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Properties of a Human Lymphoblast AP-Endonuclease Associated with Activity for DNA Damaged by Ultraviolet Light, γ-Rays, or Osmium Tetroxide†

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ABSTRACT: An endonuclease activity for UV-irradiated DNA, γ-irradiated DNA, and OsO_4-treated DNA that was partially purified from human lymphoblasts was found to have associated with it an endonuclease activity for partially depurinated DNA. When this apurinic endonuclease (Endo A) was characterized and compared with the cells' major apurinic endonuclease (Endo B), several notable differences were observed. (1) Endo A bound to oxidized DNA–Sepharose under conditions where Endo B did not. (2) Endo A did not require Mg^{2+}, retaining full activity in 10 mM ethylenediaminetetraacetic acid, while Endo B showed an absolute requirement for Mg^{2+}. (3) Whereas the nicks made in depurinated DNA by Endo B were efficient priming sites for *Escherichia coli* polymerase I, those made by Endo A were not. Further characterization of the nicks indicated that Endo A incises depurinated DNA 3′ to apurinic sites, leaving 3′-terminal deoxyribose, a poor priming site for DNA synthesis. Endo A action on UV-irradiated DNA produced nicks that resembled those it made in depurinated DNA, suggesting that the UV endonuclease activity acts through an apurinic/apyrimidinic site intermediate.

Biological systems possess several different mechanisms for coping with damage to DNA that either is caused by environmental chemicals and radiation or is spontaneously generated within the cell. Whereas some DNA lesions are repaired by direct reversal of the damage-inducing reaction, such as UV-dimer photoreactivation (Sutherland, 1978) or O^6-alkylguanine alkyl transfer (Olsson & Lindahl, 1980), the more frequent pathway for repair is by base or nucleotide excision that involves limited degradation and resynthesis of DNA. Such excision repair is initiated by lesion-specific DNA glycosylases or endonucleases. Several DNA glycosylases that remove demaged or abnormal bases from DNA without DNA strand scission have been described (Caradonna & Cheng, 1982), and endonuclease that results in release of intact nucleotides from UV-irradiated DNA has recently been demonstrated unequivocally for the uvr ABC system in *Escherichia coli* (Rupp et al., 1983). A third class of enzymes has been

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identified where DNA glycosylase and apurinic/apyrimidinic (AP) endonuclease activities are tightly associated, so that these enzymes were initially considered to be lesion-specific endonucleases (Riazuddin & Grossman, 1977; Gates & Linn, 1980). More recently it was recognized that these reactions occur as two steps, namely, base release followed by AP-endonuclease mediated strand scission (Haseltine et al., 1980; Demple & Linn, 1980), and that the AP-endonucleases in these enzyme complexes are distinct from the cell's major free AP-endonucleases.

In this report, we have reexamined an endonuclease that acts on UV- or X-irradiated PM2 DNA, but not on untreated PM2 DNA, that was previously partially purified from human lymphoblasts (Brent, 1976, 1977) (termed the UVX endonuclease). Further characterization of this enzyme preparation with respect to an apparent contaminating activity for apurinic DNA indicated that this AP-endonuclease activity differs from the major AP-endonuclease also partially purified from human lymphoblasts (Brent, 1976). In this report we describe properties of the two AP-endonuclease activities that clearly demonstrate their distinct identities. They differed in binding affinity for DNA–Sepharose, requirement for Mg^{2+}, and capacity to generate nicks that can serve as primers for DNA polymerase.

