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Purification and characterization of a uracil-DNA glycosylase from the yeast, Saccharomyces cerevisiae

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ABSTRACT

An activity which releases free uracil from bacteriophage PBS1 DNA has been purified over 10,000 fold from extracts of Saccharomyces cerevisiae. The enzyme is active on both native and denatured PBS1 DNA and is active in the absence of divalent cation, and in the presence of 1 mM EDTA. The enzyme has a native molecular weight of 27,800 as estimated by glycerol gradient centrifugation and gel filtration. Enzyme activity has been recovered after denaturation in SDS and electrophoresis in an SDS polyacrylamide gel. This analysis suggests that the enzyme consists of a single polypeptide chain of about 27,000 daltons. Normal levels of uracil-DNA glycosylase activity were found in partially purified extracts of the nitrous-acid sensitive rad18-2 mutant of yeast.

INTRODUCTION

Uracil residues introduced into DNA, either by deamination of cytosine bases or by incorporation of dUMP by DNA polymerase, can be enzymatically removed by cell extracts isolated from most microorganisms and higher cells thus far examined (1). An activity which removes uracil from DNA was originally described in partially purified extracts of Escherichia coli (2) and the enzyme was later purified to homogeneity (3). The activity of the enzyme, uracil-DNA glycosylase, which specifically hydrolyzes glycosyl base-sugar bonds of dUMP in polymeric form, is conveniently assayed by measuring the release into acid soluble material of ^{3}H-uracil moieties from ^{3}H-deoxyuridine-labeled DNA of the Bacillus phage PBS1, which contains uracil in place of thymine (4). The reaction products are free uracil and an apyrimidinic site in DNA; phosphodiester bonds are not hydrolyzed. Mutants of E. coli which are defective in uracil-DNA glycosylase activity exhibit mutator effects (5) and are sensitive to the lethal effects of nitrous acid and bisulfite (6,7).

Uracil-DNA glycosylase activities have been identified from several eukaryotic organisms (see 1 for a review), although they have generally not been highly purified. The physiological role of the enzyme in eukaryotic

cells is not known. The availability of a large number of DNA repair mutants (8) in the eukaryote Saccharomyces cerevisiae, some of which are particularly sensitive to nitrous acid (9,10), as well as the ease of isolating new mutants, offers the possibility of understanding the physiological role of uracil-DNA glycosylase in eukaryotic cells. For this reason, we have begun studies on the purification and characterization of this enzyme from S. cerevisiae. The present work describes the physical and catalytic properties of highly purified uracil-DNA glycosylase activity isolated from vegetative cells of S. cerevisiae.

MATERIALS AND METHODS

Strains and Culture Conditions

Saccharomyces cerevisiae strain A364A (MATa ade1 ade2 lys2-1 his7 gal1 tyr1 ura1 was obtained from Dr. L. Hartwell (University of Washington). Yeast strain LP1688-1C (MATα arg4-17 leu1-12 trp2 rad18-2) and the RAD+ strain LP2320-10A (MATa arg4-17 his5 lys1 RAD+) were obtained through crosses involving a rad18-2 strain obtained from Dr. C.W. Lawrence (University of Rochester). Bacteriophage PBS1 and its host Bacillus lichenoformis strain BL721215 were provided by Dr. G. Wilson (University of Rochester).

Cultures (12 L) of S. cerevisiae A364A were grown in a New Brunswick MF-100 fermentor in YPD growth medium [2 percent yeast extract (Yeast Products Inc., Clifton, NJ), 1 percent bacto-peptone (Difco) and 2 percent dextrose (Staley, Decatur, IL) pH 5.3-5.5] at 30°C with aeration (6 L/min and mechanical stirring). Cells were harvested in late logarithmic phase of growth by centrifugation and frozen at -20°C until use. Cells of LP1688-1C and LP2320-10A were grown in 1 liter cultures in YPD medium (pH 6.4-6.5) at 30°C in 2.3-liter Fernbach flasks on a gyratory shaker. Cells were harvested as for A364A.

