A Mollicute (Mycoplasma) DNA Repair Enzyme: Purification and Characterization of Uracil-DNA Glycosylase

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The DNA repair enzyme uracil-DNA glycosylase from Mycoplasma lactate (831-C4) was purified 1,657-fold by using affinity chromatography and chromatofocusing techniques. The only substrate for the enzyme was DNA that contained uracil residues, and the $K_m$ of the enzyme was $1.05 \pm 0.12 \mu M$ for dUMP containing DNA. The product of the reaction was uracil, and it acted as a noncompetitive inhibitor of the uracil-DNA glycosylase with a $K_i$ of 5.2 mM. The activity of the enzyme was insensitive to Mg$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Ca$^{2+}$, and Co$^{2+}$ over the concentration range tested, and the activity was not inhibited by EDTA. The enzyme activity exhibited a biphasic response to monovalent cations and to polyamines. The enzyme had a $pI$ of 6.4 and existed as a nonspherical monomeric protein with a molecular weight of 28,500 ± 1,200. The uracil-DNA glycosylase from Mycoplasma lactate was inhibited by the uracil-DNA glycosylase inhibitor from bacteriophage PBS-2, but the amount of inhibitor required for 50% inhibition of the mycoplasmal enzyme was 2.2 and 8 times greater than that required to cause 50% inhibition of the uracil-DNA glycosylases from Escherichia coli and Bacillus subtilis, respectively. Previous studies have reported that some mollicutes lack uracil-DNA glycosylase activity, and the results of this study demonstrate that the uracil-DNA glycosylase from Mycoplasma lactate has a higher $K_m$ for uracil-containing DNA than those of the glycosylases of other procaryotic organisms. Thus, the low G + C content of the DNA from some mollicutes and the A · T-biased mutation pressure observed in these organisms may be related to their decreased capacity to remove uracil residues from DNA.

Members of the cell-wall-deficient class Mollicutes are the smallest self-replicating organisms known (17). They possess the smallest genomes of any free-living organism (37, 39), and the G + C content of their DNA is among the lowest known (29). It has been proposed that the low G + C content of their DNA is due to an A · T-biased mutation pressure (33). It has been suggested that variations in the amounts and/or activities of enzymes involved with DNA repair could cause the biased mutation pressure in these organisms (33, 47). However, there have been few studies on DNA repair processes and the enzymes involved with such processes in these organisms (1, 12, 18).

DNA glycosylases are a group of enzymes that remove damaged bases from DNA (7, 24). One enzyme in this group, uracil-DNA glycosylase, specifically removes uracil residues from DNA that arise either by the spontaneous deamination of deoxycytidine monophosphate residues (2, 16, 27) or the misincorporation of dUTP into DNA by DNA polymerase (39, 45). Although uracil-DNA glycosylase has been purified and characterized from several procaryotic (10, 21, 23, 25, 26) and eucaryotic (6, 8, 11, 14, 15, 20, 35, 41) organisms, this enzyme has not been studied in mollicutes. Since little is known concerning the enzymology of DNA repair in these organisms and since uracil-DNA glycosylase may play a role in A · T-biased mutation pressure observed in the mollicutes (33, 47), further characterization of this enzyme seemed necessary.

In this report, we describe the purification of the uracil-DNA glycosylase from Mycoplasma lactate (831-C4). The purified enzyme was characterized with respect to substrate specificity, kinetic properties, divalent cation requirement, pH optimum, and molecular weight.

MATERIALS AND METHODS

Materials. Nonradioactive nucleotides, Blue Sepharose, double-stranded DNA-cellulose, and Poly-U Sepharose were purchased from Sigma Chemical Co., St. Louis, Mo. Polybuffer 74 and Polybuffer exchanger PBE-94 were purchased from Pharmacia Fine Chemicals, Piscataway, N.J. Endonuclease-free Escherichia coli DNA polymerase I was obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. [5-$^3$H]dCTP (28 Ci/mmol), [5-methyl-$^3$H]dTTP (63 Ci/mmol), [8-$^3$H]dATP (12 Ci/mmol), and [8-$^3$H]dGTP (9 Ci/mmol) were purchased from ICN Radiochemicals Inc., Irvine, Calif. [5-$^3$H]dUTP was purchased from Moravek Biochemicals Inc., Brea, Calif.

