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Yeast Redoxyendonuclease, a DNA Repair Enzyme Similar to *Escherichia coli* Endonuclease III†

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ABSTRACT: A DNA repair endonuclease (redoxyendonuclease) was isolated from bakers' yeast (*Saccharomyces cerevisiae*). The enzyme has been purified by a series of column chromatography steps and cleaves OsO_4-damaged, double-stranded DNA at sites of thymine glycol and heavily UV-irradiated DNA at sites of cytosine, thymine, and guanine photoproducts. The base specificity and mechanism of phosphodiester bond cleavage for the yeast redoxyendonuclease appear to be identical with those of *Escherichia coli* endonuclease III when thymine glycol containing, end-labeled DNA fragments of defined sequence are employed as substrates. Yeast redoxyendonuclease has an apparent molecular size of 38 000–42 000 daltons and is active in the absence of divalent metal cations. The identification of such an enzyme in yeast may be of value in the elucidation of the biochemical basis for radiation sensitivity in certain yeast mutants.

The genetics of the DNA repair pathways of *Saccharomyces cerevisiae* have been studied extensively. A number of loci have been identified that, when mutated, result in an increased sensitivity to chemical and physical DNA damaging agents. Of the nearly 100 different mutants so far characterized, 35 of these are radiation sensitive and have been grouped into 3 epistasis groups termed RAD3, RAD6, and RAD52 and are thought to constitute different cellular responses to ultraviolet and/or ionizing radiation-induced DNA damage [for a review, see Game (1983)]. Although a wealth of information exists concerning the genetic aspects of yeast DNA repair, there are comparatively few studies that address the biochemical characteristics of this system. Such existing investigations have focused primarily on the identification of various apurinic/apyrimidinic (AP)[1] endonuclease activities (Pinon, 1970; Chlebowicz & Jachymczyk, 1977; Armel & Wallace, 1978, 1984; Bryant & Haynes, 1978a; Futcher & Morgan, 1979; Akhmedov et al., 1982), although there are reports identifying other enzymes including uracil–DNA glycosylase (Crosby et al., 1981) and UV-specific endonucleases (Bryant & Haynes, 1978b; Bekker et al., 1980).

In consideration of the types of DNA repair enzymes that should be present in yeast, it is noteworthy that mutants in several RAD loci (*rad6*, *rad9*, *rad18*) are sensitive to both UV light and ionizing radiation (Game, 1983). Previous studies by our laboratory (Doetsch et al., 1986, 1987; Helland et al., 1986) and others (Bachetti & Benne, 1975; Gates & Linn 1977; Katcher & Wallace, 1983; Brent, 1983) have indicated that *Escherichia coli* endonuclease III and a similar mammalian enzyme, redoxyendonuclease, recognize oxidative DNA damage induced by both UV light and X-rays. Thymine glycol, a major product of ionizing radiation damage (Teoule & Cadet, 1978), and other ring-saturated, ring-cleaved, and ring-contracted base damage products appear to be substrates

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[1] Abbreviations: bp, base pair(s); Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonyl fluoride; DMSO, dimethyl sulfoxide; AP, apurinic/apyrimidinic; SDS, sodium dodecyl sulfate.

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for this group of enzymes (Breimer & Lindahl, 1984; Helland et al., 1986). The bacterial and mammalian enzymes act on such substrates via a combined *N*-glycosylase/AP endonuclease mechanism whose net effect is to remove the damaged base and cleave the phosphodiester backbone of the damaged DNA strand (Demple & Linn, 1980; Katcher & Wallace, 1983; Doetsch et al., 1986). Since this activity is conserved among bacterial and mammalian cells, we explored the possibility that other, nonmammalian eukaryotes might also contain a similar activity. In addition, because the yeast system is considered to be a representative model for eukaryotic DNA repair of radiation damage, and because certain RAD loci are responsible for processing lesions due to both UV and ionizing radiation-induced DNA damage, we sought to determine whether or not an activity similar to *E. coli* endonuclease III and the mammalian redoxyendonuclease was present in *S. cerevisiae*.

In this study, we report the purification and characterization of an endonuclease present in bakers' yeast that cleaves thymine glycol containing DNA substrates of defined sequence in a manner identical with that of *E. coli* endonuclease III. Analysis of the enzyme-generated DNA scission products on DNA sequencing gels provided a direct indication of the sites of phosphodiester backbone breakage at the level of individual nucleotides. The yeast enzyme also cleaves UV-irradiated DNA at sites of individual cytosines and thymines and, in this respect, also resembles *E. coli* endonuclease III and redoxyendonuclease isolated from human lymphoblasts. The yeast redoxyendonuclease is unlike any of the previously reported AP endonucleases and other DNA repair enzymes isolated from yeast and may provide insight into the biochemistry of the radiation repair pathways in *S. cerevisiae*.