Preparation of ^{3}H-Uracil Labeled Bacteriophage PBS1 DNA

A 100 mL culture of B. lichenoformis 721215 was grown at 37°C in Spizizen glucose minimal medium (11) to a cell density of 7.5 X 10^{8} cells/mL (OD_{590} = 1.5), phage were added at a multiplicity of 3 and 5 minutes after infection, 1 mCi of ^{3}H-deoxyuridine (20 Ci/mmol, Moravek Biochemicals, City of Industsry, CA) was added. After 90 minutes, 0.5 mL chloroform was added and cell debris was removed from the lysate by centrifugation for 15 min at 6,000 RPM in a Sorvall SS-34 rotor. The phage were collected by a 60 min centrifugation at 17,000 RPM in the SS-34 rotor,resuspended in 10 mL of phage diluent (0.15 M NaCl, 0.1 M KPO_4, pH 7.2) and centrifuged at 6,000 RPM for

15 min. The precipitate was discarded, the phage were collected from the supernate as before, resuspended in 2 mL of diluent and 2.33 g of CsCl were added. The solution was brought to 6.0 g with phage diluent and the phage centrifuged to equilibrium at 10°C for 20 hrs in a Beckman SW50.1 rotor at 27,000 RPM. The band of phage was collected and dialyzed against sterile phage diluent. The dialyzed phage (1.3 mL) were gently extracted, first with 2.6 mL and then two times with 1.3 mL of redistilled phenol saturated with phage diluent. The extracted DNA was dialyzed 4 days against 8 x 1 liter of sterile 10 mM Tris-HCl (pH 7.5) 0.1 mM EDTA at 4°C. In a typical preparation, 75 μg of PBS1 DNA with a specific activity of 54,300 cpm/μg was recovered from the 100 mL culture.

The PBS1 DNA was enzymatically digested to nucleosides and analyzed by high pressure liquid chromatography (HPLC) by Dr. D. Swinton, Department of Biology, University of Rochester. This analysis confirmed the published base composition of the DNA [72 percent A, U (4)] and indicated that 66 percent of the radioactivity was in deoxyuridine with the remaining 34 percent in deoxycytidine. Thus, the specific activity of the uridine residues in the PBS1 DNA was calculated to be 33 cpm/pmole.

Uracil-DNA Glycosylase Assay

Reactions (0.1 mL) contained: 20 mM Tris-HCl (pH 8.0), 10mM 2-mercaptoethanol, 1 mM EDTA, 0.1 μg of phage PBS1 ^{3}H-DNA (54,000 cpm/μg) and 0.0015 to 0.045 units of enzyme. The enzyme was diluted in 20 mM Tris-HCl (pH 8.0), 10 mM 2 mercaptoethanol, 1 mM EDTA and 1 mg/mL bovine serum albumin. Reactions were initiated by the addition of enzyme. After incubation for 30 min at 37°C, reactions were stopped by placing at 0°C and adding one drop of salmon sperm DNA (0.5 mg/mL) and 0.5 mL of cold 10 percent (w/v) trichloroacetic acid. After 10 min at 0°C, the precipitated DNA was removed by centrifugation at 14,000 x g for 10 min, and the ^{3}H remaining in the supernate was counted in 5 mL of Liquiscint (National Diagnostics) in a liquid scintillation counter. One unit of uracil-DNA glycosylase activity is defined as the amount catalyzing the release of 1 nmole of ^{3}H-uracil during the 30 min incubation at 37°C.

It is assumed that all of the acid soluble radioactivity produced duringthe incubation is uracil. The product formed by the purified enzyme has been identified as uracil by thin layer chromatography. It is possible that with crude fractions, some of the acid soluble radioactivity is produced by nucleases. However, when T7 ^{3}H-DNA is substituted for theuracil containing PBS1 ^{3}H-DNA in the standard reaction, no detectable acid soluble

^3H is formed, even during incubation with the crude extract.

Other Procedures

Protein concentrations were determined by the method of Lowry et al. (12) using bovine serum albumin as standard. All pH measurements were made at room temperature at a buffer concentration of 0.05 M. Salt concentrations in column fractions were determined using a conductivity meter. Denatured salmon sperm DNA-agarose was prepared essentially as described by Schaller et al. (13) using agarose purchased from Sigma (St. Louis, MO). Bacteriophage T7 DNA was prepared as previously described (14).

RESULTS

Purification

All procedures were carried out at 0-4°C, and all centrifugations were at 10,000 RPM for 20 min in a Sorvall GSA rotor unless stated otherwise. The results of the purification are shown in Table 1.