Organisms. Bacillus subtilis SB19E and SB5 and the bacteriophage PBS2 were obtained from I. Takahashi, McMaster University, Hamilton, Ontario, Canada, and R. B. Guyer, Pennsylvania State University, University Park. These organisms were grown and maintained as described previously (20, 48). Mycoplasma lactate 831-C4, which was originally designated Mycoplasma sp. (Let. 1), was isolated from lettuce by N. L. Somerson, The Ohio State University. This organism was chosen for the study since it contains low levels of alkaline DNase activity, which nonspecifically hydrolyzes DNA containing uracil residues (47). The organism was grown at 37°C in modified Edward medium (3) supplemented with 4% (vol/vol) heat-inactivated (56°C, 1 h) horse serum (Hazleton, Lenexa, Kans).

Uracil-DNA glycosylase assay. Uracil-DNA glycosylase activity was determined by using a modification of the procedure of Caradonna and Cheng (6). Briefly, the reaction mixture contained the following in a total volume of 0.2 ml: 50 mM Tris hydrochloride (pH 7.5), 100 µg of bovine serum albumin per ml, 20 mM dithiothreitol, 3 to 5 µg of calf thymus double-stranded DNA containing $[^3$H]uracil residues (specific activity, 99 Ci/µmol or uracil, 0.041 nmol of

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dUMP(μg of DNA), 10 mM EDTA, and the enzyme sample (0.2 to 9 μg of protein). DNA containing either radiolabeled uracil, cytosine, guanine, thymine, or adenine residues was prepared by nick translation with the radioactive triphosphate as described by Rigby et al. (38). After incubation at 37°C, the reactions were terminated, and the acid-soluble radioactivity was determined as described previously (6). A unit of uracil-DNA glycosylase activity was defined as the amount of enzyme required to release 1 nmol of uracil as trichloroacetic acid-soluble material per min at 37°C. Products of the reaction were determined by thin-layer chromatography as described by Beardsley and Abelson (4).

**Enzyme purification.** All buffers contained 0.1 mM phenylmethylsulfonyl fluoride to prevent proteolytic digestion of the enzyme. Unless otherwise stated, all purification procedures were performed at 4°C. Protein elution from the various matrices was monitored spectrophotometrically at 280 nm.

(i) **Crude extract.** Fifteen liters of culture (containing approximately 1.5 × 10^12 cells) was harvested during the mid-log growth phase and washed as described previously (3). The cell pellet was suspended in 150 ml of TMGE buffer (10 mM Tris hydrochloride [pH 7.5], 2 mM 2-mercaptoethanol, 10% [vol/vol] glycerol, and 1 mM EDTA), and the cells were disrupted by sonication using a Branson model 350 sonifier (four 1-min pulses with a microprobe output setting of 4, 40% duty cycle). The resulting homogenate was centrifuged (30,000 × g, 30 min), and the supernatant, designated fraction 1, was used as the source of uracil-DNA glycosylase.

(ii) **Blue Sepharose chromatography.** Fraction 1 was applied to a Blue Sepharose column (3 by 40 cm) equilibrated in TMGE buffer. Uracil-DNA glycosylase activity was eluted with a linear gradient of increasing ionic strength by using 250 ml each of TMGE buffer and TMGE buffer containing 2 M KCl. Fractions were collected in 5-ml portions. Uracil-DNA glycosylase eluted as a single peak at a KCl concentration of 0.95 M. The fractions containing the enzyme activity were pooled and dialyzed overnight against TMGE buffer (fraction 2).

(iii) **DNA-cellulose chromatography.** Fraction 2 was applied to a double-stranded DNA-cellulose column (2.5 by 20 cm) that had been equilibrated in TMGE buffer. Uracil-DNA glycosylase activity was eluted with a linear gradient of increasing ionic strength by using 250 ml each of TMGE buffer and TMGE buffer containing 2 M KCl. Fractions were collected in 5-ml portions. The enzyme was eluted as a single peak at a KCl concentration of 0.34 M. Fractions containing uracil-DNA glycosylase activity were pooled and dialyzed overnight against KG buffer (10 mM potassium phosphate [pH 7.5], containing 10% [vol/vol] glycerol) (fraction 3).

(iv) **Poly-U Sepharose chromatography.** Poly-U Sepharose chromatography was performed with a Pharmacia fast-protein liquid chromatography system. The separation was performed at 25°C, but the column and all buffers were cooled to 4°C. Fractions were collected on ice. Fraction 3 was applied to a Poly-U Sepharose column (1 to 10 cm) that had been equilibrated in KG buffer. Uracil-DNA glycosylase activity was eluted with a linear gradient of increasing ionic strength by using 80 ml each of KG buffer and KG buffer containing 1 M KCl. Fractions were collected in 4-ml portions. Uracil-DNA glycosylase activity eluted in a single peak at a KCl concentration of 0.45 M. Fractions were pooled and dialyzed overnight against IH buffer (14 mM imidazole hydrochloride [pH 8.3]) (fraction 4).