Materials and Methods

Enzymes and Chemicals. *E. coli* endonuclease III was purified as described (Doetsch et al., 1987). Human lymphoblast redoxyendonuclease was purified through the phosphocellulose chromatography step (Doetsch et al., 1987) and was further purified by Sephadex G-100 (superfine) chromatography (Lee et al., 1987). Restriction enzymes, DNA polymerase I (Klenow fragment), and T4 polynucleotide kinase were purchased from Bethesda Research Laboratories. [γ-^{32}P]ATP (specific activity 5000 Ci/mmol) and [α-^{32}P]dNTPs (specific activity 800 Ci/mmol) were purchased from Amersham. All other chemicals were of the highest commercially available purity.

DNA Nicking Assay of Yeast Redoxyendonuclease. The reaction mixture (25-μL final volume) contained 15 mM KH_2PO_4, pH 6.8, 10 mM EDTA, 10 mM 2-mercaptoethanol (buffer B) plus 40 mM KCl, 85 fmol of OsO_4-damaged pUC19 supercoiled plasmid DNA, and 0.05–10 μg of various yeast redoxyendonuclease preparations. Following incubation at 37 °C for 30 min, the reaction was terminated by addition of 3 μL of stop solution (10% SDS, 0.25% bromphenol blue, and 25% ficoll), loaded onto a 0.9% agarose minigel (Model I, Dan-Kar), and electrophoresed for 90 min at 80 V. The amounts of supercoiled (form I) and nicked (form II) DNA in a given sample were determined by densitometric scans (Bio-Rad Model 620 video densitometer) of the photographic negative of the UV light illuminated, ethidium bromide stained gel. The number of nicks per pUC19 molecule was determined by the relation $n = -\ln$ (form I fraction) (Jorgensen et al., 1987). One unit of yeast redoxyendonuclease activity is defined as 1 fmol of nicks per minute on OsO_4-damaged pUC19 DNA that contains an average of three thymine glycol sites per molecule.

Preparation of Defined-Sequence DNA Damage Substrates. A 201 bp *SalI–PvuII* restriction fragment was generated from pUC19 and was either 3′ end labeled (fragment 1) with [α-^{32}P]dNTPs and DNA polymerase I (Klenow fragment) or 5′ end labeled (fragment 2) with [γ-^{32}P]ATP and polynucleotide kinase as previously described (Doetsch et al., 1986). The end-labeled, defined sequence DNA fragments [specific activity (2–10) $\times 10^6$ cpm/μg of DNA] were isolated on preparative, nondenaturing polyacrylamide gels as previously described (Doetsch et al., 1985) and, following recovery, were resuspended in TE buffer (10 mM Tris-HCl, pH 7.5, and 1 mM EDTA). These defined-sequence DNA fragments were damaged with either OsO_4 or UV light and used as substrates for the yeast and human redoxyendonucleases and *E. coli* endonuclease III.

Osmium Tetroxide Damage of DNA Substrates. End-labeled DNA fragments of defined sequence (25–100 ng) were exposed to OsO_4 (700 μg/mL) and incubated at 70 °C for 20 min in TE buffer (100-μL final volume). The reaction was terminated by extracting 5 times with a 2-fold volume of ether, and the oxidized DNA was precipitated and resuspended in TE buffer. Supercoiled pUC19 DNA (200 μg) was reacted with OsO_4 (300 μg/mL) and incubated at 70 °C for 90 min in 100 mM NaCl, 10 mM Tris-HCl, pH 7.6, and 1 mM EDTA (600-μL final volume). The reaction was terminated as described above, and the DNA was resuspended in TE buffer. This treatment resulted in an average of three thymine glycol sites per pUC19 molecule (Jorgensen et al., 1987).

UV Light Damage of DNA Substrates. End-labeled DNA substrates (5-μL droplets on ice) were UV irradiated (254 nm) with a dose of 10000 J/m^2 from a Mineralight shortwave 34-0003-015W lamp. Irradiated DNA samples were subjected to enzyme treatments immediately after irradiation.

