Volume 5 Number 4 April 1978 Nucleic Acids Research

# Enzymes from Micrococcus luteus involved in the initial steps of excision repair of spontaneous DNA lesions: uracil-DNA-glycosidase and apurinic-endonucleases

N.V. Tomilin, O.N. Aprelikova and L.S. Barenfeld

Institute of Cytology of Academy of Sciences of the USSR, Leningrad, USSR

Received 6 March 1978

**ABSTRACT.**
Uracil-DNA-glycosidase that releases free uracil from single-stranded or double-stranded deaminated DNA and poly d(A-U) has been partially purified from *Micrococcus luteus*. The enzyme has a molecular weight of about 16,000 and can be separated from uracil-endonuclease and endonucleases (AP-endonucleases) specific for apurinic and apyrimidinic sites. Uracil-DNA-glycosidase does not act on guanine residues opposite uracil in double-stranded DNA and on xanthine in deaminated DNA. The glycosidase generates apyrimidinic sites which can serve as substrate sites for different AP-endonucleases from *M.luteus*.

The most important class of spontaneous damage to DNA involves apurinic sites resulting from heat fluctuations (1). In accordance with Arrhenius's law, logarithm of the rate constant for depurination of DNA is inversely proportional to temperature and the activation energy of the reaction is 130 kJ/mole (2). This allows to calculate that *in vivo* DNA spontaneously loses from 5000 to 10,000 purines per mammalian genome per day, so that the repair at apurinic and apyrimidinic sites may be important in the maintenance of genetic stability in undamaged cells.

Analysis of heat mutagenesis in phage T4 has led to the conclusion that cytosine deamination in DNA is another spontaneous (heat-dependent) reaction important in mutagenesis (3). The rate for deamination of cytosine in native DNA at 37°C at neutral pH is probably only 10-100 fold lower than the rate for depurination (3).

An endonuclease activity specific for apurinic sites has first been discovered in *Micrococcus luteus* (4). Recently, two isozymes of AP-endonuclease has been purified from *M.luteus*:

one (AP-endonuclease I) with a low molecular weight (about 15,000) has an associated UV-endonuclease activity (5,6), the other (AP-endonuclease II) with a higher molecular weight (about 30,000) has no such an activity (7).

In this paper a partial purification and some properties of uracil-DNA-glycosidase from M.luteus will be described. This enzyme releases uracil from deaminated DNA or poly d(A-U) resulting in apyrimidinic sites which can be cleaved by AP-endonucleases. Uracil-DNA-glycosidase from M.luteus possesses many properties in common with a similar enzyme found in E.coli (8, 9). Some data on the purification and properties of several AP-endonuclease isozymes from M.luteus will be also presented.

## MATERIALS AND METHODS

### MICROORGANISMS

All the enzymes used during this investigation were isolated from Micrococcus luteus ATCC 4698 obtained from Dr. S. Okubo. The $^{14}$C-cytosine-labeled DNA was prepared from E.coli $\chi$108 ura$^-$pyr A287 (from Dr. M.Mosevitskii), the $^{14}$C-guanine-labeled DNA was isolated from E.coli ATCC 2465 gua A21 (from Dr. B.Bachman). The plasmid colE1 DNA was extracted from E.coli JC 411 thy$^-$ (from Dr. N.Matvienko) by the method described in detail elsewhere (6).

### MEDIA AND CULTIVATION

M.luteus cells was obtained by confluent growth on fish-bone agar (Institute of Nutrient Media, Daghestan, USSR). E.coli was grown in the liquid medium M9 containing 2.5 mg/ml of casamino acids (Difco). Radioactive precursors were added: either 2-$^{14}$C-thymine (specific activity 70 mCi/mM) to a concentration of 4 $\mu$g/ml, 8-$^{14}$C-guanine (specific activity 1.8 mCi/mM) to a concentration of 50 $\mu$g/ml or 2-$^{14}$C-cytosine (specific activity 40 mCi/mM) to a concentration of 50 $\mu$g/ml.

### ISOLATION OF DNA AND TREATMENT WITH MUTAGENS

E.coli DNA was prepared by chloroform extraction (10).

Phage DNA was obtained by the phenol method. The specific radioactivity of DNA was from 5,000 to 30,000 cpm/ $\mu$g.

The DNA was treated with nitrous acid (2M $NaNO_2$) in 1M acetate buffer, pH 4.2, for 12 hours at 25$^{\circ}$C to achieve complete deamination (11). Following incubation the reaction mixture was dialysed at 4°C against 0.01 M potassium phosphate buffer, pH 6.8, containing 0.05 M NaCl.

A specific deamination of cytosine in E.coli DNA was carried out in 0.5 M $Na_2S_2O_5$, pH 5.0, for 2 hours at 25°C. Prior to incubation DNA was denatured with 0.2 M NaOH for 10 min at 25°C and neutralised with HCl. Following incubation the reaction mixture was dialysed against 0.01 M Tris-HCl buffer, pH 8.0, containing 0.05 M NaCl.