Preparation of Cell Extract

S. cerevisiae strain A364A (490 grams wet weight) was thawed to 15°C and the cell paste was added to 1.6 kg of glass beads (0.45-0.55 mm diameter) plus 60 mL Buffer A [50 mM KPO_4 (pH 8.7), 0.12 M KCl, 0.5 mM phenyl-

Table 1. Purification of DNA-uracil glycosylase from 490 g haploid wild-type strain A364A.

	STEP	VOLUME (ml)	ACTIVITY (units)	PROTEIN (mg)	SPECIFIC ACTIVITY (units/mg)
I	Extract	657	25,000	15,800	1.6
II	Protamine Sulfate	751	47,300	10,800	4.4
III	Ammonium Sulfate	200	49,100	6,160	8.0
IV	Phosphocellulose	850	36,300	1,020	35.6
V	Hydroxylapatite	150	10,900	90	121
VI	Denatured DNA Agarose	9.5	9,560	3.1	3,080
VII	Sephadex G-100	5.9	6,060	.35	17,300

Procedures were carried out as described in the text.

methylsulphonylfluoride, 1 percent (v/v) dimethylsulfoxide] in a 1 kg size plastic reagent bottle (Sigma). The bottle containing the mixture was packed with ice into a one gallon paint can and shaken in a paint shaker (model 5400, Red Devil Inc., Union, NJ) three times for 15 min each. This procedure results in at least 90 percent cell breakage and the temperature was maintained at $\leq$ 1°C.

Buffer A (165 mL) was added to the mixture, the beads were allowed to settle (gentle vibration of the bottle helps this process), and the supernate was carefully decanted. This washing procedure was repeated twice with 165 mL of Buffer A and the three supernates were pooled and centrifuged. The supernate (289 mL) was adjusted to A_{260} = 200 by addition of 302 mL of Buffer A and 66 mL of 1.0 M Tris-HCl, pH 8.0 was added (Fraction I).

Protamine Sulfate Fractionation

To 657 mL Fraction I were added 164 mL of 1 percent (w/v) protamine sulfate. The mixture was stirred for 30 min and the precipitate removed by centrifugation for 45 min at 10,500 RPM. The supernatant fluid (751 mL) was recovered (Fraction II).

Ammonium Sulfate Fractionation

To 751 mL Fraction II, 263.6 g ammonium sulfate was added over 15 min and the mixture stirred an additional 30 min. The precipitate was collected by centrifugation and resuspended in 200 mL Buffer B [25 mM KPO_4 (pH 7.2), 5 mM 2-mercaptoethanol, 10 percent v/v glycerol], (Fraction III).

Phosphocellulose Chromatography

Fraction III was diluted to 2.5 liters with Buffer B to produce a solution with a conductivity equal to that of Buffer B containing 0.04 M KCl. The diluted fraction was applied to a column of Whatman P11 phosphocellulose (19 cm^2 X 17 cm) which was equilibrated with Buffer B. The resin was washed with 600 mL of Buffer B and proteins were eluted with a 4 liter linear gradient from 0 to 0.6 M KCl in Buffer B at a flow rate of 150 mL/hr.

Uracil-DNA glycosylase activity eluted in two approximately equal peaks at about 0.2 M and 0.3 M KCl. The two peaks were pooled separately and the two pools were then combined (Fraction IV)*.

*Footnote: Throughout the subsequent purification, only a single species of uracil-DNA glycosylase activity was observed. Furthermore, when Fraction IV enzyme from either peak was rechromatographed on phosphocellulose, a single peak of activity eluted at 0.3 M KCl. Although we do not understand why two peaks of activity are observed during this first chromatography step, these results suggest that both phosphocellulose pools may represent the same enzymatic species.

Hydroxylapatite Chromatography

Fraction IV was concentrated from 850 mL to 300 mL in a Millipore Pellicon Cassette filtration unit using membranes of 10,000 molecular weight porosity. The concentrated sample was loaded onto a hydroxylapatite column (3.5 cm^2 x 16 cm) which was equilibrated with Buffer B. The column was washed with 140 mL Buffer B and eluted at 10 mL/hr with a 500 mL linear gradient from 0.025 M to 0.4 M KPO_4 (pH 7.2) buffer containing 5 mM 2-mercaptoethanol and 10 percent (v/v) glycerol. The active fractions were pooled and dialyzed against Buffer C (20 mM) Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.1 mM dithiothreitol, 10 percent v/v glycerol) containing 0.1 M NaCl(Fraction V).