Redoxyendonuclease Digestion of End-Labeled DNA Substrates. OsO_4 or UV-damaged, end-labeled DNA substrates (5–20 ng) were incubated with various amounts of yeast redoxyendonuclease (1.7–5.2 μg) or 1000 units (Cunningham & Weiss, 1985) of *E. coli* endonuclease III in buffer B plus 40 mM KCl (40-μL final volume) for 30 min, 37 °C. The amount of human redoxyendonuclease used in these experiments was approximately equivalent to the amount of yeast redoxyendonuclease. Saturating amounts of *E. coli*, yeast, and human enzymes were used; additional amounts did not produce an increase in the observed DNA scission products. Following incubation, DNA samples were extracted 3 times with phenol–chloroform–isoamyl alcohol (20:19:1 v/v), ethanol precipitated, and subjected to electrophoresis on DNA sequencing gels. The presence of yeast redoxyendonuclease in various column fractions was monitored by incubating a 10–25-μL aliquot with UV-damaged, end-labeled defined-sequence substrates under the conditions described above followed by analysis of the incubations on DNA sequencing gels. The presence of redoxyendonuclease was revealed as a series of pyrimidine-specific incisions occurring primarily at sites of cytosine on UV-irradiated DNA substrates (Doetsch et al., 1986; Helland et al., 1986).

Hot Alkali Treatment of OsO_4-Damaged DNA Substrates. When indicated, OsO_4-damaged, end-labeled DNa substrates (5–20 ng) 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 OsO_4-induced thymine glycol (Friedman & Brown, 1980).

DNA Sequencing and PAGE. The purine (G + A) and pyrimidine (C + T) specific DNA chemical cleavage reactions (Maxam & Gilbert, 1980) were performed and run alongside

each set of enzyme digestions in all experiments. Processed DNA samples were loaded onto denaturing 20% polyacrylamide–7 M urea gels and subjected to electrophoresis and autoradiography as previously described (Doetsch et al., 1985).

Purification of Yeast Redoxyendonuclease. (*A*) *Preparation of Yeast Cell Extracts.* Yeast cells (170 g dry weight) from dried, active, commercial bakers' yeast (Nabisco) were soaked at 4 °C in 150 mL of 50 mM KH_2PO_4, pH 8.7, 0.5 mM PMSF, and 1% DMSO (buffer Y) plus 0.2 M NaCl for 45 min. The resulting cell paste (approximately 200 g) was divided into three equal portions, and each portion was mixed with 210 g of glass beads (0.5-mm diameter) and approximately 50 mL of buffer Y plus 0.12 M NaCl. Each yeast cell–glass bead batch was loaded into a Bead Beater (Biospec Products, Bartlesvile, OK) and subjected to four spin bursts of 1-min duration. The cells were held in an ice bath for 4 min between each burst. The beads were separated from the disrupted cells, and the resulting yeast homogenates from each original portion were pooled (550-mL total volume), brought to 0.7 M NaCl by adding 5 M NaCl, and held on ice for 30 min to disrupt the chromatin. Buffer Y was added to bring the chromatin extracts to 0.3 M NaCl, and this material was centrifuged at 100000*g* for 30 min, 4 °C. The 100000*g* supernatants were pooled (1085-mL total volume) and constituted fraction I (Table I). Fraction I was loaded onto a DEAE-cellulose column (Whatman DE52, 4 × 40.5 cm) equilibrated with 10 mM Tris-HCl, pH 8.0, 10 mM EDTA, and 10 mM 2-mercaptoethanol (buffer A) in 0.3 M NaCl. Yeast redoxyendonuclease did not bind to the column and was eluted with the same buffer as a broad peak of activity, while most nucleic acids and some proteins are retained. The DEAE-cellulose flow-through fractions containing redoxyendonuclease activity were pooled (fraction II).

(*B*) *Phosphocellulose Chromatography.* Fraction II was dialyzed against buffer A plus 50 mM NaCl and applied to a phosphocellulose column (Whatman P11, 3.8 × 33.5 cm) equilibrated in the same buffer and washed until the absorbance at 280 nm was zero. The column was eluted with a 0.05–1.0 M NaCl linear gradient (1000 mL/1000 mL) in buffer A (12-mL fractions), and redoxyendonuclease activity was monitored as described above. The enzyme eluted in the range of 0.35–0.65 M NaCl, and the fractions containing redoxyendonuclease activity were pooled (552-mL total volume), concentrated, and dialyzed against buffer B plus 50 mM NaCl (fraction III). The 50 mM NaCl phosphocellulose wash was passed through the phosphocellulose column a second time, and the elution procedure described above was repeated, and resulted in the recovery of an additional amount of yeast redoxyendonuclease (fraction IVb).

