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A Highly Conserved Endonuclease Activity Present in *Escherichia coli*, Bovine, and Human Cells Recognizes Oxidative DNA Damage at Sites of Pyrimidines

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We have compared the sites of nucleotide incision on DNA damaged by oxidizing agents when cleavage is mediated by either *Escherichia coli* endonuclease III or an endonuclease present in bovine and human cells. *E. coli* endonuclease III, the bovine endonuclease isolated from calf thymus, and the human endonuclease partially purified from HeLa and CEM-C1 lymphoblastoid cells incised DNA damaged with osmium tetroxide, ionizing radiation, or high doses of UV light at sites of pyrimidines. For each damaging agent studied, regardless of whether the *E. coli*, bovine, or human endonuclease was used, the same sequence specificity of cleavage was observed. We detected this endonuclease activity in a variety of human fibroblasts derived from normal individuals as well as individuals with the DNA repair deficiency diseases ataxia telangiectasia and xeroderma pigmentosum. The highly conserved nature of such a DNA damage-specific endonuclease suggests that a common pathway exists in bacteria, humans, and other mammals for the reversal of certain types of oxidative DNA damage.

Cellular DNA is constantly subjected to a variety of physical and chemical agents that produce alterations requiring repair for maintenance of the genetic integrity of the cell. Agents that inflict damage on DNA through the production of active oxygen species have been implicated as endogenous initiators of an array of degenerative processes related to cancer and aging (1, 10). Several endonuclease activities present in mammalian cells and other eucaryotic cells that recognize and cleave DNA altered by oxidizing agents, ionizing radiation, or high doses of UV light have been described (2, 4, 5, 7, 24, 29, 35). Analogous activities present in *Escherichia coli* are properties of endonuclease III (6, 23). We have recently provided evidence that such activities in calf thymus are attributable to a single endonuclease activity that preferentially incises damaged DNA at sites of pyrimidines (14; D. E. Helland, R. Male, and K. Kleppe, FEBS Lett., in press).

The common targets for these enzymes of broad substrate specificity appear to be ring-saturated or ring-cleaved pyrimidines. The chemical mechanisms that produce substrates for this group of endonucleases are either oxidation or reduction reactions generated by ionizing radiation or other oxidizing agents (9). We have termed these enzymes redoxyendonucleases to emphasize the mechanisms of substrate production.

The goal of the present study was to determine whether human cells contained a redoxyendonuclease activity with a specificity directed against pyrimidines in DNA damaged with osmium tetroxide, ionizing radiation, or high doses of UV light. For these studies, end-labeled, defined-sequence DNA fragments were used as damage substrates, and the endonuclease-generated DNA scission products were analyzed by DNA-sequencing methods. Use of such substrates allows direct analysis of enzyme incision sites at the level of individual nucleotides within a sequence. We also used an endonuclease-specific DNA-nicking assay that provides an indication of the level of redoxyendonuclease activity in crude cell extracts.

We have identified and partially purified human redoxyendonuclease from HeLa and CEM-C1 lymphoblastoid cells. We also detected this enzyme in extracts of nontransformed and simian virus 40 (SV40)-transformed normal human fibroblasts and fibroblasts derived from individuals with two diseases associated with DNA repair deficiencies, ataxia telangiectasia and xeroderma pigmentosum. In each case, the sequence specificity of cleavage of damaged DNA by the human cell extracts was determined and compared with those of the bovine redoxyendonuclease and *E. coli* endonuclease III. We found that redoxyendonuclease appears to be ubiquitous in the various human cell lines examined.

A second goal of the work was to determine the extent of similarities that might exist between *E. coli* endonuclease III and the bovine and human redoxyendonuclease. The conservation of DNA repair pathways among procaryotes and eucaryotes has been suggested through comparisons of DNA repair enzymes, such as O^6-methylguanine transferase, uracil glycosylase, and 3-methyladenine-DNA glycosylase (15, 25). Such similarities indicate that the repair of certain types of DNA damage is necessary to maintain genetic integrity in a wide variety of dissimilar organisms. In this regard, we find that a very similar relationship exists in the sequence specificity of DNA cleavage mediated by the *E. coli*, bovine, and human endonucleases that recognize pyrimidines damaged by oxidizing agents.