Denatured DNA-Agrose Chromatography

Fraction V was applied at about 15 mL/hr to a column (0.79 cm^2 x 14 cm) of denatured DNA-agarose which had been equilibrated with Buffer C containing 0.1 M NaCl. The column was washed with 10 ml of this buffer and proteins were eluted at 5 mL/hr with a 100 mL linear gradient of 0.1 M to 1.0 M NaCl in Buffer C. Fractions containing uracil-DNA glycosylase activity, which eluted at 0.3 M NaCl, were pooled and dialyzed against 20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 10 mM 2-mercaptoethanol, 0.1 mM EDTA, 20 percent (v/v) glycerol (Buffer D); 9.5 mL of the dialyzed enzyme was recovered (Fraction VI).

Sephadex G-100 Chromatography

Fraction VI was layered onto a column (5.8 cm^2 x 78 cm) of Sephadex G-100 equilibrated with Buffer D. The column was eluted with Buffer D at a flow rate of 20 mL/hr and 10 mL fractions were collected. Fractions 19 to 25, containing uracil-DNA glycosylase activity, were pooled and concentrated to 5.9 mL by dialysis in Spectropor 3 dialysis tubing, first against solid polyethylene glycolP-6000, and then against Buffer B containing 50 percent (v/v) glycerol (Fraction VII). Fraction VII was stored at -20°C and lost about 30 percent of its activity in 3 months.

Purification of Uracil-DNA Glycosylase from Commercial Yeast

The described purification procedure was also used to purify uracil-DNA glycosylase from baker's yeast (Fleischmann's) purchased commercially from a local supermarket bakery supply. All aspects of the purification were identical to those for A364A cells, except that the yield of enzyme from commercial cells was approximately one-fourth that recovered from freshly grown A364A cells.

Physical Properties

1. Purity: We have purified the yeast uracil-DNA glycosylase approximately 10,000 fold. Analysis of up to 3 μg of Fraction VII by electrophoresis on SDS polyacrylamide gels revealed no visible protein bands after staining with Coomassie Blue. Since we can usually detect as little as 0.5 μg of a single protein on these gels, this result suggests that our enzyme preparation is not homogeneous.

The purified enzyme, however, is free of any detectable exonuclease activity. Incubation of 1 unit of Fraction VII enzyme with 1 nmole of native or denatured T7 ^{32}P-DNA for 60 min at 37°C, using the standar duracil-DNA glycosylase reaction with 1 mM EDTA or in the presence of 10 mMMgCl_2, produced no detectable (< 1 pmole) acid soluble radioactivity. In the presence of MgCl_2, a small amount of endonuclease activity was detected which was equally active on duplex or single stranded T7 ^{3}H-DNA. On duplex DNA, the nuclease apparently makes double stranded breaks, since the number of breaks per molecule, measured by sedimentation in an alkaline sucrose gradient, was approximately equal to the number of breaks measured in a neutral sucrose gradient. Also, when the enzyme was incubated with supercoiled pBR322 DNA, agarose gel electrophoresis showed that only linear molecules were produced. No relaxed circular DNA molecules containing a single-strand break were detected. Using either T7 DNA or pBR322 DNA, about 0.01 pmoles of double-strand breaks were produced per unit of Fraction VII enzyme during a 30 min incubation at 37°C. The same rate of cleavage was observed with single stranded T7 DNA. No detectable breaks (< 0.0002 pmoles per unit) were produced in the absence of MgCl_2.

The purified anzyme is also free of any detectable apurinic endonuclease activity. This activity was assayed according to the procedures of Ljungquist (15), using lightly depurinated (2 to 4 purines removed per molecule) PM2 DNA. No detectable breaks (< 0.01 pmoles) were introduced in this DNA during a 15 min incubation with 5 units of Fraction VII uracil-DNA glycosylase.