(*C*) *DNA–Cellulose Chromatography.* Fraction III was applied to a DNA–cellulose column (Sigma, 1.8 × 13 cm, 4.3 mg of double-stranded calf thymus DNA/g of dry cellulose) equilibrated in buffer B plus 50 mM NaCl. The column was washed with equilibration buffer (625 mL) until the absorbance at 280 nm was zero. Yeast redoxyendonuclease was eluted with buffer B plus 0.8 M NaCl (175 mL) and was concentrated and dialyzed against buffer B plus 0.5 M NaCl (fraction IVa).

(*D*) *Sephacryl S-200 Chromatography.* Fraction IVa was applied to a calibrated Sephacryl S-200 column (Pharmacia, 2.5 × 118 cm) equilibrated in buffer B plus 0.5 M NaCl and was eluted with 500 mL of the same buffer. Yeast redoxyendonuclease activity eluted as a broad peak, and the fractions containing activity were pooled (40-mL total volume), concentrated, and dialyzed against buffer B plus 0.1 M NaCl in 50% glycerol (storage buffer) (fraction Va). Fraction IVb was also subjected to an identical procedure, and the resulting pooled, redoxyendonuclease-containing fractions (40-mL total volume) were concentrated and dialyzed against storage buffer (fraction Vb).

Table I: Purification of Yeast Redoxyendonuclease[a]

step	purification step	protein (mg)	sp act. (units/mg)[b]	yield (%)[c]
I	crude extract	15841	27	
II	DEAE-cellulose	13410	73	100
III	phosphocellulose 1	326	887	29.5
IVa	DNA–cellulose	133	1433	19.4
IVb	phosphocellulose 2	145	24	0.35
Va	Sephacryl S-200 1	8.0	432	0.35
Vb	Sephacryl S-200 2	19.1	152	0.30
VI	Sephadex G-100	10.1	216	0.22

[a] Yeast redoxyendonuclease was purified by a series of column chromatography steps as described under Materials and Methods. [b] Specific activity was determined by using a nicking assay employing thymine glycol containing, supercoiled pUC19 DNA (Materials and Methods). [c] Percent yield was calculated starting with fraction II following removal of nucleic acids by DEAE-cellulose chromatography.

(*E*) *Sephadex G-100 Chromatography.* Fractions Va and Vb were combined and applied to a calibrated Sephadex G-100 (Superfine) column (Pharmacia, 1.5 × 94 cm) equilibrated with buffer B plus 0.5 M NaCl. The column was eluted with 150 mL of the same buffer, and the fractions containing the highest levels of redoxyendonuclease activity corresponded to a molecular size range of 38 000–42 000 daltons. The active fractions (14-mL total volume) were pooled, concentrated, and dialyzed against storage buffer (fraction VI).

RESULTS

Initial Purification and Properties. Previous studies with *E. coli* endonuclease III and calf thymus and human redoxyendonucleases have established that these enzymes cleave OsO_4-damaged DNA substrates at sites of thymine glycol and heavily UV-irradiated DNA primarily at sites of monobasic pyrimidine photoproducts (Doetsch et al., 1986, 1987; Helland et al., 1986; Weiss & Duker, 1987). The site specificity of DNA cleavage is nearly identical for these three enzymes, and this property was utilized to establish the presence and determine some of the properties of yeast redoxyendonuclease. Yeast redoxyendonuclease was purified from dried bakers' yeast (*S. cerevisiae*) by a series of column chromatography steps, and is summarized in Table I. For each purification step, yeast redoxyendonuclease activity was monitored on thymine glycol containing, supercoiled pUC19 DNA and either heavily UV-irradiated or thymine glycol containing ^{32}P end-labeled DNA fragments of defined sequence and compared to both *E. coli* endonuclease III and human redoxyendonuclease.

Yeast redoxyendonulcease efficiently cleaved UV or OsO_4-damaged DNA in the presence of 10 mM EDTA, indicating that divalent cations are not required for activity. The enzyme was inhibited by KCl or NaCl concentrations greater than 150 mM, a property similar to *E. coli* endonuclease III (Katcher & Wallace, 1983). The specific activity of the yeast crude extract (fraction I) is an underestimate due to the presence of nucleic acids in this preparation. For this reason, enzyme yields are given following the removal of nucleic acids, starting with fraction II (Table I). Chromatography on Sephadex G-100 (superfine) indicated that the molecular size of yeast redoxyendonuclease is 38 000–42 000 daltons.