Materials and Methods

Preparation of DNA Substrates. Procedures for the growth of bacteriophage PM2, labeling with [^{3}H]thymidine, and isolation of the radiolabeled DNA have been described (Teebor & Brent, 1981). Partially depurinated DNA containing one to two AP sites per molecule was prepared by heating at 70 °C in 0.1 M sodium citrate buffer (pH 5.5) for 20–40 min. Ultraviolet-irradiated DNA containing about one alkali-stable, endonuclease sensitive site per molecule was prepared by exposure to 254-nm light (1500 J/m^2) from a germicidal lamp at a dose rate of 500 J m^{-2} min^{-1} (determined by using a Blak-Ray ultraviolet meter). PM2 DNA in 20 mM Tris-HCl[1] (pH 7.5), 1 mM EDTA, and 0.1 M NaCl (buffer A) was γ irradiated under air at a dose rate of 800 rads/min with a ^{137}Cs irradiator. A total of 5000 rads produced approximately one alkali-stable, endonuclease-sensitive site per molecule. OsO_4-treated DNA containing about one endonuclease-sensitive site per molecule was prepared by preheating PM2 DNA in buffer A to 70 °C for 2 min, adding OsO_4 to a concentration of 0.02%, and continuing incubation at 70 °C for 5 min. After cooling to room temperature, the solution was extracted 3 times with 2 volumes of diethyl ether and finally dialyzed extensively against buffer A.

Preparation of Oxidized DNA–Sepharose 4B Affinity Column. Calf thymus DNA (Sigma type I), 500 mg in 50 mL of H_2O, was denatured by boiling and rapid cooling. After dialysis against 2 L of 0.3 M NH_4Cl buffer, pH 8.6, $KMnO_4$ was added to a concentration of 1.5 mM and the mixture stirred at 4 °C for 30 min. The reaction was stopped by addition of 1 M sodium metabisulfite until the color disappeared. This step was followed by dialysis against 2 L of 10 mM potassium phosphate, pH 8.0. The DNA so oxidized was then reacted with cyanogen bromide activated Sepharose 4B (Sigma), as described by Arndt-Jovin et al. (1975).

Endonuclease Assays. Endonuclease activity was measured by a nitrocellulose filter binding assay. Approximately 0.1 μg of [^{3}H]thymidine-labeled PM2 DNA that was partially depurinated (AP-DNA), irradiated with ultraviolet light (UV-DNA) or with γ-rays (γ-DNA), or treated with OsO_4 (OsO_4-DNA) or untreated was incubated for 30 min at 37 °C in a 50 mM Tris-HCl buffer (pH 7.5) together with 10 μL of enzyme fraction in a total volume of 60 μL. Reaction mixtures contained either 2 mM EDTA or 2 mM $MgCl_2$. The enzyme reaction was terminated by addition of 30 μL of proteinase K (1 mg/mL) in 10 mM Tris-HCl buffer (pH 7.4) with 10 mM EDTA. After 5 min of further incubation at 37 °C, 2.0 mL of 0.01 M sodium phosphate (pH 11.8) with 0.6 M NaCl was added at room temperature for 1 min to denature the DNA. In some cases the pH 11.8 incubation at room temperature was extended to 2 h. This treatment resulted in quantitative hydrolysis of AP sites. Addition of 0.25 mL of 1.0 M disodium phosphate and 2.0 mL of 0.18 M sodium citrate buffer (pH 7.0) with 1.2 M NaCl returned the solution to neutral pH. Those covalently closed DNA molecules not nicked by endonuclease (or the extended alkaline hydrolysis) were renatured under these conditions, whereas nicked molecules remained single stranded. When the solution was passed through nitrocellulose membrane filters (Schleicher & Schuell BA 85) that were subsequently washed with 0.09 M sodium citrate (pH 7.0) containing 0.09 M NaCl, the single-stranded DNA was bound to the filter while the intact double-stranded molecules passed through. Assuming that endonucleolytic nicks are introduced randomly in the population of DNA molecules, we calculated the average number of nicks per molecule (x) using the Poisson formula $x = -\ln y$, where y is the fraction of DNA remaining unnicked. A unit of endonuclease was defined as the activity producing one picomole of strand breaks in 30 min in the standard assay.