DNA hybrids containing uracil in the unlabeled strands and $^{14}$C-guanine in the complementary strands were prepared in the following manner. The unlabeled E.coli DNA treated with metabisulfite was mixed with a two-fold excess of the $^{14}$C-guanine labeled E.coli DNA. Then the DNA in the reaction mixture (the final concentration was 40 μg/ml of DNA) was denatured with 0.1 M NaOH for 10 min at 25°C, neutralised with HCl and incubated for two hours at 65°C, followed by slow cooling to room temperature.

The composition of the incubation mixture for depurination of circular plasmid colE1 $^{14}$C-DNA was: 1 volume of DNA in 0.01 M potassium phosphate buffer, pH 7.5,- 0.05 M NaCl - 0.001 M EDTA and 0.5 volume of 0.1 M $KH_2PO_4$ - 0.1 M NaCl. The reaction mixture (the final pH was 6.0) was incubated for 150 min at 70°C and cooled on ice.

UV-irradiation was carried out under the lamp BUV-60 (254 nm) at a dose rate of 0.18 J/m$^2$/sec.

ASSAYS OF AP-ENDONUCLEASES AND URACIL-DNA-GLYCOSIDASE

About 10,000 cpm of $HNO_2$-treated E.coli DNA (370 pmol of a polymeric uracil, 0.3 μg DNA) were mixed with an equal volume of the enzyme and incubated at 37°C for 2 hours in 0.01 M potassium phosphate buffer, pH 6.8, - 0.0005 M EDTA. The mixture together with unlabeled uracil was applied to a silicagel plate (150 × 150 mm) (Silufol, ČSSR). The plate was developed for 30 min in n-butanol - acetic acid - water (200:35:80). The spot of uracil was localized with the help of ultrachemiscope, cut out and its radioactivity was measured in a gas-flow counter.

One unit of enzyme activity was defined as the amount of enzyme necessary to release 15 pmoles (400 cpm) free uracil from polymeric deaminated DNA under standard conditions

To determine uracil-DNA-glycosidase activity gel filtration technique on a Sepharose 4B (Pharmacia) column can be also used.

Activity of AP-endonuclease was measured by two methods. One method is based on the release of fragments with a low molecular weight from the depurinated DNA immobilised in polyacrylamide gel (5,12). Another method is based on the measurement of relaxation of the supercoiled depurinated colE1 DNA by agarose electrophoresis technique (6,7). Further, any endonuclease activity acting on the DNA heated at acid pH will be reffered to here as AP-endonuclease.

SYNTHESIS OF POLY d(A-U)

The reaction mixture contained (in 1 ml) 50 mM potassium phosphate buffer, pH 7.5, 10 mM $MgCl_2$, 3.5 $\mu$M poly d(A-T) (poly (dA-dT)•poly (dA-dT), PL Biochemicals), 0.5 mM dATP, 0.5 mM dUTP, 0.02 mM dTTP, 40 units of the M.luteus DNA-polymerase and either 20 $\mu$Ci of $^3$H-dATP (8.6 Ci/mmol, Amersham) or 20 $\mu$Ci of $^3$H-dUTP (48 Ci/mmol, USSR). After incubation at 37°C for 17 hours the polymer was isolated by gel filtration.

DNA-polymerase was extracted from M.luteus according to the method described (13,14). The enzyme purification was completed by the affinity chromatography step.

OTHER REAGENTS AND METHODS

The composition of Buffer A was: 0.01 M potassium phosphate buffer, pH 6.5 or 7.5, - 0.001 M EDTA - 0.001 M 2-mercaptoethanol. The composition of Buffer B was: 0.01 M potassium phosphate buffer, pH 6.8, - 0.01 M 2-mercaptoethanol - 20% (v/v) of ethylene glycol. Lysozyme was obtained from Sigma, standard proteins used for calibration of Sephadex columns were obtained from Serva. Sephadex, Sepharose and dextrane blue (mol.weight of $2 \times 10^6$) were received from Pharmacia, ion-exchange cellulosesDE-32, CM-32 and P-11 were supplied by Whatman.

Protein concentration was measured by Lowry's method (15) or by the method of Waddel (16).

Radioactivity was measured in a Nuclear Chicago Mark II scintillation spectrometer and in a gas-flow counter made at Kurchatov Institute of the Atomic Energy, USSR.

## RESULTS

### Purification of the uracil-DNA-glycosidase

If not mentioned, all the procedures were carried out at 4 - 8°C. The cells were washed in Buffer A, pH 7.5 by centrifugation at 3000 × g and stored at -20°C. To 40-50 g of the cells resuspended in 200 ml of Buffer A, pH 7.5, were added 20 mg of lysozyme dissolved in water and the mixture was incubated for 30-40 min at 37°C. The lysate was cooled, sonicated (15 Kgc, 10-15 min) and centrifuged for 40 min at 25,000 × g. The transparent supernatant (Fraction I, crude extract) was treated with solid ammonium sulfate to reach 75% saturation. Within 2 hours of adding $(NH_4)_2SO_4$ the mixture was centrifuged for 10 min at 25,000 × g. The sediment was dissolved in 100 ml of Buffer A, pH 7.5, and dialyzed against 10 litres of the same buffer. 150 ml of the dialyzed material referred to as Fraction II were passed through a DE-32 cellulose column (25 × 250 mm) at a flow rate of 0.6 ml/min. More than 90% of the glycosidase activity did not absorb to DE-32 cellulose (Fraction III).