Activity on Thymine Glycol Containing DNA. Thymine glycol, a DNA damage product produced by ionizing radiation

and other oxidizing agents, can be specifically introduced into DNA by treatment with OsO_4 (Burton & Riley, 1966). To determine the sequence specificity of yeast redoxyendonuclease cleavage of DNA containing thymine glycol and to establish whether or not the yeast enzyme was similar to *E. coli* endonuclease III and human redoxyendonuclease, OsO_4-damaged 3′ and 5′ end-labeled DNA fragments of defined sequence were utilized as substrates. The enzyme-generated DNA scission products were analyzed on DNA sequencing gels and compared to each other and the base-specific DNA sequencing chemical cleavage products. This method allowed for the direct analysis of enzyme incision sites at the level of individual nucleotides within a sequence and also provides insight into the mechanism of DNA cleavage.

Yeast redoxyendonuclease cleaved 3′ end-labeled OsO_4-damaged DNA at sites of thymine glycol with a base specificity identical with that of both *E. coli* endonuclease III and human redoxyendonuclease (Figure 1A). Hot alkali treatment of 3′ end-labeled, OsO_4-damaged DNA substrates (a T-specific DNA sequencing reaction) produced a set of scission products that comigrated with the scission products of the yeast, *E. coli*, and human enzymes and hence represent identical species. DNA substrates damaged with OsO_4 alone, without further enzyme or chemical treatment, exhibited little, if any, background cleavages [not shown; Helland et al. (1986)]. *E. coli* endonuclease III, human redoxyendonuclease, and hot alkali cleavage of DNA at sites of thymine glycol result in the production of fragments containing 5′-phosphoryl groups (Demple & Linn, 1980; Katcher & Wallace, 1983; Doetsch et al., 1987). Our results indicate that this is also the case for yeast redoxyendonuclease and we conclude that thymine glycol is a substrate for this enzyme.

An identical experiment was performed with 5′ end-labeled OsO_4-damaged DNA. Yeast redoxyendonuclease produced DNA cleavage products identical with those produced by *E. coli* endonuclease III but were different compared to human redoxyendonuclease and hot alkali-generated cleavage products (Figure 1B). Although cleavage events occurred at sites of thymine glycol for each enzyme and chemical treatment, the electrophoretic mobilities of the scission products generated by the yeast and *E. coli* enzymes were shifted up with respect to the positions of the human redoxyendonuclease and hot alkali-generated scission products. *E. coli* endonuclease III produces DNA cleavage products that contain a 3′-terminal base-free sugar or modified sugar (Demple & Linn, 1980; Katcher & Wallace, 1983; Doetsch et al., 1986), and the results shown in Figure 1B indicate that such a product is also produced by yeast redoxyendonuclease. In contrast, hot alkali cleavage of thymine glycol containing DNA and the other base-specific chemical cleavage reactions produce DNA cleavage products that contain a 3′-phosphoryl group (Maxam & Gilbert, 1980). These data suggest that yeast redoxyendonuclease cleaves damaged DNA via a mechanism similar to *E. coli* endonuclease III to produce DNA cleavage products containing 5′-phosphoryl and 3′ base-free sugar groups.

Activity on UV-Damaged DNA. UV irradiation (254 nm) of 3′ end-labeled DNA of defined sequence results in the formation of monobasic photoproducts that occur primarily at positions of cytosine and less frequently at positions of thymine and are substrates for both *E. coli* endonuclease III and human redoxyendonuclease (Doetsch et al., 1987). Although the exact chemical nature of these photoproduct substrates has not been established, it is likely that they correspond to ring-saturated pyrimidine photohydrates such as 6-hydroxy-5,6-dihydrocytosine that have been previously iden-

