Proc. Nat. Acad. Sci. USA
Vol. 71, No. 5, pp. 2022–2026, May 1974

The Enzymatic Release of O^6-methylguanine and 3-methyladenine from DNA Reacted with the Carcinogen *N*-methyl-*N*-nitrosourea

(DNA repair/methylnitrosourea/endonuclease II/chemical carcinogenesis)

D. M. KIRTIKAR AND D. A. GOLDTHWAIT

Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

Communicated by Harland G. Wood, January 29, 1974

ABSTRACT **Endonuclease II (deoxyribonucleate oligonucleotidohydrolase, EC 3.1.4.30) of *Escherichia coli* has been shown to break phosphodiester bonds in alkylated DNA and depurinated DNA. The hypothesis that depurination is a step in the mechanism of the reaction with alkylated DNA is supported by *in vitro* experiments with DNA reacted with *N*-methyl-*N*-nitrosourea. Endonuclease II releases O^6-methylguanine and 3-methyladenine, but not 7-methylguanine, from DNA that has been methylated by the carcinogen *N*-methyl-*N*-nitrosourea.**

Endonuclease II (deoxyribonucleate oligonucleotidohydrolase, EC 3.1.4.30) of *Escherichia coli* is an enzyme capable of breaking phosphodiester bonds in DNA which has been reacted with the alkylating agent methyl methanesulfonate (MMS) (1–3). MMS-treated DNA contains 7-methylguanine and 3-methyladenine. Endonuclease II also recognizes depurinated and depurinated-reduced sites in DNA (4) and at high concentrations makes a limited number of single-strand breaks in native DNA from T-4 and T-7 bacteriophages (5). The enzyme hydrolyses the phosphodiester bonds in native DNA to yield 5′-phosphomonoesters (5), and indirect evidence has been presented to support the proposal that enzyme-induced chain breaks are on the 5′-phosphate side of the depurinated reduced sites (5). Because the enzyme recognizes both alkylated and depurinated sites in the DNA, it was postulated that the process of depurination was an intermediate step prior to phosphodiester bond cleavage of alkylated DNA. Enzymatic depurination by the endonuclease II preparation has now been demonstrated. This has allowed us to observe a specificity of the enzyme for some but not all of the methylated bases. This paper describes the ability of the enzyme to release O^6-methylguanine and 3-methyladenine, and the inability of the enzyme to release 7-methylguanine from DNA that has been methylated by the carcinogen *N*-methyl-*N*-nitrosourea (MNU).

MATERIALS AND METHODS

[^{3}H]Dimethyl sulfate (DMS) (specific activity 384 mCi/mmol) and [^{3}H]*N*-methyl-*N*-nitrosourea (specific activity 48 mCi/mmol) were obtained from New England Nuclear Corp., Boston. Unlabeled MNU was synthesized according to Lawley and Shah (6). Unlabeled and [^{3}H]thymine-labeled DNA from bacteriophage T4 (T4 DNA) was prepared as described previously (7). Endonuclease II was prepared and purified from *E. coli* JC 4583, using the procedure of Hadi *et al.* (5). The specific activity of the enzyme preparation used was 95 μmol of DNA released per mg per hr.

The cation exchange resin AG50W-X4 (200-400 mesh), hydrogen form, was from Bio-Rad Laboratories, California. Eastman chromagram sheets (20 × 20 cm, no. 6065, cellulose with fluorescent indicator) for thin-layer chromatography were obtained from Eastman Organic Chemicals, Eastman Kodak Co. For paper chromatography, Whatman 3MM chromatography paper was used. 1-Methyladenine, 3-methyl adenine, 7-methyladenine, and 7-methylguanine were obtained from Cyclo Chemical, Travenol Laboratories, Inc., California. Adeninehydrochloride, guanine, and thymine were from Sigma Chemical Co., St. Louis, Mo. O^6-methylguanine was a gift from Dr. P. D. Lawley.