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MATERIALS AND METHODS

Cell culture. Fibroblast cells from ataxia telangiectasia patients, cell lines GM367, GM5823, GM2052, and GM5849, were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ. GM5849 is an SV40-transformed cell line derived from GM5823 cells (same as AT5BI). The GM5849 line is hypersensitive to X-rays, bleomycin, and neocarzinostatin (20) compared with other SV40-transformed fibroblast lines. A normal human fibroblast strain, IMR90, and the SV40-transformed normal fibroblast lines GM637 and AG2804B were obtained from B. Royer-Pokora, Dana-Farber Cancer Institute. Cell line M1 (30) is a cloned SV40-transformed fibroblast line derived from a patient with xeroderma pigmentosum (XP12RO). Normal fibroblast strains AG1521 and AG1522 were a gift of R. Reynolds, Harvard School of Public Health. All fibroblasts were maintained in Dulbecco modified Eagle medium (DME) supplemented with 15% fetal calf serum at 37°C in a 5% CO_2 atmosphere. Human epithelial carcinoma cells (HeLa) were grown in spinner culture in Dulbecco modified Eagle medium plus 10% fetal calf serum. CEM-C1 human lymphoblast cells were grown in suspension in RPMI 1640 medium plus 10% fetal calf serum. All cells were cultured at 37°C in a 5% CO_2 atmosphere and were harvested in the logarithmic growth phase.

Assay of redoxyendonuclease. The standard assay mixture of 100 μl contained buffer A (10 mM Tris hydrochloride [pH 8.0], 10 mM EDTA, 10 mM 2-mercaptoethanol) plus 40 mM KCl and 100 fmol of UV-irradiated (1,000 J/m^2), ^{3}H-labeled φX174 replicative form I (RFI) DNA (27). Following the addition of 1 to 15 μl of redoxyendonuclease sample, the mixture was incubated at 37°C for 30 min. Conversion of RFI to nicked RFII DNA was determined by the nitrocellulose filter binding assay (27). Samples containing 100 fmol of unirradiated φX174 RFI [^{3}H]DNA were incubated with each redoxyendonuclease sample as described above. Endonuclease activity on undamaged DNA substrate (background) was subtracted from the activity obtained on UV-damaged substrate. Background activity for cell extracts ranged from 1 to 10% of the activity obtained with UV-damaged substrate. One unit of redoxyendonuclease is defined as the amount of enzyme necessary to produce 1 fmol of UV-specific nicks under these conditions.

Preparation of cell extracts. Endonuclease was prepared from cultured cell lines essentially as described previously for calf thymus endonuclease (18; Helland et al., in press). In brief, cells (ca. 5×10^9) were washed in phosphate-buffered saline and suspended in 0.1 M NaCl–50 mM Tris hydrochloride (pH 8.0)–1 mM phenylmethysulfonyl fluoride (PMSF) and homogenized in a Dounce homogenizer, pestle B. The NaCl concentration was then adjusted to 0.7 M with 5.0 M NaCl, and the viscous solution was mixed slowly for 10 min. The NaCl concentration was then reduced to 0.3 M by the addition of homogenization buffer without NaCl. The extract was centrifuged for 40 min at 40,000 rpm in a Beckman 70.1 Ti rotor. The clear supernatant was applied to a DEAE-cellulose column equilibrated in buffer A plus 0.3 M NaCl, and the enzyme was eluted with the same buffer as a single broad peak of activity. The enzyme is not bound to DEAE-cellulose under these conditions, while most nucleic acids and some proteins are retained.

Phosphocellulose chromatography. The enzyme-containing fractions that had been passed through DEAE-cellulose (21 mg) were dialyzed against buffer A plus 50 mM NaCl, loaded onto a phosphocellulose column (Whatman P11, 2.5 by 12.5 cm) equilibrated in the same buffer, and washed until the absorbance at 280 nm was zero. Enzyme was eluted with a 300-ml gradient of NaCl (0.05 to 0.75 M) in the same buffer. Fractions of 12 ml were collected, and enzyme activity was determined as above. Protein concentrations were determined by the method of Bradford (3).