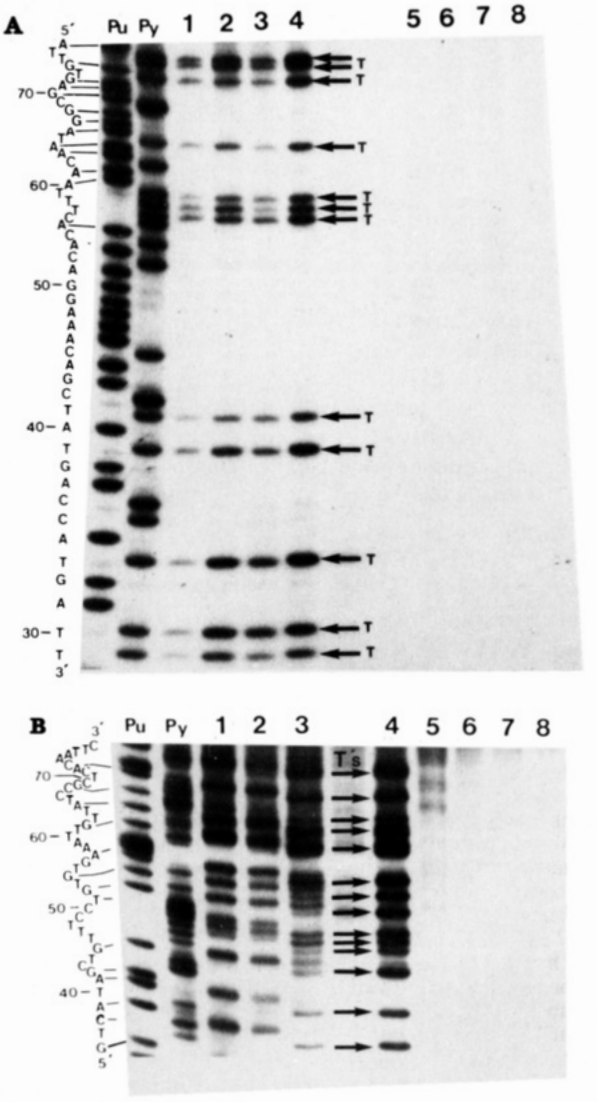

FIGURE 1: Sequence specificity of yeast redoxyendonuclease cleavage of thymine glycol containing DNA. (A) 3′ end-labeled DNA fragment 1 was damaged with OsO_4 under conditions producing thymine glycol as the predominant base damage (Materials and Methods). OsO_4-damaged (lanes 1–4) or undamaged (lanes 5–8) DNA fragments were reacted with hot alkali (lanes 1 and 8) or incubated with 1000 units of *E. coli* endonuclease III (lanes 2 and 7), 5.2 μg of yeast redoxyendonuclease from fraction Va (lanes 3 and 6), or 6.7 μg of human redoxyendonuclease (lanes 4 and 5). DNA reaction products were subjected to electrophoresis on DNA sequencing gels followed by autoradiography. The purine (Pu) and pyrimidine (Py) DNA sequencing reactions were run alongside the enzyme reaction lanes. Lane 1 (hot alkali) represents a T-specific DNA sequencing reaction (Friedman & Brown, 1980). Arrows indicate enzyme incisions at sites of thymine glycol. The amounts of radioactivity loaded into the sample lanes were as follows: 17 kcpm, lane 1; 78 kcpm, lane 2; 74 kcpm, lane 3; 65 kcpm, lane 4; 64 kcpm, lane 5; 70 kcpm, lane 6; 88 kcpm, lane 7; 18 kcpm, lane 8. The majority of radioactivity in each lane corresponded to uncleaved, full-length DNA fragments and was located at the top of the gel (not shown). Base numbering starts from the 3′ end-labeled terminus of the restriction fragment. (B) 5′ end-labeled DNA fragment 2 was reacted with OsO_4 as in (A). OsO_4-damaged (lanes 1–4) or undamaged (lanes 5–8) DNA fragments were incubated with *E. coli* endonuclease III (lanes 1 and 8), 2.6 μg of yeast redoxyendonuclease from fraction VI (lanes 2 and 7), human redoxyendonuclease (lanes 3 and 6), or hot alkali (lanes 4 and 5). Analysis of DNA scission products was as described in (A). Arrows indicate chemical cleavage at sites of thymine glycol. The amounts of radioactivity loaded into the sample lanes were as follows: 61 kcpm, lane 1; 57 kcpm, lane 2; 60 kcpm, lane 3; 74 kcpm, lane 4; 84 kcpm, lane 5; 66 kcpm, lane 6; 68 kcpm, lane 7; 58 kcpm, lane 8. Base numbering is from the 5′ end-labeled terminus of the restriction fragment.