(v) **Chromatofocusing chromatography.** Fraction 4 was applied to a PBE-94 column (1.5 by 40 cm) equilibrated in IH buffer. Uracil-DNA glycosylase activity was eluted from the matrix by using 300 ml of Polybuffer 74 (pH 5.0). Fractions were collected in 4-ml portions. Uracil-DNA glycosylase activity eluted as a single peak at a pH of 6.4. Fractions containing uracil-DNA glycosylase activity were pooled and stored at 4°C (fraction 5).

**PBS-2 uracil-DNA glycosylase inhibitor.** The PBS-2 uracil-DNA glycosylase inhibitor was partially purified from PBS-2-infected *B. subtilis* as described previously (9). Inhibitor activity was determined in both a HEPS (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)-buffered system (HEP) and a Tris-buffered system (TRI). The HEP system contained the following in a total volume of 0.2 ml: 70 mM HEPS-KOH (pH 7.8), 1 mM EDTA, 1 mM dithiothreitol, 0.1% bovine serum albumin, 4 μg of [3H]uracil-labeled calf thymus DNA (specific activity, 66.4 μCi/μmol of uracil, 0.053 nmol of [3H]dUMP/μg of DNA), uracil-DNA glycosylase (0.002 to 0.003 U), and inhibitor (0.05 to 3.5 μg of protein). The TRI system contained the following in a total volume of 0.2 ml: 50 mM Tris hydrochloride (pH 7.5), 2 mM dithiothreitol, 10 mM EDTA, 0.1% bovine serum albumin, 4 μg of [3H]uracil-labeled calf thymus DNA (specific activity, 66.4 μCi/μmol of uracil, 0.053 nmol of [3H]dUMP/μg of DNA), uracil-DNA glycosylase (0.002 to 0.003 U), and inhibitor (0.05 to 3.5 μg of protein). The inhibitor was preincubated with uracil-DNA glycosylase for 15 min at 37°C in the appropriate buffer system, and the reaction was started by the addition of substrate. A unit of PBS-2 inhibitor was defined as the amount of inhibitor required to neutralize a unit of *B. subtilis* uracil-DNA glycosylase under our assay conditions.

**Molecular weight determination.** The molecular weight of uracil-DNA glycosylase was determined by sucrose gradient ultracentrifugation and gel filtration chromatography (30, 40). Sucrose gradient centrifugation was carried out in a linear 5 to 20% (wt/vol) sucrose gradient (50 mM Tris hydrochloride [pH 7.5], 2 mM 2-mercaptoethanol, 100 mM NaCl) at 40,000 rpm for 28 h in an SW41-Ti rotor (Beckman Instruments, Inc.) at 4°C. Approximately 150 μg of each of the protein standards (bovine serum albumin, ovalbumin, chymotrypsigenin A, and lysozyme) and 0.034 U of the purified *M. lactucae* uracil-DNA glycosylase were layered on separate gradients. After centrifugation, each gradient was fractionated into 0.4-ml fractions, and each fraction was assayed for either protein and/or enzyme activity and sucrose concentration. Stokes' radius was determined as described previously (30) with a Sephadex G-75 column (1.5 by 60.5 cm) equilibrated in 10 mM Tris hydrochloride (pH 8.0) containing 0.2 M KCl. The column was calibrated with bovine serum albumin (M, 67,000), ovalbumin (M, 43,000), chymotrypsigenin A (M, 25,000), and lysozyme (M, 13,400). The subunit composition of uracil-DNA glycosylase was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 12.5% polyacrylamide gels as described previously (5) with a Bio-Rad Mini-Protein II apparatus. Before electrophoresis a sample of fraction 5 was concentrated approximately 25-fold by precipitation with ammonium sulfate (80% saturation). The precipitate was resuspended in TMGE buffer and dialyzed against this buffer for 12 h at 4°C. After electrophoresis at 200 V for 45 min, gels were either stained for proteins by the silver staining procedure of Tunon and Johansson (42) or reconstituted to detect enzyme activity by the procedure of Domena et al. (15).