Methylation of T4 DNA with [^{3}H]DMS was done according to Smith *et al.* (8) at a DMS to DNA nucleotide molar ratio of 7:1 and according to Uhlenhopp and Krasna (9) at a ratio of 10:1. For methylation of DNA with MNU, the procedure of Lawley and Shah (6) was followed. The unlabeled or [^{3}H]-thymine-labeled T4 DNA in 0.2 M Tris · HCl buffer (pH 8.0) was treated in the dark with labeled or unlabeled MNU at 37°. After a 1-hr incubation, cold solutions of sodium acetate (2.5 M, 0.1 volume) and ethanol (95%, 2 volumes) were added and the tube contents mixed gently. After 1 or 2 hr at 0°, the DNA was spooled out on a glass rod and washed 5 or 6 times with small aliquots of cold 95% ethanol. Methylated DNA was solubilized in 0.05 M Tris · HCl buffer (pH 8.0) at 0°. The DNA was stable for at least 2 weeks at 4°, as judged by single-strand breaks.

The paper chromatographic separation of methylated bases was as follows. Whatman 3MM paper and the solvent of Lawley and Thatcher (10) [2-propanol:concentrated $NH_4OH:H_2O$ (7:1:2) by volume] were used. Alcohol-soluble material, from the reaction mixture of DNA incubated with or without endonuclease II, was mixed with authentic methylated bases and chromatographed. Bases were identified by UV absorption and the radioactivity was determined. No correction was made for quenching. Recovery of radioactivity from paper chromatograms was usually around 50%.

The thin-layer chromatography was done as follows. The solvent system [2-methyl-1-propanol:0.8 M boric acid:14.8 M NH_4OH (100:14:0.4, by volume)] is a modification of the solvent used in the paper chromatographic separation of methylated ribonucleotides by Al-Arif and Sporn (11). The R_F values of the methylated and nonmethylated bases in this system relative to adenine (R_F = 1.0) are as follows: guanine,

---

Abbreviations: MNU, *N*-methyl-*N*-nitrosourea; MMS, methyl methanesulfonate; DMS, dimethyl sulfate; T4 DNA, DNA from bacteriophage T4; EDTA, ethylenediaminetetraacetic acid.

TABLE 1. *Enzymatic release of 3-methyladenine from DNA treated with [³H]DMS*

Experiment	Method	Endonuclease II present	Counts recovered 7-methylguanine	3-methyladenine
1	Paper chromatography (10)	−	7,100	4,080
		+	7,640	17,320
2	Column chromatography (6)	−	13,100	5,150
		+	12,800	15,200

The incubation mixtures contained per 0.5 ml, 21.6 nmol of T4 DNA methylated with [³H]DMS (specific activity 9453 cpm/nmol of nucleotide with 11.2 nmol of [³H]methyl per mol of DNA nucleotide), 1×10^{-4} M β-mercaptoethanol, 1×10^{-4} M 8 hydroxyquinoline, 5×10^{-2} M Tris·HCl (pH 8.0), and 0.2 units enzyme where indicated. After 30 min at 37°, ethylenediaminetetraacetic acid (EDTA) at a final concentration of 2×10^{-2} M was added. The DNA from each incubation mixture was precipitated at 0° with 33% ethanol in the presence of 50 μg of unlabeled T4 DNA and 0.5 M sodium chloride.

Experiment 1. An aliquot of 50 μl from the alcohol soluble fraction was chromatographed with added methylated bases. The numbers given represent the total counts in the alcohol-soluble fraction recovered with the specific alkylated bases.

Experiment 2. The total alcohol-soluble fraction was chromatographed with added alkylated bases. The total counts recovered in the peaks containing the indicated bases are given. For details of the two chromatographic procedures, see *Materials and Methods.*

−, no enzyme present.

+, enzyme present.

0.328; 1-methylguanine, 0.487; 7-methylguanine, 0.578; 1-methyladenine, 0.603; 7-methyladenine, 0.725; 3-methyladenine, 0.856; adenine, 1.000; 9-methyladenine, 1.081; O^6-methylguanine, 1.261; thymine 1.30; and O^6-methyl-2-deoxyguanosine, 1.40. UV-absorbing spots were cut out and the cellulose powder was scraped off and extracted with 0.1 or 0.2 ml of 0.001 N HCl. Then 0.05 ml of concentrated NH_4OH was added and the radioactivity was determined in an aliquot. Recovery of radioactivity from plates varied from 73 to 82%.