Enzyme Purifications. Both AP-endonuclease and UVX-endonuclease activities were purified from cultured human lymphoblasts (CCRF-CEM line). Suspension cultures were maintained in exponential growth in roller bottles in Eagle's minimum essential medium supplemented with 5% newborn calf serum (KC Biological). Eight liters of cell suspension at 10^6 cells/mL was harvested by low-speed centrifugation (300g for 10 min). All subsequent operations were at 4 °C. The cells were washed with phosphate-buffered saline (pH 7.2) and finally resuspended in 50 mM Tris-HCl buffer (pH 7.5) with 2 mM EDTA, 1 mM β-mercaptoethanol, and 0.1 M NaCl. The protease inhibitors aprotinin (Sigma, 30 trypsin inhibitor units/L) and phenylmethanesulfonyl fluoride (0.2 mM) were added to the extraction buffer immediately before its use. The cells were disrupted by intermittent sonication for 3 min and centrifuged at 140000g for 45 min. The supernatant, termed fraction I, was dialyzed against 0.2 M $(NH_4)_2SO_4$ and 0.2 M potassium phosphate (pH 7.0), loaded on a DEAE-cellulose column (Whatman DE-23, 25 × 4.7 cm diameter) that was equilibrated with the same buffer, and eluted with this buffer at 30 mL/min. Nucleic acids were retarded by this column, and the peak of nucleic acid free protein emerging first constituted fraction II. This material was fractionally precipitated by addition of saturated ammonium sulfate solution. The precipitates were redissolved in 10 mM Tris-HCl (pH 7.5), 2 mM EDTA, 1 mM β-mercaptoethanol, 0.15 M NaCl, 10% glycerol, and 0.02% sodium azide (buffer B) and extensively dialyzed against buffer B. The protein precipitated between 20 and 40% saturation was termed fraction IIIA and that from 40–60% was termed fraction IIIB. Most of the AP-endonuclease activity (90%) was in fraction IIIB, and a majority of the UVX-endonuclease activity was in fraction IIIA.

In further purification, fraction IIIA was loaded on a column (3.7 cm × 2.5 cm) of oxidized DNA–Sepharose 4B. This

[1] Abbreviations: Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

Table I: Purification of Endo A

purification step	fraction no.	vol (mL)	[protein] (mg/mL)	sp enzyme act. (units × 10^{-3}/mg of protein) UV-DNA	sp enzyme act. (units × 10^{-3}/mg of protein) intact DNA	sp act. corrected for nonspecific endonuclease	purification factor	% act. recovered
crude extract	I	53	19.5	98	9.3	88.7	1	100
DEAE-cellulose chromatography	II	100	5.0	124	12	112	1.26	61
ammonium sulfate fractionation	IIIA	4.5	23.5	652	2.6	649	7.3	75
oxidized DNA-Sepharose chromatography	IVA	24	0.1	9200	220	8980	101	23

column, prequilibrated with buffer B, was extensively washed with buffer B (about 150 mL) and then eluted with a linear gradient (200 mL) of 0–1 M KCl in buffer B. The UV-endonuclease activity that eluted between 0.3 and 0.5 M KCl was pooled and concentrated 2–3-fold by ultrafiltration (Amicon YM10). This material (fraction IVA) contained the partially purified UVX-endonuclease and will be referred to as Endo A.

The AP-endonuclease in fraction IIIB was further purified by gel filtration on Sephadex G-75-SF and subsequently by phosphocellulose chromatography as described previously (Brent, 1976). The peak of AP-endonuclease activity that was eluted from phosphocellulose at about 0.1 M NaCl was fraction VB and is identical with the AP-endonuclease partially purified previously (Brent, 1976). This preparation represents the major cellular AP-endonuclease and will be referred to as Endo B.

Assay for DNA Synthesis on Nicked PM2 DNA. Approximately 0.1–1.0 μg of [^{3}H]thymidine-labeled PM2 DNA, either partially depurinated, UV irradiated, or untreated, was incubated at 37 °C with sufficient endonuclease to nick all of the susceptible sites. With Endo A the reaction mixture contained 50 mM Tris-HCl (pH 7.5) and 2 mM EDTA in 150 μL. With Endo B the reaction volume of 165 μL contained 50 mM Tris-HCl (pH 7.5), 1.8 mM EDTA, and 2.4 mM $MgCl_2$. With exonuclease III (which is also AP-DNA-specific endonuclease VI of *E. coli*) the 165-μL reaction mixture contained 50 mM Tris-HCl (pH 7.5), 1.8 mM EDTA, 6 mM MgCl, and 0.05 unit of exonuclease III (Bethesda Research Laboratories). In general, an initial 10-min reaction with Endo A or the enzyme buffer was terminated by heating to 70 °C for 3 min. The reaction mixture was then adjusted with buffer containing $MgCl_2$ and incubated for a further 10 min at 37 °C with Endo B, exonuclease III, or appropriate buffer, the reaction again being stopped by heating at 70 °C for 3 min.