**Other procedures.** Protein was determined by using the Coomassie blue dye binding method as recommended by...
Bio-Rad Laboratories with bovine serum albumin as the standard. Radiolabeled uracil containing RNA was prepared from *M. lacteae* (831-C4) by growing the cells (8 × 10^8) in Edwards medium containing 0.05 μCi of [5-^3H]uridine per ml for 48 h at 37°C. The DNA and RNA from *M. lacteae* (831-C4) were purified by our modification of the technique of Marmur as described by Maniatis et al. (28). Alkaline DNase and endonuclease activities were determined as described by Pollack and Hoffmann (36) with calf thymus DNA containing [1^4C]thymidine residues (23,000 cpm/μg of DNA) for the DNase assay and plasmid pBR322 for the endonuclease assay.

### RESULTS

**Purification of uracil-DNA glycosylase.** The summary of a typical purification of the uracil-DNA glycosylase is shown in Table 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated that the fraction 5 preparation contained two protein species (Fig. 1A). The major protein species exhibited a molecular weight of 28,500 ± 2,000 (five determinations) and a minor protein species with a molecular weight of 41,000 ± 2,000 (two determinations). Since we can detect bands containing 5 ng of protein, we have estimated that these two proteins composed 99% of the protein in these preparations. The major protein species was identified as uracil-DNA glycosylase by renaturation of the sodium dodecyl sulfate-polyacrylamide gel (Fig. 1B). The *R*_f for both the major protein species and the renatured uracil-DNA glycosylase activity was 0.72. Enzymatic analyses of fraction 5 preparations demonstrated that they were free of other enzymatic activities such as alkaline DNase and endonuclease.

There was no significant loss of uracil-DNA glycosylase activity in fraction 5 preparations stored at 4 or -20°C for at least a month. However, repeated freezing and thawing of the enzyme resulted in a significant loss of activity (data not shown).

**Substrate specificity and kinetic analysis.** The purified uracil-DNA glycosylase did not exhibit any significant hydrolysis of DNA containing [3H]adenine, [3H]thymine, [3H]cytosine, or [3H]guanine residues or [3H]uridine-labeled RNA from *M. lacteae* (data not shown). The only substrate for the enzyme was DNA containing [3H]uracil residues, and only [3H]uracil was released during the reaction as determined by thin-layer chromatography. There was no significant difference in the rate of hydrolysis of calf thymus or *M. lacteae* DNA containing [3H]uracil residues, nor was there a significant difference in the rate of hydrolysis of native or heat-denatured DNA (data not shown).

The initial velocity of the enzyme was measured by using native calf thymus DNA containing different amounts of [3H]uracil residues (Fig. 2A). Under the conditions of the assays, less than 50% of the substrate was hydrolyzed. The results were converted into a Lineweaver-Burk plot, and the Michaelis constant (K_m) was estimated to be 1.05 ± 0.12 μM for dUMP containing DNA (Fig. 2B). Uracil acted as an inhibitor of the uracil-DNA glycosylase. The mechanism of uracil inhibition of uracil-DNA glycosylase was examined as described by Dixon and Webb (13) with various concentrations of uracil in different series of fixed substrate concentrations. Uracil acted as a noncompetitive inhibitor of the enzyme with a K_i of 5.2 mM (Fig. 2B).

**Biochemical and physical characteristics.** The effect of the monovalent cations K^+*, Na^+*, and NH_4^+ on uracil-DNA glycosylase activity is shown in Table 2. At concentrations between 20 and 100 mM, all of the monovalent cations stimulated the activity of the enzyme. However, at concentrations greater than 200 mM, there was a slight inhibition of enzyme activity.

Uracil-DNA glycosylase did not require a divalent cation for activity. In the absence of EDTA the addition of Mg^2+, Mn^2+, Zn^2+, Cu^2+, Ca^2+, and Co^2+ over the concentration range of 1 to 5 mM did not have any effect on the activity of the enzyme (data not shown).

Polyamines had a significant effect on the activity of the uracil-DNA glycosylase (Fig. 3). With native calf thymus DNA as the substrate, spermidine stimulated the enzyme activity to the greatest degree (150%), whereas putrescine stimulated the enzyme the least (25%). The enzyme exhibited a biphasic response to spermine, with maximum stimulation (75%) occurring at 50 μM; higher concentrations caused a slight decrease in the activity of the enzyme. Under the conditions that we employed, the polyamines did not cause any significant release of [3H]uracil from the DNA in the absence of uracil-DNA glycosylase (data not shown).