Column chromatography was as follows. The procedure of Lawley and Shah (6) was used. Dowex 50 AG 50W (X4, H^+ form, 200-400 mesh) was converted to NH_4^+ form, and a column of 60 cm × 1.5 cm was equilibrated with 0.3 M ammonium formate (pH 6.65). Alcohol-soluble material and acid hydrolysates of alcohol-insoluble material from DMS-treated or MNU-treated DNA samples, with or without endonuclease treatment, were chromatographed with 3 mg of each of the authentic methylated bases. The column was eluted with 0.3 M ammonium formate (pH 6.65), 110 fractions of 6.4–6.5 ml each, and then with 1.0 M ammonium formate (pH 6.65). The UV absorbance at 260 nm of each fraction was determined. The position of each marker base on the column was confirmed by comparing the UV spectrum of the peak fractions with authentic base solutions. For the radioactivity determinations, 0.5-ml aliquots were counted. Recovery of radioactivity from the column was about 80–90%. The ratio of counts to UV absorption for the fractions containing either O^6-methylguanine or 3-methyladenine was constant. The ratios for the fractions containing 7-methylguanine and 7-methyladenine were not constant because unlabeled adenine, as well as the unlabeled 7-methylpurines, had been added.

TABLE 2. *Analysis of methylated bases in DNA reacted with increasing concentrations of MNU*

Areas of thin-layer chromatography	Ratio of MNU to DNA nucleotide 1:1	5:1	10:1	25:1	50:1	100:1
	(Percentage of total counts recovered)					
Origin	0.58	1.46	0.92	0.232	0.384	0.311
I	0.288	0.17	0.306	0.232	0.165	0.0622
II	5.28	7.3	3.06	1.54	1.095	0.622
7-methyl guanine	48	60.8	73.3	73.3	71.7	74.6
1-methyl-adenine	1.44	1.58	2.29	3.08	5.47	6.85
7-methyl-adenine	0.624	2.44	1.53	1.31	2.19	1.24
3-methyl-adenine	9.13	7.3	12.22	10.08	15.11	11.9
O^6-methyl-guanine	9.13	7.8	3.06	1.93	2.19	2.95
III	25.455	11.204	3.214	2.468	2.119	1.461

Unlabeled T4 DNA (250 nmol) in 1.0 ml was reacted (see *Materials and Methods*) with [³H]MNU (specific activity 1 mCi/mM) at the ratios indicated. The yield of radioactivity in alkylated DNAs was 74–82%. The alkylated DNA samples after alcohol precipitation and washings were solubilized in 0.1 ml of 0.05 M Tris·HCl (pH 8.0) at 0°. Aliquots of 10 μl were hydrolyzed with an equal volume of 0.2 N HCl at 70° for 30 min. Methylated bases were added and the mixtures were chromatographed on thin-layer chromatography plates (see *Materials and Methods*). The R_F values of areas I, II, and III relative to adenine were 0.2, 0.3, and >1.3, respectively. Areas I and II were between the origin and 7 methylquanine and area III was the area between O^6-methylguanine and the solvent front.

Determination of DNA single-strand breaks was done by centrifugation in alkaline sucrose density gradients (3). The number average molecular weight (M_n) was calculated. *E. coli* rRNA was used as a marker and the S value of each fraction was calculated according to Studier (12). The molecular

TABLE 3. *Single-strand breaks in DNA treated with MNU as a function of enzyme concentration*

Endonuclease II units	Total single-strand breaks	Enzyme-induced breaks
0	81	—
0.030	112	31
0.075	190	109
0.150	197	116
0.225	216	135
0.300	289	208

[³H]Thymine-labeled T4 DNA (1850 nmol, specific activity about 400 ³H cpm/nmol) in 2.0 ml was reacted with unlabeled MNU at a ratio of 10:1. Incubation mixtures of 0.25 ml contained 24 nmol of [³H]MNU-treated T4 DNA, 1×10^{-4} M β-mercaptoethanol, 1×10^{-4} M 8-hydroxyquinoline, 5×10^{-2} M Tris·HCl (pH 8.0), and 0.03–0.3 units of enzyme where indicated. After 60 min at 37°, the reaction was terminated by adding EDTA and sodium dodecyl sulfate to a final concentrations of 2×10^{-2} M and 0.25%, respectively. An aliquot of 0.25 ml was centrifuged through 3.6 ml of 5–20% alkaline sucrose density gradients at 32,000 rpm at 20° for 3 hr in an SW 56 rotor. Molecular weights and single-strand breaks were calculated as described in *Materials and Methods.*