Purification of *E. coli* endonuclease III. Endonuclease III of *E. coli* was purified from a strain carrying plasmid pRPC54, which results in 100-fold overproduction of the enzyme. Cells were disrupted by sonication in 50 mM Tris hydrochloride (pH 8.0)–200 mM NaCl–2.5 mM EDTA–1 mM dithiothreitol (DTT)–0.1 mM PMSF. After centrifugation at 48,000 × *g* for 30 min, the supernatant was recovered (fraction I). Polymin-P was slowly added to fraction I with gentle stirring to a final concentration of 0.5%. After 1 h the mixture was centrifuged at 48,000 × *g* for 30 min. The supernatant was recovered, and ammonium sulfate was added slowly with stirring to a final concentration of 100% saturation. After 1 h the mixture was centrifuged at 48,000 × *g* for 30 min, and the precipitate was suspended in buffer B (100 mM potassium phosphate [pH 6.6], 0.1 mM DTT). This mixture was dialyzed overnight against buffer B and applied to a column of phosphocellulose. The column was washed with 100 ml of buffer B, and the absorbed proteins were eluted with a 1-liter gradient of potassium phosphate (pH 6.6) from 100 to 500 mM with 0.1 mM DTT. The most active fractions were pooled (fraction II). Fraction II was applied to a Sephadex G-75 column equilibrated with 50 mM potassium phosphate (pH 6.6)–150 mM NaCl. The most active fractions were pooled (fraction III). Fraction III was concentrated by vacuum dialysis against 100 mM potassium phosphate (pH 6.6) and applied to a Mono S column. The absorbed protein was eluted with a 20-ml gradient of 0 to 430 mM NaCl in the same buffer. Active fractions were pooled (fraction IV). Fraction IV was dialyzed against 100 mM potassium phosphate (pH 6.6)–200 mM NaCl–1 mM EDTA–0.1 mM DTT–50% glycerol for long-term storage at −20°C. Fraction IV was greater than 90% pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) and Coomasie blue staining. A second band of protein constituted the remainder of the protein in fraction IV.

Preparation of defined-sequence DNA damage substrates. A 112-base-pair *Sal*I-*Pvu*II restriction fragment (fragment A) generated from pUC18 (36) was 3′-end labeled (positive strand) with the Klenow fragment of DNA polymerase I and [α-^{32}P]deoxynucleotide triphosphates (3,000 Ci/mmol; New England Nuclear Corp.) and isolated on preparative polyacrylamide gels was described previously (13). The 3′-end-labeled 211-base-pair negative-strand fragment (fragment B) was also used as a DNA damage substrate. End-labeled DNA fragments were suspended in 10 mM Tris hydrochloride (pH 7.5)–1 mM EDTA (TE buffer).

Redoxyendonuclease digestion of end-labeled DNA damage substrates. 3′-end-labeled DNA substrates were incubated with 5 μl of redoxyendonuclease isolated from HeLa, CEM-Cl, or calf thymus cells or 2,000 U of *E. coli* endonuclease III (12) in buffer A plus 40 mM KCl (total volume, 40 μl) for 30 min at 37°C. Following incubation, DNA samples were extracted three times with phenol-chloroform-isoamyl alcohol (20:19:1, vol/vol/vol), ethanol precipitated, and subjected to PAGE.

UV irradiation of DNA substrates. DNA substrates in TE buffer were divided into 5-μl droplets on Parafilm and placed on ice. Droplets were UV-irradiated (254 nm) to a dose of 10,000 J/m^2 with a General Electric 15T8 germicidal lamp.

DNA substrates were subjected to enzyme or chemical treatments immediately following irradiation.

Ionizing radiation damage of DNA substrates. End-labeled DNA fragments were gamma-irradiated with a dose of 22 krad from a ^{137}Cs source (Gamma-Cell) in 10 mM KH_2PO_4 (pH 7.0)–10 mM KI as described previously (22). Following irradiation, DNA samples were ethanol precipitated and suspended in TE buffer.

Osmium tetroxide damage and hot alkali treatment of DNA substrates. End-labeled DNA fragments were brought to an osmium tetroxide concentration of 400 μg/ml in a volume of 100 μl and incubated at 70°C for 20 min. The reaction was terminated by extracting three times with 200 μl of ether to remove the osmium tetroxide. DNA samples were ethanol precipitated and suspended in TE buffer. When indicated, osmium tetroxide-treated DNA substrates were treated with 1 M piperidine in a volume of 100 μl at 90°C for 30 min. Under these conditions DNA is quantitatively cleaved at sites of osmium tetroxide-produced thymine glycols (16).