The enzyme exhibited a narrow pH optimum, with maximal activity occurring over the pH range of 7.2 to 7.6. Only 30 and 40% of the uracil-DNA glycosylase activity remained at pH 6.1 and 8.0, respectively (data not shown).
FIG. 2. Initial velocity studies of uracil-DNA glycosylase. (A) Rate of uracil release from uracil-containing DNA. Initial velocities were determined by using the standard assay, except that the concentration of dUMP in the DNA was varied from 0.24 to 1.75 µM. The amount of purified enzyme used was 0.002 U per assay. (B) Double-reciprocal plot of initial velocity. (C) Effect of uracil on uracil-DNA glycosylase activity. The standard enzyme assay was employed by using a series of fixed DNA concentrations with a dUMP concentration in the DNA between 0.24 and 0.97 µM and variable concentrations of free uracil from 0.1 to 7.6 mM. Approximately 0.002 U of purified uracil-DNA glycosylase was used per assay. Data were plotted as described by Dixon and Webb (13). Concentration of dUMP in DNA (symbols): ●, 0.24 µM; ○, 0.39 µM; ■, 0.49 µM; □, 0.58 µM; △, 0.73 µM; ▲, 0.97 µM.

In a 5 to 20% sucrose gradient containing 100 mM NaCl the *M. lactucae* uracil-DNA glycosylase sedimented as a single peak of activity with a sedimentation coefficient of 2.54. The Stokes radius was calculated at 3.4 nm by using gel filtration chromatography. Assuming a partial specific volume of 0.725 cm³/g, a molecular weight of 33,500 was calculated by using the Svedberg equation. The frictional coefficient (f/f₀) was calculated to be 1.51, indicating that the uracil-DNA glycosylase has a nonspherical shape.

**Uracil-DNA glycosylase inhibitor.** The effect of the partially purified PBS-2 uracil-DNA glycosylase inhibitor on the activities of uracil-DNA glycosylases in crude cellular extracts from *B. subtilis*, *E. coli*, and *M. lactucae* is shown in Fig. 4. Regardless of the buffer system employed, the enzyme from *M. lactucae* was more resistant to inhibition by the PBS-2 inhibitor than the enzymes from *B. subtilis* and *E. coli*. The amounts of inhibitor required to cause a 50% inhibition of the uracil-DNA glycosylase activity in the HEP system were 1.20, 0.55, and 0.15 µg, respectively, for *M. lactucae*, *E. coli*, and *B. subtilis*; in the TRI system, the amounts were 1.25, 0.65, and 0.20 µg. This difference was not due to differences in the amounts of enzyme used in the assays, nor was it due to the proteolytic digestion of the inhibitor in the extracts from *M. lactucae*. Similar results were observed with the purified *E. coli* and *M. lactucae* uracil-DNA glycosylases (data not shown).

**DISCUSSION**

The uracil-DNA glycosylase from *M. lactucae* was purified approximately 1,657-fold by using a combination of affinity and chromatofocusing techniques. Two protein spe-

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**TABLE 2. Effect of monovalent cations on the activity of**
**M. lactucae** uracil-DNA glycosylase**

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* Assays were performed as described in Materials and Methods with 0.02 U of fraction 5 purified uracil-DNA glycosylase. Values represent the average of three determinations ± standard deviations. The salts that were employed were KCl, NaCl, and (NH₄)₂SO₄. ND, Not determined.

**FIG. 3. Effect of polyamines on uracil-DNA glycosylase activity.** Reactions were performed as described in the text with native calf thymus DNA as the substrate. Reaction mixtures contained 0.003 U of the purified enzyme. Reaction mixtures were incubated at 37°C for 60 min. Symbols: ▲, putrescine; □, spermine; ○, spermidine.
composition and molecular weight (10, 23, 26), and stimulation by monovalent cations (10, 23, 26). Conversely, the uracil-DNA glycosylase from *M. lactucae* differed from other procaryotic uracil-DNA glycosylases in that it was more resistant to inhibition by the PBS-2 uracil-DNA glycosylase inhibitor (22), by monovalent cations (10, 23, 26), by the divalent cations Fe$^{2+}$, Zn$^{2+}$, and Co$^{2+}$ (10), and by uracil (10, 21, 23, 26), had no preference for single-stranded DNA as a substrate (10, 23), and had a 40- to 1,000-times-higher $K_m$ for dUMP containing DNA (10, 21, 23, 26).