The incised ^{3}H-labeled DNA in 155 μL of the endonuclease reaction mixture was next incubated for up to 30 min at 37 °C in a reaction mixture (final volume 375 μL) containing 1×10^{-4} M dATP, dGTP, and dCTP, 4×10^{-6} M dTTP, 7×10^{-3} M $MgCl_2$, 7×10^{-2} M potassium phosphate, 1×10^{-2} M mercaptoethanol, 1 μCi of [α-^{32}P]dTTP (800 Ci/mmol), and 3 units of *E. coli* polymerase I (Bethesda Research Laboratories). At various times during the reaction, 60-μL samples were removed, added to 30 μL of proteinase K [1 mg/mL in 10 mM Tris-HCl (pH 7.4) and 10 mM EDTA], and incubated for 5 min at 37 °C. Each sample was then denatured, renatured, and filtered through nitrocellulose filters, which were then assayed for radioactivity as described above for the endonuclease assay. In this assay, all ^{32}P incorporated into DNA is bound to the filter because DNA synthesis occurs only on nicked DNA molecules and only nicked DNA binds to the filter. Since intact DNA molecules do not bind, the fraction of total ^{3}H that bound to the filter afforded a measure of the number of nicks per molecule, as described for the endonuclease assay.

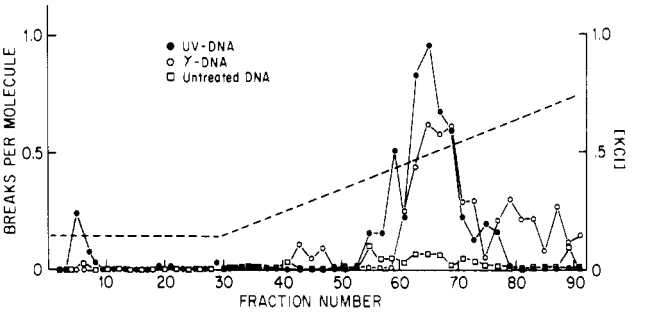

FIGURE 1: Elution profile of UVX-endonuclease fraction IIIA from oxidized DNA–Sepharose. Endonuclease activity was determined in the presence of 2 mM EDTA with UV-DNA (●), γ-DNA (○), or untreated DNA (□) as substrates.

Results

Purification of UVX-Endonuclease. The endonuclease activity for irradiated DNA was purified essentially as described before (Brent, 1976), except that $KMnO_4$-oxidized DNA covalently linked to Sepharose 4B was substituted for the UV-irradiated DNA–agarose affinity column previously used. The rationale for this change was based on the production of radiation-type products such as 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol) in DNA by this mild oxidation treatment (Frenkel et al., 1981) and the likelihood that the human lymphoblast endonuclease for irradiated DNA acted on such nondimer lesions. In practice the UVX-endonuclease bound only marginally more tightly to the oxidized-DNA column; however, the main advantage of the column was that it bound virtually none of the major Mg^{2+}-requiring AP-endonuclease (Endo B). Figure 1 shows the elution profile of fraction IIIA from the oxidized DNA–Sepharose column. Assayed with 2 mM EDTA, a peak of endonuclease active on UV-irradiated DNA bound to the column and was eluted by about 0.5 M KCl. Coeluting with this activity was a peak of endonuclease active on γ-irradiated DNA (Figure 1) and AP-DNA (not shown). The activity for untreated DNA was minimal. Little endonuclease activity washed through the column as assayed in the absence of $MgCl_2$; however, in the presence of low Mg^{2+} concentrations (2 mM) a large amount of AP-endonuclease was demonstrable in the wash-through fractions (data not shown). The endonuclease activities bound to the column were unaffected by the presence of Mg^{2+} (see below). Table I summarizes the results of a typical purification. The UV-endonuclease was purified over 100-fold with 10–20% yield.