2. Molecular Weight and Subunit Composition: The molecular weight of the native uracil-DNA glycosylase has been determined from the sedimentation coefficient and the Stokes radius by the method of Siegel and Monty (16). The protein has a sedimentation coefficient of 2.7 S, determined by zonal sedimentation in a glycerol gradient, and a Stokes radius of 25 Å, determined by gel filtration on Sephadex G-100. If we assume that the protein has a partial specific volume of 0.725 cm^3/g, a molecular weight

of 27,800 is calculated from these data. We have also recovered uracil-DNA glycosylase activity after denaturation in SDS and electrophoresis on an SDS-polyacrylamide gel, using a procedure developed by Hager and Burgess (17). The analysis of Fraction VII enzyme revealed a single band of activity with a mobility corresponding to a protein of molecular weight 26,900 ± 2,000 (fig. 1). We conclude that the yeast uracil-DNA glycosylase consists of a single polypeptide chain and has a molecular weight of about 27,000.

Enzymatic Properties

1. Requirements for Activity: The yeast uracil-DNA glycosylase exhibited a broad pH optimum around pH 7.5-8.0, with 60 percent of maximal activity remaining at pH 7.0 or 8.5. The enzyme is active in the presence of 1 mM EDTA and is only slightly stimulated (50 percent to 70 percent) by

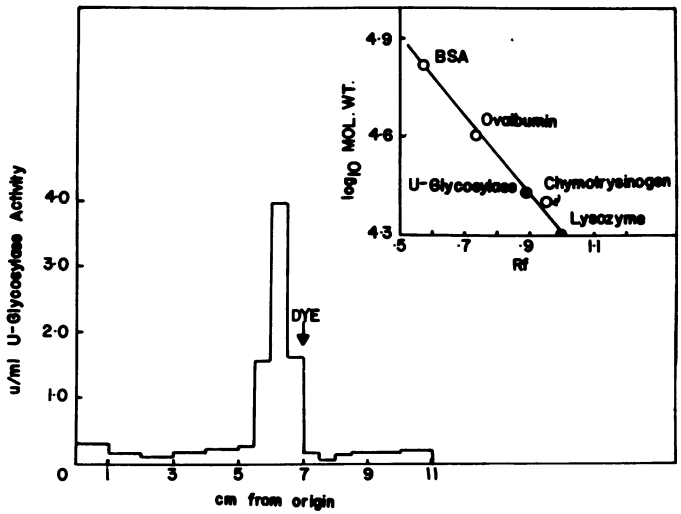

Figure 1. SDS-polyacrylamide gel analysis of yeast uracil-DNA glycosylase

Uracil-DNA glycosylase (20 units) was electrophoresed on a 5 percent SDS-polyacrylamide gel according to the procedure of Weber and Osborn (18). The gel was cut into 0.5 cm (or 1 cm) slices and enzyme activity was recovered as described by Hager and Burgess (17). In brief, the procedure involved eluting the protein from the crushed gel slice and removing the SDS during acetone precipitation. The protein pellet was resuspended in a small volume of 6 M guanidinium-HCl and was then diluted 50-fold to a volume of 1.25 mL (2.5 mL for the 1 cm slices) and assayed for enzyme activity. The recovery of activity was 32 percent. No protein bands were observed when a parallel gel was stained with Coomassie blue.

the addition of 10 mM $MgCl_2$. The addition of 10 mM $CaCl_2$ to the standard reaction causes a 95 percent inhibition of the enzyme. The addition of NaCl to the standard reaction causes first a slight stimulation of activity (50 percent at 50 mM) and then inhibition (40 percent at 200 mM).

At saturating substrate concentrations the uracil-DNA glycosylase is equally active using either native or denatured PBS1 DNA. When the concentration of PBS1 DNA was altered in the standard reaction mixture, 50 percent of the maximal enzyme activity was observed at 0.13 μg/mL for native PBS1 DNA and at 0.06 μg/mL for denatured PBS1 DNA. Since PBS1 DNA contains 72 percent A U base pairs (4), these data indicate that the Km for dUMP residues in native and denatured PBS1 DNA is 0.14 μM and 0.065 μM, respectively.

The enzyme is inhibited by the product, uracil. Fifty percent inhibition is obtained when 0.5 mM uracil is added to the standard reaction.

2. Identification of the Reaction Product: An aliquot of a standard uracil-DNA glycosylase reaction containing ^{3}H-PBS1 DNA was analyzed by thin layer chromatography to identify the reaction product. The radioactivity released from the DNA by the enzyme chromatographed as a single peak with a mobility corresponding to that of free uracil. Although 34 percent of the radioactivity in our PBS1 DNA preparation is contained in cytosine residues (see Methods), no ^{3}H-cytosine was released from the PBS1 DNA by the yeast uracil-DNA glycosylase.