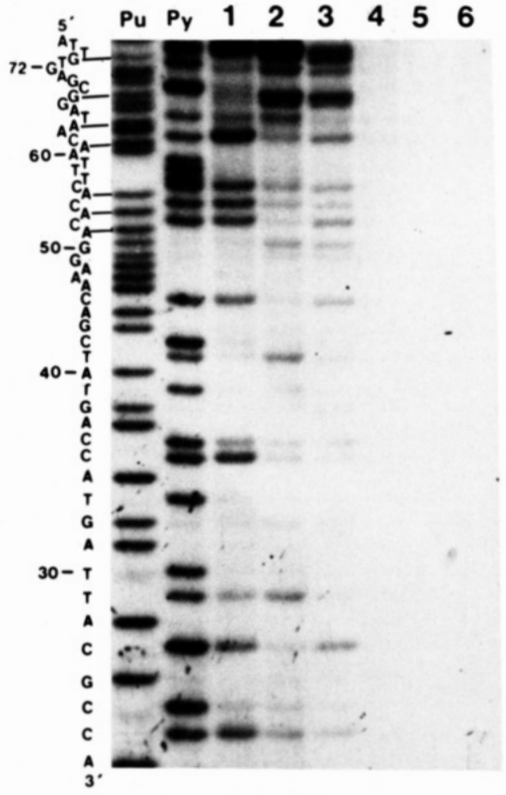

FIGURE 2: Sequence specificity of yeast redoxyendonuclease cleavage of UV-irradiated DNA. 3′ end-labeled DNA fragment 1 was UV irradiated (254 nm) with a dose of 10000 J/m^2 (Materials and Methods). UV-damaged (lanes 1–3) or undamaged (lanes 4–6) DNA fragments were incubated with *E. coli* endonuclease III (lanes 1 and 6), 1.7 μg of yeast redoxyendonuclease from fraction VI (lanes 2 and 5), or human redoxyendonuclease (lanes 3 and 4). Analysis of DNA scission products and the inclusion of DNA sequencing reactions were the same as described in the Figure 1A legend. The amounts of radioactivity loaded into the sample lanes were as follows: 67 kcpm, lane 1; 59 kcpm, lane 2; 49 kcpm, lane 3; 69 kcpm, lane 4; 62 kcpm, lane 5; 67 kcpm, lane 6. The majority of radioactivity in each lane corresponded to uncleaved, full-length DNA fragments and was located at the top of the gel (not shown). Base numbering is the same as in Figure 1A.

tified in UV-damaged DNA (Fisher & Johns, 1976; Kochetkov & Budovskii, 1972). To determine whether or not yeast redoxyendonuclease also recognized such photoproducts, UV-irradiated, 3′ end-labeled DNA substrates were incubated with the yeast, *E. coli*, and human enzymes. The sequence specificity of DNA cleavage mediated by these three enzymes was determined by analysis of the reaction products on a DNA sequencing gel. Yeast redoxyendonuclease cleaved UV-irradiated DNA primarily at sites of pyrimidines as did endonuclease III and human redoxyendonuclease (Figure 2). Cleavages were also observed at certain sites of guanine (e.g., G67 and G68), a result previously observed for both endonuclease III (Helland et al., 1986) and human redoxyendonuclease (Doetsch et al., 1988). The nature of the guanine photoproduct is not known. UV-damaged DNA not subjected to enzyme treatment exhibited no detectable background cleavages (not shown; Doetsch et al., 1986). Most base positions of cleavage were common to all 3 enzymes and were observed within the 55 bp sequence analyzed (A23–A77) at the following 19 sites: C24, C27, T29, C35, C36, T41, C45, G49, G50, C52, C54, C56, C61, T64, G67, G68, G72, G74, and T75. However, the yeast, *E. coli* and human enzymes exhibited some differences with respect to the extent of cleavage at a number of base positions within the sequence. For example, at base position T75 (bands at the top of lanes 1–3), more extensive cleavage is observed for the *E. coli* and yeast enzymes compared to the human enzyme, whereas at C45 only weak cleavage is observed for yeast redoxyendonuclease compared to endonuclease III and human redoxyendonuclease. At other positions (e.g., C36), the extent of cleavage is nearly similar for all three enzymes. These results suggest that the extent of cleavage on UV-damaged DNA mediated by the yeast, *E. coli*, and human enzymes may depend on the particular sequences flanking a given photoproduct and varies from enzyme to enzyme.