Further purification was carried out by two methods. Method I (see Table I) yields uracil-DNA-glycosidase with a high specific activity (up to $8 \times 10^5$ units/mg of protein) but contaminated with AP-endonuclease and/or uracil-endonuclease. Uracil-DNA-glycosidase isolated by method 2 has a smaller specific activity ($3 \times 10^4$ units/mg of protein) but is free of AP-endonuclease and uracil-endonuclease activity.

According to method 1 Fraction III was dialyzed against 6 litres of Buffer B and applied to a column (35 × 60 mm) of single-stranded uracil-DNA-cellulose equilibrated with the same buffer. The elution was carried out with 2 × 150 ml of a 0.0 to 0.7 M NaCl linear gradient in Buffer B. Uracil-DNA-glycosidase (Fraction IV-1) was eluted in a wide peak between 0.1 and 0.5 M NaCl. Fractions containing activity were combined (60-100 ml), dialyzed against 2.5 litres of Buffer B and concentrated by ultrafiltration through a Diaflo UM-10 membrane. Purification by affinity chromatography was repeated once more. The active fractions eluting between 0.3 and 0.4 M NaCl were pooled to form Fraction V-1 and stored at -10°C. The elution pattern of uracil-

DNA-glycosidase after affinity rechromatography is shown in Fig.1. A summary of the purification procedure (method 1) is given in Table 1.

According to method 2, 100 ml of Fraction III were dialyzed against Buffer A, pH 6.5, and applied to a column (25× 250 mm) of CM-32 cellulose equilibrated with the same buffer. About 10 per cent of uracil-DNA-glycosidase are bound to CM-cellulose at a low ionic strength and then may be eluted between 0.1 and 0.2 M NaCl giving Fraction IV-2. At step IV-2 uracil-DNA-glycosidase is separated from UV-endonuclease eluting between 0.2 and 0.25 M NaCl. The active fractions were pooled, concentrated and placed on a Sephadex G-150 column (35× 850 mm). The enzyme was eluted with Buffer A, pH 7.5, containing 0.2 M NaCl. Uracil-DNA-glycosidase was eluted in the range of 16,000 molecular weight(Fraction V-2) together with an AP-endonuclease III isozyme. The specific activity of uracil-DNA-glycosidase (Fraction V-2) was about 16,000 units/mg, the protein concentration was 0.032 mg/ml. The active fractions were concentrated, dialyzed against Buffer B and then applied to an uracil-DNA-cellulose column (10× 20 mm). The elution was performed with 2× 65 ml of a 0.0 to 0.7 M NaCl linear gradient in the same buffer. The specific activity of the enzyme (Fraction VI-2) at this stage was about 30,000 units/mg, the protein concentration was 0.026 mg/ml. The enzyme was free of the UV-endonuclease and AP-endonuclease activities.

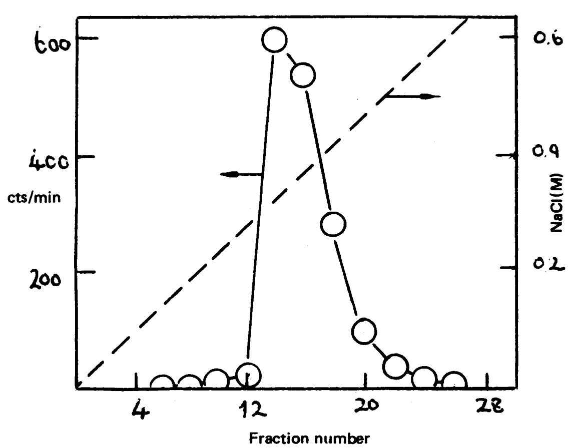


Fig. 1. Elution pattern of uracil-DNA-glycosidase from a DNA-cellulose column. For experimental details see "Results".

Table 1. Purification of uracil-DNA-glycosidase (method 1)

| Step | Fraction | Specific activity (units/mg) | Purification factor | Protein (mg/ml) |
|---|---|---|---|---|
| crude extract | I | 5600 | 1 | 8.5 |
| $(NH_4)_2SO_4$ fractionation | II | 2300-5300 | 1 | 7.5-13 |
| DEAE-cellulose | III | 18,000 | 3.2 | 1 |
| uracil-DNA-cellulose | IV-1 | 55,000 | 10 | 0.006 |
| uracil-DNA-cellulose rechromatography | V-1 | 800,000 | 143 | 0.003 |

Some properties of the uracil-DNA-glycosidase.