The removal of uracil residues from PBS1 DNA presumably leaves apyrimidinic sites in the DNA. The phosphodiester bonds at such sites are quantitatively hydrolyzed during a 4 hr incubation in 1 M glycine-NaOH (pH 12.8) at 25°C (19). However, less than 10 percent of these sites are cleaved during sedimentation at 4°C in an alkaline sucrose gradient (19). In an attempt to demonstrate that the yeast uracil-DNA glycosylase introduces apyrimidinic sites into PBS1 DNA, we have analyzed treated DNA by alkaline sucrose gradient sedimentation before and after a 4 hr incubation at pH 12.8 and 25°C (fig. 2). Our preparation of PBS1 ^{3}H-DNA (control) has an average sedimentation coefficient ($s^\circ_{20,w}$) of about 39 S in alkali, relative to 37 S for T7 DNA (20) and apparently contains some apyrimidinic or apurinic sites, since the DNA sediments slightly slower after the 4 hr incubation at pH 12.8. When the PBS1 DNA was treated withuracil-DNA glycosylase (0.002, 0.02 or 0.08 units) the rate of sedimentationin alkali was somewhat reduced but incubation of the treated DNA at pH 12.8 did not significantly affect the rate of sedimentation. This result suggests that

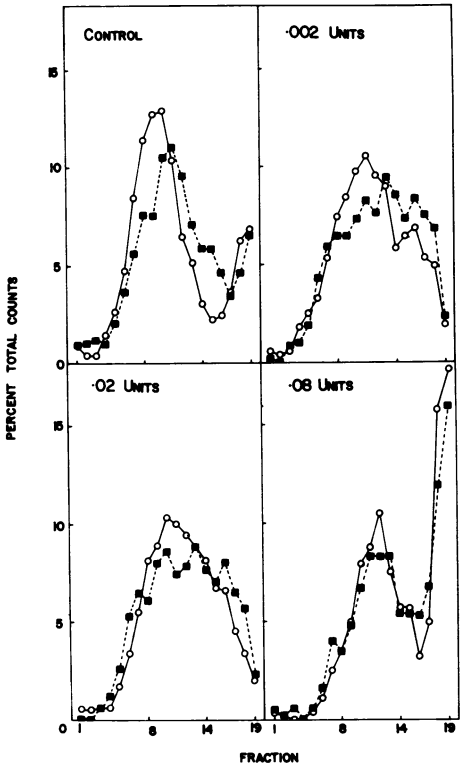

Figure 2. Alkaline sucrose gradient analysis of PBS1 ^{3}H-DNA treated with uracil-DNA glycosylase

PBS1 ^{3}H-DNA was incubated for 60 min at 37°C in a standard reaction (0.2 mL) containing the indicated quantities of yeast uracil-DNA glycosylase. The reaction was placed on ice and two 25 µl portions were withdrawn and added to an equal volume of 2.0 M glycine-NaOH, pH 13.1 (final pH 12.8). One sample (O) was immediately diluted with 50 µl of cold water, layered on a 3.7 mL 5-20 percent linear sucrose gradient containing 0.1 N NaOH, 0.9 M NaCl, 0.1 mM EDTA, 0.015 percent sarkosyl, and centrifuged for 70 min at 60,000 RPM and 5°C in a Beckman model SW60 rotor. Fractions (0.2 mL) were collected from the bottom of the tube, neutralized with 0.1 mL of 0.2 N HCl and counted in 3 mL of liquid scintillation cocktail (Liquiscint, National Diagnostics). The other sample ( ) was incubated at 25°C for 4 hrs and was then diluted with an equal volume of cold water and analyzed as just described by sedimentation on a sucrose gradient. An aliquot of the reaction mixture containing 0.08 units of enzyme was analyzed for release of acid-soluble ^{3}H as described in the text, and it was determined that 12.5 percent of the uracil had been released from the PBS1 DNA. Phage T7 DNA ($s^{\circ}_{20,w}$ = 37 S) sedimented to fraction 10 in a parallel sucrose gradient (not shown).

our enzyme preparation might be introducing breaks into the PBS1 DNA. However, the enzyme does not contain any detectable apurinic endonuclease activity. Furthermore, the number of breaks or apyrimidinic sites observed in the treated PBS1 DNA was much smaller than expected from the amount of uracil released from the DNA. For example, during the incubation with 0.08 units of uracil-DNA glycosylase, 12.5 percent of the uracil residues were released from the PBS1 DNA. Since this represents 4.5 percent of the bases in the DNA, if these uracil residues were removed at random, the average distance between apyrimidinic sites in the DNA should be only 22 nucleotides. Even after the 4 hr incubation at pH 12.8, about 50 percent of this treated PBS1 DNA had a sedimentation coefficient > 20 S, indicating that half of the DNA remained in polymers larger than 9,000 nucleotides.