TABLE 4. *The percent of the total counts present on a thin-layer chromatogram recovered in different bases as a function of enzyme concentration*

Enzyme units	0	0	0.02	0.04	0.08	0.20
Incubation temperature	0°	37°	37°	37°	37°	37°
Incubation time	0′	60′	60′	60′	60′	60′
Area of thin-layer chromatogram			*Percent of total counts*			
Origin	92.9	84.60	78.10	71.0	65.40	61.60
I	0.09	0.76	0.46	0.22	0.47	0.39
7-methylguanine	2.87	5.97	6.00	5.90	5.93	5.70
1-methyladenine	0.30	0.58	0.99	1.47	1.72	1.99
7-methyladenine	0.16	0.42	0.36	0.55	1.11	1.60
3-methyladenine	2.12	4.64	9.59	15.36	18.80	19.40
II	0.26	0.67	1.25	1.14	1.42	1.74
O^6-methylguanine	1.07	1.94	2.43	4.20	4.95	7.41
III	0.23	0.28	0.55	0.32	0.35	0.29

Unlabeled T4 DNA (3000 nmol) in 2.0 ml was reacted with [^{3}H]MNU (specific activity 48 mCi/mM) at MNU:DNA nucleotide ratio of 7:1; after alcohol precipitation and washing (see *Materials and Methods*), the alkylated DNA was solubilized in 1.0 ml of 0.05 M Tris·HCl (pH 8.0) at 0°. For enzymatic hydrolysis, 0.1 ml reaction mixtures containing 20 nmol [^{3}H]-MNU-T4 DNA (specific activity 2500 ^{3}H cpm/nmol of nucleotide, with 23.7 mmol of [^{3}H]methyl per mol of DNA nucleotide), 1×10^{-4} M β-mercaptoethanol, 1×10^{-4} M 8 hydroxyquinoline, 5×10^{-2} M Tris·HCl (pH 8.0), and enzyme as indicated were incubated at 37° for 1 hr. The reactions were terminated with EDTA at a final concentration of 0.02 M. The aliquot of 20 μl (approximately 5000 ^{3}H cpm), supplemented with methylated bases, from each sample was chromatographed separately on thin-layer sheets (see *Materials and Methods*). Area I was the area with an R_F relative to adenine of 0.2–0.3; area II had an R_{F_A} of 0.981; and area III was the area from the O^6-methylguanine spot to the solvent front. The total counts recovered from all the sections of the thin-layer plate were taken as 100%.

weight of 59.5×10^6 (13) was used for the T4 DNA single strands. Radioactivity was determined by use of a Packard Tri-Carb Liquid Scintillation Counter.

RESULTS

The enzymatic depurination of alkylated bases was observed first with DNA reacted with [^{3}H]DMS. This reagent methylates the N-3 position of adenine and the N-7 position of guanine in an approximate ratio of 1:4 (10). Table 1 shows that when DNA, labeled with DMS, was incubated with endonuclease II, there was release of 3-methyladenine, but there was no significant release of 7-methylguanine. This enzymatic release of 3-methyladenine into an alcohol-soluble form was detected by isolation of the methylated bases either by paper or column chromatography. There was considerable nonenzymatic release of both the adenine and guanine derivatives due to the increased lability of the glycosidic bond when the bases are alkylated. The bond of the adenine derivative is more labile than that of the guanine derivative (14).

