johns (1976a,b), Cadet and Vigny (1990)]. UV-radiation-induced formation of analogous purine products in DNA has not been reported. Although it is generally recognized that the deleterious effects of UV radiation can be attributed to the major dimeric products, there is evidence that other less frequently occurring photoproducts may also be mutagenic (LeClerc et al. 1984, Romac et al. 1989). In addition, some DNA repair enzymes recognize and cleave UV-irradiated DNA at sites of monomeric products occurring at any of the four DNA bases. However, the nature of guanine and adenine monomeric photoproducts cleaved by these enzyme activities remained unknown (Doetsch et al. 1988, Helland et al. 1986, Gossett et al. 1988). Thus, the identification of UV-radiation-induced purine photoproducts is an important step towards the characterization of such repair enzymes as well as in defining the role of these monomeric products in the deleterious effects of UV radiation. In this work, we studied the formation of monomeric products that may be generated in DNA upon UV-irradiation in addition to those previously known monomeric products.

Aerated DNA solutions were exposed to UV radiation of 254 nm or 310 nm at doses from 50 to 10000 J/m². Control and UV-irradiated DNA samples were analyzed by GC/MS-SIM for modified DNA bases. The formation of three products, namely FapyAde, FapyGua and 5-OH-5,6-diHThy, was observed at doses starting from 100 J/m². Nine other

products were also identified and quantified. However, their amounts were increased significantly over control levels at the highest dose 10000 J/m² only. Figure 7 illustrates the dose-yield plots of FapyAde in the dose range from 50 to 1000 J/m² and from 50 to 10000 J/m². Significant increases in the control amount of FapyAde were observed at the dose range from 100 to 10000 J/m². Above 200 J/m², the yield deviated from linearity reaching a plateau between the doses 5000 and 10000 J/m². At 310 nm, FapyAde was the only modified base formed in appreciable amounts (Figure 8). Figures 9 and 10 illustrate the dose-yield plots of FapyGua and 5-OH-5,6-diHThy, respectively. In contrast to FapyAde and FapyGua, a linear increase in the yield of 5-OH-5,6-diHThy in the entire dose range was observed. Dimethylsulfoxide (DMSO), a typical hydroxyl radical scavenger, at a concentration of 0.5 M had no effect on product yields.

The results of this study demonstrated for the first time that exposure of DNA in aqueous solution to far UV radiation at low doses causes formation of three monomeric base products, namely FapyAde, FapyGua and 5-OH-5,6-diHThy. These compounds are well known base products of DNA, which are produced in DNA upon exposure to ionizing radiation [reviewed in Téoule (1987) and Dizdaroglu (1982)]. In this work, however, the use of DMSO clearly excluded the involvement of $\cdot\text{OH}$ in product formation. Mechanisms underlying formation of FapyAde and FapyGua may be analogous to those proposed for hydration of pyrimidines (Fisher and Johns, 1976a,b, Cadet and Vigny, 1990). Hydration of the imidazole ring of purines across the N(7)-C(8) double bond with the hydroxyl group located at C(8) may yield purine hydrates 7-hydro-8-hydroxyadenine and 7-hydro-8-hydroxyguanine. These compounds being hemiorthoamides are sensitive to hydrolysis and may yield formamidopyrimidines FapyAde and FapyGua, respectively (Steenken 1989). The formation of 5-OH-5,6-diHThy may be an indication for alternative hydration of pyrimidines with the hydroxyl group located at C(5) of the pyrimidine ring. A number of previous studies have reported that DNA repair enzyme preparations recognize and cleave UV-irradiated DNA at sites of both adenine and guanine without elucidation of the nature of modifications (Doetsch et al. 1988, Helland et al. 1986, Gossett et al. 1988). Both FapyAde and FapyGua are substrates for the Fpg protein (Boiteux et al. 1992). Hence, the previously reported "A-cutter" and "G-cutter" activities on UV-irradiated DNA can now be accounted for by the presence of enzymes cleaving DNA at sites of UV-radiation-induced FapyAde and FapyGua.