Fraction III of uracil-DNA-glycosidase has a pH optimum at 7.5; at pH 6.0 and 8.0 approximately 85% of the maximal activity was observed. Uracil-DNA-glycosidase activity towards deaminated DNA treated with nitrous acid and poly d(A-U) is not stimulated by the presence of 10 mM $MgCl_2$ and is not inhibited by the low concentration of EDTA (1 mM). It should be noted that uracil-endonuclease from M.luteus is not stimulated by the presence of 5 to 10 mM $MgCl_2$ at pH 7.0 and pH 9.25 and not inhibited by 1mM EDTA either.

As Fig.2 illustrates, the uracil-DNA-glycosidase (Fraction V-1) elutes from a Sephadex G-100 column after chymotrypsinogen A and immediatly before cytochrome C, that is in the range of about 16,000 molecular weight. Using Sephadex G-150, the enzyme (Fraction V-2) was found to have practically the same molecular weight which indicates that the both methods (1 and 2) yield one and the same enzyme. As seen below, uracil-endonuclease from M.luteus is copurified with AP-endonuclease II (molecular weight 33,000) upon gel filtration, and hence, has a higher molecular weight compared to uracil-DNA-glycosidase.

Uracil-DNA-glycosidase activity towards E.coli DNA treated with sodium bisulfite

It is clear that uracil-DNA-glycosidase can recognize uracil in the geteroduplex U:G pairs in double-stranded DNA deaminated with nitrous acid. As it is known, that depending on conditions, the U:G pairs in synthetic polyribonucleotides may be both of intra- and extrahelical conformation (26).

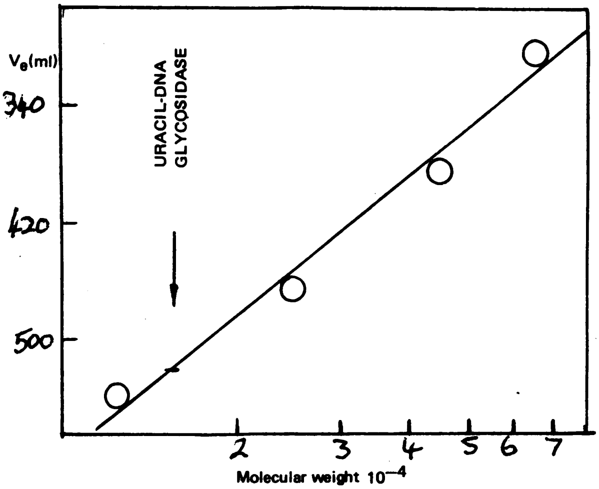


Fig. 2. Gel filtration of uracil-DNA-glycosidase on a Sephadex G-100 column (35×900 mm). Fraction V-1 of the enzyme (10 ml) was applied to the column and eluted with Buffer A, pH 7·5 – 0·2 M NaCl. (–o–) – $V_e$ of the reference proteins: cytochrome C (12,500), egg albumin (45,000) and bovine serum albumin (67,000). Arrow indicates $V_e$ of uracil-DNA-glycosidase.

If uracil-DNA-glycosidase recognizes extrahelical base pair conformation it must work symmetrically, releasing not only uracil but also guanine from double-stranded DNA containing the U:G pairs. To check this possibility we have prepared hybrids containing uracil in the unlabeled strands deaminated with bisulfite and $^{14}$C-guanine in the complementary untreated strands. The introduction of alkali-labile sites (apurinic sites and breaks) in the labeled strands of the hybrids by uracil-DNA-glycosidase (Fraction V-1) was analyzed by sedimentation on alkaline sucrose gradients. Single-strand breaks in the $^{14}$C-guanine labeled strands were not detected. However, as can be seen from Fig.3, the enzyme introduces single-strand breaks in the $^{14}$C-guanine-labeled DNA treated with sodium bisulfite. Thus, it may be concluded that the DNA base mismatching is unessential for uracil-DNA-glycosidase. The enzyme recognizes uracil not only in the heteroduplex U:G pairs in double-stranded DNA treated with nitrous acid, and in single-stranded DNA treated with bisulfite, but also in the homoduplex U:A pairs in poly d(A-U).

The possibility of the release of xanthine, a product of guanine deamination, from the DNA was also checked. For this purpose the $^{14}$C-guanine-labeled E.coli DNA was treated with $NaNO_2$ and incubated with Fraction V-1 of uracil-DNA-glycosidase. The incubation mixture was placed on a silicagel plate together with unlabeled xanthine, and the plate was developed. Radioactivity has not been found in the spot of xanthine.

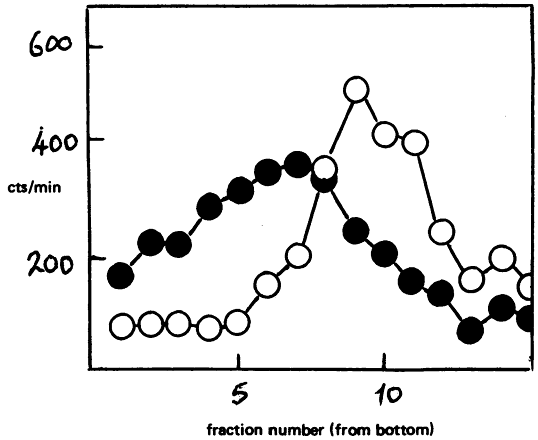


Fig. 3. Effect of uracil-DNA-glycosidase on sodium bisulfite-treated $^{14}$C-guanine-labeled single-stranded DNA. 1200 units/ml of the enzyme (Fraction V-1) (—o—); no enzyme (—•—) for 90 min at 37°C. The reaction was stopped by adding an equal volume of 0.5 M NaOH, a 0.2 ml sample was placed on a 4.7 ml alkaline sucrose gradient (5), centrifuged in an SW-65 rotor for 17 hr at 22,000 rpm at 20°C. The gradients were fractionated and acid-insoluble radioactivity was measured.