DNA sequencing reactions and PAGE. The purine (G+A) and pyrimidine (C+T) sequencing reactions (26) were performed, and the cleavage products were subjected to electrophoresis in parallel lanes alongside each set of samples in all experiments. Processed DNA samples were loaded onto denaturing 20% polyacrylamide–7 M urea gels and subjected to PAGE and autoradiography as described previously (13).

RESULTS

E. coli endonuclease III and the bovine redoxyendonuclease are relatively small (25 to 30 kilodaltons) and possess no divalent cation requirements (17; Helland et al., in press). Redoxyendonuclease assays can be performed in the presence of 10 mM EDTA. This property allows quantitation of these enzymes in crude extracts, since contaminating Mg^{2+}-dependent endonucleases are completely inhibited (18; Helland et al., in press). Previous studies with both *E. coli* endonuclease III (29) and several mammalian endonucleases (7, 19, 28; Helland et al., in press) have demonstrated that enzyme activity may be rapidly and quantitatively assessed by incubation of endonuclease preparations with UV-irradiated supercoiled plasmid or phage RFI DNA followed by determination of the conversion of covalently closed circular (form I) DNA to nicked circular DNA (form II). As previously described for calf thymus and mouse plasmacytoma extracts (18, 19), high doses (500 J/m^2 or greater) are required to produce substantial endonuclease activity above background levels, indicating that the UV damage recognized by the endonuclease is not a cyclobutane pyrimidine photoproduct. The nucleotide sites of endonuclease incision cannot, however, be determined by this method.

To identify redoxyendonuclease activity in human cells, we used two criteria for the identification of this activity in human cell extracts; the ability of extracts to introduce nicks specifically into damaged, supercoiled DNA in the presence of 10 mM EDTA and to incise end-labeled defined-sequence DNA damage substrates at sites of pyrimidines in a pattern similar to that of the previously described redoxyendonucleases of *E. coli* and calf thymus.

Endonuclease activity specific for UV irradiated DNA was measured in extracts from several different types of cultured human cells. Extracts from each cell line were prepared in an identical fashion as described in Materials and Methods. Endonuclease activity was measured by the binding of nicked φX174 RF [^{3}H]DNA to nitrocellulose filters (19). Endonuclease activity specific for UV-irradiated DNA

TABLE 1. Redoxyendonuclease activity[a] in mammalian cell lines

Redoxyendonuclease source	Activity (U/mg of protein)
Calf thymus	12,361
HeLa	5,261
PC1	812
PC2	2,820
Lymphoblasts (CEM-C1)	17,543
PC1	10,892
PC2	13,362
Fibroblasts	
AG2804[b]	628
IMR90	342
GM637[b]	714
AG1521	442
AG1522	457
Xeroderma pigmentosum (M1)[b]	442
Ataxia telangiectasia	
GM5849[b]	371
GM2052	542
GM367	442

[a] Cell extracts were prepared and assayed for redoxyendonuclease activity on UV-damaged, ^{3}H-labeled φX174 RFI DNA substrates and for protein concentration as described in Materials and Methods. Background activity on undamaged DNA substrate was subtracted from activity on UV-damaged DNA substrate to obtain redoxyendonuclease activity.

[b] SV40-transformed fibroblast cell lines.

was detected in each of the extracts tested (Table 1). Activities ranged from 342 U/mg of protein in extracts of IMR90 cells to 17,543 U/mg of protein in CEM-C1 cells. Equivalent levels of redoxyendonuclease activity were present in both primary fibroblast cell lines and SV40-transformed fibroblast cell lines. Somewhat higher levels of activity were also present in the human cell line HeLa, derived from an epithelial carcinoma, and CEM-C1 calls, derived from lymphoblasts. These results suggest that redoxyendonuclease activity is broadly distributed among cells derived from various malignant and nonmalignant tissues.