In conclusion, the formation of three novel monomeric products was observed in DNA in aqueous solution upon exposure to UV-radiation at low doses. The formation of formamidopyrimidines indicates that hydration of purines in addition to well-known hydration of pyrimidines occurs in UV-irradiated DNA. It appears that thymine is also susceptible to hydration across the C(5)-C(6) double bond, but with the hydroxyl group located at C(5). Mechanisms proposed in the past for UV-radiation-induced hydration of pyrimidines may also hold for purines. Mechanistic aspects of formation of formamidopyrimidines and thymine hydration with the

Figure 7: Dose-yield plot of 4,6-diamino-5-formamidopyrimidine. A: in the range of 0-1 kJ/m² of UV irradiation at 254 nm, B: in the range of 0-10 kJ/m² of UV irradiation at 254 nm. Each data point represents the mean of three values that were obtained by measurement of three independently prepared DNA samples. Error bars represent standard deviations. 1 nmol of a modified base/mg of DNA corresponds to ≈ 32 modified bases per 10^5 DNA bases.

Figure 8: Dose-yield plot of 4,6-diamino-5-formamidopyrimidine in the range of 0-10 kJ/m² of UV irradiation at 310 nm. Other details as in Figure 7.

Figure 9: Dose-yield plot of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) [UV irradiation at 254 nm].

Figure 10: Dose-yield plot of 5-hydroxy-5,6-dihydrothymine (5-OH-5,6-diHThy) [UV irradiation at 254 nm].

hydroxyl group located at C(5) need to be investigated further.

This work has been accepted as an accelerated publication in *Biochemistry* (Doetsch et al. 1994).

D. STUDIES OF DNA REPAIR

Our measurement technique facilitates the investigation of the enzymatic repair of DNA damage. Because a magnitude of modified DNA bases are measured in the same DNA sample at the same time, it permits precise determination of excision (or nonexcision) of both pyrimidine- and purine-derived lesions from DNA by a DNA repair enzyme. Kinetics of excision can also be determined. Using this unique approach, which has been developed in my laboratory, we recently studied the substrate specificities of several DNA glycosylases. These enzymes remove modified bases from DNA by hydrolyzing the *N*-glycosylic bond between the sugar and the modified base as the first step in the base excision repair of DNA [reviewed in Sancar and Sancar (1988), Wallace (1988)]. In the previous three year period of this project, we had studied the substrate specificity of the *Escherichia coli* Fpg protein (formamidopyrimidine-DNA glycosylase). The results were published in *Biochemistry* (Boiteux et al. 1992) and also reported in the previous report of accomplishments. In this period, we examined several additional glycosylases including *E. coli* endonuclease III, uracil DNA *N*-glycosylase and T4 endonuclease V. The objective was to determine whether these enzymes possess any activity for the magnitude of modified bases that we can measure in irradiated DNA.

I. SUBSTRATE SPECIFICITY OF THE *E. COLI* ENDONUCLEASE III

Endonuclease III from *E. coli* has been shown to possess both a DNA *N*-glycosylase activity and an abasic (apurinic and/or apyrimidinic) site nicking activity (Demple and Linn 1980, Cunningham and Weiss 1985). The *nth* gene coding for endonuclease III has been cloned and sequenced (Cunningham and Weiss 1985). The *N*-glycosylase activity of endonuclease III removes a number of pyrimidine lesions from double-stranded DNA damaged by free radicals, oxidizing agents and ultraviolet radiation [reviewed in Sancar and Sancar (1988), Ramotor and Demple (1993)]. These lesions include urea, 5,6-dihydrothymine, thymine glycol, 5-hydroxy-5-methylhydantoin, methyltartronylurea, an unidentified hydroxydihydrothymine, an unidentified cytosine photoproduct, and thymine and uracil hydrates. Because of the analytical techniques used, the excision of only one or a limited number of lesions has been assessed at a time. Thus, a broad substrate specificity of this enzyme covering both pyrimidine- and purine-derived modified DNA bases has not been established. In this work, we studied the excision by *E. coli* endonuclease III of pyrimidine- and purine-

derived lesions from DNA, which was damaged by ionizing radiation in the absence or presence of oxygen.