**AP-endonucleases from *M.luteus***

The endonuclease activity of *M.luteus* towards DNA heated at acid pH can be separated into five components by ion-exchange chromatography and gel filtration. A summary of the purification procedure is given in Fig.4. Purification of AP-endonucleases I and II has been described in detail elsewhere (5,6, 7). Separation of AP-endonucleases I, III and IV by carboxymethylcellulose chromatography is shown in Fig.5. AP-endonuclease III elutes at about 0.15 M NaCl, contains a low level of UV-endonuclease activity, has a molecular weight of about 14,000 (gel filtration), is unstable upon storage, and contrary to other AP-endonucleases is inhibited by $MgCl_2$. AP-endonuclease I elutes at 0.2 M NaCl together with UV-endonuclease I (7). Carboxymethylcellulose rechromatography results in congruent profiles of the two activities. It has been shown (5,6) that AP-endonuclease I and UV-endonuclease I activities are associated with one and the same protein. AP-endonuclease IV elutes from a carboxymethylcellulose column at 0.26 M NaCl.

AP-endonuclease V is copurified with an endonuclease specific for alkali-stable lesions in $\gamma$-irradiated DNA but differs from the latter enzyme (17,18). AP-endonuclease I and II are clearly specific for apurinic sites in the DNA heated at acid pH (6,7). The number of breaks induced in depurinated *colE1* DNA by AP-endonuclease V is practically equal to that induced by AP-endonuclease I (Table 2) and hence AP-endonuclease V is also specific for apurinic sites. Single-strand breaks in-

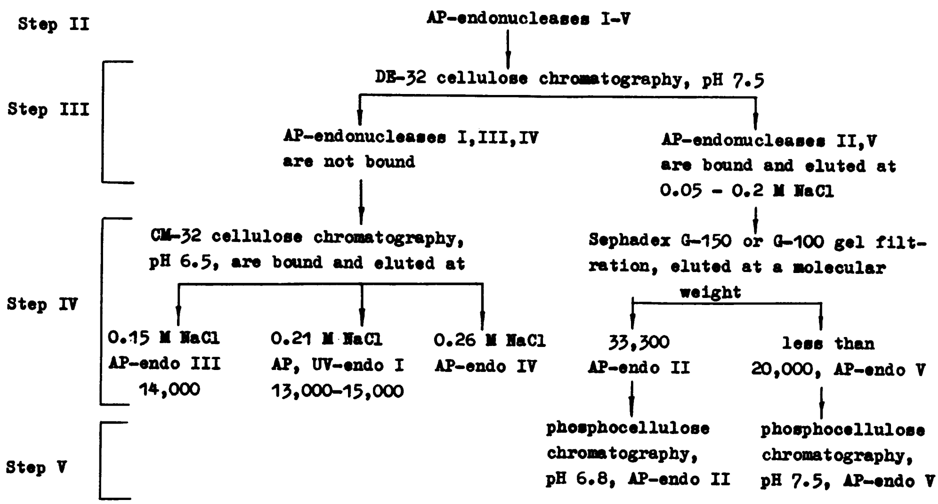


Fig. 4. Purification of AP-endonucleases from M. luteus.

duced by AP-endonucleases I and V (Table 2) and by AP-endonuclease II (7) can be repaired in vitro by DNA-polymerase from M.luteus and phage T4 ligase and, therefore have a 3'OH-5'$PO_4$ structure. The specificity of AP-endonucleases I and V towards apurinic and apyrimidinic sites is supported by the data presented in the following section as well.

Table 2. Excision repair of depurinated (70°C, 150 min, pH 6.0) colE1 DNA.

| Variant | Number of breaks per colE1 DNA molecule |
|---|---|
| AP-endonuclease I | 1.30 |
| AP-endonuclease I + DNA-polymerase + ligase | 0.34 |
| AP-endonuclease V | 1.35 |
| AP-endonuclease V + DNA-polymerase + ligase | 0.64 |

Experimental conditions have been described in detail elsewhere (7). The concentration of AP-endonuclease I was 20 μg/ml and that of AP-endonuclease V was 15 μg/ml. The data represent the average number of breaks obtained from two experiments. T4 polynucleotide ligase was a generous gift of Dr. V.Tanyashin and Dr. A.Solonin.