TABLE 5. *The percent of the total counts present on a thin-layer chromatogram which were recovered in different bases as a function of time*

	Endonuclease II present	Incubation time (min)				
		0	15	30	45	60
Origin	−	85.6	81.0	75.5	70	68.2
	+	86.0	75.1	70.0	61	53.6
I	−	0.30	0.28	0.95	0.98	1.25
	+	0.14	0.40	1.08	1.22	1.92
7-methylguanine	−	7.03	8.90	9.45	14.00	15.9
	+	7.16	8.20	9.00	13.95	13.70
1-methyladenine	−	0.09	0.45	0.85	0.98	0.68
	+	0.23	0.62	1.38	1.83	2.37
7-methyladenine	−	1.70	2.36	3.50	3.16	3.28
	+	1.80	3.78	4.00	4.68	4.65
3-methyladenine	−	2.80	3.56	4.87	6.00	5.16
	+	2.88	7.20	7.46	10.60	13.40
II	−	—	0.59	0.59	0.65	0.66
	+	—	0.65	1.08	—	2.02
O^6-methylguanine	−	1.48	2.10	2.38	3.23	3.05
	+	1.58	2.90	4.40	4.94	5.04

The incubation mixtures of 0.1 ml contained 20 nmol of [^{3}H]MNU-treated T4 DNA (specific activity 2500 ^{3}H cpm/nmol of nucleotide; alkylated as described in Table 4), 1×10^{-4} M β-mercaptoethanol, 1×10^{-4} M 8-hydroxyquinoline, 5×10^{-2} M Tris·HCl (pH 8.0), and 0.2 units of enzyme where indicated. The reactions were terminated with EDTA after 0-, 15-, 30-, 45-, and 60-min incubations at 37°. The contents were concentrated about 4-fold under vacuum at 20°. Aliquots of 20 μl containing approximately 5000 cpm plus alkylated bases were subjected to thin-layer chromatography. Area I was a UV spot with R_{F_A} 0.2–0.3. Area II was a UV spot with R_{F_A} 0.98. These have not been identified.

−, no enzyme present.

+, enzyme present.

MNU is a very strong mutagen and carcinogen while MMS and DMS are weak mutagens and carcinogens (15–17). All three agents react with DNA to give 3-methyladenine and 7-methylguanine. However, MNU also reacts to give O^6-methylguanine while MMS and DMS do not produce levels that are detected easily (18). Experiments were therefore done with [*methyl*-^{3}H]MNU. This compound in high concentration produced many single strand breaks, in the absence of endonuclease II; at increasing ratios of MNU:DNA nucleotide, increasing MNU-induced strand breaks were observed: 1:1, 7 breaks; 5:1, 48 breaks; 10:1, 75 breaks; 15:1, 101 breaks; and 30:1, 152 breaks. A ratio of MNU to DNA nucleotide of 10:1 was used for the enzymatic experiments.

The relative ratios of bases methylated with [^{3}H]MNU were examined by gentle acid hydrolysis (10) and thin-layer chromatography. The results in Table 2 show that at low ratios of MNU to DNA nucleotide there is more alkylation of the O-6 position of guanine relative to the other derivatives than at high ratios. Alkylation of the N-1 and N-7 positions of adenine was also observed. Radioactive compounds present on the chromatogram from the origin to the position of 7-methylguanine (R_{F_A} 0.2–0.3) and also beyond O^6-methylguanine ($R_{F_A} > 1.3$) have not been identified.

The production of single-strand breaks as a function of enzyme concentration was studied by determining the number average molecular weight of the DNA in sucrose gradients be-