Aliquots of irradiated DNA samples were incubated with either the native enzyme or the heat-inactivated enzyme and subsequently ethanol-precipitated. As controls, other aliquots of DNA samples were precipitated in the incubation buffer without prior treatment with the enzyme. All pellets were analyzed by GC/MS-SIM after acidic hydrolysis and trimethylsilylation. The supernatant fractions of the same samples were analyzed after lyophilization and trimethylsilylation without acidic hydrolysis. Sixteen and twelve modified bases were identified and quantified in DNA samples irradiated under anoxic and oxic conditions, respectively. These were 5,6-diHThy, 5,6-diHUra, 5-OH-5-MeHyd, alloxan, 5-OH-Hyd, 5-OH-Ura, 5-OH-Cyt, 5-OH-6-HThy, 5-OH-6-HUra, 5-OHMeUra, Thy glycol, 5,6-diOH-Ura, FapyAde, 8-OH-Ade, 2-OH-Ade, FapyGua and 8-OH-Gua. Under oxic irradiation conditions, 5,6-diHThy, 5,6-diHUra, 5-OH-6-HThy and 5-OH-6-HUra were not observed. The uracil derivatives 5,6-diHUra, 5-OH-Hyd, 5-OH-Ura, 5-OH-6-HUra and 5,6-diOH-Ura detected in hydrolyzates in DNA are products resulting from $\cdot$ OH attack on cytosine as previously reported (Téoule and Cadet 1978, Dizdaroglu 1985, Téoule 1987).

The results showed the excision of 5,6-diHThy, 5,6-diHUra, 5-OH-5-MeHyd, alloxan, 5-OH-6-HThy, 5-OH-6-HUra, Thy glycol and Ura glycol (detected as 5-OH-Ura). 5-OH-Ura detected in supernatant fractions may have resulted from dehydration of Ura glycol (and/or from deamination and dehydration of Cyt glycol). This means that Ura glycol (and/or Cyt glycol), not 5-OH-Ura has been excised by the enzyme. No excision of purine-derived lesions was observed. As examples, Figure 11 illustrates the results obtained with Ura glycol (detected as 5-OH-Ura) and 5,6-diHUra. The structures of the pyrimidine-derived lesions excised by endonuclease III are illustrated in Figure 12.

In conclusion, the results of this study showed that the *E. coli* endonuclease III excises a number of pyrimidine-derived DNA lesions formed by ionizing radiation-generated free radicals under anoxic or oxic conditions. We confirmed previously described excision of 5,6-diHThy, 5-OH-5-MeHyd and Thy glycol. We also discovered novel activities of endonuclease III to recognize and excise four cytosine-derived lesions and another thymine-derived lesion, extending its substrate specificity.

This work has been published in *Biochemistry* (Dizdaroglu et al. 1993).

II. A NOVEL ACTIVITY OF URACIL DNA N-GLYCOSYLASE

Uracil DNA N-glycosylase (UNG) excises uracil, which is formed in DNA by deamination of cytosine (Lindahl et al. 1977). Recently, UNG has been reported to recognize and excise an $\cdot$ OH-induced modified DNA base, namely 5-hydroxyuracil (Hatahet et al. 1994). An oligonucleotide containing 5-hydroxy-2'-deoxyuridine was used as

Figure 11. Excision of 5,6-dihydrouracil (5,6-diHUrA) and uracil glycol (Ura glycol) from DNA by endonuclease III. 1, unirradiated DNA; 2, γ -irradiated DNA; 3, γ -irradiated DNA after incubation with the inactivated endonuclease III; 4, γ -irradiated DNA after incubation with the native endonuclease III. Black bars: pellets; light bars: supernatant fractions. Error bars represent standard deviations (1 nmol of a modified base/mg of DNA corresponds to ≈ 32 modified bases per 10^5 DNA bases).

Figure 12. Structures of modified bases excised from DNA by endonuclease III (uracil derivatives may exist in DNA as their cytosine-derived analogues).

a substrate. In this work, we wished to determine whether UNG possesses activity for modified bases in irradiated DNA.

Irradiated DNA samples were treated with active or inactivated UNG and then analyzed as described above in the case of endonuclease III. The results showed that, of the multitude of base lesions, only 5,6-dihydroxyuracil was excised from DNA by active UNG. 5,6-Dihydroxyuracil is the enol form of isodialuric acid [6-hydroxy-2,4,5(1*H*,3*H*,6*H*)-pyrimidinetrione], which is the prevalent form in aqueous solution or in the crystalline state (Behrend and Roosen 1889). Isodialuric acid is converted into its enol form during derivatization, and thus it is detected as 5,6-dihydroxyuracil by GC/MS (Dizdaroglu 1985). In this work, 5,6-dihydroxyuracil was detected in DNA pellets as well as in the supernatant fractions. Its cytosine-derived analogue 5,6-dihydroxycytosine was not observed.