**Uracil-DNA-glycosidase generates substrate sites for AP-endonucleases.**

Uracil-DNA-glycosidase purified by method I (Fraction V-1) is found to contain a considerable activity of AP-endonuclease; it induces 0.65 breaks in depurinated (70°C, 150 min, pH 6.0) colE1 DNA for 20 min at 37°C. Uracil-DNA-glycosidase (Fraction VI-2) is separated completely from AP-endonucleases and UV-endonuclease by method 2.

The elution patterns of the $^3$H-dATP-labeled poly d(A-U) from a Sepharose 4B column shown in Fig. 6a indicate that there is no degradation of poly d(A-U) induced by Fraction VI-2 of uracil-DNA-glycosidase which is free of AP-endonuclease activi-

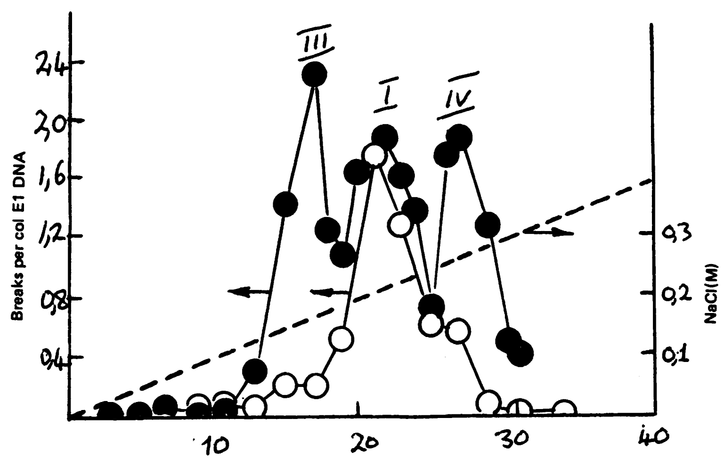


Fig. 5. Separation of AP-endonucleases I, III and IV by Carboxymethylcellulose chromatography. 250 ml of uracil-DNA-glycosidase (Fraction III) were passed through a column (25×250), washed with 150 ml of Buffer A, pH 6.5, and the proteins were eluted with 2×500 ml of a 0 to 0.5 M NaCl linear gradient in the same buffer. The endonucleolytic activity was determined by agarose slab gel electrophoresis: depurinated (70°C, 150 min, pH 6.0, —•—) or UV-irradiated (10 J·m$^{-2}$, —o—) colE1 DNA were used as substrates.
I, III and IV – AP-endonucleases I, III and IV.

ty, while Fraction V-1 which contains AP-endonuclease activity, induces degradation of the polymer. The elution profile of poly d(A-U) incubated without the enzymes (not shown) is coincided with that incubated with Fraction VI-2.

Analogous data were obtained with the $^3$H-dUTP-labeled po-

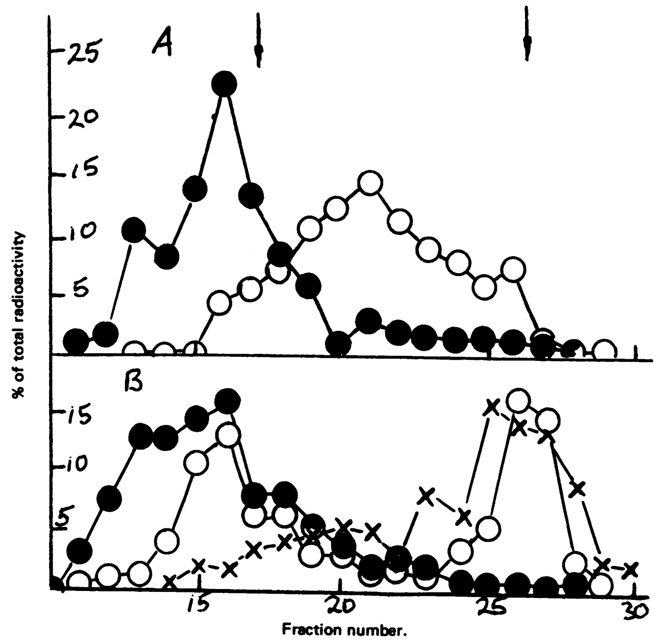


Fig. 6. The effect of uracil-DNA-glycosidase and AP-endonuclease I on poly d(A-U). A $-^{3}$H-dATP-labeled poly d(A-U) with 600 units/ml of uracil-DNA-glycosidase (Fraction VI-2) (—•—) or Fraction V-1 (—o—). B — $^{3}$H-dUTP-labeled poly d(A-U) without enzymes (—•—), with 600 units/ml of uracil-DNA-glycosidase (Fraction VI-2) (—o—), with Fraction VI-2 and 12 $\mu$g/ml of AP-endonuclease I (—x—). Other experimental details and designations are the same as in the legend to Fig. 7.

ly d(A-U) (Fig.6b). Fraction VI-2 of uracil-DNA-glycosidase induces the release of uracil (radioactivity in monomer fractions) without breakage of the polymer. Adding AP-endonuclease I to the reaction mixture results in both depolymerization of poly d(A-U) and the release of uracil. The action of AP-endonucleases II and V resultsin degradation of the $^{3}$H-dATP-labeled poly d(A-U) in the presence of uracil-DNA-glycosidase (Fraction VI-2) as well (Fig.7a,b). Contrary to AP-endonucleases I and V, AP-endonuclease II acts on the $^{3}$H-dATP-labeled poly d(A-U) in the absence of uracil-DNA-glycosidase but shows no glycosidase activity. Certainly, our preparation of AP-endonuclease II possesses uracil-endonuclease activity.