Redoxyendonuclease activity in radiation-sensitive human cell lines. We wished to establish the presence or absence of redoxyendonuclease activity in extracts of DNA repair-deficient human cells. Cells isolated from ataxia telangiectasia patients are highly sensitive to the lethal effects of ionizing radiation (33) and radiomimetic drugs (31, 32, 34) and may be deficient in an activity that repairs damage by such agents. Cells derived from individuals with xeroderma pigmentosum are highly sensitive to UV light (11) and lack the ability to repair cyclobutane dimers and possibly other types of UV-induced DNA photoproducts (15). Mammalian and *E. coli* redoxyendonucleases recognize pyrimidine photoproducts induced by high doses of UV light (14). The UV photoproducts recognized by redoxyendonuclease are neither cyclobutane pyrimidine dimers nor (6-4)pyrimidine dimers because they occur at either isolated or adjacent pyrimidines. The exact nature of these UV photoproducts has not yet been established.

The level of redoxyendonuclease activity was determined in cell line M1, an SV40-transformed fibroblast line derived from a patient with xeroderma pigmentosum (complementation group A) that retains marked sensitivity to killing by UV

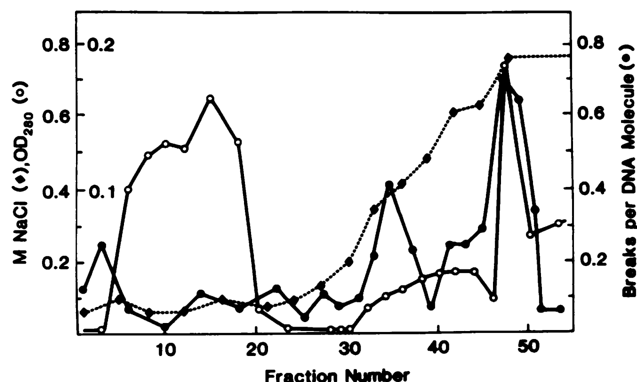

FIG. 1. Phosphocellulose chromatography of redoxyendonuclease from human lymphoblasts. CEM-Cl cells (ca. 5×10^9) were cultured, and redoxyendonuclease was extracted as described in Materials and Methods. The enzyme-containing fractions passing through DEAE-cellulose were collected, and chromatography on phosphocellulose was performed as described in Materials and Methods. Fractions containing enzyme activity (fractions 33 to 37, PC1, and fractions 41 to 51, PC2) were pooled and dialyzed against storage buffer (50% glycerol, 10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA, 0.1 M NaCl). Breaks per DNA molecule correspond to the fraction of ϕX174 DNA converted to nicked molecules.

light (30). M1 cell extracts had a level of redoxyendonuclease equivalent to that present in normal and SV40-transformed fibroblast lines (Table 1). We also determined the level of redoxyendonuclease activity in three ataxia telangiectasia cell lines, two fibroblast lines (GM2052 and GM367), and an SV40-transformed fibroblast line (GM5849). Each of these cell lines is markedly sensitive to killing by X-rays and bleomycin (20). The levels of redoxyendonuclease measured in extracts of these cells were all within the range measured for extracts from normal cells. These experiments indicate that the sensitivity of xeroderma pigmentosum cells to UV light and the sensitivity of ataxia telangiectasia cells to gamma irradiation is not due to a gross deficiency in the level of redoxyendonuclease activity.

Phosphocellulose chromatography resolves two forms of redoxyendonuclease in human cell extracts. To determine whether the human redoxyendonuclease was similar to the recently characterized enzyme from calf thymus, human cell extracts were subjected to phosphocellulose chromatography. Phosphocellulose chromatography is the initial purification step for the calf thymus redoxyendonuclease, which elutes from phosphocellulose in two peaks of activity at approximately 0.2 M (PC1) and 0.5 M PC2) NaCl. When extracts from CEM-C1 (Fig. 1) and HeLa (data not shown) cells were subjected to chromatography under the same conditions, a similar resolution of two peaks of activity was observed. The functional significance of two apparently different forms of redoxyendonuclease is unknown. Preliminary experiments have not determined any difference in the behavior of these two forms, either by gel exclusion chromatography or by sequence specificity of cleavage of UV-irradiated or osmium tetroxide-treated DNA.