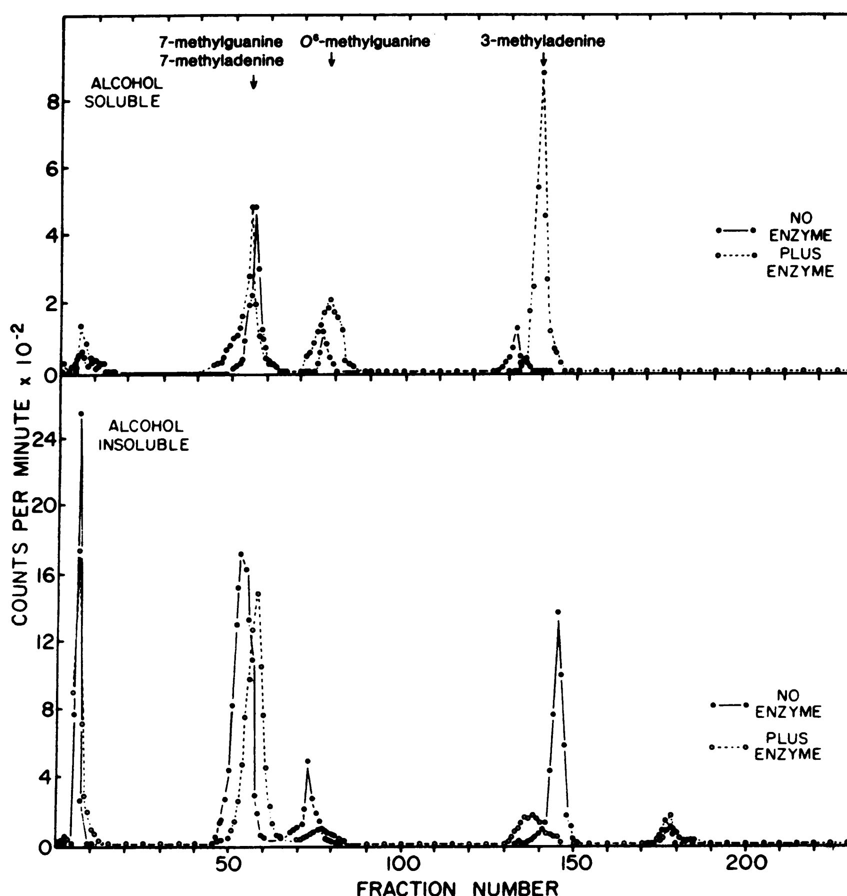

FIG. 1. The nonenzymatic and enzymatic release of alkylated bases (alcohol-soluble fraction) from DNA (alcohol-insoluble fraction) treated with [^{3}H]MNU. The incubation mixtures of 0.5 ml contained 100.0 nmol of [^{3}H]MNU-treated T4 DNA (alkylated as described in Table 4; specific activity 2500 cpm/nmol of nucleotide), 1×10^{-4} M β-mercaptoethanol, 1×10^{-4} M 8-hydroxyquinoline, 5×10^{-2} M Tris·HCl (pH 8.0), and 1.0 unit of enzyme (where indicated). After 60 min at 37°, the reactions were terminated as in Table 4. The DNA was precipitated with 33% ethanol in the presence of about 60 μg of unlabeled T4 DNA and 0.25 M sodium acetate at 0°. Methylated bases were added to each fraction which was then chromatographed on a Dowex AG 50W-X-4 [NH_4^+] column as described in *Materials and Methods*. In the alcohol-soluble fraction, radioactivity was found in the void volume amounting to 0.8% and 1.6% of the total radioactivity of the nonenzyme and enzyme-treated material, respectively; the respective values for the alcohol-insoluble fraction were 16.3% and 18.4%. Counts were also isolated in 1-methyladenine only in the alcohol-insoluble fraction which amounted to 2.0% and 3.1% for the non-enzyme and the enzyme-treated material, respectively. (The numbers on the ordinate have been multiplied by 10^{-2}.)

fore and after treatment with endonuclease II. The results in Table 3 show that with increasing enzyme concentration there are increasing numbers of single-strand breaks. No single-strand breaks occur in native DNA under these conditions until the level of enzyme exceeds 0.075 units (5).

The release of bases as a function of enzyme concentration is shown in Table 4. In this experiment, an aliquot of the total reaction mixture was placed on the thin-layer plate. The enzymatic release of both 3-methyladenine and O^6-methylguanine is apparent, as well as the release of 1-methyladenine and 7-methyladenine. No release of 7-methylguanine was catalyzed by the enzyme. O^6-methylguanine, but not O^6-methyl deoxyguanosine, was liberated by the enzyme as determined by thin-layer chromatography.

The release of bases as a function of time is shown in Table 5. Again, the enzymatic release of 3-methyladenine, O^6-methylguanine, 1-methyladenine, and 7-methyladenine is evident. When the enzyme-catalyzed release of each base was calculated and plotted as a function of time, the release of 3-methyladenine occurred at a rate approximately four times that of the other bases.