Figure 13 (upper graph) shows the amounts of isodialuric acid in pellets and supernatant fractions. The amount of isodialuric acid excised from DNA by active UNG corresponded to its amount found in the supernatant fractions. Detectable levels of isodialuric acid were also detected in the supernatant fractions of the samples incubated without the enzyme or with inactivated enzyme. However, the amounts were significantly smaller than that found in the supernatant fractions of the samples incubated with the active enzyme. Middle graph in Figure 13 illustrates the time course of excision of isodialuric acid by active UNG. The excision progressed up to 1 h of incubation, reaching a plateau thereafter. A plot of these data with time versus the logarithm of the ratio of the initial amount (a_0) to the remaining amount (a) of isodialuric acid in DNA shows that the excision follows first-order kinetics (Figure 13, lower graph). The excision was also determined as a function of the substrate concentration. Figure 14 (upper graph) shows a Lineweaver-Burk plot of the data obtained. This plot indicated an apparent K_m of ≈ 85 nM and an apparent V_{max} of ≈ 0.44 nM/min. Lower graph in Figure 14 illustrates the excision of isodialuric acid as a function of the enzyme amount.

The results of this work provided evidence for a novel activity of UNG that excises isodialuric acid (5,6-dihydroxyuracil) from DNA. The results also showed that fifteen other modified bases, which are produced in DNA along with isodialuric acid, are not substrates for UNG. The excision of isodialuric acid means that the non-aromaticity of a substrate may not be a limiting factor for UNG. Previous work has shown that isodialuric acid was not a substrate for *N*-glycosylases such as *E. coli* Fpg protein or endonuclease III, although the majority of the DNA base lesions were substrates for these enzymes (see above and Boiteux et al. 1992, Dizdaroglu et al. 1993). Isodialuric acid is a product of cytosine, which is formed in DNA by reactions of $\cdot\text{OH}$ [reviewed in Téoule and Cadet (1978), Téoule (1987), and Dizdaroglu (1992)] or by non-radical pathways upon exposure of DNA to oxidizing agents (Dizdaroglu et al. 1986, Akman et al. 1990). Recently, we reported the formation of this product in genomic DNA of cultured mammalian cells [reviewed in Dizdaroglu (1992)] and in genomic DNA of animals

isodialuric acid

5,6-dihydroxyuracil

Figure 13. Excision of isodialuric acid (5,6-dihydroxyuracil) from DNA by UNG, **upper graph:** 1, unirradiated DNA; 2, irradiated DNA; 3, irradiated DNA after incubation with the inactivated UNG; 4, irradiated DNA after incubation with the native UNG. Black bars: pellets; light bars: supernatant fractions, **middle graph:** time course of excision, **lower graph:** time course of excision where a_0 and a are the initial amount and the remaining amount of isodialuric acid in DNA, respectively. Error bars represent standard deviations ($n = 3$).

Figure 14. Excision of isodialuric acid (5,6-dihydroxyuracil) from DNA by UNG, **upper graph:** Lineweaver-Burk plot of the excision as a function of the substrate concentration, $[P]$: concentration of the excised isodialuric acid, $[S]$ initial concentration of isodialuric acid, **lower graph:** the excised amount of isodialuric acid as a function of the enzyme amount.

in vivo (Toyokuni et al. 1994, Kasprzak et al. 1992, 1994) that were exposed to agents causing oxidative DNA damage. Moreover, chromatin samples isolated from human cancerous tumors have been found to contain this compound in greater amounts than those isolated from surrounding normal tissues (Olinski et al. 1992, Jaruga et al. 1994). The biological effects of isodialuric acid are not known. The fact that a repair enzyme exists for its efficient removal from DNA may attest to its blocking of DNA synthesis or miscoding during DNA synthesis. The base-pairing characteristics of this compound and the mechanisms underlying its excision by UNG remain to be determined.