Human calf thymus, and *E. coli* redoxyendonucleases cleave DNA at sites of thymine glycol. The substrate specificities of redoxyendonucleases from human, bovine, and bacterial cells were determined by using DNA of defined sequence containing a specific type of base damage. For these purposes, 3'-end-labeled DNA was reacted with osmium textroxide under conditions resulting in the production of thymine glycol as the primary base damage (8). Such DNA damage substrates were digested with HeLa, CEM-C1, calf thymus, and *E. coli* redoxyendonucleases. The redoxyendonuclease-generated DNA scission products were analyzed on DNA sequencing gels and compared with the base-specific Maxam and Gilbert DNA-sequencing fragments (Fig. 2). Osmium tetroxide-damaged DNA was also treated with hot alkali, a procedure that cleaves DNA at sites of thymine glycol and is a thymine-specific DNA-sequencing reaction (16). All four redoxyendonuclease preparations produced a similar pattern of DNA cleavage at sites of thymine (Fig. 2). DNA damaged with osmium tetroxide and incubated without enzyme in reaction buffer did not produce DNA scission products (20) (data not shown). The electrophoretic mobilities of the enzyme-generated DNA scission products were the same for all the redoxyendonucleases and identical to the hot-alkali-generated chemical cleavage DNA products. We conclude that redoxyendonucleases from human, bovine, and bacterial sources recognize and cleave DNA at sites of thymine glycol in a similar manner.

Human redoxyendonuclease cleaves gamma-irradiated DNA at damaged cytosines and thymines. The substrate specificities of human, bovine, and bacterial redoxyendonucleases were compared by using end-labeled DNA substrates damaged with ionizing radiation (22 krads). Such DNA substrates contain numerous types of damage, including ring-saturated, ring-cleaved, and ring-contracted pyrimidines (9). As previously reported (18, 21, 22), end-labeled DNA damaged with ionizing radiation alone produced a uniform base cleavage pattern of faint bands of equal intensity at all base sites, indicative of direct strand breakage (18, 20–22) (data not shown). Redoxyendonuclease cleavage of ionizing-radiation-induced pyrimidine base damage was evident as dark bands superimposed on the fainter background of uniform strand breakage (Fig. 3). Comparison of the enzyme-generated DNA scission products revealed that the HeLa, CEM-C1, bovine, and bacterial redoxyendo-

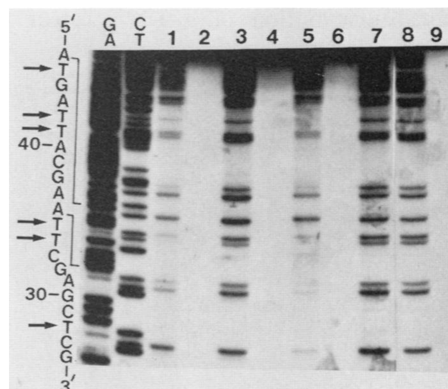

FIG. 2. Redoxyendonuclease cleavage of DNA at sites of thymine glycol. 3'-end-labeled restriction fragment B was reacted with osmium tetroxide under conditions producing thymine glycol as the predominant base damage (Materials and Methods). Damaged (lanes 1, 3, 5, 7, and 8) and undamaged (lanes 2, 4, 6, and 9) DNA samples were incubated with HeLa redoxyendonuclease (PC2 fraction) (lanes 1 and 2), CEM-C1 redoxyendonuclease (PC2 fraction) (lanes 3 and 4), calf thymus redoxyendonuclease (lanes 5 and 6), *E. coli* endonuclease III (lanes 8 and 9), or hot alkali (lane 7). Treated DNA samples were subjected to electrophoresis on sequencing gels, followed by autoradiography. The purine-specific (G+A) and pyrimidine-specific (C+T) DNA sequencing reactions were loaded adjacent to the enzyme reaction lanes. Arrows indicate redoxyendonuclease incision at sites of thymine glycols. Base numbering starts from the 3'-end-labeled terminus of the restriction fragment.

nucleases cleaved DNA damaged with ionizing radiation at the same cytosine and thymine base sites (Fig. 3). Although the overall pattern of redoxyendonuclease cleavage of damaged DNA was similar for each of the four enzyme preparations, the extent of cleavage (band intensities at sites of pyrimidines) varied somewhat among the different enzyme sources. Such differences can be attributed to differences in the specific activities of the redoxyendonuclease preparations and differences in the amount of radioactivity loaded in the gel lanes, but might also be due to minor differences in the sequence specificity of cleavage by redoxyendonucleases from various species. From this experiment it is apparent that the substrate specificities of redoxyendonucleases are similar regardless of the cell source.