Effect of Mg^{2+} on Endo A and Endo B. The difference between AP Endo A and AP Endo B in their response to

Table II: Effect of $MgCl_2$ on Endonuclease Activity[a]

addition	incubation time (min)	average no. of nicks per PM2 DNA molecule: Endo A	Endo B
none	0	0	0
	5	0.26	0
	10	0.54	0
	15	0.97	0
	20	1.31	0.03
EDTA (2 mM)	0	0.02	0
	5	0.21	0
	10	0.56	0
	15	0.77	0
	20	1.24	0.02
$MgCl_2$ (2 mM)	0	0.02	0.04
	5	0.35	0.30
	10	0.64	0.57
	15	0.88	0.80
	20	1.29	1.12

[a] Partially depurinated PM2 DNA was incubated with Endo A or Endo B in 50 mM Tris-HCl buffer, pH 8.0. Values for controls incubated with buffer have been subtracted.

Table III: Substrate Specificities of Endo A and Endo B

substrate	enzyme activity[a]: Endo A[b]	Endo B[c]
AP-DNA	22.8	29.4
UV-DNA[d]	8.7	0
γ-DNA[d]	9.9	0.6
OsO_4-DNA[d]	4.8	0
untreated DNA[d]	0	0

[a] Activities are expressed as moles $\times 10^{-15}$ of strand breaks produced in 30 min by 10 μL of enzyme fraction. Values for controls incubated without enzyme have been subtracted. [b] Assayed with 2 mM EDTA. [c] Assayed with 2 mM $MgCl_2$. [d] All assays except with AP-DNA included a postincubation for 2 h at pH 11.8 to hydrolyze any alkali-labile sites.

$MgCl_2$ is shown in Table II. The relative reaction rates were determined with or without the addition of $MgCl_2$ at 2 mM or EDTA at 2 mM. Whereas Endo B showed no activity except in the presence of the $MgCl_2$, Endo A activity was independent of $MgCl_2$ concentration. Concentrations of EDTA up to 10 mM did not noticeably affect AP Endo A activity (data not shown).

Substrate Specificities of Endo A and Endo B. Endo A displayed activity not only for UV-DNA and γ-DNA but also for OsO_4-treated DNA, consistent with the notion that it acts on thymine glycol type products (Table III). These activities like that of the AP-endonuclease neither required nor were stimulated by Mg^{2+}. In contrast, Endo B was entirely specific for AP-DNA (Table III). Since the activities for the irradiated and OsO_4-treated DNA might have resulted from the AP-endonuclease activity in Endo A acting on AP sites present in the damaged DNA, the assays shown in Table III included an extended alkaline hydrolysis such that all preexisting AP sites were nicked and enzyme-mediated nicks necessarily occurred at alkali-stable, non-AP sites.

Priming Activity of AP-Endonuclease-Incised DNA. Figure 2 shows the rates of DNA synthesis primed by partially depurinated PM2 DNA that was incised with Endo A, Endo B, or *E. coli* exonuclease III. Whereas all three enzymes nicked all of the AP sites in the DNA, only nicks made by Exo III and Endo B were good primers for *E. coli* DNA polymerase I. The failure of Endo A to yield nicks that were adequate primer sites for Pol I could have been due to (i) the presence of a 3′-terminal phosphate at the nick or (ii) the presence of