Discussion

Yeast redoxyendonuclease recognizes and cleaves DNA at sites of thymine glycol and UV light induced base damage. The base specificity and products of DNA strand cleavage mediated by yeast redoxyendonuclease appear to be identical with those produced by *E. coli* endonuclease III when thymine glycol containing DNA fragments are utilized as substrates (Figure 1). *E. coli* endonuclease III acts on damaged DNA via a combined *N*-glycosylase/AP endonuclease mechanism (Demple & Linn, 1980; Katcher & Wallace, 1983; Doetsch et al., 1986) that may involve a β-elimination reaction (Bailly & Verly, 1987). The net effect of such a mechanism is the production of the type of DNA scission products observed for both the *E. coli* and yeast enzymes. The possibility that yeast redoxyendonuclease also processes damaged DNA via an *N*-glycosylase/AP endonuclease mechanism is currently under investigation. The observed similarities between the yeast and *E. coli* enzymes suggest that in addition to thymine glycol, yeast redoxyendonuclease may recognize a similar variety of other ring-saturated, ring-cleaved, and ring-contracted products that are recognized by endonuclease III following exposure of DNA to ionizing radiation and oxidizing agents (Breimer & Lindahl, 1984, 1985).

The yeast, *E. coli*, and human enzymes used in this study are also similar with respect to the base specificity of cleavage of UV-irradiated DNA but differ with respect to the extent of cleavage at most of these UV-induced damage sites (Figure 2). These differences can be attributed to different affinities for particular UV photoproducts and may also reflect contribitions by various base sequences flanking a potential photoproduct substrate. Such differences between the yeast, *E. coli*, and human enzymes with respect to the extent of cleavage at a given damage site were not as obvious when OsO_4-damaged DNA was employed as a substrate. OsO_4-damaged substrates contained thymine glycol nearly exclusively as the base damage product, and it appears that all three enzymes as well as the chemical (hot alkali) method cleaved DNA at most sites of thymine glycol to a similar extent.

Yeast redoxyendonuclease and *E. coli* endonuclease III possess no requirement for divalent cations, are inhibited by high salt concentrations, and produce identical cleavage products. Armel and Wallace (1984) have described five chromatographically distinct yeast AP endonucleases. One of these, endonuclease E, is similar to yeast redoxyendonuclease with respect to molecular size (37000 daltons) and was found to be slightly active against OsO_4-damaged, supercoiled DNA. However, in contrast to yeast redoxyendonuclease, endonuclease E required Mg^{2+}, was resistant to high salt concentrations, and was found to be a class II AP endonuclease. Yeast redoxyendonuclease differs from the other previously described yeast UV endonucleases (Bryant & Haynes, 1978b; Bekker et al., 1980) and AP endonucleases (Pinon, 1970; Chlebowicz & Jachymczyk, 1977; Bryant & Haynes, 1978a; Futcher & Morgan, 1979; Akhmedov et al., 1982; Armel & Wallace, 1984) with respect to molecular size, divalent cation

and salt requirements, and other properties.

The purification of the yeast redoxyendonuclease resulted in a preparation with a relatively low specific activity that decreased following DNA–cellulose and Sephacryl S-200 chromatography (Table I). The reasons for such a decrease in activity are unknown at present, but it is possible that yeast redoxyendonuclease loses activity following the removal of certain proteins that contribute to the stability of the enzyme. DNA–cellulose chromatography of human redoxyendonuclease also results in a preparation that rapidly loses activity (unpublished results).

The identification of a yeast enzyme that recognizes and cleaves DNA at sites of thymine glycol and UV-induced base damage suggests that such an activity may mediate a role in initiating the repair of DNA damaged by ionizing and ultraviolet radiation. Mutants belonging to the yeast RAD52 epistasis group exhibit sensitivity to X-rays, and several mutants from the RAD6 epistasis group (*rad6*, *-9*, and *-18*) are both UV and X-ray sensitive (Game, 1983). Whether or not any such mutants are deficient in redoxyendonuclease activity remains to be determined. The DNA sequencing methodologies employed in this investigation can be utilized to specifically assess the presence of redoxyendonuclease in crude cellular extracts. Such an approach should facilitate the screening of extracts of various yeast DNA repair mutants for the presence of redoxyendonuclease and may provide insight into the biochemical aspects of yeast DNA repair. Enzymes similar to yeast redoxyendonuclease including *E. coli* endonuclease III, human redoxyendonuclease, and others found in various bacterial and mammalian cells appear to be highly conserved among prokaryotes and eukaryotes. Given the wealth of information that is available with respect to the genetics of yeast DNA repair, an understanding of the regulation and role of yeast redoxyendonuclease may also provide insights into the genetics of DNA repair of other organisms including humans.

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