Sequence specificity of DNA cleavage by redoxyendonucleases from repair-deficient cell lines. Although the total level of redoxyendonuclease was equivalent to that in normal cells in the ataxia telangiectasia and xeroderma pigmentosum cell lines examined, such cells might contain an endonuclease with altered specificity of DNA cleavage. To test this possibility, UV-irradiated DNA fragments were treated with redoxyendonuclease from ataxia telangiectasia, xeroderma pigmentosum, or calf thymus cells. For each of these cell extracts the overall pattern of sequence specificity of DNA cleavage was similar (Fig. 4). However, as in the experiments with ionizing-radiation-damaged DNA substrates (Fig. 3), the extent of cleavage differed somewhat among the ataxia telangiectasia, xeroderma pigmentosum, and calf thymus redoxyendonuclease preparations, probably reflecting the differences in specific activity among these preparations. When osmium tetroxide-treated DNA was the substrate, cleavage specificity by normal and repair-deficient cell extracts was indistinguishable (data not shown).

DISCUSSION

This work provides insight into the substrate specificity and biological distribution of a DNA repair enzyme,

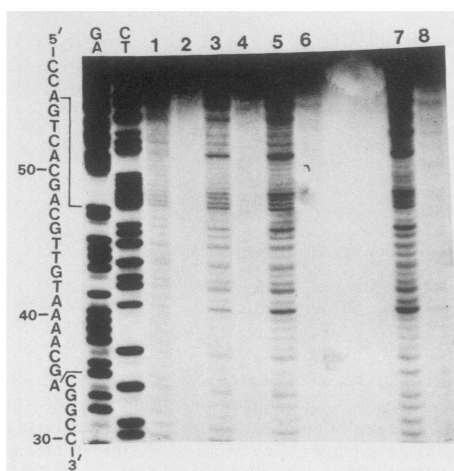

FIG. 3. Redoxyendonuclease cleavage of DNA damaged with ionizing radiation. 3'-end-labeled restriction fragment A was exposed to a dose of 22 krads from a ^{137}Cs source. Irradiated (lanes 1, 3, 5, and 7) and unirradiated (lanes 2, 4, 6, and 8) DNA samples were incubated with HeLa (PC2 fraction) (lanes 1 and 2), CEM-C1 (PC2 fraction) (lanes 3 and 4), or calf thymus (lanes 5 and 6) redoxyendonuclease or *E. coli* endonuclease III (lanes 7 and 8). Treated DNA samples were analyzed as described in the legend to Fig. 2. Bases are numbered from the 3'-end-labeled terminus of the restriction fragment.

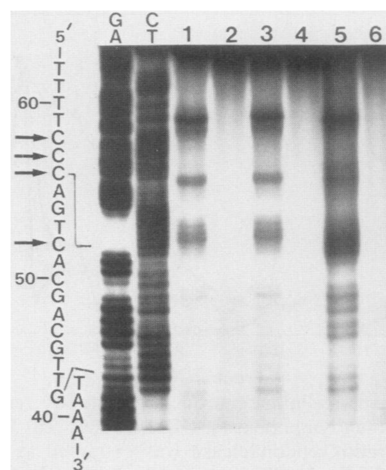

FIG. 4. Cleavage of UV-irradiated DNA by redoxyendonuclease from ataxia telangiectasia and xeroderma pigmentosum cells. 3'-end-labeled restriction fragment A was UV-irradiated as described in Materials and Methods. Irradiated (lanes 1, 3, and 5) and unirradiated (lanes 2, 4, and 6) DNA samples were incubated with extracts prepared from ataxia telangiectasia cells (GM5849) (lanes 1 and 2), xeroderma pigmentosum cells (M1) (lanes 3 and 4), or calf thymus redoxyendonuclease (lanes 5 and 6). Treated DNA samples were subjected to gel electrophoresis and autoradiography as described in the legend to Fig. 2. Arrows indicate the sites of redoxy-endonuclease incision at sites of pyrimidine base damage. Base numbering starts from the 3'-end-labeled terminus of the restriction fragment.