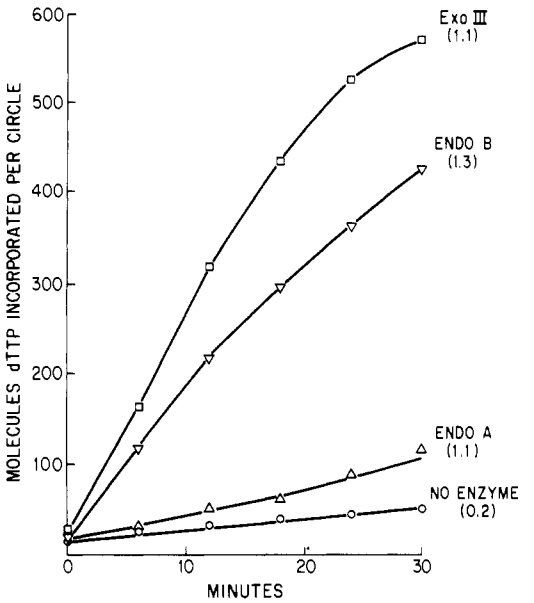

FIGURE 2: Priming activity of nicks generated by AP-endonuclease. PM2 DNA containing one to two AP sites per molecule was treated with enough Endo A (△), Endo B (▽), or *E. coli* Exo III (□) to nick all the AP sites. Enzyme-free buffer (○) served as the control. Rates of DNA synthesis were measured under conditions where the DNA primer was limiting. Numbers in parentheses represent nicks per PM2 molecule.

an AP site 5′ to the nick. The first of these possibilities was tested by incubating the incised DNA with *E. coli* alkaline phosphatase; however, such treatment did not improve the priming activity while similar treatment of PM2 DNA containing two to three nicks per molecule made by micrococcal nuclease (Sigma) converted 3′-phosphate to 3′-hydroxyl termini and resulted in a very large increase in priming activity (data not shown).

To test the second possibility, we treated Endo A incised DNA with Exo III before assaying the DNA synthesis rate. Figure 3A shows that this enzyme does convert the DNA to an efficient primer without producing more nicks. Since Exo III incises 5′ to AP sites (Weiss, 1976) to yield efficient polymerase I priming sites, we conclude that Endo A cleaves at the 3′ side of AP sites and Exo III acts by cleaving the resultant 3′-terminal AP residues. Although the 3′–5′-exonuclease activity of Exo III would extent the nicks to gaps, one would not expect this to affect the initial *rates* of DNA synthesis under conditions where the number of 3′-hydroxyl nucleoside termini is the limiting factor. When used to treat DNA preincised by Endo A, Endo B also converted the nicks to efficient primers for DNA synthesis (Figure 3B).

Endonuclease for UV-DNA. The endonuclease activity for UV-DNA that is present in Endo A was examined in parallel experiments. Like the AP-endonuclease activity, it was not stimulated by $MgCl_2$ and was unaffected by 10 mM EDTA. Experiments to determine the effectiveness of nicked UV-DNA as a primer for DNA synthesis were complicated by rapid cessation of DNA synthesis. This was presumed to be due to inhibition of DNA polymerase by large numbers of pyrimidine dimers relative to the nondimer lesions acted on by this UV-endonuclease (Setlow et al., 1963). However, when DNA synthesis rates were determined over relatively shorter time periods, the priming activity of nicks in UV-DNA could be determined. Figure 4 shows that UV-endonucleolytic nicks made in UV-DNA resemble those made by the AP-endonuclease activity in that they are poor primers for DNA synthesis but are converted to efficient primers by Exo III.