The stoichiometry of the enzymatic reaction was examined using a column chromatographic procedure for the identification of the methylated bases. The bases isolated in the alcohol-soluble and insoluble fractions in the absence and presence of enzyme are shown in Fig. 1. Table 6 is a summary of the data. It will be seen that the amounts of O^6-methylguanine and 3-methyladenine released from the alkylated DNA by the enzyme are balanced by the amounts of these bases disappearing from the DNA. Some enzymatic release of 7-methyladenine (Tables 4 and 5) could account for the change in the column fractions which contain both this base and 7-methylguanine.

DISCUSSION

Enzymatic recognition of alkylated DNA and subsequent phosphodiester bond hydrolysis was observed in extracts first by Strauss (19). The purification of endonuclease II of *E. coli* in our laboratory was pursued initially because of the desire to find a non-specific nuclease which might be involved in recombination (3). MMS was used as the alkylating agent and evidence was presented to support the hypothesis that

TABLE 6. *Stoichiometry of the enzyme reaction with DNA alkylated with MNU—a summary of the data in Fig. 1*

	Percentage of the total counts in the reaction mixture					
	Alcohol-soluble fraction			Alcohol-insoluble fraction		
Fractions	− Enzyme	+ Enzyme	Δ	− Enzyme	+ Enzyme	Δ
O^6-methylguanine	1.4	6.8	+5.4	7.8	2.8	−5.0
3-methyladenine	1.8	13.7	+11.9	21.1	7.0	−14.1
7-methylguanine plus 7-methyladenine	7.0	9.0	+2.0	41.8	37.8	−4.0

−Enzyme, without endonuclease II in the reaction mixture.
+ Enzyme, with endonuclease II in the reaction mixture.

the enzyme recognized alkylated DNA bases (2). Further studies showed that the enzyme also recognized depurinated DNA (4). The hypothesis that depurination was an intermediate step in phosphodiester bond breakage by this enzyme preparation is supported by the data presented in this paper. The liberation of methylated bases on incubation of [*methyl*-^{3}H]DNA with the enzyme preparation, under conditions which result in phosphodiester bond cleavage, has been demonstrated. However, we have not yet established the stoichiometry of bases released versus phosphodiester bonds broken. It is also not yet clear whether the 1600-fold purified enzyme preparation is a single enzyme with two sites, one for depurination and the second for phosphodiester bond breakage. Alternatively, the preparation could contain two separate enzymes which have remained together throughout the extensive purification. The latter hypothesis might explain the findings of Verly *et al.* (20, 21), who have used our procedure for purification but who claim that the preparation is active only on depurinated DNA. The activity of the enzyme on 3-methyladenine only is an alternative explanation.

The enzyme has specificity for some of the alkylated bases but does not recognize others. *In vivo* studies by Lawley and Orr (22) showed that 7-methylguanine was not removed from DNA of *E. coli* reacted with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, but that 3-methyladenine and O^6-methylguanine were excised. The *in vitro* data presented here are comparable with the *in vivo* results of Lawley and Orr (22), and also of Prakash and Strauss (23) who observed that *B. subtilis* was able to go through several generations without loss of alkyl groups (presumably 7-methylguanine). An increased rate of release of 3-methyladenine was observed *in vivo* (22) as well as in our *in vitro* experiments. Specificity of an *E. coli* preparation had been observed for 3-alkyladenine, and not 7-alkylguanine by Papirmeister *et al.* (24). O^6-methylguanine is found in DNA alkylated with MNU but only to a small extent in DNA alkylated with MMS (25). MNU is a strong mutagen and carcinogen while MMS acid has weak activity. This association made by Loveless (18) and supported by the demonstration of Gerchman and Ludlum (26) that there is aberrant base pairing with a polymer containing O^6-methylguanine suggests that the mechanism of carcinogenesis by MNU is through a mutation by improper base pairing during DNA replication. If this is the case, then endonuclease II is the first defined enzyme to recognize a potentially carcinogenic base in DNA.