This work has been carried out in collaboration with Dr. Paul W. Doetsch of the Emory University, Atlanta, Georgia. It has been submitted to *Biochemistry* for publication (Zastawny et al. 1994).

III. A NOVEL ACTIVITY OF T4 ENDONUCLEASE V

In UV-radiation-resistant organisms such as *M. luteus* and phage T4-infected *E. coli*, a DNA repair enzyme, which specifically cleaves UV-irradiated DNA at pyrimidine dimers, has been discovered [reviewed in Friedberg et al. 1981, Sancar and Sancar (1988)]. This enzyme named T4 endonuclease V hydrolyses the glycosylic bond of the 5'-pyrimidine of the dimer and then the phosphodiester bond at the 3'-side of the generated apyrimidic site. Studies indicated a strict specificity of the enzyme activity for UV-radiation-induced dimers in DNA. Thus far, no other activity of this enzyme has been reported.

In this work, we wished to determine whether T4 endonuclease V possesses activity for any monomeric base lesions in DNA, which are produced by ionizing radiation or by UV-radiation. Irradiated DNA samples were incubated with native or inactivated T4 endonuclease V and then analyzed by GC/MS-SIM. As a result, we discovered a novel activity of this enzyme that excises 4,6-diamino-5-formamidopyrimidine (FapyAde) from DNA. Other modified bases that were present in DNA along with FapyAde were not recognized by this enzyme. Figure 15 (upper graph) illustrates the excision of FapyAde from γ -irradiated DNA. The time course of excision by native T4 endonuclease V is shown in lower graph in Figure 15. The excision progressed up to 2 h of incubation, with the excised amount of FapyAde remaining constant thereafter. Figure 16 (upper graph) shows that the excision of FapyAde by T4 endonuclease V follows first-order kinetics. The excision was also determined as a function of the substrate concentration. A Lineweaver-Burk plot of the data is shown in Figure 16 (lower graph). This plot indicated an apparent K_m of ≈ 1600 nM and an apparent V_{max} of ≈ 4 nM/min.

We concluded from this work that T4 endonuclease V possesses a specificity not only for UV-radiation-induced dimers, but also for a UV-radiation-induced monomeric product in DNA. FapyAde is also a substrate for the Fpg protein (Breimer and Lindahl 1985,

Boiteux et al. 1992). FapyAde is a typical $\cdot\text{OH}$ -induced product of adenine in DNA [reviewed in Steenken (1989), Dizdaroglu (1992)]. As was mentioned above, we recently discovered the formation of this compound in DNA by far UV-radiation (254 nm and 310 nm) at low doses. We also demonstrated the formation of FapyAde in genomic DNA of cultured cells [reviewed in Dizdaroglu (1992)], and of animals *in vivo* (Mori et al. 1993, Toyokuni et al., 1994; Kasprzak et al., 1992, 1994) that were exposed to ionizing radiation or other DNA damaging agents. Moreover, human cancerous tumors have been found to contain FapyAde in greater amounts than surrounding normal tissues (Olinski et al. 1992, Jaruga et al. 1994). The biological significance of FapyAde is not known. The existence of two repair enzymes for its excision from DNA may be indicative of its biological importance.

This work is in preparation for publication. It has been carried out in collaboration with Dr. Stephen Lloyd of the Texas University Medical Branch at Galveston, Texas.

Figure 15. Excision of FapyAde from DNA by T4 endonuclease V. **upper graph:** 1, unirradiated DNA; 2, irradiated DNA; 3, irradiated DNA after incubation with the inactivated T4 endonuclease V; 4, irradiated DNA after incubation with the native T4 endonuclease V. Black bars: pellets; light bars: supernatant fractions. **lower graph:** time course of excision. Error bars represent standard deviations ($n = 3$).

Figure 16: Excision of FapyAde from DNA by T4 endonuclease V, **upper graph:** time course of excision where a_0 and a are the initial amount and the remaining amount of FapyAde in DNA, respectively, **lower graph:** Lineweaver-Burk plot of the excision as a function of the substrate concentration, $[P]$: concentration of the excised FapyAde, $[S]$ initial concentration of FapyAde.

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F. PUBLICATIONS ACKNOWLEDGING THE SUPPORT FROM THE DEPARTMENT OF ENERGY IN THIS PERIOD OF THE PROJECT

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