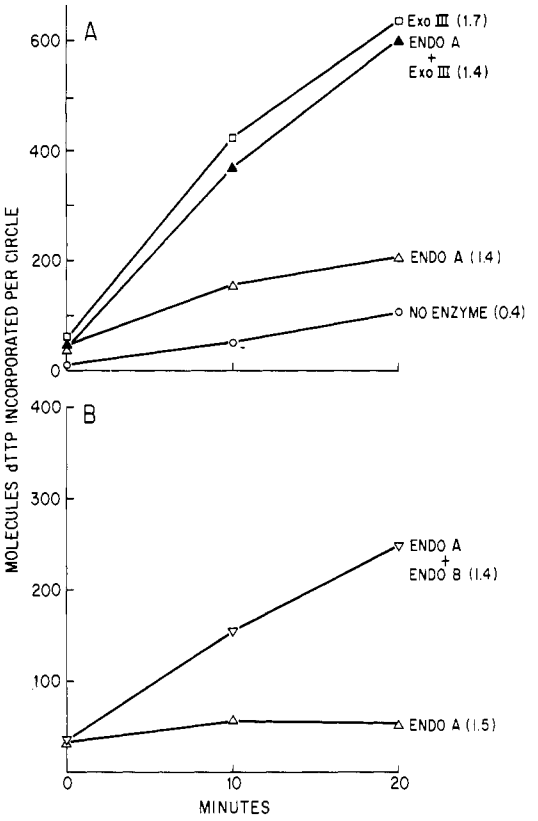

FIGURE 3: Effect of (A) Exo III or (B) Endo B on priming activity of Endo A generated nicks. PM2 DNA containing one to two AP sites per molecule was treated with (A) Endo A alone (△) and Endo A followed by Exo III (▲). Exo III alone (□) and buffer (○) served as controls. (B) DNA was treated with Endo A alone (△) or Endo A followed by Endo B (▽). Numbers in parentheses indicate nicks per circle.

Exo III alone makes a limited number of incisions in the UV-DNA, consistent with previous observations that UV radiation induces low levels of AP sites (Rahn, 1979). The majority of sites nicked by Endo A are not susceptible to Exo III and hence are not AP sites.

Discussion

Both prokaryotic and mammalian cells have been shown to contain multiple species of AP endonucleases, which have been divided into two classes: those enzymes that produce efficient primers for DNA polymerase and those that do not (Lindahl, 1982; Warner et al., 1980). The two AP endonucleases from human cultured lymphoblasts (Endo A and Endo B) likewise can be distinguished on this basis. In addition, their activities differ in (i) capacity to bind to oxidized DNA–Sepharose 4B and (ii) requirement for Mg^{2+}. The association of the AP-endonuclease in Endo A with the activities for irradiated and oxidized DNA also sets it apart from Endo B, although at this preliminary stage of purification such association may be fortuitous.

Endo A resembles in many respects the endonuclease purified from mouse plasmacytoma cells by Nes (1980a) that acts on UV-DNA and OsO_4-treated DNA, as well as on AP-DNA. This enzyme also is insensitive to divalent ions and separable from the major Mg^{2+}-requiring AP-endonuclease (Nes, 1980b). Endonuclease III from *E. coli* (Gates & Linn, 1977; Demple & Linn, 1980) also closely resemble Endo A in its substrate specificity for UV-, γ-, OsO_4-, and AP-DNAs, as also does an X-ray endonuclease purified from *E. coli* by Wallace et al. (1981). These bacterial activities appear to be due to the combined action of a glycosylase that acts on

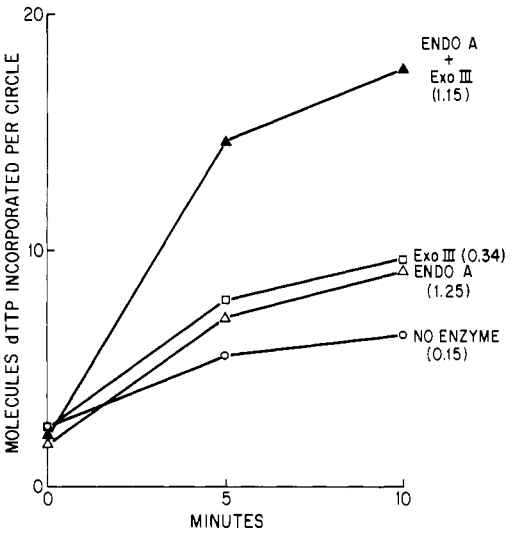

FIGURE 4: Priming activity of nicks generated in UV-irradiated DNA by Endo A. PM2 DNA containing one to two UV-endonuclease sensitive sites per molecule was treated with enough Endo A alone (△) or Exo III alone (□) to produce maximum nicking. The same treatments were carried out sequentially with Endo A followed by Exo III (▲). DNA synthesis rates were measured in the standard assay. Numbers in parentheses are nicks per circle.

thymine glycol type products in irradiated or oxidized DNA together with AP-endonuclease that is responsible for DNA strand scission.