A surprising result was obtained when AP-endonuclease III was added to the reaction mixture containing the $^{3}$H-dATP-labeled poly d(A-U) and Fraction VI-2 of uracil-DNA-glycosidase. As can

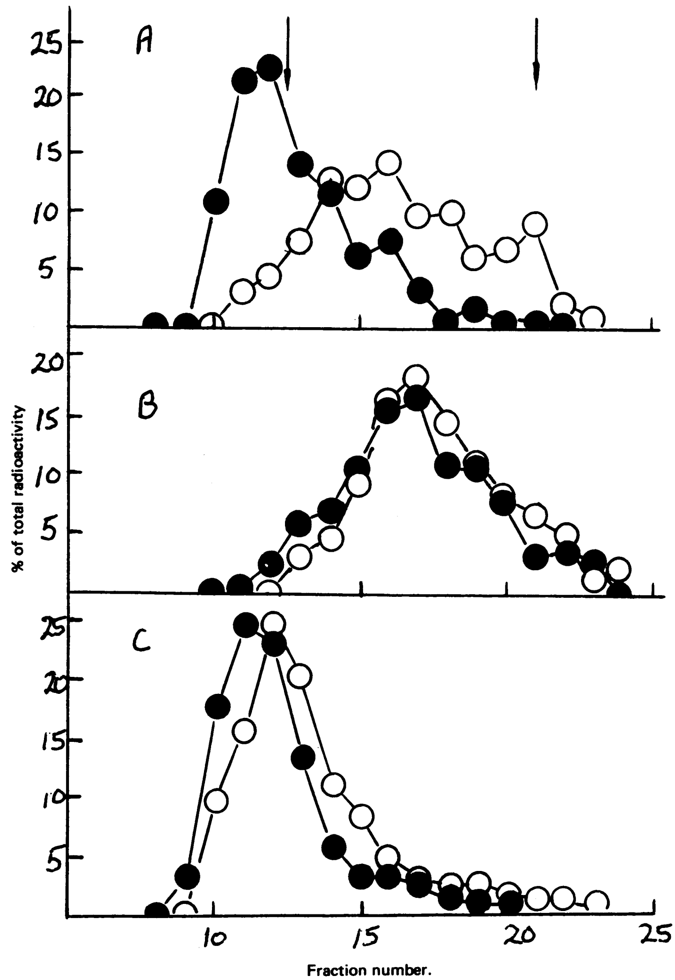


Fig. 7. Uracil-DNA-glycosidase-induced substrate sites in $^{3}$H-dATP-labeled poly d(A-U) for different AP-endonucleases. The composition of the incubation mixture (total volume 200 $\mu$l) was: 3000 c.p.m. of $^{3}$H-poly d(A-U), 600 units/ml of uracil-DNA-glycosidase (Fraction VI-2) and 2.5 $\mu$g/ml of AP-endonuclease V (A), or 1 $\mu$g/ml of AP-endonuclease II (B), or 40 $\mu$g/ml of AP-endonuclease III (C), 0.005 M Tris-HCl buffer, pH 8.0, 0.0005 M EDTA. After 60 min incubation at 37°C the mixture was applied to a Sepharose 4B column (6x70mm) and eluted with 0.01 M Tris-HCl buffer, pH 8.0 – 0.001 M EDTA. 120 $\mu$l fractions were collected into filter paper discs ($\phi$ 2.5 cm), dried and radioactivity was counted.
(—●—) – AP-endonuclease (V, II and III in A, B and C, respectively); (—○—) – AP-endonuclease (V, II, III in A, B, C, respectively) with uracil-DNA-glycosidase (Fraction VI-2). Arrows indicate the elution position of dextran blue (2x10$^{6}$, left) and dTMP (350, right).

be seen from Fig.7c neither AP-endonuclease III alone nor the endonuclease together with the glycosidase induces substantional degradation of poly d(A-U). This may indicate that AP-endonuclease III cannot recognize apyrimidinic sites formed by uracil-DNA-glycosidase.

## DISCUSSION

The properties of uracil-DNA-glycosidase from M.luteus described here are in accord with those of analogous enzyme from E.coli (9,19). It is evident, that the two glycosidases differ from other repair enzymes such as UV-endonucleases, AP-endonucleases and uracil-endonucleases. Uracil-DNA-glycosidase from M.luteus liberates uracil from both the DNA deaminated by nitrous acid and sodium bisulfite, and from poly d(A-U) generating substrate sites for AP-endonucleases (i and V). The uracil-DNA-glycosidase is not stimulated by $MgCl_2$, has an optimum at slightly alkaline pH and a molecular weight of about 16,000. The enzyme does not act on guanine residues opposite uracil in double-stranded DNA and on xanthine in deaminated DNA.