This DNA is not resistant to the enzyme. After incubation with 1 unit of uracil-DNA glycosylase, none of this large DNA is observed during sedimentation in the alkaline gradient. These results suggest that the yeast uracil-DNA glycosylase may act in a processive manner to remove most of the uracil residues from some DNA strands and leave other strands relatively intact. This mode of action could also explain our failure to detect alkaline sensitive sites in the treated DNA. If the enzyme removes uracil residues from the DNA in clusters, these clusters of apyrimidinic sites would be supremely sensitive to alkaline hydrolysis and might be hydrolyzed during the alkaline sedimentation.

Uracil-DNA Glycosylase Activity in the rad18-2 Mutant of Yeast

In addition to showing extreme sensitivity to UV irradiation, the yeast mutant rad18 is unusually sensitive to nitrous acid and shows highly increased nitrous acid mutagenesis (10). This suggests that this mutant may be defective in repair of damage caused by the deamination of cytosine residues in the DNA. We have examined uracil-DNA glycosylase activity in this mutant. Extracts of the rad18-2 strain LP1688-1C contained normal levels of the enzyme and purification through Step IV revealed no alteration of the uracil-DNA glycosylase in the mutant.

DISCUSSION

Uracil-DNA glycosylase activities have been purified to various degrees from a wide variety of organisms. The activity we purified from the yeast S. cerevisiae is similar to uracil-DNA glycosylases characterized so far from E. coli (3), B. subtilis (21), calf thymus (22) and human cells

(23,24). The proteins all have similar molecular weights and pH optima and except for the enzyme obtained from leukemia patients (25), all are active in the presence of EDTA.

Uracil, which is not normally found in DNA except in a few bacteriophages, can nevertheless arise in DNA principally by two different mechanisms: spontaneous deamination of cytosine to uracil and misincorporation of dUMP by DNA polymerase. The deamination of cytosine to uracil in double stranded DNA occurs at 0.3-0.5 percent of the rate observed with single stranded DNA (1), but some of the DNA in growing cells will be single stranded, due to unwinding that occurs during replication and transcription. Growing cells may therefore have appreciable amounts of uracil in their DNA, which, if it is not removed, could result in CG to TA transition mutations. Consistent with this notion is the observation that uracil-DNA glycosylase mutants (*ung*$^-$) of *E. coli* show enhanced spontaneous mutator activity and are more sensitive to nitrous acid, which causes deamination of cytosine to uracil, then are *ung*$^+$ strains (5). Similar results have been found in mutants of *B. subtilis* (26).

Double mutants defective in both dUTPase activity and endonuclease II (exonuclease III) (*dut*$^-$ *xth*$^-$), the major apurinic endonuclease found in *E. coli*, are inviable at temperatures above 30°C, probably due to an accumulation of apyrimidinic sites in the DNA (27). The addition of the *ung*$^-$ mutation suppresses this defect and *dut*$^-$ *xth*$^-$ *ung*$^-$ strains grow well (27), presumably because uracil can no longer be removed as efficiently from the DNA in these uracil-DNA glycosylase mutants, resulting in fewer unrepaired apyrimidinic sites. These observations suggest that uracil-DNA glycosylase plays an important role *in vivo* in *E. coli* in removing uracil from DNA.

The yeast *rad18* mutant, which is sensitive to UV and nitrous acid, shows enhanced spontaneous mutability (28) and enhanced nitrous acid induced mutations (10), was found to have normal levels of uracil-DNA glycosylase activity. Although we expect the yeast uracil-DNA glycosylase to function *in vivo* by keeping the level of uracil in DNA at a minimum, further insight into the role of the yeast enzyme *in vivo* awaits the identification of a mutant strain deficient in uracil-DNA glycosylase activity and characterization of its phenotype.

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