It has been reported that monovalent cations can modulate the activity of uracil-DNA glycosylase (10, 23, 26). The modulation of the activity of the *M. lactucae* uracil-DNA glycosylase was not dependent upon a particular monovalent cation but rather upon ionic strength. At low ionic strengths (ionic strength of 0.1 or below), the enzyme activity was stimulated, whereas at greater ionic strengths (ionic strength of 0.15 or greater), the activity of the enzyme was inhibited. Thus variations in the ionic strength of the microenvironment during DNA replication or repair may play a role in modulating uracil-DNA glycosylase by promoting the binding of the enzyme to DNA either by increasing the processivity of the enzyme or by changing the structural conformation of the DNA, which gives rise to a better or worse substrate for the enzyme.

The polyamines have also been reported to modulate uracil-DNA glycosylase activity from *Micrococcus luteus* (23) and human lymphocytes (6). Our results demonstrated that the response of the *M. lactucae* uracil-DNA glycosylase to polyamines was similar to that observed with the enzyme from human lymphocytes. Whereas low concentrations of spermidine stimulated enzyme activity, concentrations greater than 50 μM inhibited activity. Conversely, spermidine stimulated the enzyme activity over the concentration range of 25 to 400 μM, whereas putrescine caused a slight stimulation of enzyme activity over the concentration range of 25 to 100 μM but no significant inhibition of activity at higher concentrations. Caradonna and Cheng (6) postulated that the polyamines (spermine) modulated uracil-DNA glycosylase activity by binding both to the enzyme and to DNA. At low concentrations, spermine binds to the enzyme, causing a conformational change in the enzyme that is reflected by a higher affinity of the enzyme for the substrate; at higher concentrations, spermine binds to DNA, creating a substrate that has a lower affinity for the enzyme. This hypothesis can explain how polyamines modulate uracil-DNA glycosylase activity, it can also explain why different polyamines have differential effects on uracil-DNA glycosylase activity. However, further studies are necessary to determine whether polyamines exhibit differential binding to uracil-DNA glycosylases and whether they are synthesized in members of the class Mollicutes.

With the possible exception of *Drosophila melanogaster* (19) and some mollicutes (47), all organisms that have been examined possess uracil-DNA glycosylase activity. Recently it has been demonstrated that there are highly conserved regions both at the DNA level and the amino acid level between the uracil-DNA glycosylases from *E. coli* (43), *Saccharomyces cerevisiae* (35), herpes simplex virus (32), and human placenta (34). The PBS-2 uracil-DNA glycosylase specifically inhibits the activity of uracil-DNA glycosylases from both procaryotic and eucaryotic sources (22), and a recent study has demonstrated that the inhibition of enzyme activity is due to the binding of the inhibitor to the enzyme (44). This suggests that the inhibitor protein must recognize an amino acid sequence that is conserved in the
various uracil-DNA glycosylases. Thus, although neither the DNA sequence for the gene encoding for the uracil-DNA glycosylase from *M. lactucae* nor the amino acid sequence of the protein is known, our results with the PSB-2 uracil-DNA glycosylase inhibitor suggest that there may be some homology, at least at the amino acid level, between the uracil-DNA glycosylase from *M. lactucae* and uracil-DNA glycosylases from other organisms.

It has been suggested that mollicutes must be metabolically constrained by their limited genome size (29) and that they may represent minimal cellular life forms that are capable of self-replication (31). Since deamination of cytosine residues in DNA occurs under physiological conditions (29), the inability to remove uracil residues from DNA would result in A·T transition mutations and ultimately in a decrease in the G+C content of DNA. We reported previously that *Mycoplasma gallisepticum* (31% G+C), *Mycoplasma capricolum* (25% G+C), and *Ureaplasma urealyticum* (26% G+C) lack uracil-DNA glycosylase activity (47) and that all members of the genera *Mycoplasma* and *Ureaplasma* lack dUTPase activity (46). The results of this study demonstrate that, whereas *M. lactucae* (30% G+C) possesses uracil-DNA glycosylase activity, the *Km* of the enzyme for uracil-containing DNA is 40 to 1,000 times greater than that reported for other uracil-DNA glycosylases. These results suggest that the mollicutes may be less efficient than other organisms in their ability to remove uracil residues from DNA. Thus, although further studies are necessary, it is possible that the low G+C content of the DNA from these mollicutes and the A·T biased mutation pressure observed in these organisms may be related to their decreased capacity to remove uracil residues from DNA.

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