redoxyendonuclease. It has previously been shown by us that calf thymus cells contain this enzyme (18). Our present study demonstrates that an enzyme with the same broad substrate specificity is present in a wide variety of human cells. Of the bases in cellular DNA, the pyrimidines are particularly susceptible to alteration by reaction with reduced oxygen species, such as the hydroxyl radicals that are produced by ionizing radiation, during phagocytosis, and at low levels by normal metabolic processes (1). These reactions produce a variety of products derived from thymine and cytosine in which the aromaticity of the heterocyclic ring is destroyed (9). These products include oxidations of thymine at the 5,6 bond to yield the various stereoisomers of thymine glycol and hydroxy-dihydrothymine, reduction at the same position to yield dihydrothymine, and ring fragment products such as urea residues. The 5,6 double bond of the cytosine bases is similarly susceptible to attack by hydroxyl radicals resulting in addition across the double bond. Therefore, most but not all products of reduction or oxidation of the pyrimidine bases in DNA result in the loss of aromaticity and also a loss of planarity for the altered base. This loss of planarity should produce a local distortion in the conformation of the DNA and may also interfere with normal hydrogen bonding between complementary bases.

In view of the large number of agents that produce damage to pyrimidines at the 5,6 double bond, it is not surprising that cells have evolved efficient enzyme systems for coping with such damage and that such repair systems might be very similar from species to species and tissue to tissue. In *E. coli*, the principal enzyme responsible for recognition of ring-saturated and ring-fragmented pyrimidines is endonuclease III. This enzyme is a single polypeptide with two distinct activities, a DNA glycosylase and an apurinic-apyrimidinic (AP) endonuclease that hydrolyzes the DNA at the base-free site resulting from glycosylase action. We have

recently reported the substrate specificity (18) and mechanism of action (14) of an enzyme from calf thymus that is similar in many respects to the endonuclease III of *E. coli*. This calf thymus enzyme also contains both a thymine glycol glycosylase and AP endonuclease activities. This report establishes that enzymes with similar substrate specificity are present in cells of several human tissue types. The possibility of more than one human endonuclease combining the substrate specificites of *E. coli* endonuclease III and the bovine redoxyendonuclease cannot be ruled out until a homogeneous human redoxyendonuclease becomes available.

A puzzling feature of DNA repair enzymes from mammalian cells has been the presence of enzyme activities that incise DNA following damage by either UV light, X rays, or oxidizing agents such as $KMnO_4$, OsO_4, and H_2O_2 (7, 24, 27, 28). The dose response for induction of enzyme-sensitive sites by UV light indicated that the sites could be neither cyclobutane nor (6-4)pyrimidine dimers (unpublished results). We have recently demonstrated, through the use of DNA-sequencing techniques, that calf thymus redoxyendonuclease cleaves UV-irradiated DNA at altered cytosines (14). Therefore, the apparently broad specificity of damage recognized by these enzymes is due to their recognition of a number of different chemical lesions that occur at pyrimidine sites. The sequence specificity of cleavage results from the characteristic ratio of cytosine to thymine damage produced by each agent. UV irradiation produces predominantly damage to cytosine. Gamma irradiation and H_2O_2 produce roughly equal amounts of cytosine and thymine damage, and osmium tetroxide produces predominantly altered thymines.

The present results eliminate a gross deficiency in redoxyendonuclease activity as the cause of either xeroderma pigmentosum or ataxia telangiectasia, both hereditary diseases associated with increased radiation sensitivity. The overall level of redoxyendonuclease activity and the sequence specificity of cleavage of UV-irradiated or osmium tetroxide-treated DNA are similar for extracts from normal and DNA repair-deficient cell lines. A more subtle difference between the redoxyendonucleases of normal and repair-deficient cells cannot be eliminated until the primary structure, isoenzyme composition, and subcellular location of redoxyendonucleases are defined in normal and DNA repair-deficient cells.

The distribution of redoxyendonuclease in bacterial, bovine, and human cells indicates a broad species distribution for this enzyme. We suspect that activities in cells from other species that have been characterized only by their activity against a single type of damaged oxidized pyrimidine (e.g., thymine glycol) are actually properties of redoxyendonuclease, an enzyme with a much broader substrate specificity. The conservation of activities that recognize oxidative pyrimidine damage suggests that the repair of such damage may be critical for maintaining the genetic and biological integrity of cells.

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