The observations on Endo A and Endo B are also similar to observations by Kuhnlein et al. (1978) on two AP endonucleases in human cultured fibroblasts. These enzymes, termed class I and class II AP-endonucleases by Mosbaugh & Linn (1980), produced either nonprimer nicks or efficient-primer nicks. Unlike Endo A the fibroblast class I AP-endonuclease is stimulated by Mg^{2+}; it is not known if it acts on irradiated or oxidized DNA. Endo B also resembles the major AP-endonuclease purified from HeLa cells (Kane & Linn, 1981) and from human placenta (Shaper et al., 1982; Grafstrom et al., 1982) except the latter enzyme incises both 5′ and 3′ to the AP site and does not cleave 3′-terminal AP residues. The data presented here do not preclude the possibility that Endo B acts to some extent by incising 3′ to AP sites; in fact, the priming activity of Endo B nicks in Figure 2 is less than expected compared with that of Exo III mediated nicks suggesting that some nonpriming termini are formed.

The identity of the radiation-induced lesions recognized by Endo A is not yet known. On the basis of their low frequency (Rahn, 1979), they are not pyrimidine dimers, while their occurrence in DNA irradiated by γ-rays and UV light and oxidized by OsO_4 suggests they might by thymine glycol type derivatives. The similarity of properties shown by Endo A in polymerase priming assays with UV-DNA and AP-DNA suggests that the same activity, namely, AP-endonuclease, is responsible for the incision function in both substrates. This implies that the UV lesions are recognized and initially acted on by a DNA glycosylase.

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Registry No. Endo A, 61811-29-8; UV endonuclease, 81611-73-6.

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Chemical Inducers of Differentiation Cause Conformational Changes in the Chromatin and Deoxyribonucleic Acid of Murine Erythroleukemia Cells†

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ABSTRACT: The chemical inducers of murine erythroleukemia cell differentiation, dimethyl sulfoxide, sodium butyrate, and ethionine, elicited conformational changes in the DNA and chromatin of treated cells. The chromatin from dimethyl sulfoxide treated and butyrate-treated cells exhibited circular dichroic spectra different from that of the noninduced control. The molar ellipticity $[\theta]_{282.5}$ in isotonic saline decreased from 4900 deg·cm^2·dmol^{-1} for control chromatin to 3800 and 3600 deg·cm^2·dmol^{-1} for dimethyl sulfoxide treated and butyrate-treated chromatin, respectively, while that from ethionine-treated chromatin remained virtually unchanged (5400 deg·cm^2·dmol^{-1}). Increasing the ionic strength to 2.5 or 5 M with NaCl resulted in a substantial, uniform, decrease in molar ellipticity. Thermal denaturation profiles of high molecular weight DNA prepared from each of the inducer-treated cells showed a pronounced hyperchromic shift but no change in T_m when compared to control DNA. Circular dichroic spectra of the DNA indicated a decrease in ellipticity $[\theta]_{277}$ from 9600 deg·cm^2·dmol^{-1} to 8900, 8300, and 8800 deg·cm^2·dmol^{-1} for ethionine, dimethyl sulfoxide, and butyrate treated cells, respectively. Treatment of the DNA with 3 M NaCl canceled the UV and CD differences. These measurements indicate an increased stacking of bases or an increased compactness of the DNA from induced cells. Concomitant with specific modifications such as hypomethylation of DNA, the data can be interpreted in terms of conformational changes in chromatin resulting from core histone acetylation.

The processes that mediate differentiation of murine erythroleukemia cell(s) (MELC) involve biochemical mechanisms that are not fully understood. Certain postsynthetic modifications of the macromolecular constituents of chromatin have been implicated. Inducers of MELC differentiation such as dimethyl sulfoxide (Me_2SO), butyrate, and ethionine have been shown to cause DNA hypomethylation, and this has been correlated with an increase in the expression of the globin gene (Christman et al., 1977; Van Der Ploeg et al., 1980; Weintraub et al., 1981). The possible relevance of DNA methylation to gene expression has been extensively reviewed (Drahovsky & Boehm, 1980; Razin & Riggs, 1980; Doerfler, 1981). The mechanisms by which these inducers of differentiation may cause DNA hypomethylation, however, are not entirely clear. Ethionine is a well-documented DNA methylase inhibitor (Cox & Irving, 1977) that interferes, as well, with lysine methyltransferase catalyzed histone methylation (Baxter & Byvoet, 1974). Me_2SO and butyrate, on the other hand, have not been

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