Unpublished experiments in this laboratory* indicate that endonuclease II also recognizes lesions in DNA produced by X-irradiation. It is probable that *Micrococcus luteus* extracts, which have been observed by Setlow and Carrier (27) to recognize x-irradiated DNA, contain an enzyme with properties similar to endonuclease II. Careful studies by Minton and Friedberg† have shown that the preparation of endonuclease II used for the experiments presented here does not recognize damage in DNA due to high levels of UV irradiation. It is apparent that endonuclease II of *E. coli* represents a new DNA repair system which may be specific for purines. An investigation of the action of the enzyme on DNA reacted with other carcinogens is underway.

This research was supported by grants from the NIH (CA-11322) and the Health Fund of Greater Cleveland. D.A.G. is an NIH Research Career Investigator (K06-GM—21,444). The authors wish to thank Dr. P. D. Lawley for many helpful suggestions and Dr. A. Al-Arif for extensive help in the synthesis and handling of MNU.

1. Friedberg, E. C. & Goldthwait, D. A. (1968) *Cold Spring Harbour Symp. Quant. Biol.* **33**, 271–275.
2. Friedberg, E. C. & Goldthwait, D. A., (1969) *Proc. Nat. Acad. Sci. USA* **62**, 934–940.
3. Friedberg, E. C., Hadi, S. M. & Goldthwait, D. A., (1969) *J. Biol. Chem.* **244**, 5879–5889.
4. Hadi, S. M. & Goldthwait, D. A. (1971) *Biochemistry* **10**, 4986–4994.
5. Hadi, S. M., Kirtikar, D. M., & Goldthwait, D. A., (1973) *Biochemistry* **12**, 2747–2754.
6. Lawley, P. D. & Shah, S. A. (1972) *Biochem. J.* **128**, 117–132.
7. Melgar, E. & Goldthwait, D. A., (1968) *J. Biol. Chem.* **243**, 4401–4408.
8. Smith, K. D., Armstrong, J. L. & McCarthy, B. J. (1967) *Biochim. Biophys. Acta* **142**, 323–330.
9. Uhlenhopp, E. L. & Krasna, A. L. (1971) *Biochemistry* **10**, 3290–3295.
10. Lawley, P. & Thatcher, C. J. (1970) *Biochem. J.* **116**, 693–707.
11. Al-Arif, A. & Sporn, M. B. (1972) *Anal. Biochem.* **48**, 386–393.
12. Studier, F. W. (1965) *J. Mol. Biol.* **11**, 373–390.
13. Lang, D. (1970) *J. Mol. Biol.* **54**, 557–565.
14. Lawley, P. D. & Brookes, P. (1963) *Biochem. J.* **89**, 127–138.
15. Druckrey, H., Steinhoff, D., Preussman, R. & Ivancovic, S. (1964) *Z. Krebsforsch.* **66**, 1.
16. Swann, P. F. & Magee, P. N. (1968) *Biochem. J.* **110**, 39–47.
17. Loveless, A. & Hampton, C. L. (1969) *Mutat. Res.* **7**, 1–12.
18. Loveless, A. (1969) *Nature* **223**, 206–207.
19. Strauss, B. S. (1962) *Proc. Nat. Acad. Sci. USA* **48**, 1670–1675.
20. Verly, W. G. & Paquette, Y. (1970) *Fed. Can. Soc. Biol.*, 685.
21. Paquette, Y., Crine, P. & Verly, W. G. (1972) *Can. J. Biochem.* **50**, 1199–1209.
22. Lawley, P. D. & Orr, D. J. (1970) *Chem-Biol. Interactions* **2**, 154–157.
23. Prakash, L. & Strauss, B. (1970) *J. Bacteriol.* **102**, 760–766.
24. Papirmeister, B., Dorsey, J. K., Davison, C. L. & Gross, C. L. (1970) *Fed. Proc.* **29**, 726.
25. Lawley, P. D. & Shah, S. A. (1972) *Chem-Biol. Interactions* **5**, 286–288.
26. Gerchman, L. L. & Ludlum, D. B. (1973) *Biochim. Biophys. Acta* **308**, 310–316.
27. Setlow, R. B. & Carrier, W. L. (1973) *Nature New Biol.* **241**, 170–172.

* D. M. Kirtikar, J. Slaughter, and D. A. Goldthwait.
† K. Minton and E. C. Friedberg.