It is shown that different AP-endonucleases, which induce breaks in DNA heated at slightly acid pH, can be isolated from M.luteus and some of them (AP-endonucleases I, II and V) induce breaks that can be repaired by DNA-polymerase from M.luteus and phage T4 ligase. AP-endonucleases I and V recognize apurinic and apyrimidinic sites. AP-endonuclease III cannot cleave poly d(A-U) at apyrimidinic sites formed by uracil-DNA-glycosidase. Thus, it is possible that AP-endonuclease III is specific for heat-induced lesions other than the apurinic sites. Evidence is presented for the existence of uracil-endonuclease in M.luteus. Thus M.luteus as well as E.coli (19) possesses two pathways of the excision repair of uracil in the DNA; one is mediated by uracil-DNA-glycosidase and AP-endonucleases, another - by uracil-endonuclease. This can explain the normal growth of E.coli cells deficient in uracil-DNA-glycosidase (21).

Calculations show that the activity of uracil-DNA-glycosidase in one cell of M.luteus is sufficiently high to release from DNA 10-100 uracil residues per hour. According to Baltz et al. (3), the rate for spontaneous deamination of cytosine at

$37^{o}C$ in a cell is $4 \times 10^{-8}$ /G:C pair/day. This gives about 0.01 cytosine deamination per M.luteus genome per hour. Thus, the activity of uracil-DNA-glycosidase in M.luteus is at least 1000 times higher than it is necessary to repair uracil arising from spontaneous deamination of cytosine. The enzyme may be also used for the release of uracil incorporated into DNA during replication (20).

Excision repair of spontaneous DNA lesions (apurinic sites and uracil) may be very important in the maintenance of genetic stability of mammalian cells. Really, apurinic-endonuclease activity is altered in group A and group D Xeroderma pigmentosum cells (22) and is reduced in a line of Ataxia teleangiectasia (23). These defects might account for the neurological disorders in these patients (24). Uracil-DNA-glycosidase has now also been found in mammalian cells (25).

## REFERENCES


1 Zamenhof S., (1960), Proc.Natl.Acad.Sci.USA, 46, 101-105
2 Lindahl T. and Nyberg B., (1972), 13, 3405-3410
3 Baltz R.H., Bingham P.M. and Drake J.W., (1976), Proc.Natl. Acad.Sci.USA, 73, 1269-1273
4 Strauss B.S. and Robbins M., (1968), Biochim.Biophys.Acta, 461, 68-79
5 Tomilin N.V., (1974), Molekuljarnaya Biologiya (Russian), 8, 557-568
6 Tomilin N.V., Paveltschuk E.B. and Mosevitskaya T.V., (1976), Eur.J.Biochem., 69, 265-272
7 Tomilin N.V. and Barenfeld L.S., (1977a), Biochimia (Russian) 42, 985-993
8 Lindahl T., Proc.Natl.Acad.Sci.USA, (1974), 71, 3649-3653
9 Lindahl T., Ljungquist S., Siegert S., Nyberg B. and Sperens B., (1977), J.Biol.Chem., 252, 3286-3294
10 Marmur J., (1961), J.Mol.Biol., 3, 208-218
11 Matsuda M. and Ogoshi H., (1966), Biochim.Biophys.Acta, 119, 210-212
12 Hadi S.N. and Goldthwait D.A., (1971), Biochemistry, 10, 4986-4995
13 Hamilton L., Mahler I. and Grossman L., (1974), Biochemistry, 13, 1886-1898
14 Tomilin N.V., Paveltschuk E.B. and Mosevitskaya T.V., (1977), Tsitologija (Russian), 19, 417-426
15 Lowry O.H., Rosenbrough N.J., Farr A.E. and Randall R.L., (1951), J.Biol.Chem., 192, 265-275
16 Murphy J.B. and Kies M.W., (1960), Biochim.Biophys.Acta, 45, 382-385
17 Tomilin N.V. and Barenfeld L.S., (1977b), Biochimia (Russian) 42, 1173-1183
18 Tomilin N.V. and Barenfeld L.S., (1977), Int.J.of Rad.Biol., in the press

19 Gates F.T. and Linn S., (1977), J.Biol.Chem., 252, 1647-1653
20 Tye B.K., Nyman P.O., Lehman I.R., Hochhauser S. and Weiss B. Proc.Natl.Acad.Sci.USA, (1977), 74, 154-157
21 Duncan B.K., Rockstron P.A. and Warner H.R., (1976), Fed. Proc., 35, 1493 (Abstr. 685)
22 Kühnlein U., Penhoet E.E. and Linn S., (1976), Proc.Natl. Acad.Sci.USA, 73, 1169-1173
23 Tomilin N.V., Aprelikova O.N. and Barenfeld L.S., unpublished observations.
24 McKusick V.A., (1970), Mendelian inheritance in man. The John Hopkins Press, Baltimore and London
25 Lindahl T., (1976), Mutat.Res., 46, 138 (A)
26 Lomant and Fresco J.R., (1975), In: Progress in Nucleic Acids Research and Molecular Biology, 15, 185-218